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Effects of isoflavones containing soy protein isolate compared with fish protein on serum lipids and susceptibility of low density lipoprotein and liver lipids to in vitro oxidation in hamsters

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The effects of dietary soy protein isolate (SPI), ethanol-extracted SPI (E-SPI) low in isoflavones, and fish protein (FP) on the concentration of blood lipids and the susceptibility of low density lipoprotein (LDL) to copper-induced oxidation were compared in male golden Syrian hamsters fed a moderate hypercholesterolemic semi-purified diet for 10 weeks. SPI, E-SPI, and FP were incorporated into the isonitrogenous experimental diets as protein sources. The SPI group exhibited significantly lower serum total cholesterol concentration compared with the E-SPI group ($P < 0.05$*) and the FP group (*$P < 0.01$*). Both the SPI and E-SPI groups showed lower LDL cholesterol (*$P < 0.001$ *and* $P < 0.05$*, respectively) and less LDL apolipoprotein B (*$P < 0.01$*) compared with the FP group. The distribution pattern of serum lipoprotein cholesterol fractions of the SPI and E-SPI groups were similar to each other, but different from that of the FP group. The lysine/arginine ratio of the three diets was significantly correlated with serum total cholesterol concentration (*$r = 0.462$*,* $P = 0.023$*). The resistance of LDL to copper-induced oxidation was greater in the SPI group than in the E-SPI and FP groups as assessed by the lower concentrations of thiobarbituric acid-reactive substances (TBARS) and the longer lag time required for the formation of conjugated dienes (*$P < 0.01$*). Livers of hamsters fed the FP diet had a higher amount of TBARS than those of hamsters fed SPI (*$P < 0.01$*) and E-SPI (*$P < 0.05$*) diets. The SPI diet showed sparing effects on α-tocopherol contents in both serum and liver. It seems likely that soy isoflavones protect the circulating and membrane lipids by sparing α-tocopherol and endogenous antioxidants.* (J. Nutr. Biochem. 10: 631–637, 1999) *© Elsevier Science Inc. 1999. All rights reserved.*

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Introduction

In a recent study,[1] we found that Taiwanese vegetarians had a lower concentration of plasma total and low density lipoprotein (LDL) cholesterol than control omnivores. LDL of the vegetarians was less susceptible to oxidative modification in vitro. However, these vegetarians did not consume more vegetables and fruits than did the omnivores on per caput per day basis, although their intake was slightly higher on per kilogram body weight basis. Therefore, it seemed that the dietary intakes of vitamin C, β-carotene, flavonoids (i.e., catechin), and dietary fiber could not possibly contribute to the significantly greater resistance of LDL of the vegetarians to in vitro oxidation. However, in addition to the dietary saturated fat and cholesterol, there was a significant difference in intake of soybean products between the vegetarians and omnivores. Thus, we presume that the dietary soybean products may be responsible for the decreases in

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the concentrations of blood lipids and for the susceptibility of LDL to oxidation in vitro.

It is well known that the nature of dietary protein could influence cholesterol metabolism in animals and humans.[2] To date, most studies have focused on the hypercholesterolemic effect of casein versus soybean protein, and little attention has been paid to the effects of other kinds of dietary proteins. In general, animal proteins are considered to be hypercholesterolemic when compared with plant proteins.[2] The effect of fish protein (FP) on blood lipids was investigated in rats[3] and rabbits.[4] Bergeron and Jacques[4] demonstrated that the serum cholesterol level of rabbits fed FP was intermediate and not different than that of subjects fed casein or soy protein. Iritani et al.[5] reported that feeding FP to rats had a hypocholesterolemic effect equivalent to that of soy protein rather than casein. Thus, FP has been shown to induce variable effects on serum cholesterol level compared with casein and soy protein.[6] Although the hamster is an appropriate animal model for the study of cholesterol metabolism,[7] little data are available to compare the effect of FP and soy protein on blood lipids in hamsters.

