Soyasaponins Lowered Plasma Cholesterol and Increased Fecal Bile Acids in Female Golden Syrian Hamsters

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A study was conducted in hamsters to determine if group B soyasaponins improve plasma cholesterol status by increasing the excretion of fecal bile acids and neutral sterols, to identify group B soyasaponin metabolites, and to investigate the relationship between a fecal group B soyasaponin metabolite and plasma lipids. Twenty female golden Syrian hamsters, 11–12 weeks old and 85–125 g, were randomly assigned to a control diet or a similar diet containing group B soyasaponins (containing no isoflavones), 2.2 mmol/kg, for 4 weeks. Hamsters fed group B soyasaponins had significantly lower plasma total cholesterol (by 20%), non–high-density lipoprotein (HDL) cholesterol (by 33%), and triglycerides (by 18%) compared with those fed casein ($P < 0.05$). The ratio of total cholesterol to HDL cholesterol was significantly lower (by 13%) in hamsters fed group B soyasaponins than in those fed casein ($P < 0.05$). The excretion of fecal bile acids and neutral sterols was significantly greater (by 10[illegible]% and 85%, respectively) in soyasaponin-fed hamsters compared with those fed casein ($P < 0.05$). Compared with casein, group B soyasaponins lowered plasma total cholesterol levels and non-HDL cholesterol levels by a mechanism involving greater excretion of fecal bile acids and neutral sterols. Hamsters fed group B soyasaponins statistically clustered into two fecal soyasaponin metabolite–excretion phenotypes: high excreters ($n = 3$) and low excreters ($n = 7$). When high and low producers of this soyasaponin metabolite were compared for plasma cholesterol status, the high producers showed a significantly lower total-cholesterol-to-HDL-cholesterol ratio compared with the low producers (1.38 ± 0.7 vs. 1.59 ± 0.13; $P < 0.03$). Greater production of group B soyasaponin metabolite in hamsters was associated with better plasma cholesterol status, suggesting that gut microbial variation in soyasaponin metabolism may influence the health effects of group B soyasaponins. Exp Biol Med 230:472–478, 2005

Key words: group B soyasaponin; soyasaponin metabolite

Soyasaponins are one of the major classes of phytochemicals associated with soy protein. The primary saponins in soybeans are group A and group B soyasaponins because of their aglucones, soyasapogenols A and B (1, 2). The content of group B soyasaponins in whole soybean seeds is about 60%–75%, by weight of total soyasaponins (3, 4). Soybeans, isolated soy protein, and soy products provide soyasaponins at approximately 1.4–5.9 μmol/g, 9.5–10.6 μmol/g, and 1.5–4.5 μmol/g on a dry weight basis, respectively (3, 5, 6).

Soyasaponins may lower plasma cholesterol concentrations (7–10). Compared with those fed casein, plasma total cholesterol was significantly decreased in male Sprague-Dawley rats fed soy protein isolate, either intact or depleted of isoflavones, but containing 49% of the amount of saponins present in the diet based on isolated soy protein (10). Rats fed a 1% cholesterol diet with 1% purified soyasaponins had significantly lower plasma and hepatic total cholesterol levels compared with those fed the 1% cholesterol diet (7).

The potential mechanisms responsible for the hypocholesterolemic effects of soyasaponins or synthetic saponins have been investigated (7, 11–13). The synthetic saponins pamaqueside and tiqueside significantly decreased the intestinal absorption of cholesterol, compared with a control diet (12). Oakenfull *et al.* (7) found that 1% soyasaponins significantly increased fecal bile acid and neutral sterol excretion compared with a control diet without soyasaponin. The cholesterol-lowering effects of saponins may be mediated by the decreased intestinal absorption of cholesterol or the enhanced fecal excretion of bile acids or neutral sterols.

Little is known about the metabolism and bioavailability of soyasaponins (14–17). In an *in vitro* fermentation system, soybean saponins were hydrolyzed to soyasapogenols (i.e., aglycone saponins), partially hydrolyzed forms, and sugars by colonic microflora (15, 16). *In vivo*, neither soyasaponins nor soyasapogenols were detected in the blood of rats, mice, and chicks (14) or in human urine (17).

