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Human Fecal Metabolism of Soyasaponin I

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Abstract

The metabolism of soyasaponin I (3-O-[alpha-L-rhamnopyranosyl-beta-D-galactopyranosyl-beta-D-glucuronopyranosyl]olean-12-ene-3beta,22beta,24-triol) by human fecal microorganisms was investigated. Fresh feces were collected from 15 healthy women and incubated anaerobically with 10 mmol soyasaponin I/g feces at 37 degrees C for 48 h. The disappearance of soyasaponin I in this in vitro fermentation system displayed apparent first-order rate loss kinetics. Two distinct soyasaponin I degradation phenotypes were observed among the subjects: rapid soyasaponin degraders with a rate constant k = 0.24 +/- 0.04 h(-)(1) and slow degraders with a k = 0.07 +/- 0.02 h(-)(1). There were no significant differences in the body mass index, fecal moisture, gut transit time, and soy consumption frequency between the two soyasaponin degradation phenotypes. Two primary gut microbial metabolites of soyasaponin I were identified as soyasaponin III (3-O-[beta-D-galactopyranosyl-beta-D-glucuronopyranosyl]olean-12-ene-3beta,22beta,24-triol) and soyasapogenol B (olean-12-ene-3beta,22beta,24-triol) by NMR and electrospray ionized mass spectroscopy. Soyasaponin III appeared within the first 24 h and disappeared by 48 h. Soyasapogenol B seemed to be the final metabolic product during the 48 h anaerobic incubation. These results indicate that dietary soyasaponins can be metabolized by human gut microorganisms. The sugar moieties of soyasaponins seem to be hydrolyzed sequentially to yield smaller and more hydrophobic metabolites.

Keywords

College of Veterinary Medicine, Soyasaponin; metabolism; human gut microorganisms

Disciplines

Food Science | Human and Clinical Nutrition | Other Animal Sciences

Comments

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The metabolism of soyasaponin I (3-O-[α-L-rhamnopyranosyl-β-D-galactopyranosyl-β-D-glucuronopy-ranosyl]olean-12-ene-3β,22β,24-triol) by human fecal microorganisms was investigated. Fresh feces were collected from 15 healthy women and incubated anaerobically with 10 mmol soyasaponin I/g feces at 37 °C for 48 h. The disappearance of soyasaponin I in this in vitro fermentation system displayed apparent first-order rate loss kinetics. Two distinct soyasaponin I degradation phenotypes were observed among the subjects: rapid soyasaponin degraders with a rate constant $k = 0.24 \pm 0.04$ h^{-1} and slow degraders with a $k = 0.07 \pm 0.02$ h^{-1}. There were no significant differences in the body mass index, fecal moisture, gut transit time, and soy consumption frequency between the two soyasaponin degradation phenotypes. Two primary gut microbial metabolites of soyasaponin I were identified as soyasaponin III (3-O-[β-D-galactopyranosyl-β-D-glucuronopyranosyl]olean-12-ene-3β,-22β,24-triol) and soyasapogenol B (olean-12-ene-3β,22β,24-triol) by NMR and electrospray ionized mass spectroscopy. Soyasaponin III appeared within the first 24 h and disappeared by 48 h. Soyasapogenol B seemed to be the final metabolic product during the 48 h anaerobic incubation. These results indicate that dietary soyasaponins can be metabolized by human gut microorganisms. The sugar moieties of soyasaponins seem to be hydrolyzed sequentially to yield smaller and more hydrophobic metabolites.

KEYWORDS: Soyasaponin; metabolism; human gut microorganisms

INTRODUCTION

Saponins are a family of steroid or triterpenoid glycosides found in a wide variety of plants. Soyasaponins are triterpenoid glycosides with one or two polysaccharide chains mainly present in legumes (*1*). Significant amounts of soyasaponins are found in soybeans and soy products between 0.5 and 114 μmol/g (*2*). The potential relationship of soyasaponins to health effects has been suggested. Soyasaponins seem to be significant contributors to the cholesterol lowering effect of soy consumption (*3*, *4*). Soyasaponins showed anticarcinogenic activity against various tumors or tumor cell lines (*5*−*8*) and have been shown to have hepatoprotective and antiviral activities (*9*−*11*).

It is important to understand the intestinal metabolism and absorption of soyasaponins because humans obtain soyasaponins from the diet. The forms of soyasaponins that are most likely to be involved in the potential health effects of these compounds are not known. Most studies investigating the biological effects of soyasaponin have been limited to in vitro experiments and a few animal studies. Data on the bioavailability and absorption of saponins are scarce. Gestetner et al. (*12*) reported that ingested

soyasaponins were hydrolyzed to aglycones by nonspecific glycosidases of cecal microflora in chicks, rats, and mice. Karikura et al. (*13*) observed that ginseng saponins were hydrolyzed into smaller molecules with removal of the sugar moieties in the rat intestine. However, no study has reported the fate of soyasaponins or explored their possible metabolites in humans.

