Kidney International, Vol. 63 (2003), pp. 1230–1239

Evidence that soyasaponin B_b retards disease progression in a murine model of polycystic kidney disease

Diana J. Philbrick, Dominique P. Bureau, F. William Collins,[1] and Bruce J. Holub

Departments of Human Biology and Nutritional Sciences and Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada; and the Eastern Cereal and Oilseed Research Centre (ECORC), Ottawa, Ontario, Canada

Evidence that soyasaponin B_b retards disease progression in a murine model of polycystic kidney disease.

Background. We reported a lessened cyst growth in the *pcy* mouse model of polycystic kidney disease (PKD) when mice were fed a soy protein isolate (SPI)–based diet and hypothesized that the soyasaponins may be associated with this therapeutic effect. The effects of feeding a saponin-enriched alcohol extract (SEAE) from SPI, an isoflavone- and saponin-enriched soy supplement (Novasoy 400®), or a 99.5% pure soyasaponin B_b powder on cyst growth are reported here.

Methods. The therapeutic effects of the soyasaponins were studied in 60-day-old male *pcy* mice in two separate, 90-day feeding trials. In the first study, mice were fed either a casein-based (control) diet, a diet in which SPI replaced the casein or the control diet supplemented with SEAE. In the second study, mice were fed the control diet unsupplemented or supplemented with either a soyasaponin- and isoflavone-enriched soy product (Novasoy 400®) or a 99.5% pure soyasaponin B_b powder.

Results. In study 1, kidney weight, water content, and plasma creatinine and urea levels were markedly reduced in the SEAE-fed animals compared to tissues from the control group; likewise, mice fed the SPI-based diet showed a decreased plasma creatinine, but only a slightly reduced plasma urea. In study 2, kidney weight, water content, plasma creatinine and urea levels were significantly reduced in mice fed the soyasaponin B_b powder and the Novasoy-400® supplement, compared to controls.

Conclusion. Soyasaponin B_b can impede kidney enlargement and cyst growth in the *pcy* mouse model of PKD. Further studies are needed to determine its most effective dose and mechanism of action.

Our studies in polycystic kidney disease (PKD) have concentrated on various dietary interventions to attenuate cyst growth in the DBA/2 FG-pcy *(pcy)* mouse, an accepted animal model of PKD [1]. We have reported that changes associated with disease progression, including total kidney weight, kidney weight relative to body weight, mean cyst volume, and organ water content, were more moderate in kidneys from mice fed a soy protein isolate (SPI), compared to animals fed a casein-based diet [2]. This finding is consistent with reports of a beneficial therapeutic effect of soy-based diets on tissue morphometry and the prolongation of renal function in human kidney disease [3–6], as well as the extended survival and moderation of the pathologic changes in several animal models of kidney disease, including PKD [7–10].

The component of SPI retarding cystogenesis and extending tissue viability and extending functioning capacity in the *pcy* mouse kidney has not been identified. Isoflavones, naturally present in SPI, have been suggested as the potentially active component(s) of SPI. These reportedly function as tyrosine kinase inhibitors, reducing the amount of activated growth factors available for stimulating cyst growth in organ culture and curtailing further progression of cystic disease in a mouse model of the autosomal-recessive form of PKD [11–14]. On the other hand, we have reported that supplementation of a casein-based diet with pure genistein in amounts several times higher than those levels present in SPI had little effect on renal cyst development in the *pcy* mouse [2].

SPI also contains saponins and it is possible that any effect it has is associated with these high-molecular-weight glycosides. Saponins are widely distributed in the plant kingdom and consist of a diverse group of nonpolar triterpene or steroid glycosides, linked to one or two polar oligosaccharides. Five forms of these amphiphilic compounds (two forms belonging to the A and three to the B groups of soyasaponins) have been reported in wild (*Glycine soja*) and cultivated *(G. max)* species of soybean which may contain 0.2% to 0.8% of their dry weight as soyasaponins, depending on plant strain, growing locale, and the amount carried over during the preparation of the SPI [15–19]. Despite their relatively minute amounts in foods, the soyasaponins are reported to have

[1] ECORC contribution number, 991435.

Key words: saponin-enriched alcohol extract (SEAE), soyasaponin B_b, cyst growth in PKD.

Received for publication June 3, 2002
and in revised form October 24, 2002
Accepted for publication November 21, 2002

significant effects on food intake [20], plasma cholesterol levels [21, 22], epithelial cell proliferation [23], and cell membrane permeability [24] in animals.