In the present study, purified group B soyasaponins fed in amounts relevant to those found in a soy protein–based diet were hypothesized to improve plasma total cholesterol status and non-HDL cholesterol status by increasing the

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excretion of fecal bile acids and neutral sterols. The study was also designed to identify putative gut microbial metabolites of group B soyasaponins and investigate the relationship between these metabolites and plasma lipid status in female hamsters.

Materials and Methods

Diets. Crude group B soyasaponins were generously donated by Dr. Mark Berhow (USDA, National Center for Utilization of Agricultural Products, Peoria, IL). The soyasaponin fractions of crude group B soyasaponins were analyzed by high-performance liquid chromatography (HPLC; Ref. 5).

All dietary ingredients except rice flour were obtained from Harlan Teklad (Madison, WI). Rice flour was purchased from Bioserve (Frenchtown, NJ). Two treatments were fed: group B soyasaponins or casein as control. The amount of group B soyasaponins fed in this study was similar to the amount of soyasaponins in a diet based on a 25% isolated soy protein diet (2.2 mmol/kg). The daily dose of group B soyasaponins was approximately 128 mg/kg body wt. Soyasaponin contents in the group B soyasaponin diet were 1.36 mg soyasaponin I/g diet and 1.04 mg soyasaponins II/g diet. No isoflavones were detected in the group B soyasaponin diet. The control diet for hamsters (18) was formulated to contain approximately 37% of energy as fat (i.e., 23% coconut oil, 5% safflower oil, 9% soybean oil), 25% casein, and 0.1% cholesterol. Rice flour was used as a carbohydrate source to replace cornstarch in the standard rodent diet because rice flour prevents chronic diarrhea, which causes a high rate of mortality for hamsters (18).

Animals. The study protocol was approved by the Committee on Animal Care, Iowa State University. Twenty female golden Syrian hamsters, 11–12 weeks old and 85–125 g, were purchased from Harlan Teklad and individually housed in a temperature-controlled room (23°C) with a 12:12-hr light:dark cycle. Hamsters, with the same average body weight in each group, were randomly assigned to two treatments. Hamsters had free access to food and water during the 4-week experimental period. Body weights were measured weekly and food intakes were measured daily. At the end of the feeding period, diets were withdrawn from hamsters 16–18 hrs before they were sacrificed under CO_2. Blood was collected by cardiac puncture and centrifuged at 5000 *g* for 15 mins at 4°C to prepare plasma that was stored at –20°C until analysis.

Plasma Lipid Analysis. Plasma total cholesterol and HDL cholesterol concentration were measured with Sigma diagnostics kits (Sigma Chemical Co., St. Louis, MO). The diagnostic kit reagent mixture contained cholesterol oxidase (concentration >200 U/l), cholesterol esterase (>500 U/l), peroxidase (>300 U/l), 4-aminoantipyrine (0.25 m*M*), hydroxybenzoic acid (10 m*M*), and buffer (50 m*M*). One ml of this mixture was used per 10 µl of hamster plasma. Plasma triglycerides were measured with ThermoTrace kits (ThermoTrace Ltd., Melbourne, Australia). Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol and represented low-density lipoprotein (LDL) + intermediate-density lipoprotein (IDL) + very low density lipoprotein (VLDL) cholesterol.

Analysis of Fecal Bile Acids and Neutral Sterols. Hamsters were put in metabolic cages for 24 hrs to collect feces during the last 2 or 3 days of the feeding period. Quantitation of bile acids and neutral sterols in feces was measured by gas-liquid chromatography according to the method of Batta *et al.* (19). Two hundred µl n-butanol containing cholic acid and 5α-cholestane (internal standards) and 50 µl of concentrated hydrochloric acid were added to 15 mg of freeze-dried fecal samples and to the standards (i.e., 10 µg and 20 µg of each bile acid and neutral sterol). The mixture was heated at 60°C for 4 hrs, and solvents were evaporated under nitrogen at 60°C. The butyl-esterified bile acids, neutral sterol standards, and fecal samples were reacted with 100 µl of Sil-prep (trimethyl silylation reagent; Alltech Associates Inc., Deerfield, IL) for 30 mins at 55°C, and solvents were evaporated under nitrogen at 55°C. Trimethyl silyl ether derivatives of samples and standards were resuspended in 200 µl of hexane and centrifuged to remove fecal debris, and 2 µl were injected onto the gas-liquid chromatography column.