There are a number of biologically active compounds found in soy products other than the protein itself, which has been associated with lowering blood lipids.[8] The major isoflavones (phytoestrogens) in soybeans are genistein and daidzein. The hypocholesterolemic effect of isoflavones was noted in mice,[9] hamsters,[10] nonhuman primates,[11] and humans.[12] In addition, isoflavones with antioxidant activity were reported to inhibit in vitro oxidative modification of LDL by endothelial cells[13] or in the presence of copper.[14] Tikkanen et al.[15] suggested that intake of soy-derived antioxidants may provide protection against oxidative modification of human LDL. Kubow et al.[16] demonstrated that dietary proteins could modulate tissue lipid peroxidation concentrations in hamsters. However, to date, there has been no report comparing the effect of soybean protein and FP on the susceptibility of LDL to in vitro oxidation. At the same time, no study has been performed to verify whether ethanol-extract of soy protein is partially responsible for the antioxidant effect observed in ex vivo.

In the present study, we used the semi-purified diets that were isonitrogenous and equal in fat and cholesterol contents to compare the effect of intact soy protein isolate (SPI), low-isoflavone ethanol-extracted SPI (E-SPI), and FP on serum lipids and oxidation related parameters in serum and liver of hamsters.

Materials and methods

Animals

Male golden Syrian hamsters, aged 4 weeks and obtained from the National Laboratory Animal Breeding and Research Center, National Science Council (NSC; Taipei, Taiwan), were housed in plastic cages (4 per cage) in a temperature-controlled room (22 ± 1°C) with a 12-hour light:dark cycle. They were fed laboratory rodent chow (Ralston Purina, St. Louis, MO USA) for 6 weeks before being assigned to one of three dietary treatment groups (n = 8 per group) on a weight-randomized basis and fed a test diet for 10 weeks. Hamsters were given free access to the test diets and water, and body weights were recorded weekly. All animal experimental procedures followed the *Guide for the Care and Use of Laboratory Animals* (NSC).

Diets

Three kinds of test diets that varied in protein source were prepared. The protein sources were SPI (Supro 670, Protein Technologies International, St. Louis, MO USA), E-SPI, and FP prepared from salmon fillets, which were purchased in a local supermarket. The frozen fish fillets were cut into small pieces, lyophilized, and subsequently delipidated with diethyl ether in a Soxhlet apparatus for 24 hours. Crude FP was obtained by evaporating the remaining solvent in a hood and grinding the remainder into fine powder. Isoflavones were extracted from SPI with 20 volumes of ethanol by stirring at 60°C for 2 hours. This procedure was repeated twice more with recovered ethanol, and E-SPI was obtained after removal of ethanol. The contents of isoflavones were determined by high performance liquid chromatography (HPLC) analysis according to the method of Wang et al.[17] The SPI was found to contain 1.24 mg of genistein and 0.61 mg of daidzein per gram. The E-SPI contained 0.21 mg of genistein and 0.06 mg of daidzein per gram. Protein (N × 6.25) was measured by the Kjeldahl method, and the dietary level of the three test proteins was adjusted to 18% at the expense of sucrose. Compositions of the three diets are shown in *Table 1*. Rice flour was used as the major carbohydrate source to avoid the "wet tail" disease.[18] We chose 0.1% cholesterol addition to mimic human cholesterol consumption and to induce hypercholesterolemia. The crystalline cholesterol was dissolved in oil before being added. L-methionine was added to the SPI and E-SPI diets to match the content of sulfur-containing amino acids of the FP diet. To maximize the potential antioxidant effects of isoflavones, α-tocopherol acetate (3 IU/kg of diet), which is a potent antioxidant, was used to meet the minimum requirement for hamsters.[19] The vitamin E-stripped soybean oil was prepared according to the method of Liu and Huang.[20] Diets were stored at −20°C before use and fresh diets were provided daily.

Sample preparation

At the end of the 10-week feeding period, the diet was removed 15 hours prior to anesthetizing the animals with pentobarbital. After whole blood was drawn by cardiac puncture, liver was removed, blotted, and weighed. Blood was collected in sterile tubes without anticoagulant and was centrifuged at 2,000 × g for 20 minutes at 4°C to obtain serum. Aliquots of the serum samples were stored at −70°C until the analyses of α-tocopherol, bilirubin, and total antioxidant status within 1 month. EDTA, phenylmethylsulfonyl fluoride (PMSF), and sodium azide were added to the remaining serum samples to prevent oxidative modification and decomposition for isolation of lipoproteins. Liver homogenate was prepared with 5 volumes of ice-cold 1.15% kCl in 0.01 mol/L phosphate buffer, pH 7.4. Portions of the homogenates were measured immediately for the thiobarbituric acid-reactive substances (TBARS) and α-tocopherol. Another portion of the homogenate was centrifuged at 9,000 × g for 20 minutes, and the supernatant was immediately stored at −70 °C until the analyses of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities within 1 month. The same lobe of each hamster's liver was used for a particular assay.