The present study was designed to investigate whether soyasaponins were metabolized by human gut microorganisms using a model fecal incubation system and to identify gut metabolites of soyasaponins. Soyasaponin I (**Figure 1**), as a representative soyasaponin, was examined in this study because it is the predominant form of soyasaponin in heat-treated soy products (*2*). Individual variability in soyasaponin metabolism by human gut microflora was evaluated, and soyasaponin catabolic phenotype among human subjects was characterized. Gut microorganism populations and bacterial enzyme activities have been known to be affected by factors such as genetic background, dietary habits, and physical activity in humans (*14*). Therefore, the influence of individual status, such as body composition, ethnicity, gut transit time (GGT), and soy consumption on fecal metabolism of soyasaponins, was examined as well. The information obtained in this study will help to predict soyasaponin bioavailability in humans and provide

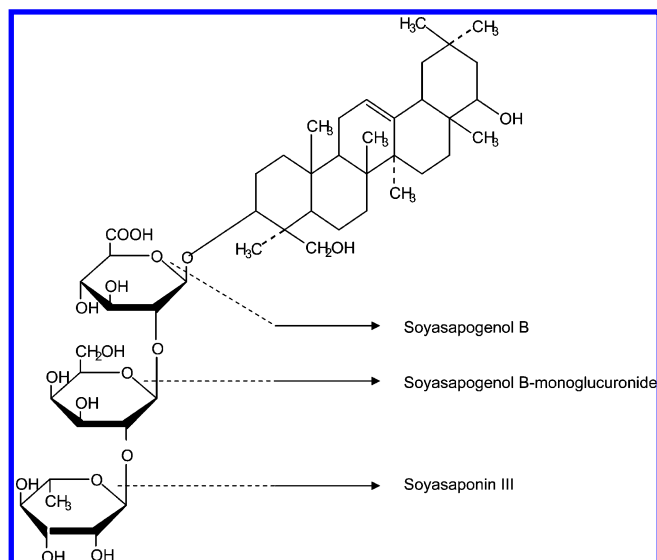**2690** *J. Agric. Food Chem.,* Vol. 52, No. 9, 2004

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Figure 1. Structures of soyasaponin I and its metabolites.

evidence of the presence of soyasaponin metabolites in the human intestine.

MATERIALS & METHODS

Preparation of Soyasaponin I and III and Soyasapogenol B Standards. Soyasaponin I was isolated using the method of Hu et al. (*2*) from soy germ donated by Acatris (Minneapolis, MN). Soyasaponin III was produced by hydrolyzing 100 mg of soyasaponin I in 1 N hydrochloric acid−dioxane (1:1, v/v) by refluxing for 1 h. The reaction mixture was neutralized by 10 N sodium hydroxide and desalted with a high capacity C_{18} SPE column (Alltech Associates Inc., Deerfield, IL) eluting sequentially with 150 mL of water and 100 mL of methanol. Crude soyasaponin III was obtained in the methanol fraction. A yield of 43 mg of soyasaponin III was obtained by further purification with a semipreparative high-performance liquid chromatography (HPLC) system as previously described (*2*). Soyasapogenol B was produced by hydrolyzing 100 mg of crude group B soyasaponins (generously provided by Dr. Mark Berhow, National Center for Agricultural Utilization Research, USDA, Peoria, IL) in 3 N hydrochloric acid by refluxing for 3 h. The reaction mixture was neutralized, desalted as described above, and then purified with the semipreparative HPLC system using 70% aqueous acetonitrile with 1 mM ammonium acetate at a 2 mL/min flow rate. A yield of 24 mg of purified soyasapogenol B was obtained as a white amorphous powder.

The structural identity of the purified soyasaponin standards was confirmed by electrospray ionized (ESI) mass spectroscopy and ^{1}H and ^{13}C NMR analyses. ESI spectra were acquired in the positive Q1MS mode on a Finnigan TSQ 700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) fitted with a Finnigan ESI interface. ^{1}H NMR and ^{13}C attached proton test NMR spectra were acquired on a Varian VXR-300 spectrometer (Varian Inc., Palo Alto, CA). The samples were dissolved in chloroform-d_6 or dimethyl sulfoxide-d_6 (Cambridge Isotope Laboratories, Inc., Andover, MA) with tetramethylsilane (Cambridge Isotope Laboratories, Inc.) as an internal standard. The spectra obtained for these purified compounds were in good agreement with those reported by Baxter (*15*) and Kudou et al. (*16*).

Anaerobic Incubation of Soyasaponin I with Human Fecal Microflora. The subjects were 15 healthy women recruited from Iowa State University and the surrounding community. They were 18−52 years of age, with a body mass index (BMI) of 23.0 ± 5.8 kg/m^2, and had not used any medication for 3 months prior to and during the study. The subjects included ten Caucasians, four Chinese, and one Asian Indian. The study protocol was reviewed and approved by Iowa State University Human Subjects in Research Committee in 2001.

Brain−heart infusion media (BHI) was prepared according to Zheng (*17*). Soyasaponin I was added to BHI media in a concentration of 1.6 μmol/mL. All media were saturated with CO_2 and autoclaved.

The subjects were instructed to avoid soyasaponin-containing foods for 4 days prior to fecal collection as a washout period. One fresh stool from each subject was collected into a sterile plastic bag at the end of the washout period. Feces were manually homogenized in the sealed bag. Ten grams of feces was added to 25.0 mL of sterilized BHI media and mixed by vortexing. The fecal suspension (10 mL) was inoculated into 25.0 mL of BHI media containing soyasaponin I to give an initial concentration of 10 μmol soyasaponin I/g feces. The mixture was incubated anaerobically at 37 °C for 48 h. Parallel incubation was performed without soyasaponin I present in the media for each subject as a negative control. Two types of positive controls were included as follows: BHI media containing soyasaponin I without feces and BHI media containing soyasaponin I cultured with autoclaved fecal suspension. Culture aliquots were taken at 0, 4, 8, 12, 24, 36, and 48 h and immediately frozen at −20 °C until analysis.