Analysis was performed using a Varian Chrompak CP-Sil 5 CB Low Bleed/MS fused silica capillary column, 25 *M* × 0.25-mm ID × 0.25-mm film thickness (Supelco Park, Bellefonte, PA) installed in a Hewlett Packard 6890 gas chromatograph equipped with a flame-ionization detector and autosampler. The carrier gas was helium at a flow rate of 1.5 ml/min, the injector temperature was 260°C, and the detector temperature was 290°C. Peaks were identified by comparing retention times with standards (i.e., hyodeoxycholic acid, deoxycholic acid, hyocholic acid, cholic acid, chenodeoxycholic acid, ursodeoxycholic acid, ursocholic acid, lithocholic acid, coprostanol, campesterol, stigmasterol, stigmastanol, cholestanone, cholesterol, cholestenone, cholestane, β-sitosterol, lanosterol, lathosterol; Steraloids Inc., Newport, RI). The weight percentage of each bile acid and neutral sterol was determined by integration of the peak areas.

Extraction of Fecal Samples for Soyasaponin Metabolites. A method of Hu *et al.* (16) was used. One gram of ground fecal sample was weighed and extracted with 50 ml of 70% ethanol at room temperature for 2 hrs. The extract was filtered through Whatman filter paper (Whatman, Florham Park, NJ). The filtrate was then collected in a round-bottom flask and dried in a rotary evaporator (Buchler Instrument, Fort Lee, NJ). The dried residues were dissolved in 5 ml of 20% methanol and slowly applied onto a preconditioned Sep-Pak cartridge (C18; Waters Corp., Milford, MA). To remove the water-soluble impurities, the cartridge was washed with 5 ml of distilled water followed by 5 ml of 30% methanol. The

Table 1. Group B Soyasaponins Lowered Plasma Total Cholesterol and Non-HDL Cholesterol in Female Hamsters[a]

Treatment	Total cholesterol (m*M*)	HDL cholesterol (m*M*)	Non-HDL cholesterol (m*M*)[b]	Ratio of total cholesterol to HDL cholesterol	Triglyceride (m*M*)
Casein	6.33 ± 0.32[c]	3.75 ± 0.53	2.58 ± 0.31[c]	1.69 ± 0.04[c]	2.67 ± 0.24[c]
Group B soyasaponin	5.14 ± 0.69[d]	3.43 ± 0.69	1.71 ± 0.39[d]	1.50 ± 0.10[d]	2.18 ± 0.17[d]

[a] Values represent means ± SD. $n = 10$. Within a column, means with different superscripts are different ($P < 0.05$).
[b] Represents the LDL + IDL + VLDL fractions (by difference: total – HDL).

eluent was recovered in 1 ml of HPLC-grade methanol and then vortexed and filtered through a 0.45-mm polytetrafluoroethylene filter (Alltech Associates Inc., Deerfield, IL) prior to HPLC analysis.

Group B soyasaponin metabolite was determined by HPLC (5). Elution was at a flow rate of 1 ml/min with 0.05% trifluoroacetic acid in Milli-Q water (solvent A; Millipore Co., Bedford, MA) and acetonitrile (solvent B). After injecting the 50-µl sample, solvent B was linearly increased from 73% to 100% over 35 mins. The solvent was recycled to 73% over 4 mins. The column temperature was 30°C, and analytes were monitored from 190–350 nm using a photodiode array detector.

Preparation of Soyasapogenol B and Identification of Soyasaponin Metabolite. Soyasapogenol B was prepared according to Hu *et al.* (16) to compare with soyasaponin metabolite. The chemical structure of soyasaponin metabolite was identified by ^{1}H nuclear magnetic resonance (^{1}H-NMR), ^{13}C Attached Proton Test NMR (^{13}C-NMR), electrospray-ionized (ESI) mass spectroscopy, and infrared absorbance analyses. The ^{1}H-NMR and ^{13}C-NMR spectra were acquired on a Varian VXR-300 spectrometer (Varian Inc., Palo Alto, CA). A Finnigan TSQ 700 triple quadrupole mass spectrometer, equipped with a Finnigan ESI interface, was used in the positive Q1MS mode (Finnigan MAT, San Jose, CA). The sample was dissolved in methanol-d_4 or choloroform-d_6 (Cambridge Isotope Laboratories, Inc., Andover, MA). The ^{1}H-NMR and ^{13}C-NMR spectra of soyasapogenol B were in good agreement with those reported by Kudou *et al.* (20) and Hu *et al.* (16).