Biochemical analyses

Three lipoprotein fractions were isolated from serum by ultracentrifugation: very low density lipoprotein (VLDL), density (d) < 1.019 kg/L; LDL, 1.019 < d < 1.063 kg/L; and high density lipoprotein (HDL), 1.063 < d < 1.21 kg/L. The concentrations of

Table 1 Composition of the experimental diets

Ingredients	Diets* g/kg diet SPI	E-SPI	FP
SPI†	190		
E-SPI‡		182	
Fish protein§			188
Rice flour	428	428	428
Sucrose	141	146	143
Vitamin E-stripped soybean oil	44	47	47
Lard‖	93	93	93
Alphacel	48	48	48
Mineral mix**	40	40	40
Vitamin mix††	10	10	10
L-methionine	2.9	2.9	
Choline chloride	2	2	2
Crystalline cholesterol	0.905	0.905	0.905
Energy (MJ)	16.8	16.9	16.8

*Semipurified diets contained (wt%): protein, 18%; carbohydrate, 51%; fat, 14%. The polyunsaturated to saturated fatty acid (P/S) ratio was 1.
†Intact Supro 670: 86.8% protein, 0.42% fat. The amino acid composition of the soy protein isolate (SPI) (g/kg): Ala, 43; Arg, 76; Asp, 116; Cys, 13; Gluc, 191; Gly, 42; His, 26; Ile, 49; Leu, 82; Lys, 63; Met, 13; Phe, 52; Pro, 51; Ser, 52; Thr, 38; Trp, 13; Tyr, 38; Val, 50.
‡Ethanol-extracted SPI Supro 670 (E-SPI): 90.6% protein, 0.1% fat.
§Fish protein (FP): 87.7% of salmon protein, 0.1% fat. The amino acid composition of salmon protein (g/kg): Ala, 53; Arg, 59; Asp, 88; Cys, 20; Glu, 126; Gly, 39; His, 24; Ile, 43; Leu, 74; Lys, 82; Met, 29; Phe, 45; Pro, 32; Ser, 34; Thr, 41; Trp, 11; Tyr, 34; Val, 47.
‖Lard contained 1.02 g/kg of cholesterol.
**AIN-76 mineral mixture (g/kg): calcium phosphate dibasic, 500; sodium chloride, 74; potassium citrate monohydrate, 200; potassium sulfate, 52; magnesium oxide, 24; manganese carbonate, 3.5; ferric citrate, 6; zinc carbonate, 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01; chromium potassium sulfate, 0.55.
††Vitamin E-free AIN-76 vitamin mixture (mg/kg): thiamine HCl, 600; riboflavin, 600; pyridoxine HCl, 700; nicotinic acid, 3,000; Ca pantothenate, 1,600; folic acid, 200; biotin, 20; vitamin B_{12}, 1; vitamin A (250,000 IU/g), 1,600; vitamin D_3 (400,000 IU/g), 250; menaquinone, 50. α-Tocopherol acetate (250 IU/g) 1.2 g was added to 1 kg of vitamin E-free AIN-76 vitamin mixture to meet the minimum requirement for hamsters.

cholesterol and triacylglycerol in serum and lipoproteins were determined using commercial enzymatic kits (E. Merck, Darmstadt, Germany). Serum free cholesterol was measured by an enzymatic kit (Boehringer Mannheim Diagnostics, Mannheim, Germany). The concentration of apolipoprotein B (apoB) in LDL fraction was determined by the enzyme linked immunosorbent assay (ELISA) method.[21] For ELISA, an affinity-purified rabbit polyclonal antibody against hamster apoB was produced in our laboratory. A purified LDL standard separated by gel filtration (Superose 6 HR 10/30 column, Pharmacia LKB, Uppsala, Sweden) was used to calibrate the assay. Serum concentration of total bilirubin was assessed using a Merckotest reagent kit (E. Merck). Total antioxidant capacity of serum was determined by using reagents supplied by Randox Diagnostics (County Antrim, UK). α-Tocopherol of serum was measured by HPLC procedure as described previously.[22] α-Tocopherol of liver homogenate was measured according to the method of Liu and Huang.[20] TBARS concentration in liver homogenate was assessed after incubation at 37°C for 1 hour without or with 10 μmol/L ferrous sulfate as described by Hu et al.,[23] using tetra-butyl ammonium malondialdehyde as standard. The activity of GSH-Px was analyzed according to the method of St. Clair and Chow.[24] The activity of SOD was determined using a commercial kit (Randox Diagnostics). Protein concentrations of LDL and liver homogenates were analyzed by a modified Lowry method[25] with bovine serum albumin as standard.

