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Soy Leaf Lowers the Ratio of Non-HDL to HDL Cholesterol in Hamsters

HING MAN HO,† LAI KWOK LEUNG,† FRANKY L. CHAN,‡ YU HUANG,§ AND ZHEN-YU CHEN*,†

Food & Nutritional Sciences Programme, Department of Biochemistry, Department of Anatomy, Department of Physiology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, The People's Republic of China

The present study was to examine effect of soy leaf powder (SLP) and soy leaf ethanol extract (SLEE) on serum lipoproteins in hamsters. The control group was fed a semisynthetic diet containing 0.1% cholesterol, while the tested groups were maintained on the same diet but supplemented with 3% SLP or the equivalent amount of SLEE derived from 3% SLP for 4 weeks. SLP supplementation led to a trend of lowering serum total cholesterol (TC) and nonhigh density lipoprotein cholesterol (non-HDL-C), with HDL-C being unaffected, whereas incorporation of SLEE into the diet led to an elevated level of HDL-C and a lower level of non-HDL-C with TC being unchanged. Both SLP and SLEE supplementation caused favorably a decrease in the ratio of non-HDL-C to HDL-C. The present results demonstrate that not only soybean seeds but also soy leaves are cardioprotective, by favorably modulating serum lipid profile.

KEYWORDS: Cholesterol; hamster; HDL cholesterol; kaempferol; LDL cholesterol; soybean; soy leaf

INTRODUCTION

Soybean (*Glycine max* L. Merr.) has incorporated into human diet for over five thousand years. Traditionally, soybean foods consumed are categorized into two major types, the fermented and the nonfermented. The former includes soy sauce, miso, natto, paste, and fermented tofu. The latter are mainly tofu, soymilk, soybean sprouts, soy flour, and various forms of soybean proteins. Health benefits of soybean products have been extensively investigated. Consumption of soybean has been shown to prevent certain cancers (*1*, *2*), reduce the risk of osteoporosis in women (*3*, *4*), lower serum cholesterol (*5*, *6*), exhibit an antiatherosclerotic activity (*7*, *8*), and decrease the risk of coronary heart disease (*9*−*11*).

The active components in soybeans responsible for these beneficial effects are attributable to a group of isoflavones abundant in soybean seeds, namely daidzein, glycitein, and genistein (*12*). Total genistein and daidzein account for about 0.2% of dry raw soybean seed (*13*). The soy leaf has not been part of the human diet. We previously compared the flavonoid profile between soybean seeds and soy leaf and found that soybean seeds were most abundant in malonyl-genistin, followed by malonyl-daidzin, genistin, daidzin, genistein, and daidzein, in decreasing order (*14*). In contrast, soy leaf contained only trace amounts of malonyl-genistin and genistin, but it was rich in kaempferol glycosides (*14*). The present study was carried out further to explore the effect of soy leaf powder (SLP) and soy leaf ethanol extract (SLEE) on serum lipid profile using hamsters as an animal model.

MATERIALS AND METHODS

Preparation of Soy Leaf Powder and Ethanol Extract. Soy leaf was collected from a farm located in Meng County of the Henan Province of China during August 2000. The fresh leaf was then dried, and the SLP was prepared by grinding dry leaf into powder. A portion of powder (300 g) was extracted with 1.8 L of 70% ethanol three times at 50 °C. Ethanol was evaporated under vacuum to yield 14.5 g of SLEE, which was incorporated into the diet as described below.

Quantification of Soy Leaf Fiber. Determination of soy leaf fiber was carried out using the Official Methods of Analysis of the Association of Official Analytical Chemists (*15*). In brief, this method determined soy leaf fiber by using a combination of enzymatic and gravimetric methods. Triplicate samples of soy leaf powder (1g/sample) were subjected to sequential enzymatic digestion by heat stable α-amylase, protease, and amyloglucosidase, to remove starch and protein. For the total soy leaf fiber, enzymatic digestate was treated with alcohol to precipitate soluble fiber before filtering. The total fiber residue was washed with alcohol and acetone, dried, and weighed. For insoluble dietary fiber, enzymatic digestate was filtered, and the residue was washed with warm water, dried, and weighed. For the soluble soy leaf fiber, the filtrate and washes were combined, precipitated with alcohol, filtered, dried, and weighed.