Statistical Analysis. Statistical analyses were conducted with the Statistical Analysis System (Release 8.2; SAS Institute Inc., Cary, NC). Values were expressed as means ± SD. The results were analyzed by one-way ANOVA, and differences between treatments were determined by the least-significant-difference test. An α of 0.05 was used to determine statistically significant differences.

Cluster analysis was performed to classify fecal soyasaponin metabolite excretion phenotypes and was aimed at sorting into groups based on the degree of association between the two objects. Linear regression analysis was also performed to determine the strength of relationship between plasma lipid status and fecal soyasaponin metabolite production ($P < 0.05$ was used as the criterion for significant correlations).

Results

Hamster Body Weights and Food Intakes. Daily food intakes did not differ between casein-fed hamsters and group B soyasaponin–fed hamsters (7.5 ± 1.2 g vs. 7.2 ± 1.5 g). Treatment groups had similar initial mean body weights (casein, 111 ± 10 g; group B soyasaponin, 112 ± 11 g). There were no differences in gain weights between hamsters fed casein or group B soyasaponins (14 ± 3 g vs. 16 ± 7 g).

Effects of Group B Soyasaponins on Plasma Lipid Concentrations. Total cholesterol, non-HDL cholesterol, and triglyceride concentrations, and the total-cholesterol-to-HDL-cholesterol ratio, were significantly less (by 20%, 33%, 18%, and 13%, respectively) in hamsters fed group B soyasaponins ($P < 0.05$) compared with casein-fed hamsters (Table 1). There were no differences in HDL cholesterol concentrations between treatments (Table 1).

Fecal Bile Acids and Neutral Sterols. There were significant effects of treatment on bile acid and neutral sterol outputs (Table 2). Mean value of total fecal bile acid and neutral sterol outputs was significantly greater in hamsters fed group B soyasaponins ($P < 0.05$) compared with those fed casein (Table 2). Fecal excretion of cholic acid, cholestane, coprostanol, lathosterol, and campesterol was significantly greater in hamsters fed group B soyasaponins than in those fed casein ($P < 0.05$; Table 2). Hyodeoxycholic acid, hyocholic acid, ursodeoxycholic acid, and lanosterol

Table 2. Group B Soyasaponins Increased Daily Fecal Excretion of Bile Acids and Neutral Sterols (µmol) in Female Hamsters[a]

	Casein	Group B soyasaponin
Total bile acids	22.5 ± 10.3[b]	46.2 ± 21.9[c]
Cholic acid	20.8 ± 12.7[b]	43.5 ± 25.6[c]
Total neutral sterols	29.2 ± 4.7[b]	54.1 ± 8.7[c]
Cholestane	6.8 ± 2.6[b]	9.7 ± 4.1[c]
Coprostanol	1.8 ± 0.6[b]	6.1 ± 3.4[c]
Lathosterol	15.1 ± 9.5[b]	25.9 ± 14.4[c]
Campesterol	1.3 ± 0.3[b]	3.4 ± 1.6[c]

[a] Values represent means ± SD. $n = 10$. Within a column, means with different superscripts are different ($P < 0.05$).

were not detected. Other bile acids (i.e., deoxycholic acid, chenodeoxycholic acid, ursocholic acid, lithocholic acid) and neutral sterols (i.e., cholesterol, cholestanone, cholesterone, stigmasterol, β-sitosterol, stigmastanol) were not significantly different between treatments.

Fecal Soyasaponin Metabolite Excretion. The retention time of a putative soyasaponin metabolite was approximately 22 mins on the HPLC system, whereas the retention time of soyasapogenol B was approximately 15 mins. According to a cluster analysis ($P < 0.05$), hamsters fed group B soyasaponins sorted into two fecal soyasaponin metabolite excretion phenotypes: high excreters ($n = 3$) and low excreters ($n = 7$; Fig. 1).