The samples were thawed to room temperature (RT) before soyasaponin analysis. A 2.0 mL aliquot of each sample was dispersed in 8 mL of methanol in a 15 mL polypropylene centrifuge tube and shaken at RT for 30 min. The suspension was centrifuged at 15 000g for 10 min. The supernatant was removed, and the precipitate was resuspended in 10 mL of methanol. After the mixture was vortexed for 15 min, the sample suspension was centrifuged again. After the second centrifugation, the two supernatants were combined and evaporated to dryness under reduced pressure at RT. The residue was resuspended in 5 mL of 20% methanol and loaded onto a preconditioned Sep-Pak cartridge (classic short-body C_{18}, Waters Corp., Milford, MA). The cartridge was washed with 5 mL of 5% methanol. Soyasaponin was eluted with 2.0 mL of HPLC grade methanol. The extract was vortexed and subjected to thin-layer chromatography (TLC) and HPLC. Samples were extracted and analyzed in duplicate. The logarithm of the remaining soyasaponin I concentration was plotted vs incubation time. The soyasaponin I disappearance reaction rate loss constant k and half-life $t_{1/2}$ were calculated according to Labuza and Riboh (*18*).

TLC analyses were performed on the LK6F silica gel plates (Whatman, Hillsboro, OR). Each set of fecal incubation samples per subject was analyzed using two different solvent conditions: one was developed with *n*-butanol−ethanol−aqueous ammonia (5:5:4, v/v), and the other was developed with hexanes−ethyl acetate (2:1, v/v). Soyasaponins were detected after spraying acetic acid−sulfuric acid−anisaldehyde (100:2:1, v/v) and heating at 120 °C for 10 min.

Soyasaponin I concentration and metabolite formation were determined by HPLC using the method previously reported (*2*). The mobile phases were 0.05% trifluoroacetic acid in water (solvent A) and 0.05% trifluoroacetic acid in acetonitrile (solvent B). The gradient elution was carried out as: solvent B held at 37% for 3 min, increased from 37 to 40% in 12 min, then solvent B increased to 48% in 25 min, and finally solvent B increased to 100% in 1 min and held at 100% for 2 min. The gradient program recycled back to the initial state of 37% solvent B in 5 min. The column temperature was 30 °C. The injection volume was 50 μL. The flow rate was 1 mL/min, and the UV absorbance was monitored from 190 to 350 nm.

To monitor the formation of more hydrophobic metabolites in the culture, the samples were analyzed with the same HPLC system using a different gradient program: solvent B increased linearly from 73 to 100% in 35 min and then solvent B recycled back to 73% in 4 min.

Identification of Soyasaponin I Metabolites. The metabolites of soyasaponin I formed in the culture were isolated as described below. Five grams of fresh human feces was anaerobically incubated in 50 mL of BHI media containing 50 mg of soyasaponin I at 37 °C for 48 h. Two 25 mL aliquots were taken at 12 and 48 h separately. Each sample was fractionated with a high capacity C_{18} SPE column (Alltech Associates Inc.) by successively eluting with 150 mL of water, 150 mL of 30% aqueous methanol, and 100 mL of methanol. The eluted fractions containing metabolites were further separated on preparative TLC (PK6F, Whatman) to give 12 mg of metabolite I and 20 mg of metabolite II. The metabolites were analyzed by analytical HPLC and TLC. Their R_f values on TLC and retention times on HPLC were compared with authentic standards.

Fecal incubation samples at each time point from the selected subjects were analyzed by LC-atmospheric pressure chemical ionization (ApCI)-MS to further confirm the chemical identity of the metabolites. The

analyses were conducted on a Hewlett-Packard HPLC system (Agilent Technologies, Wilmington, DE) coupled with a triple quadrupole LC-MS-MS mass spectrometer (VG Biotech, Manchester, U.K.). Sample separation was carried out under the same HPLC gradient programs as described above except that the flow rate was 0.525 mL/min. The injection volume ranged from 5 to 20 μL depending on the concentration of the analytes in the solution. The effluent was delivered to the electrospray source configured with a corona discharge pin. Nitrogen gas was used as the nebulizing and auxiliary gas for the mass spectrometer. The parameters applied to MS were as follows: corona discharge voltage, 3 kV; cone voltage, 30 V; ApCI probe temperature, 350 °C; source temperature, 120 °C; scan time, 2 s; and interscan time, 0.1 s. The full scan mass spectra over m/z range of 200−1100 amu were acquired on the eluted analytes. Before analysis, the mass spectrometer was tuned and calibrated for the range of m/z 200−1100. The soyasaponin standards were dissolved in methanol and analyzed to obtain authentic mass spectra prior to sample analysis.

Statistical Analysis. All results were reported as the mean ± standard deviation (SD) or mean ± mean square of error (MSE). Statistical analyses were performed with an SAS system (Version 8.1, SAS Institute Inc., Cary, NC). The kinetics of soyasaponin I metabolism were analyzed by general linear regression. The phenotypic pattern in human subjects was identified by average linkage cluster analysis (*19*). A general linear model was used to analyze the differences in BMI, stool moisture, GGT, and soy consumption frequency between soyasaponin degradation phenotypes. Statistical significance was set at α = 0.05 for all analyses.