Diets. The hypercholesterolemic diet previously described by Sanders and Sandaradura (*16*) was used with modification. The control diet was prepared by mixing the following ingredients: casein, 200 g; lard, 100 g; coconut oil, 100 g; cornstarch, 418 g; sucrose, 100 g; AIN-76

* Corresponding author. Telephone: (852) 2609-6382. Fax: (852) 2603-7246. E-mail: zhenyuchen@cuhk.edu.hk.
† Department of Biochemistry.
‡ Department of Anatomy.
§ Department of Physiology.

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mineral mix, 40 g; AIN-76A vitamin mix, 20 g; DL-methionine, 1 g; and cholesterol, 1 g. The ingredients were purchased from Harlan Teklad (Madison, WI), except for lard, which was obtained from the local market, and coconut oil, DL-methionine, and cholesterol, which were purchased from Sigma Chemical (St. Louis, MO). The SLP diet was prepared by mixing the control diet with 3% soy leaf powder. The SLEE diet was similarly prepared by mixing the control diet with the amount of ethanol extract derived from 3% soy leaf powder used in the SLP diet. The control, SLP, and SLEE diets (1 kg) were each mixed with 300 mL of gelatin solution (20 g/L). Once the gelatin had set, the diet was cut into ~20-g cubed portions and stored in a freezer at −20 °C.

Animals. Male Syrian golden hamsters (3 mo; 125−140 g, *Mesocricetus auratus*, The Chinese University of Hong Kong, Shatin, Hong Kong) were randomly divided into three groups ($n = 12$/each) and housed in an animal room at 23 °C with 12:12-h light/dark cycles. Frozen diets were given to hamsters daily, and the uneaten portion was discarded. Food intake was measured daily, and body weight was recorded twice a week. The hamsters were allowed access to food and tap water ad libitum. The total fecal output of each hamster was manually separated from other bedding materials and was pooled in each week. At the end of 4 weeks, all hamsters were sacrificed after food deprivation for 14 h. The blood was collected via the abdominal aorta. After clotting, the blood was centrifuged at 1500*g* for 10 min, and serum was then collected. The liver was removed, washed with saline, and stored at −80 °C for determination of cholesterol content. The protocol was reviewed and approved by the Committee of Animal Ethics, The Chinese University of Hong Kong.

Determination of Serum Total Cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C), and Triacylglycerols (TG). Several enzymatic kits were purchased from Sigma (St. Louis, MO) to measure serum TG (catalog no. 336-20) and TC (catalog no. 352-20). HDL-cholesterol was isolated and determined by precipitation of the apolipoprotein B-containing lipoproteins with sodium phosphotungstate-magnesium chloride, using the commercial Sigma kit (HDL-C; catalog no. 352-4). Non HDL-C was calculated as the difference between TC and HDL-C.

Determination of Liver Cholesterol. Total lipids were extracted from 300 g of liver with the addition of 1 mg of stigmastanol (Sigma, St. Louis, MO) as an internal standard, using chloroform/methanol (2:1, v/v). The lipid extracts were saponified with 6 mL of 1 mol/L NaOH in 90% ethanol at 90 °C for 1 h, and the nonsaponified substances including cholesterol were converted to their trimethylsilyl (TMS)-ether derivatives by a commercial TMS reagent (Sigma, St. Louis, MO). The analysis of cholesterol TMS-ether derivative was performed in a fused silica capillary column (SAC-5, 30 m × 0.25 mm, i.d.; Supelco, Inc., Bellefonte, PA) using a Shimadzu GC-14 B gas−liquid chromatograph (GLC) equipped with a flame ionization detector (Shimadzu, Kyoto, Japan) as previously described (*17*).

Quantification of Fecal Neutral and Acidic Sterols. Individual fecal neutral and acidic sterols were quantified as previously described (*17*). In brief, stigmasterol (0.5 mg in 1 mL of chloroform) as an internal standard for neutral sterols was added to a fecal sample (300 mg). The sample was then saponified, using 9 mL of 1 mol/L NaOH in 90% ethanol, containing 0.5 mg of hyodeoxycholic acid in 2 mL of 1N NaOH as an internal standard for acidic sterols (Sigma, St. Louis, MO). The total neutral sterols were extracted, using 8 mL of cyclohexane, and were then converted to their corresponding TMS-ether derivatives for GLC analysis.