Effects of Group B Soyasaponin Metabolite on Plasma Lipid Concentrations. Linear-regression analysis demonstrated that plasma total cholesterol ($P < 0.02$), non-HDL cholesterol ($P < 0.004$), and the ratio of total cholesterol to HDL cholesterol ($P < 0.004$) were significantly correlated with peak area of fecal group B soyasaponin metabolite (Fig. 2a, b, and c), whereas plasma HDL-cholesterol and triglycerides were not correlated with fecal soyasaponin metabolite peak area. There were no significant differences in plasma total cholesterol, HDL cholesterol, non-HDL cholesterol, or triglyceride concentrations between high and low producers of soyasaponin metabolite (Table 3). But, high producers of soyasaponin metabolite showed improved plasma lipid status (total cholesterol:HDL cholesterol) compared with low producers of the soyasaponin metabolite ($P < 0.03$; Table 3).

Identification of Soyasaponin Metabolite. The ^{1}H-NMR (400 MHz) spectra of soyasaponin metabolite showed signals (Fig. 3A) at δ 5.53 (m, 4H), 2.96 (t, $J = 6.0$ Hz, 2H), 2.46 (t, $J = 7.6$ Hz, 3H), 2.25 (d, $J = 6.4$ Hz, 4H), 1.78 (s, 3H), 1.50 (d, $J = 15.2$ Hz, 24H), and 1.06 (m, 6H). The ^{13}C-NMR (100 MHz) spectrum of soyasaponin metabolite exhibited carbon signals (Fig. 3B) at 177.9, 131.1, 131.0, 129.3, 129.2, 37.9, 35.9, 35.1, 32.8, 31.3, 31.0, 30.9, 30.8, 30.7, 30.5, 30.4, 28.3, 26.7, 26.3, 19.8, 14.6, and 11.9 ppm. The ^{13}C-NMR spectrum at 131.1, 130.0, 129.3, 129.2, and 177.9 ppm indicated the presence of two double bonds and

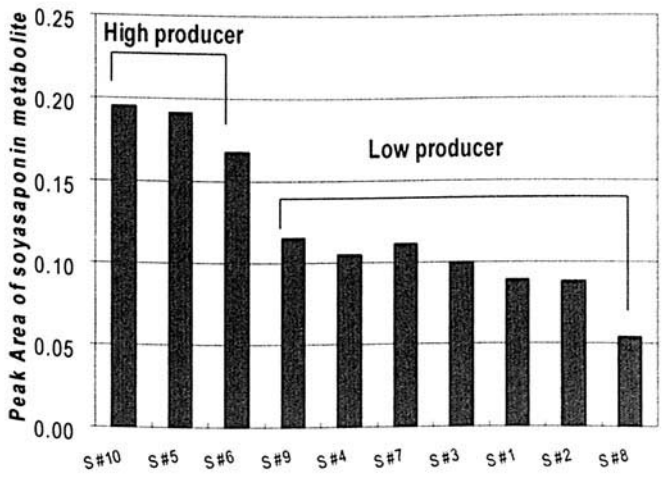

Figure 1. Fecal soyasaponin metabolite excretion phenotypes, as quantified by HPLC analysis. S# denotes individual animal identification numbers.