RESULTS AND DISCUSSION

The LC-MS chromatograms of standard soyasaponin I (3-O-[α-L-rhamnopyranosyl-β-D-galactopyranosyl-β-D-glucuronopyranosyl]olean-12-ene-3β,22β,24-triol), soyasaponin III (3-O-[β-D-galactopyranosyl-β-D-glucuronopyranosyl]olean-12-ene-3β, 22β,24-triol), and soyasapogenol B (olean-12-ene-3β,22β,24-triol) are shown in **Figure 2**. The ESI spectra of individual compounds gave a primary ion peak at m/z 944 [M + H]$^+$ for soyasaponin I, m/z 819 [M + Na]$^+$ for soyasaponin III, and m/z 459 [M + H]$^+$ for soyasapogenol B. Their ESI mass spectra and NMR data were in good agreement with the literature (*15*, *16*). The mass spectra of these standards by LC-ApCI-MS are shown in **Figure 3**. Their retention times, R_f values, and ApCI-MS fragmentation patterns are summarized in **Table 1**.

The metabolism of soyasaponin I by intestinal microflora was examined using an in vitro static fecal fermentation model. The TLC profile for soyasaponin I incubation showed that soyasaponin I gradually disappeared and two major metabolites with higher R_f values appeared in the culture over 48 h (**Figure 4**). This indicates that soyasaponin I was converted to more hydrophobic metabolites I and II. Metabolite II appeared to be the primary product in the culture after 24 h of incubation. Disappearance of soyasaponin I was not observed in the cultures inoculated with autoclaved fecal material or without fecal material indicating that degradation of soyasaponin I was caused by viable fecal microorganisms and was probably enzymatic in nature (**Figure 4**).

The HPLC chromatograms of cultured fecal samples at 12 and 48 h are shown in **Figure 2C,E**, respectively. Metabolite I was detected along with soyasaponin I using the gradient program of Hu et al. (*2*). The retention time of soyasaponin I was 25.9 min on HPLC and an R_f value of 0.60 on TLC when using the 1-butanol−ethanol−ammonia solvent system. Metabolite I had a retention time of 29.9 min on HPLC and a R_f value of 0.63 on TLC. The scanned mass spectrum showed peaks at m/z 797.3 [M + H]$^+$, 599.4 [M-rhm-gal-2H$_2$O + H]$^+$, 441.3 [aglycone − OH]$^+$, 423.3 [aglycone − OH − H$_2$O]$^+$, and 405.3 [aglycone − OH − 2H$_2$O]$^+$. Metabolite II had a

retention time of 27.4 min on HPLC under the more hydrophobic HPLC gradient program and a R_f value of 0.43 on TLC using the hexanes−ethyl acetate solvent system. The scanned ApCI mass spectrum of metabolite II showed peaks at m/z 441.3 [aglycone − OH]$^+$, 423.3 [aglycone − OH − H$_2$O]$^+$, and 405 [aglycone − OH − 2H$_2$O]$^+$. The HPLC, TLC, and MS spectral data of metabolites I and II matched those of soyasaponin III and soyasapogenol B, respectively.

These results suggest that the main metabolic pathway of soyasaponin I is the hydrolysis of terminal rhamnose from the sugar chain of soyasaponin I to produce soyasaponin III, then further hydrolysis of remaining sugars to yield soyasapogenol B. However, soyasapogenol B-monoglucuronide, one of the hypothesized metabolites of soyasaponin I, was not observed during incubation in our study, which may be due to greater gut microbial glucuronidase than rhamnosidase activity (*20*). The hydrolysis of glucuronic acid from the aglycones may occur so quickly that soyasapogenol B-monoglucuronide was not detected in our hands. On the basis of these data, it is reasonable to infer that other individual soyasaponins could be metabolized by gut microorganisms similarly; that is, the terminal sugars could be hydrolyzed in a stepwise manner, and the aglycones with structural characteristics of each soyasaponin would result.

The metabolism of saponins by microorganisms has been reported. Makkar et al. (*21*) demonstrated that quillaja triterpenoid saponins disappeared when incubated with cattle rumen liquor in vitro, suggesting the metabolism of saponins by rumen microorganisms. Gestetner et al. (*12*) incubated the cecal and colon contents of rats, chicks, and mice with soyasaponins in vitro and detected soyasaponins and soyasapogenols in the culture after a 3 h incubation. However, they did not report whether any intermediate decomposition products of soyasaponins were detected. Hasegawa et al. (*22*, *23*) reported the anaerobic metabolism of ginseng saponins by human fecal microorganisms. They demonstrated that ginsenosides were converted into smaller molecules by stepwise cleavage of terminal sugars. Our results demonstrate that human intestinal microorganisms have the ability to metabolize soyasaponins. Gestetner et al. (*12*) showed that the nonspecific glycosidases purified from rat cecal microorganisms were able to liberate glucose, galactose, arabinose, rhamnose, and glucuronic acid from soyasaponins during incubation. Thus, mammalian gut microbial enzymes had the ability to hydrolyze various soyasaponin glycosidic bonds. Human intestinal bacteria, especially *Lactobacilli*, *Bacteroides*, and *Bifidobacteria* species, possess glycosidase and β-glucuronidase activities (*15*, *24*). α-L-Rhamnosidase is reportedly produced by some strains of human intestinal *Bacteroides* (*20*, *25*). These bacterial enzymes might play a major role in liberating the aglycones from sugar-conjugated soyasaponins in the human gut. Food residue remains in the large intestine 24 h or longer. Microbes in the large bowel would have sufficient time to interact with and hydrolyze soyasaponins. Further study will be needed to identify the bacterial species in the human intestinal tract that metabolize soyasaponins.