After the cyclohexane extraction, 1 mL of 10 mol/L NaOH was added to the remaining aqueous layer and heated at 120 °C for 3 h. After cooling, 1 mL of distilled water and 3 mL of 3 N HCl were added, followed by extraction with 7 mL of diethyl ether twice. The diethyl ether layers were then pooled, followed by addition of 2 mL of methanol, 2 mL of dimethoxypropane, and 40 μL of concentrated HCl (12 mol/L). After standing overnight at room temperature, the solvents were dried down, and the acidic sterols were similarly converted to their TMS-ether derivatives at 60 °C for GLC analysis.

Statistical Analysis. Data are expressed as mean ± SD. The analysis of variance (ANOVA), followed by Student's *t*-test (two-tailed) was used where applicable for statistical evaluation of significant differences among the control, SLP, and SLEE groups using Sigmastat (Jandel Scientific Software, San Rafael). Differences were considered significant when $p < 0.05$.

Table 1. Effect of SLP and SLEE Supplementation in Diet on TG, TC,HDL-C, the Ratio of Non-HDL-C to HDL-C, and Hepatic Cholesterol Level in Hamsters[a]

	control	SLP	SLEE
serum TG (mg/dL)	203.3 ± 52.2	208.8 ± 47.2	228.8 ± 40.3
serum TC (mg/dL)	183.6 ± 9.3	174.9 ± 23.2	184.8 ± 11.0
serum HDL-C (mg/dL)	95.8 ± 7.0[b]	97.5 ± 11.4[b]	104.8 ± 10.4[c]
non-HDL-C (mg/dL)	87.8 ± 5.4[b]	77.3 ± 13.9[c]	80.1 ± 12.3[b]
non-HDL-C/HDL-C	0.92 ± 0.08[c]	0.79 ± 0.11[d]	0.78 ± 0.18[d]
hepatic cholesterol (mg/g)	24.9 ± 4.1[c]	30.4 ± 4.2[b]	30.3 ± 2.8[b]

[a] Data are expressed as mean ± SD, $n = 12$. [b,c] Means at the same row differ significantly, $p < 0.05$. [c,d] Means at the same row differ significantly, $p < 0.01$.

RESULTS

Fiber Content of Soy Leaf. SLP contained 55.3% insoluble dietary fiber and 4.6% soluble dietary fiber. When 3% SLP was added to the diet, total dietary fiber accounted for 1.8% of the diet by weight, with insoluble fiber and soluble fiber being 1.7% and 0.1% of diet, respectively. SLEE contained 0.5% soluble fiber but no insoluble fiber. When SLEE was added to the diet, the soluble fiber was not significant (0.001% of diet).

Body Weight and Food Intake. The initial average body weight of control, SLP, and SLEE groups was 132.4−132.7 g. After 4 weeks of feeding, the final average body weight was 146.3 (control), 148.8 (SLP), and 149.1 g(SLEE). No significant differences in body weight gain were observed among the three groups. The average food intake for three groups ranged 6.4−6.6 g/day. There were no significant differences among three groups.

Serum Lipids. Addition of SLP and SLEE into the diet did not affect the levels of serum TG (**Table 1**). Incorporation of SLP led to a trend of lowering serum TC and non-HDL-C, with HDL-C being unaffected. In contrast, incorporation of SLEE into the diet led to an elevated level of HDL-C and a lower level of non-HDL-C, with TC being unchanged. The major observation was that the ratio of non HDL-C to HDL-C was drastically reduced by 14.1% in the SLP group and 15.2% in the SLEE hamsters, compared with that of the control ($p < 0.01$).

Liver Lipids. No significant difference in the average liver weight was found among all the three groups (control, 4.9 g; SLP, 5.0 g; SLEE, 5.1 g), but the SLP and SLEE groups had hepatic cholesterol levels significantly higher than the control group ($p < 0.05$, **Table 1**).