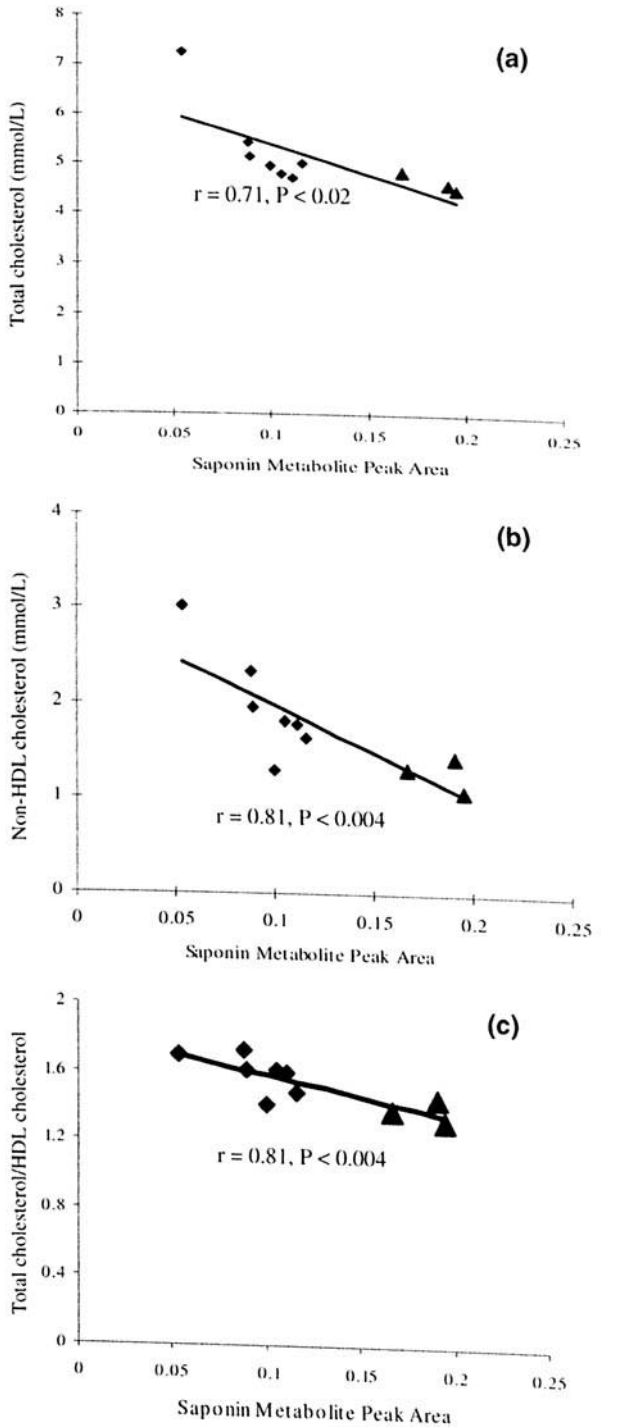

Figure 2. A linear-regression analysis of the relationship between the fecal soyasaponin metabolite peak area and plasma lipid status in hamsters fed group B soyasaponins. High producers (▲) and low producers (♦) of soyasaponin metabolite were distinguished according to cluster analysis ($P < 0.05$). (a) Total cholesterol. (b) Non-HDL cholesterol. (c) The ratio of total cholesterol to HDL cholesterol.

Table 3. High Producers of a Soyasaponin Metabolite Showed Improved Plasma Lipid Status[a]

	Total cholesterol (m*M*)	HDL cholesterol (m*M*)	Non-HDL cholesterol (m*M*)[b]	Ratio of total cholesterol to HDL cholesterol	Triglyceride (m*M*)
High producers (n = 3)	4.68 ± 0.18	3.39 ± 0.18	1.28 ± 0.18	1.38 ± 0.07[c]	1.73 ± 0.49
Low producers (n = 7)	5.35 ± 0.88	3.37 ± 0.47	1.98 ± 0.55	1.59 ± 0.13[d]	2.38 ± 0.71

[a] Values represent means ± SD. Within a column, means with different superscripts are different ($P < 0.03$).
[b] Represents the LDL + IDL + VLDL fractions (by difference: total – HDL).

one carbonyl-stretching band. The positive mode ESI spectra of soyasapogenol B and soyasaponin metabolite (Fig. 3C) were ion peak at m/z 332 $[M + 5CH_3OH + 2Na]^{2+}$ and m/z 393 $[M\text{-}COOH]^+$, respectively. The carboxyl group of soyasaponin metabolite was confirmed with its infrared absorbance spectrum, absorbing in the region of 1600–1800 cm^{-1}. The molecular formula and weight of soyasaponin metabolite were estimated by elemental analysis, $C_{30}H_{46}O_2$ and 438, respectively (the molecular weight is a calculated value based on the molecular formula). The putative structure of this soyasaponin metabolite is shown in Figure 4.