LDL oxidation in vitro

Dialyzed LDL (100 mg protein/L phosphate buffer solution) was incubated with $CuSO_4$ (final concentration: 5 μmol/L) and the lag time formation of conjugated dienes was determined by continuously measuring the increase in absorbance at 234 nm with a spectrophotometer with a thermostat (Hitachi, Tokyo, Japan) at 37°C.[26] The rate of lipid peroxidation during the propagation phase (Rp) was calculated as described by Puhl et al.[26] After incubation with 5 μmol/L of $CuSO_4$ at 37°C for 2 hours, the LDL oxidation was terminated by refrigeration and addition of 0.1 mol EDTA/L, and the extent of LDL oxidation was measured by the TBARS assay.[27]

Statistical analysis

Data are presented as mean ± SD. Comparison of variations among the three groups was made by the nonparametric Kruskall-Wallis range test, and comparisons between pairs of groups were based on the Bonferroni multiple comparisons test. For all analytic procedures, a *P*-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using the Statistical Package for the Social Science (SPSS) for Windows, version 7.0 (SPSS Inc., Chicago, IL USA).

Results

Food intake, growth, and liver weight

The mean food intakes of hamsters fed the SPI, E-SPI, and FP diets were 8.5 ± 0.8, 8.6 ± 0.9, and 8.6 ± 0.9 g per day, respectively, per animal. The initial body weights of the SPI, E-SPI, and FP groups were 124.9 ± 16.5, 125.1 ± 14.2, and 125.5 ± 12.8 g, respectively. The body weight gains of the SPI, E-SPI, and FP groups were 20.5 ± 8.0, 21.4 ± 9.0, and 21.2 ± 7.9 g/10 wk, respectively. The liver weights as percentages of body weight of the three groups were 4.2 ± 0.3%, 4.1 ± 0.2%, and 4.2 ± 0.3%, respectively. There were no significant differences among groups for any of these variables.

Lipoprotein lipid and apoB concentrations

The SPI group had the lowest concentrations of total cholesterol, LDL cholesterol (LDL-C), LDL-apoB, and LDL-C/HDL-C ratio; the FP group had the highest values for these parameters, and the E-SPI group showed intermediate values in general (*Table 2*). There was no significant difference in concentration of VLDL-C among the three groups. LDL-C was particularly higher in the FP group ($P < 0.05$) compared with the other groups. The E-SPI group had a significantly higher concentration of HDL-C compared with the SPI group ($P < 0.05$), but the ratio of LDL-C/HDL-C was not significantly different between the two groups. Regardless of isoflavone content, the two soy protein diets were associated with a significant decrease in the serum concentration of free cholesterol ($P < 0.05$). The serum total triacylglycerol concentrations (mean ± SD) of hamsters fed SPI, E-SPI, and FP-based diets were 1.8 ± 0.7, 2.6 ± 1.1, and 2.5 ± 1.9 mmol/L respectively ($P > 0.05$). The VLDL triacylglycerol concentrations (mean ± SD) of

Table 2 Serum concentrations of cholesterol and LDL-apoB in hamsters

Lipids	Dietary groups		
	SPI	E-SPI	FP
Total cholesterol (mmol/L)	4.92 ± 0.43[a,b]	5.86 ± 0.53[a]	6.16 ± 1.04[b]
Free cholesterol (mmol/L)	1.27 ± 0.25[b]	1.54 ± 0.20[a]	1.96 ± 0.27[a,b]
VLDL-C (mmol/L)	1.11 ± 0.34	0.98 ± 0.35	1.40 ± 1.05
LDL-C (mmol/L)	1.00 ± 0.29[d]	1.35 ± 0.25[a]	1.82 ± 0.42[a,d]
HDL-C (mmol/L)	2.82 ± 0.35[a]	3.54 ± 0.46[a]	2.96 ± 0.65
LDL-apoB (mg/L)	66.2 ± 7.5[b]	75.1 ± 16.2[c]	112.7 ± 31.3[b,c]
LDL-C:HDL-C (mol/mol)	0.35 ± 0.09[b]	0.39 ± 0.11[a]	0.66 ± 0.24[a,b]