Fecal Neutral and Acidic Sterols. Total fecal neutral sterols refer to a sum of cholesterol, coprostanol, coprostanone, dihydrocholesterol, campesterol, and β-sitosterol (**Table 2**). The total fecal neutral sterols were significantly elevated in the SLP group, compared with that in the control over the time. No statistical differences were observed between the control and SLEE group, except for week 4, when total out fecal neutral sterol excretion was greater in the SLEE group (1.7 mg/day/hamster) compared with that of the control hamsters (**Table 2**). In general, the SLP group had greater excretion of cholesterol than the control in weeks 3 and 4. No difference in fecal cholesterol was found between the SLEE and the control hamsters. For dihydrocholesterol, the SLP group had greater excretion than the control hamsters in weeks 1, 2, and 4. Compared with that of the SLEE, excretion of dihydrocholes-

Table 2. Effect of SLP and SLEE Supplementation in Diet on Fecal Output of Neutral Sterols (mg/day/hamster)[a]

	control	SLP	SLEE
	Week 1		
coprostanol	0.61 ± 0.15	0.63 ± 0.03	0.67 ± 0.12
coprostanone	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
cholesterol	0.29 ± 0.05	0.29 ± 0.05	0.30 ± 0.07
dihydrocholesterol	0.31 ± 0.04^{c}	0.37 ± 0.01^{b}	0.33 ± 0.04^{bc}
campesterol	0.69 ± 0.06^{c}	0.90 ± 0.03^{b}	0.84 ± 0.05^{b}
sitosterol	0.10 ± 0.02^{d}	0.31 ± 0.02^{b}	0.18 ± 0.04^{c}
total	2.00 ± 0.25^{c}	2.50 ± 0.08^{b}	2.32 ± 0.26^{bc}
	Week 2		
coprostanol	0.50 ± 0.29	0.54 ± 0.08	0.46 ± 0.09
coprostanone	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
cholesterol	0.28 ± 0.06^{c}	0.34 ± 0.04^{b}	0.30 ± 0.06^{bc}
dihydrocholesterol	0.19 ± 0.02^{c}	0.25 ± 0.02^{b}	0.20 ± 0.03^{c}
campesterol	0.35 ± 0.10^{c}	0.53 ± 0.04^{b}	0.46 ± 0.04^{c}
sitosterol	0.08 ± 0.02^{d}	0.33 ± 0.03^{b}	0.16 ± 0.01^{c}
total	1.41 ± 0.47^{c}	1.99 ± 0.08^{b}	1.59 ± 0.19^{c}
	Week 3		
coprostanol	0.52 ± 0.21	0.58 ± 0.10	0.48 ± 0.07
coprostanone	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
cholesterol	0.27 ± 0.03^{c}	0.35 ± 0.06^{b}	0.31 ± 0.02^{bc}
dihydrocholesterol	0.25 ± 0.05^{bc}	0.29 ± 0.03^{b}	0.23 ± 0.02^{c}
campesterol	0.35 ± 0.06^{d}	0.59 ± 0.08^{b}	0.47 ± 0.04^{c}
sitosterol	0.10 ± 0.02^{d}	0.43 ± 0.05^{b}	0.20 ± 0.02^{c}
total	1.49 ± 0.30^{c}	2.23 ± 0.26^{b}	1.69 ± 0.10^{c}
	Week 4		
coprostanol	0.49 ± 0.16	0.56 ± 0.06	0.59 ± 0.11
coprostanone	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
cholesterol	0.22 ± 0.02^{c}	0.30 ± 0.02^{b}	0.28 ± 0.03^{b}
dihydrocholesterol	0.21 ± 0.06^{c}	0.27 ± 0.01^{b}	0.23 ± 0.03^{c}
campesterol	0.25 ± 0.05^{d}	0.50 ± 0.04^{b}	0.44 ± 0.04^{c}
sitosterol	0.05 ± 0.01^{d}	0.35 ± 0.02^{b}	0.16 ± 0.01^{bc}
total	1.22 ± 0.28^{d}	1.98 ± 0.08^{b}	1.70 ± 0.17^{c}

[a] Data are expressed as mean ± SD, $n = 12$. [b–d] Means at the same row differ significantly, $p < 0.05$.

Table 3. Effect of SLP and SLEE Supplementation in Diet on Fecal Output of Acidic Sterols (mg/day/hamster)[a]