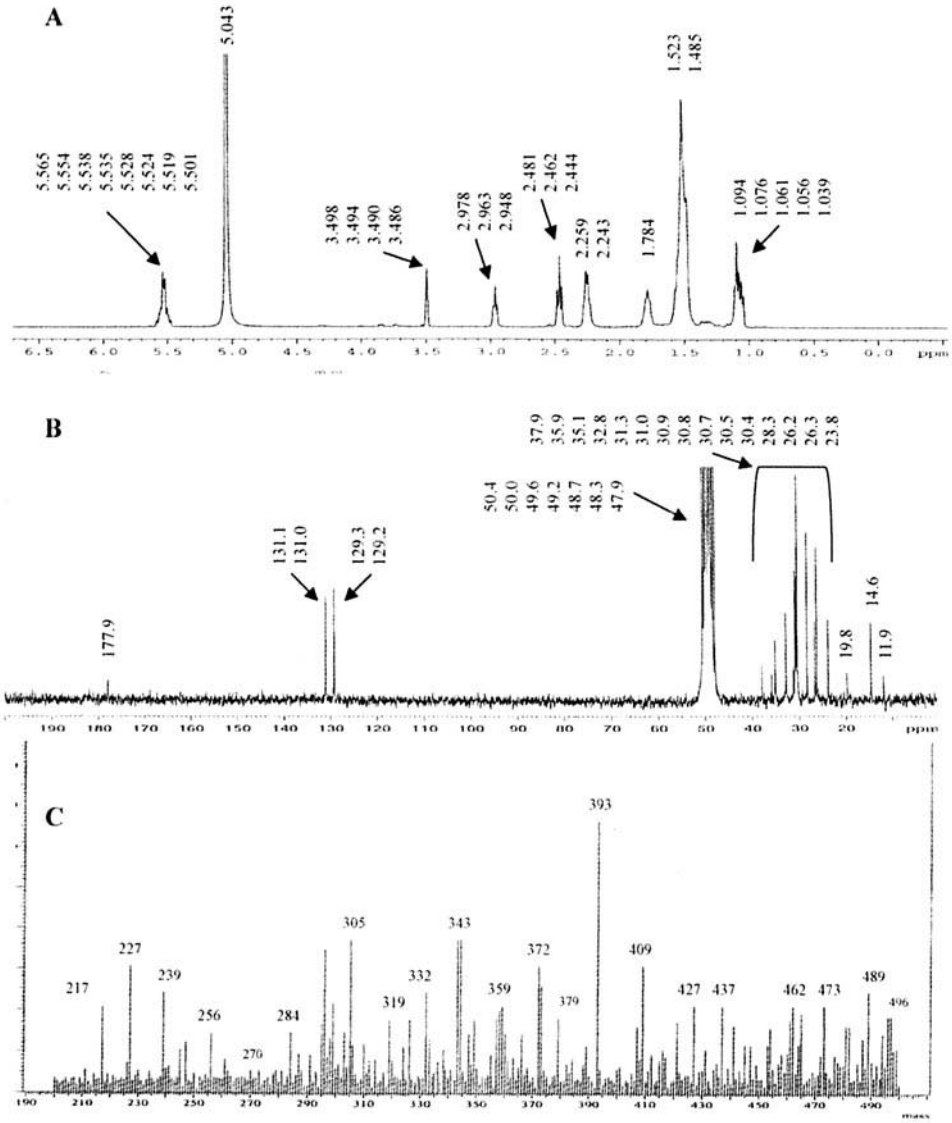

Figure 3. (A) The ^{1}H-NMR spectra of soyasaponin metabolite (ppm noted for peaks are printed above each peak). (B) The ^{13}C-NMR spectra of soyasaponin metabolite (ppm noted for peaks are printed above each peak). (C) The ESI spectra of soyasaponin metabolite (the molecular mass of each fragment is noted on the figure).

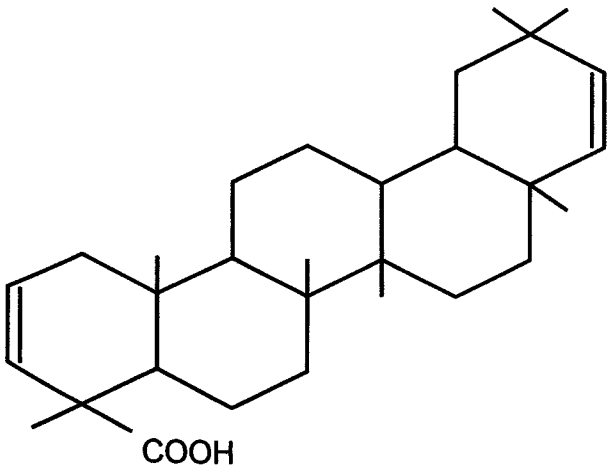

Figure 4. The putative structure of soyasaponin metabolite.