Data are presented as mean ± SD of eight animals in each group. Within a row, values with the same letter superscripts are significantly different: [a]$P < 0.05$, [b,c]$P < 0.01$, and [d]$P < 0.001$ by the Bonferroni multiple comparisons test.
LDL–low density lipoprotein. apoB–apolipoprotein B. SPI–soy protein isolate. E-SPI–ethanol extracted SPI. FP–fish protein. VLDL–very low density lipoprotein. C–cholesterol. HDL–high density lipoprotein.

these hamsters were 1.3 ± 0.6, 1.9 ± 1.0, and 1.9 ± 1.8 mmol/L respectively ($P > 0.05$).

LDL and liver lipid oxidation products

Oxidation kinetic of LDL was determined by measuring the lag time of conjugated diene formation and the amount of TBARS. *Table 3* and *Figure 1* showed the effects of SPI, E-SPI, and FP on the susceptibility of LDL to copper-induced oxidation. The SPI group had a significantly lower amount of TBARS in LDL than did the FP group ($P < 0.01$). No significant difference in LDL TBARS content was observed between E-SPI and the other two groups. LDL isolated from the hamsters of the SPI group had significantly longer lag time compared with that isolated from the E-SPI and FP groups ($P < 0.01$). The propagation rate (Rp) of the SPI group was significantly slower than that of the FP ($P < 0.01$) group. However, no significant difference in Rp was observed between the E-SPI and FP groups. In general, the resistance of LDL to copper-induced oxidation was always highest in SPI group, lowest in the FP group, and intermediate in E-SPI group. When the liver homogenate was incubated in the absence of ferrous iron, the amount of TBARS in the SPI group was significantly lower than that in the E-SPI and FP groups. However, the addition of 10 μmol ferrous iron/L to liver homogenate to facilitate lipid oxidation resulted in no significant difference in TBARS production among the three groups.

Serum and liver antioxidant status

Albumin, uric acid, ascorbic acid, α-tocopherol, bilirubin, and unidentified antioxidants in serum could contribute to the total antioxidant capacity.[28] The SPI group had significantly higher serum total antioxidant capacity ($P < 0.05$) than the other two groups, but its concentration of α-tocopherol was only higher than that of the FP group ($P < 0.01$; *Table 4*). The differences between the E-SPI and FP groups were not significant. Recently, bilirubin was shown to inhibit lipid oxidation in isolated LDL exposed to lipophilic peroxyl radicals.[29] However, there was no significant difference in serum concentration of bilirubin among the three groups. The liver homogenate of the SPI group had a significantly higher concentration of α-tocopherol than that of the E-SPI ($P < 0.01$) and FP ($P < 0.001$) groups. There were no significant differences in SOD and GPx activities of the liver among the three groups.

Discussion

The present study showed that hamsters fed soy protein diets, regardless of their contents of isoflavones, had lower concentrations of LDL-C and LDL-apoB compared with hamsters fed a FP-based diet. Several mechanisms were suggested to contribute to the cholesterol-lowering effect of soy protein, including enhancement of bile acid excretion, increased tissue LDL receptor activity, and reduced absorption of dietary cholesterol.[30] However, the exact mechanism by which soy protein intake decreases plasma cholesterol levels is still not fully understood.

Interest in the potential role of soy isoflavones as a hypocholesterolemic agent was raised because of their estrogenic activity.[31] In addition to soy isoflavones, a number of other components that were shown to have

Table 3 Metal-catalyzed peroxidation assays with LDL and liver of hamsters

	Dietary groups		
	SPI	E-SPI	FP
LDL			
TBARS (nmol MDA/mg LDL protein)	47.0 ± 17.2[b]	62.9 ± 12.5	80.1 ± 24.2[b]
Conjugated diene formation			
Lag time (min)	53.6 ± 4.5[b,c]	40.2 ± 9.6[b]	41.2 ± 4.3[c]
Rp (nmol diene/min/mg protein)	8.7 ± 1.8[b]	11.1 ± 1.4	12.3 ± 2.2[b]
Liver			
TBARS (without Fe^{2+})	2.13 ± 0.38[b]	2.42 ± 0.41[a]	3.04 ± 0.59[a,b]
TBARS (with Fe^{2+}) (nmol MDA/g tissue)	10.84 ± 1.19	12.26 ± 1.04	12.06 ± 1.59