In our in vitro incubation study, soyasaponin III and soyasapogenol B were identified as the major microbial metabolites of soyasaponin I, implying their possible existence in the human gut. A few studies investigated the biological activity of these soyasaponin metabolites. The variety and number of sugars attached to soyasapogenols have profound impact on their activity. Ikeda et al. (*26*) found that soyasaponins with a disaccharide group such as soyasaponin III and IV had more
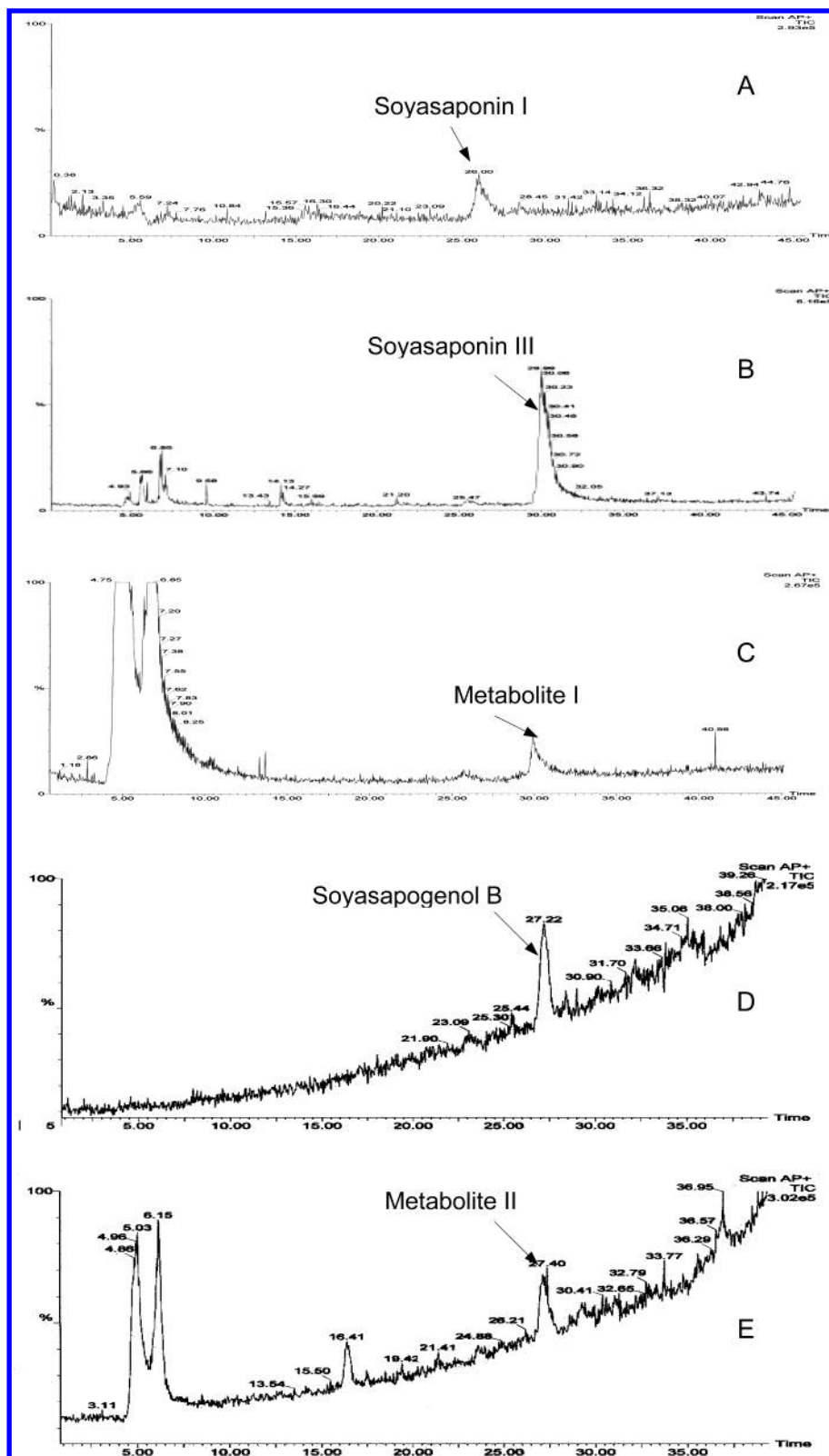
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Figure 2. Ion chromatograms of soyasaponin I (**A**), soyasaponin III (**B**), and soyasapogenol B (**D**) standards with a positive mode ApCI-MS detection. The chromatograms of 12 and 48 h fecal incubation extracts from one subject are shown in panels **C** and **E**, respectively.

potent hepatoprotective activity against immunologically induced damage than those with a trisaccharide group such as soyasaponin I and II and soyasapogenol B in the primary cultured rat hepatocytes. Soyasapogenol B had more potent activity than its various glycosides in suppressing 2-acetoxy-acetylaminofluorene-induced genotoxicity in Chinese hamster ovary cells (*27*). Soyasapogenol B showed antiproliferative

activity in human breast cancer cell lines MCF-7 and MDA-MB-231 in vitro (*28*). Therefore, the bioavailability and potential biological activities of these soyasaponin metabolites deserve further characterization due to their probable presence in the human lower intestinal tract.