Discussion

Group B soyasaponins lowered plasma total cholesterol, non-HDL cholesterol, triglycerides, and the total-cholesterol-to-HDL-cholesterol ratio in female hamsters (Table 1). Greater fecal excretion of bile acids and neutral sterols was observed in hamsters fed soyasaponins compared with those fed casein (Table 2). These results were similar to those seen in rats fed the 1% cholesterol diet plus 1% purified soyasaponins, which significantly lowered plasma (26%) and hepatic (33%) total cholesterol by increasing the excretion of fecal bile acids and neutral sterols compared with the 1% cholesterol diet. In the present study, the significant reduction of non–HDL cholesterol levels and triglyceride levels in hamsters fed soyasaponins may be a consequence of decreased hepatic cholesterol reserve as a result of the increased fecal excretion of bile acids and neutral sterols. Taken together, these data show that the cholesterol-lowering effects of group B soyasaponins were mediated by increased fecal output of bile acids and neutral sterols, indicating the inhibition of intestinal reabsorption of bile acids.

Limited studies reported fecal excretion of bile acids and neutral sterols in hamsters (21–24). Trautwein *et al.* (22) reported that male golden Syrian hamsters fed 6% psyllium for 5 weeks had significantly greater fecal concentrations of coprostanol, cholesterol, deoxycholic acid, lithocholic acid, ursodeoxycholic acid, and chenodeoxycholic acid than those fed cellulose. Fecal excretion of bile acids (i.e., lithocholic acid, deoxycholic acid, chenodeoxycholic acid) and neutral sterols (i.e., cholestanol, coprostanol) was significantly increased in male hamsters fed soy protein isolate for 2 weeks compared with those fed casein (24). Trautwein *et al.* (25) investigated the bile-acid profile from the gallbladders of male and female hamsters fed a gallstone-inducing diet and 0.4% cholesterol. Female hamsters showed a significantly greater percentage of cholate and a lower percentage of chenodeoxycholate than males. In the present study, total fecal bile acid and neutral sterol outputs were significantly greater (by approximately two-fold each) in hamsters fed group B soyasaponins than in those fed casein, and they comprised mainly cholic acid and lathosterol. The effects of diet on fecal-bile acid and the neutral-sterol profile, and the significance of this profile to cholesterol metabolism, need further study.

Few studies reported the metabolic fate of soyasaponins (14–17). *In vitro*, Gurfinkel and Rao (15) found that group A and B soyasaponins were hydrolyzed by colonic microflora to produce soyasapogenols A and B. Hu *et al.* (16) identified two gut microbial metabolites of soyasaponin I, soyasaponin III, and soyasapogenol B when human feces were incubated in brain-heart infusion media with soyasaponin I (10 μmol/g feces) for 48 hrs at 37°C. *In vivo*, Gestetner *et al.* (14) could not detect soyasaponins or soyasapogenols in blood of rats, mice, and chicks. Only soyasapogenol B was detected in human feces (i.e., 8.4% of ingested soyasaponins was recovered) after the ingestion of 434 μmol group B soyasaponins. Neither soyasapogenol B nor other soyasaponins were found in human urine (17). Thus, soyasaponins seemed not to be absorbed, but were metabolized by intestinal microorganisms and excreted in the feces.

In the present study, a new metabolite of group B soyasaponins was detected in hamster feces after a 4-week feeding period, but neither soyasaponins nor soyasapogenol B were found. Ingested soyasaponins were further metabolized or degraded by intestinal microflora. By cluster analysis, two fecal soyasaponin metabolite excretion phenotypes were identified: high and low. When high and low producers of a putative soyasaponin metabolite were compared, the high producers of soyasaponin metabolite showed a significantly lower total-cholesterol-to-HDL-cholesterol ratio ($P < 0.03$; Table 3). A nonsignificant reduction in plasma non-HDL cholesterol ($P < 0.07$) was observed in the high producers of soyasaponin metabolite. Perhaps if a larger number of animals were fed, the presence of this saponin metabolite might be associated with a significant improvement in the plasma non-HDL cholesterol concentration. The greater presence of soyasaponin metabolite did not affect fecal bile acid or neutral sterol excretion (data not shown), indicating that this metabolite, if important to cholesterol status, acted by a different mechanism than did parent soyasaponins.

In conclusion, group B soyasaponins lowered plasma-cholesterol levels by a mechanism involving the greater excretion of fecal bile acids and neutral sterols compared with casein. Greater production of soyasaponin metabolite in hamsters was associated with improved plasma cholesterol status. This study indicated that understanding the health effects of soyasaponins will require more knowledge of the metabolic fate and actions of metabolites of soyasaponins in animals and humans.

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