Data are presented as mean ± SD of eight animals in each group. Within a row, values with the same letter superscripts are significantly different: [a]$P < 0.05$, [b,c]$P < 0.01$, and [d]$P < 0.001$ by the Bonferroni multiple comparisons test.
LDL–low density lipoprotein. SPI–soy protein isolate. E-SPI–ethanol extracted SPI. FP–fish protein. TBARS–thiobarbituric acid-reactive substances. Rp–the rate of lipid peroxidation during the propagation phase.

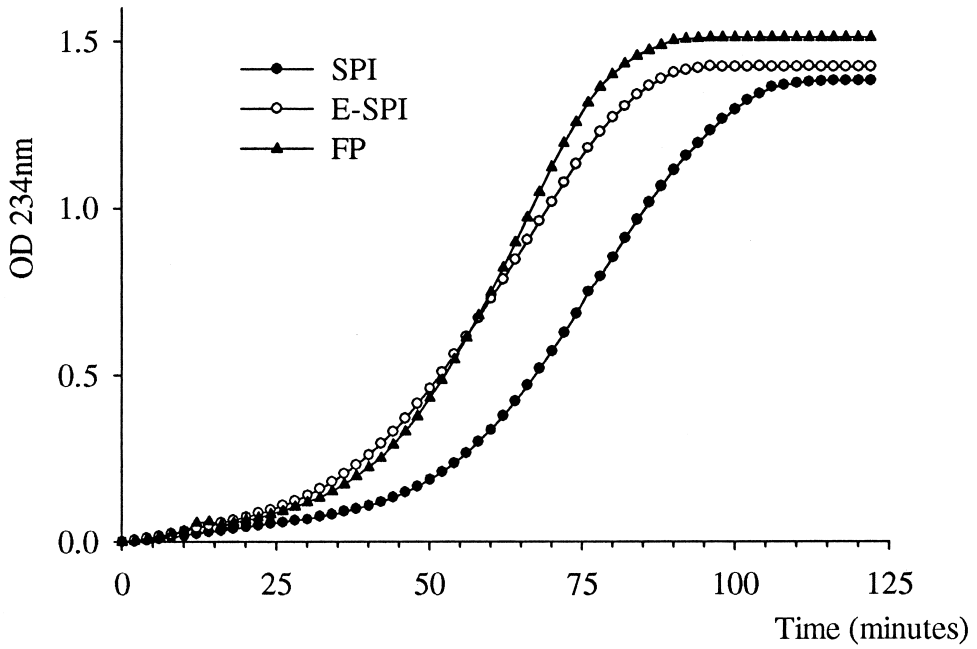

Figure 1 Formation of conjugated dienes in low density lipoproteins (LDL) from hamsters consuming the soy protein isolate (SPI;–●–), ethanol-extracted SPI (E-SPI;–○–), and fish protein (FP;–▲–) diets in the presence of copper. LDL (100 mg protein/L) in PBS containing 5 μmol/L $CuSO_4$ was incubated at 37°C for 2 hours and the optical density was continuously monitored at 234 nm. The initial absorbance was set to 0, and the absorbance was recorded at 2-minute intervals. Values were represented as mean of eight LDL samples for each group.

cholesterol-lowering effect, such as saponins, also could be removed from soy protein isolate by extraction with ethanol. Although the E-SPI diet resulted in a higher serum total cholesterol concentration than that of SPI diet in the present study, there was no significant difference in fractional serum cholesterol distribution between SPI and E-SPI groups. In contrast, Balmir et al.[10] showed that the addition of ethanol-acetone extract of soy to a SPI diet had no significant effect on blood lipids in hamsters. Differences in composition of the experimental diets used in the two studies may account for this discrepancy. The diet used in the present study contained supplementary cholesterol (0.1%) and L-methionine (0.29%), whereas the diet used by Balmir et al.[10] did not contain such supplements. It may be that the hyperlipidemic animals (induced by higher dietary methionine[32] and cholesterol) are able to respond to the ethanol extract of soy protein (serum total cholesterol concentrations of SPI group: present study, 4.92 mmol/L; Balmir et al.'s study, 3.44 mmol/L). In general, the present results were consistent with the finding of Anthony et al.,[11] who reported that isoflavones were effective in decreasing serum total cholesterol concentration in rhesus monkeys. However, further investigations are required to assess an independent and favorable effect of isoflavones on blood lipids.