	control	SLP	SLEE
	Week 1		
lithocholic acid	0.19 ± 0.01^{c}	0.31 ± 0.03^{b}	0.21 ± 0.02^{c}
deoxycholic acid	0.07 ± 0.07^{c}	0.24 ± 0.10^{b}	0.10 ± 0.05^{c}
chenodeoxycholic acid	0.28 ± 0.15^{bc}	0.28 ± 0.04^{b}	0.20 ± 0.04^{c}
cholic acid	0.08 ± 0.01	0.10 ± 0.03	0.10 ± 0.02
ursodeoxycholic acid	0.06 ± 0.03	0.05 ± 0.01	0.03 ± 0.01
total	0.69 ± 0.16^{c}	0.98 ± 0.13^{b}	0.65 ± 0.06^{c}
	Week 2		
lithocholic acid	0.25 ± 0.01^{c}	0.29 ± 0.03^{b}	0.23 ± 0.02^{c}
deoxycholic acid	0.08 ± 0.11	0.14 ± 0.04	0.17 ± 0.08
chenodeoxycholic acid	0.17 ± 0.01	0.19 ± 0.01	0.18 ± 0.04
cholic acid	0.11 ± 0.02	0.14 ± 0.02	0.13 ± 0.02
ursodeoxycholic acid	0.03 ± 0.01^{c}	0.06 ± 0.02^{b}	0.04 ± 0.01^{c}
total	0.64 ± 0.09^{c}	0.81 ± 0.04^{b}	0.74 ± 0.04^{bc}
	Week 3		
lithocholic acid	0.36 ± 0.05^{c}	0.48 ± 0.04^{b}	0.35 ± 0.03^{c}
deoxycholic acid	0.20 ± 0.12	0.18 ± 0.10	0.18 ± 0.02
chenodeoxycholic acid	0.39 ± 0.04	0.45 ± 0.07	0.38 ± 0.06
cholic acid	0.12 ± 0.04	0.14 ± 0.02	0.12 ± 0.01
ursodeoxycholic acid	0.06 ± 0.01	0.04 ± 0.02	0.04 ± 0.01
total	1.13 ± 0.13^{c}	1.29 ± 0.11^{b}	1.07 ± 0.09^{c}
	Week 4		
lithocholic acid	0.31 ± 0.06^{c}	0.53 ± 0.14^{b}	0.40 ± 0.05^{bc}
deoxycholic acid	0.12 ± 0.08^{c}	0.10 ± 0.02^{b}	0.11 ± 0.01^{b}
chenodeoxycholic acid	0.29 ± 0.06^{c}	0.44 ± 0.12^{b}	0.37 ± 0.05^{b}
cholic acid	0.11 ± 0.04	0.12 ± 0.02	0.09 ± 0.02
ursodeoxycholic acid	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
total	0.85 ± 0.21^{c}	1.22 ± 0.26^{b}	1.02 ± 0.09^{bc}

[a] Data are expressed as mean ± SD, $n = 12$. [b,c] Means at the same row differ significantly, $p < 0.05$.

terol in SLP group was greater only in weeks 2, 3, and 4. No significant difference in excretion of dihydrocholesterol was found between the control and SLEE group over time (**Table 2**). In general, the SLP group excreted campesterol the most, while the control excreted it the least. For sitosterol, significant differences in excretion were observed among the three groups, with the SLP group excreting the most, followed by the SLEE group.

The acidic sterols measured included cholic, deoxycholic, lithcholic, and ursodeoxycholic acids (**Table 3**). Similar to the result observed for neutral sterols, the total acidic sterols in the SLP groups were significantly elevated, except for the SLEE hamsters, compared with that in the control group. In general, excretion of lithocholic acid was greater in the SLP group, compared with that of the other two groups. A significant difference in excretion of deoxycholic acid was found between the SLP and the control group in weeks 1 and 4. Greater excretion of chenodeoxycholic acid was observed in the SLP and SLEE groups, compared with that of the control in week 4. Fecal cholic acid in the SLP group was slightly higher than that in the control, but the difference was statistically insignificant (**Table 3**).

DISCUSSION

The present study demonstrated that supplementation of SLP and SLEE in the diet did not decrease the levels of serum total TG and TC, but it caused a significant decrease in the ratio of non-HDL-C to HDL-C, suggesting that incorporation of SLP and SLEE into the diet cause the redistribution of serum cholesterol. The ratio of LDL-C to HDL-C is commonly calculated to assess the risk of coronary heart disease (CHD), on the basis of the evidences that an elevated LDL-C concentration is atherogenic (*18*), whereas a higher level of HDL-C is cardioprotective (*19*). In the present study, no attempt was made to determine LDL-C using the formula LDL-C = TC − HDL-C − (TG/5), because it is only applicable to humans. Non-HDL-C was therefore calculated instead, because LDL-C accounts for most cholesterol in non HDL-C of hamsters. If the data could transfer to humans, the decrease in the ratio of non-HDL-C to HDL-C indicated that SLP and SLEE supplementation in the diet would favorably modulate the serum lipid profile, as observed for soybean protein.