The metabolism of soyasaponin I showed a biphasic pattern during 48 h of incubation in the in vitro culture model (**Figure**
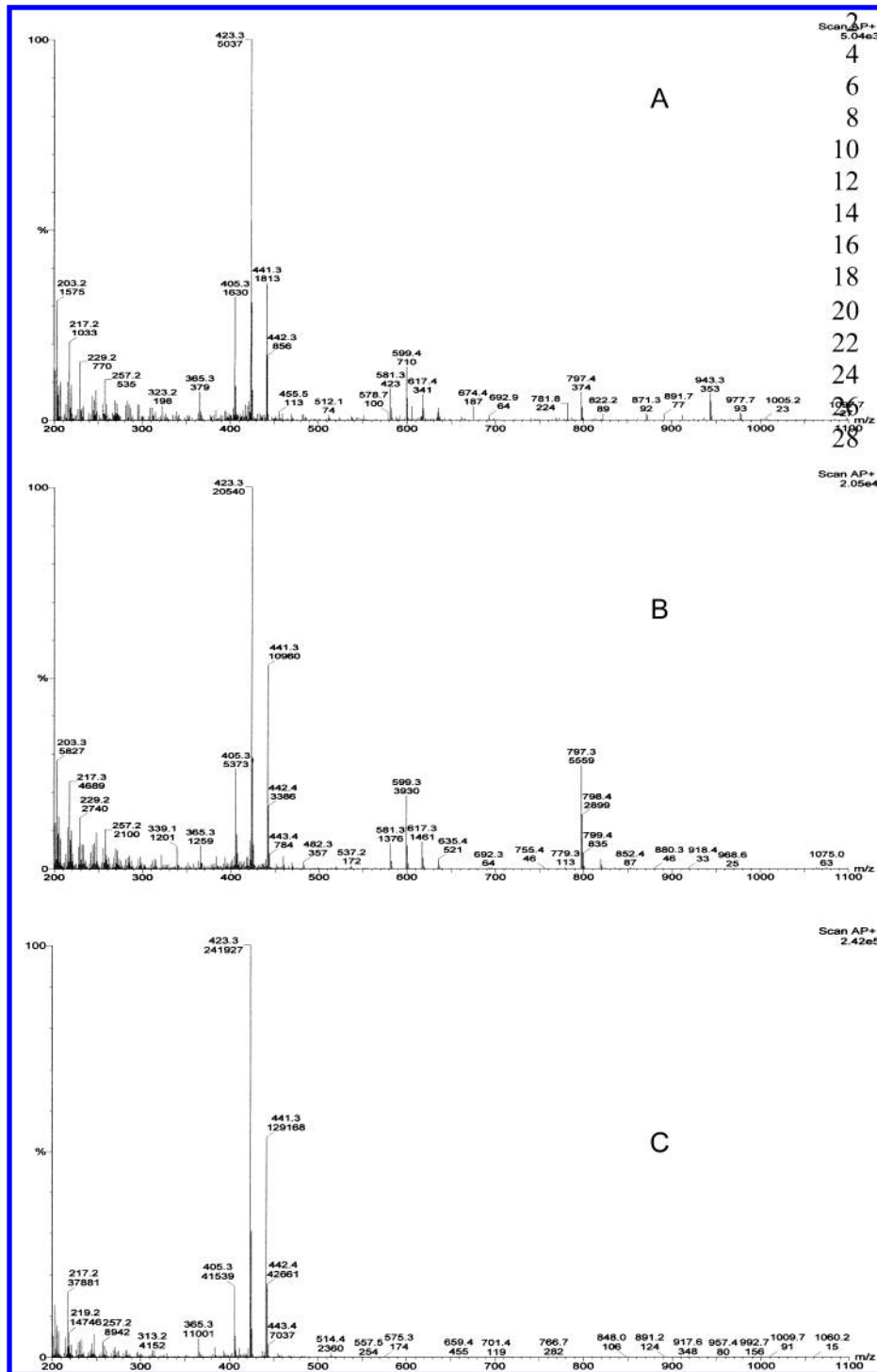
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Figure 3. Positive mode ApCI mass spectra of soyasaponin I (**A**), soyasaponin III (**B**), and soyasapogenol B (**C**).

5). No significant degradation of soyasaponin I was observed during the first 4 h. Soyasaponin I content significantly decreased from 4 to 48 h in the culture system. The absence of soyasaponin I metabolism at the early stage of incubation may be because the microorganisms need time to adapt to the culture environment before they produce sufficient enzymes to metabolize soyasaponins. Soyasaponin III was produced in fecal incubations for 13 out of 15 subjects. Soyasapogenol B was detected in fecal incubations for all of the subjects.

The rate loss of the natural logarithm of soyasaponin I concentration over the incubation time gave a linear relationship for all subjects (**Figure 6**), indicating that disappearance of soyasaponin I followed apparent first-order kinetics. The disap-

pearance of soyasaponin I in the presence of human gut microorganisms varied among subjects. The average disappearance rate loss constant was 0.15 ± 0.10 h^{-1} and half-lives $t_{1/2}$ of soyasaponin I in the culture ranged from 2.2 to 19.1 h among the subjects. Two distinct soyasaponin I disappearance phenotypes were observed among the subjects according to rate constants (**Figure 6**). The rate constants and half-lives of the two phenotypic groups were significantly different ($P < 0.05$): rapid soyasaponin degraders ($n = 7$) having a rate constant of $k = 0.24 \pm 0.04$ h^{-1} and $t_{1/2} = 3.16 \pm 0.62$ h; slow soyasaponin degraders ($n = 8$) having a $k = 0.07 \pm 0.02$ h^{-1} and $t_{1/2} = 11.15 \pm 4.34$ h. The data demonstrate that the ability of gut microorganisms to metabolize soyasaponins varied among our