In spite of removal of most isoflavones and saponins by ethanol extraction, the E-SPI diet was able to decrease the concentration of LDL-C and the ratio of LDL-C/HDL-C compared with the FP diet. The amino acid composition of soy protein has been investigated for its effect on blood lipids and its role in atherosclerosis prevention.[33,34] Kritchevsky et al.[33] showed that a significant positive correlation was observed between the lysine/arginine ratio of diet and serum total cholesterol concentration. Kurowska and Carroll[34] also reported that lysine and methionine contents of the diets seemed closely correlated with their

Table 4 Antioxidant status in serum and liver in hamsters

	Dietary groups		
	SPI	E-SPI	FP
Serum			
TAC (mmol/L)	0.98 ± 0.09[a,b]	0.81 ± 0.06[a]	0.82 ± 0.08[b]
α-Tocopherol (μmol/L)	12.10 ± 3.01[c]	9.61 ± 3.48	7.38 ± 1.04[c]
Bilirubin (μmol/L)	2.57 ± 0.04	2.41 ± 1.31	2.48 ± 1.39
Liver			
α-Tocopherol (nmol/g tissue)	63.8 ± 6.4[c,d]	49.6 ± 9.1[c]	42.0 ± 7.5[d]
SOD activity (U/mg protein)*	9.97 ± 1.69	10.15 ± 1.43	8.95 ± 1.77
GPx activity (U/mg protein)*	1.13 ± 0.06	1.10 ± 0.25	1.03 ± 0.13

Definition of unit (U) of enzyme activity: 1 unit of superoxide dismutase (SOD) = 50% inhibition of reduction of cytochrome c (i.e., a rate of 0.0125 absorbance unit per minute) at pH 7.8 at 25°C. 1 unit of glutathione peroxidase (GPx) = decrease of 1 μmol of NADPH per minute at pH 7.2 at 37°C. Data are presented as mean ± SD of eight animals in each group. Within a row, values with the same letter superscripts are significantly different: $^{a,b}P < 0.05$, $^{c}P < 0.01$, and $^{d}P < 0.001$ by the Bonferroni multiple comparisons test.
SPI–soy protein isolate. E-SPI–ethanol extracted SPI. FP–fish protein. TAC–total antioxidant capacity.

hypercholesterolemic effect. In the present study, the lysine/arginine ratio of the three diets (SPI and E-SPI, 0.83; FP, 1.39) showed a significant positive correlation with serum total cholesterol concentration ($r = 0.462$, $P = 0.023$) by a linear regression analysis. This correlation suggests that the amino acid composition of dietary protein may be at least partially responsible for its effect on serum cholesterol concentration.

Because the oxidative modification of LDL plays a potential role in early atherogenic events,[35] dietary supplementation with antioxidants may reduce risk of atherosclerosis. The present study showed that LDL isolated from hamsters fed the SPI diet had a longer lag time of conjugated diene formation and lower TBARS concentration. It seems likely that dietary isoflavones provided protection against oxidative modification of LDL, although there is no direct explanation for the mechanism. Plasma concentrations of isoflavones were found to be higher,[36] as were its urinary excretions,[37] in vegetarians than in the control omnivores. Moreover, isoflavones were shown to incorporate into LDL at isoflavonoid:LDL molar ratios ranging between 1:400 and 1:600.[15] It seems reasonable to assume that the elevated circulating isoflavones may reduce the formation of lipid hydroperoxides in LDL particles, and thus reduce the initiation rate of, and prolong the lag time for, LDL oxidation in the group of hamsters fed the SPI diet. In the present study, isoflavones had no effect on the enzymatic antioxidant system or the activities of SOD and GPx of liver. On the other hand, α-tocopherol contents of serum and liver homogenate were highest in the SPI group, suggesting that isoflavones spared this antioxidant. Our findings suggest that the intake of soy isoflavones enhances the resistance of LDL to oxidation and contributes to antioxidant defense, as well as reduces the consumption of α-tocopherol in both serum and liver of hamsters.

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