The effect of SLP on serum lipid profile is possibly attributable to its rich fiber content (59.9%). This is reflected from characteristic changes in the serum lipid profile associated with consumption of dietary fiber (*20*). First, supplementation of SLP had a trend of lowering the TC level, with HDL-C remaining unchanged (**Table 1**). Second, a decrease in non-HDL-C/HDL-C was accompanied with consumption of SLP, simply because dietary fiber decreases mainly LDL-C and thereby the ratio of non-HDL-C to HDL-C. It is noteworthy that the change in serum lipid profile associated with SLEE is different from that with supplementation of SLP. The former increased HDL-C and decreased non-HDL-C, with TC remaining unaffected, whereas the latter decreased TC (not significant) and non-HDL-C with HDL-C remained unchanged. This suggests that effect of SLP and SLEE on serum lipids cannot be solely attributable to dietary fiber, and there must be some unknown active ingredients in SLEE that are also responsible. It is speculated that isoflavones may be the active ingredients, even if they are quantitatively minor in soy leaf. A previous study showed that supplementation of soy isoflavones in the

diet could decrease non-HDL-C and increase HDL-C (*9*), because genistein can bind to estrogen receptors (*21*) to exert estrogenic activity and may share the LDL-reducing and HDL-increasing effect of human estrogen (*22*−*24*). Therefore, soy leaf isoflavones may be one of the active ingredients that were partially responsible for the observed effect of SLEE.

The inhibition of cholesterol absorption in the intestine may be possibly one of the factors that are responsible for favorable modification of the blood lipoprotein profile. The inhibition of cholesterol absorption is reflected from the observation that fecal content of cholesterol was greater in the SLP group, compared with that of the control (**Table 2**). It is known that cholesterol in the large intestine is partially degraded to its metabolites by intestinal microflora (*25*). Therefore, the total neutral sterols were measured as a whole. The results demonstrated that the SLP excreted the total neutral sterols most, while the control hamsters did least (**Table 2**). It is interesting to note that the amount of sitosterol in feces of SLP group was in an order of SLP > SLEE > control, because that soy leaf contains sitosterol (19 mg/g). It is believed that sitosterol may compete for absorption with cholesterol, thus reducing cholesterol absorption and increasing the excretion of cholesterol.

The major metabolites of cholesterol are bile acids. Greater excretion of bile acids could also lead to a lower level of serum cholesterol. Microbial conversion of primary bile acids and cholic and chenodeoxycholic acids leads to production of secondary bile acids. The bile acids should be considered as a whole rather than as individuals. In this connection, the SLP group excreted a greater amount of total fecal bile acids, compared with the control and SLEE hamsters.

Up-regulation of LDL-receptors may be an alternative mechanism responsible for the HDL-C raising or non-HDL-C-decreasing activity of SLP and SLEE. This reflects a greater amount of hepatic cholesterol in the SLP and SLEE groups than in the control hamsters (**Table 1**). This is also in agreement with the observation that the HDL-C level was elevated in the SLEE group, implying that it accelerated the reversal cholesterol transport from the peripheral parts of the body back to the liver for degradation. The rate of cholesterol metabolized by the liver to form the acidic sterols might not be as rapid as the rate of cholesterol entering into the liver, leading to elevation in hepatic cholesterol.

The present study is the first report to demonstrate the beneficial effect of soy leaf on serum lipoprotein. In addition to dietary fiber, the other active ingredients of soy leaf remain unexplored. We previously studied the composition of soy leaf and found that the ethanol extract contained mainly genistin, kaempferol, and its glycosides (*14*). The preliminary data demonstrated that they possessed the free radical scavenging activity comparable to that of (−)-epigallocatechin gallate, an antioxidant in green tea. We also demonstrated that soy leaf extract was cardioprotective by causing favorably endothelium-independent relaxation in the rat carotid artery ring (*26*). However, kaempferol glycosides, accounting for 48% of the extract in weight, were not the ingredients, and neither was genistin responsible for the observed relaxation. We are currently using various chromatographic techniques in an attempt to identify the active component(s) present in soy leaf that have cardiovascular relaxative properties.

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