Table 1. TLC, HPLC, and Mass Spectral Data of Soyasaponin I and III and Soyasapogenol B

	molecular weight[a]	TLC R_f	HPLC retention time	ApCI-mass spectra (positive mode)
soyasaponin I $C_{48}H_{78}O_{18}$	943.1	0.60 (butanol–ethanol–ammonia)	25.9 min by program 1	943.8 $[M + H]^+$, 797.4 $[M\text{-rhm} + H]^+$, 599.4 $[M\text{-glc-gal-}2H_2O + H]^+$, 441.3 $[\text{aglycone} - OH]^+$, 423.4 $[\text{aglycone} - OH - H_2O]^+$, 405 $[\text{aglycone} - OH - 2H_2O]^+$ on ApCI
soyasaponin III $C_{42}H_{68}O_{14}$	797.0	0.63 (butanol–ethanol–ammonia)	29.9 min by program 1	797.3 $[M + H]^+$, 599.4 $[M\text{-rhm-gal-}2H_2O + H]^+$, 441.3 $[\text{aglycone} - OH]^+$, 423.3 $[\text{aglycone} - OH - H_2O]^+$, 405.3 $[\text{aglycone} - OH - 2H_2O]^+$ on ApCI
soyasapogenol B $C_{30}H_{50}O_3$	458.6	0.43 (hexane: ethyl acetate)	27.2 min by program 2	441.3 $[\text{aglycone} - OH]^+$, 423.3 $[\text{aglycone} - OH - H_2O]^+$, 405.4 $[\text{aglycone} - OH - 2H_2O]^+$ on ApCI

[a] The molecular weight is a calculated value based on the molecular formula.
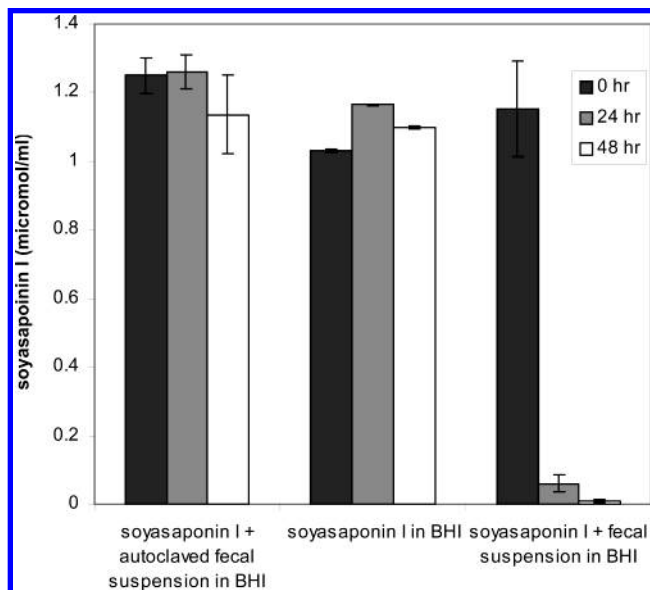

Figure 4. Comparison of in vitro metabolism of soyasaponin I in fecal fermentation system among different experimental treatments. Each bar represents the value of the mean ± SD of three replicates.
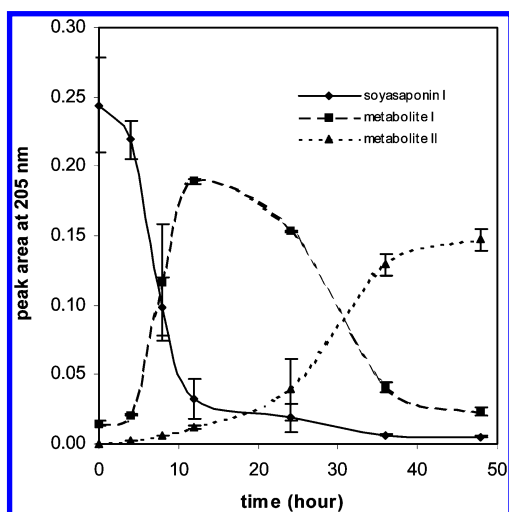

Figure 5. In vitro metabolism of soyasaponin I in fecal fermentation system measured by HPLC. Disappearance of soyasaponin I and appearance of its metabolites I and II were monitored at UV 205 nm. Each point represents the value of the mean ± SD of three replicates.

subjects and that two soyasaponin metabolic phenotypes were present. If the ability to metabolize soyasaponins in the gut was a stable characteristic in humans, enhanced bioavailability and bioactivity of soyasaponins might be expected among the

subjects exhibiting relatively longer soyasaponin half-life in the intestinal tract, whereas the subjects metabolizing soyasaponins rapidly would more likely experience effects of the metabolites.

The two groups of subjects having different soyasaponin I disappearance phenotypes are likely to have different gut microbial enzyme activities. Gut microbial population and fecal enzyme activities may be influenced by genetic background of individuals, dietary factors, physical activity, and gut motility (*29*). We examined the relationship of BMI, fecal moisture, GGT, and soy consumption frequency to the ability of the subjects' feces to metabolize soyasaponins. The subjects' BMI ranged from 18.1 to 30.4 kg/m². Fecal moisture ranged from 47.2 to 78.3%. GGTs ranged from 38 to 168 h. There were no significant differences in BMI and fecal moisture between the two soyasaponin metabolic phenotypes (**Table 2**). The slow soyasaponin degraders seemed to have longer GGTs than rapid soyasaponin degraders although this was not statistically significant at $\alpha = 0.05$. Zheng et al. (*17*) reported that GGT differed between high and low isoflavone degradation phenotypes among subjects who were recent Chinese immigrants to the U.S., and rapid GGT coupled with low fecal isoflavone degradation produced greater isoflavone bioavailability. According to their self-reported soy consumption, five subjects usually consumed soy foods more than once per week, five subjects consumed soy foods more than once a month but less than once per week, and five consumed soy less than once a month or not at all. Soy consumption frequencies did not differ according to soyasaponin metabolic rate. However, self-reported soy consumption may not have been accurate. Many food products contain soy ingredients that the subjects may not have realized and did not report. A detailed dietary assessment might be needed in the future in order to elucidate the role of dietary factors in soyasaponin metabolism. Overall, the absence of a significant difference in these factors between the two soyasaponin degradation phenotypes might also be due to the small sample size in our study, limiting statistical power. To further examine the influence of genetic and dietary factors as well as GGT on soyasaponin metabolism, a larger population group must be studied.

Our present study may provide insight into the role of gut microorganisms in soyasaponin bioavailability and biological potency. The potential of soyasaponins to lower blood cholesterol has been proposed. Soyasaponins could form insoluble complexes with cholesterol and interfere with reabsorption of bile acids and cholesterol by forming mixed micelles (*3, 30*). Soyasaponins were anticarcinogenic in an animal model of colon cancer (*7*). The colon cancer inhibiting activity of soyasaponins may be partly due to antimutagenic, cytotoxic, and/or dif-
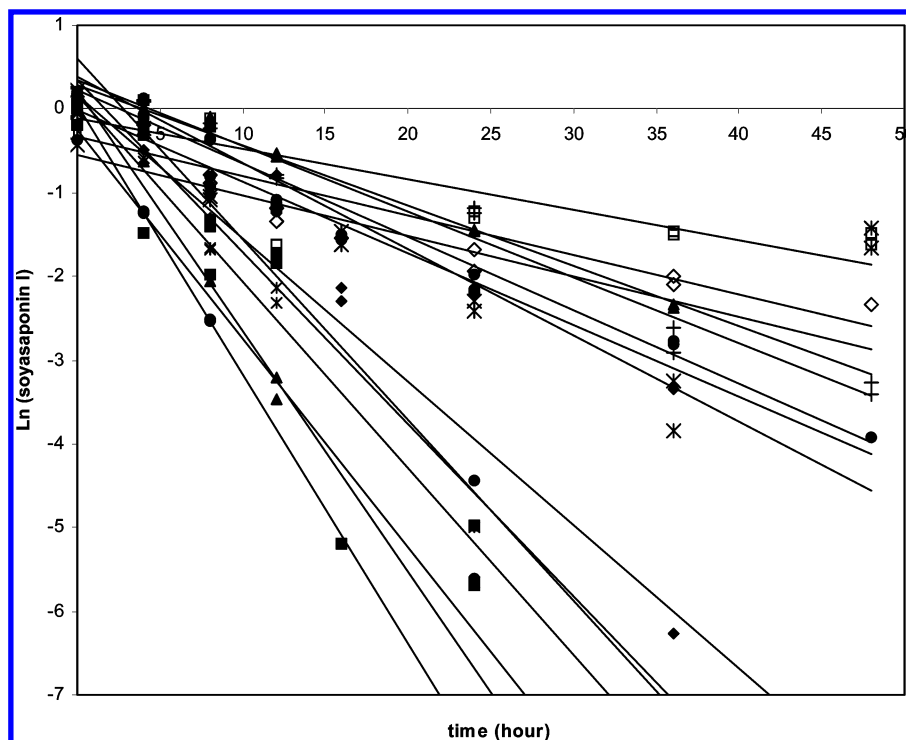
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Figure 6. Rate loss of soyasaponin I vs incubation time from all of the subjects ($n = 15$). Different symbols represent different subjects.

Table 2. Comparison of BMI, GGT, Stool Moistures, and Rate Loss of Soyasaponin I between Two Soyasaponin Metabolic Phenotypes[a]

phenotype	rate constant (h^{-1})	half-life (h)	BMI (kg/m²)	GGT (h)	stool moisture (%)
slow ($n = 8$)	0.07 ± 0.02^a	3.16 ± 0.62^a	24.4 ± 7.3	130.6 ± 40.7	60.3 ± 5.3
rapid ($n = 7$)	0.24 ± 0.05^b	11.15 ± 4.34^b	21.4 ± 3.4	69.7 ± 38.2	65.3 ± 10.4

[a] Values are expressed as the mean ± SD. The phenotype was distinguished by cluster analysis. The values in the same column with different superscripts are significantly different ($P < 0.05$).

ferentiation-inducing effects observed in cancer cell lines (*8,27, 31*). Soyasaponins could reduce the formation of secondary bile acids in the gut, which probably contributes to colon cancer inhibition by soyasaponins because secondary bile acids are known colon cancer promoters (*32*). Thus, slow soyasaponin degraders would be able to retain original soyasaponins longer in the gut to interact with cholesterol and bile acids, therefore having greater cholesterol lowering and colon cancer inhibiting effects from ingested soyasaponins. On the other hand, rapid soyasaponin degraders might have higher concentrations of soyasapogenols in the gut from soy ingestion, thus experiencing a greater bioavailability and impact from these soyasaponin aglycones.

The data from this study indicate metabolism of soyasaponin I by human gut microbial enzymes to soyasapogenol B and soyasaponins with fewer or no sugars attached to the aglycone. It is reasonable to infer that other forms of soyasaponins may be metabolized in the gut in the same manner. Two distinguishable soyasaponin metabolic phenotypes, slow and rapid soyasaponin degraders, were observed among human subjects in our study, implying that different people might experience different biological effects from soyasaponin ingestion. It is important to further characterize the factors that influence interindividual variation in the ability to metabolize soyasaponins in the gut. In addition, studies of the biological activities of soyasaponins and their gut metabolites are needed to evaluate the health promoting role of dietary soyasaponins.

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