In study 1, the effects of supplementing a casein-based diet with a crude soyasaponin-enriched alcohol extract (SEAE) from SPI were compared to those changes found in animals fed an unsupplemented casein-based diet, which reportedly accelerates disease progression [2]. In study 2, the effects of supplementing a casein-based diet with pure soyasaponin B_b, the predominant soyasaponin found in the SEAE, as well as the effects of supplementing the casein-based diet with an isoflavone-enriched commercial product (Novasoy 400®; ADM, Decator, IL, USA) containing this soyasaponin were examined.

METHODS

Preparation of a crude extract containing saponins (SEAE) (study 1)

The SEAE was extracted from the SPI (SuproPro® 675 HG, Protein Technologies International, St. Louis, MO, USA) using a modified procedure of Kitagawa et al [25]. Briefly, the SPI was stirred with methanol overnight at room temperature followed by heating for 6 to 8 hours and removal of the methanol in vacuo.

The resulting soy velasse was redissolved in a water: n-butanol solvent system (1:1,v/v), and the phases allowed to separate. The butanol upper layer, containing the saponins, was dried in vacuo. The residue was then redissolved in a small amount of methanol and the saponins precipitated by the dropwise addition to a large volume of ethyl acetate, followed by filtration and air drying. The yield of the off-white powder containing the saponins was 1.4% of the total weight of the SPI used.

Chemical characterization of the SEAE and SPI

Analyses of the SPI isoflavone content were done using a modified procedure described by Collins et al [26]. Briefly, the SPI was refluxed with acidified (0.1% acetic acid, vol/vol) aqueous 80% ethanol (EtOH) during heating; after cooling to room temperature, it was centrifuged, the supernatant decanted, and the pellet re-extracted twice with additional acidified 80% EtOH. The combined supernatants were then filtered and the filtrate evaporated in vacuo.

The SEAE and isoflavones recovered from the SPI were resuspended in either acidified (0.1% acetic acid) aqueous 50% EtOH or 50% isopropyl alcohol, respectively, prior to their separation by hydrophobic interaction chromatography on Octyl Sepharose CL-4B (Amersham Pharmacia Biotech, Piscataway, NJ, USA). For this step, a 30 mL bed volume gravity flow column of Octyl Sepharose CL-4B, pre-equilibrated in the appropriate solvent, was used to remove residual hydrophobic impurities (i.e., "defatting"). Subsequently, the resuspended mixture was applied to the column and the column washed with three bed volumes of the respective resuspension solvent and the eluate concentrated in vacuo. Under these conditions, the eluate contained all the saponins and isoflavones, while the more lipophilic "fatty" components were absorbed by the column. The resulting "defatted SPI fraction" was then reapplied to a similar column of Octyl Sepharose CL-4B gel and eluted with three bed volumes of acidified aqueous 25% EtOH to give an isoflavone-enriched subfraction. Under these conditions, the soyasaponins were retained on the column and subsquently eluted using acidifed aqueous 80% EtOH to give a soyasaponin-enriched subfraction.

Analyses of the soyasaponins in the SEAE utilized both thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). For the TLC analyses, the soyasaponins were separated on MK $C_{18:0}$ reverse-phase plates (Whatman International, Ltd., Maidstone, England) and visualized by spraying with 0.5% p-anisaldehyde (vol/vol) in ethanol:concentrated sulfuric acid (95: vol/vol) followed by heating at 100°C for 3 minutes (a characteristic rose-red color appears when soyasaponins are present). The HPLC system consisted of a Hypersil ODS column (250 × 4 mm, 5 μm particle size) coupled to a gradient delivery system (Thermo, Finnigan, Mississauga, Ontario, Canada) and an evaporative light scattering detector (HPLC-ELSD; Varex MKIII, Alltech Associates, Inc., Deerfield, IL, USA).

Identification of the individual saponins in the SEAE and SPI was done by comparative mass spectroscopy using an authentic standard isolated and identified according to Collins et al [26]. Mass spectroscopy ion flow injection analyses (FIA) indicated the presence of a compound with a molecular weight of 942, identical to an authentic standard of soyasaponin B_b as reported by Shiraiwa, Harada, and Okubo [18], and as soyasaponin I by Fuzzati et al [27]. Mass spectroscopic analyses of the SEAE showed the presence of three small peaks eluting at 12, 22, and 27 minutes into the run and a much larger peak eluting at approximately 17 minutes. Comparison of these analyses with published data allowed identification of the first small peak, eluting at 12 minutes as soyasaponin B_a, the compound eluting at 22 minutes as soyasaponin B_c and the large peak eluting at 17 minutes as soyasaponin B_b. Quantitation of the total saponins was expressed as microgram or milligram soyasaponin B_b equivalents.

Separation of the isoflavones in the SPI and SEAE was done using the same column and solvent delivery system as that described for the soyasaponins, except that the isoflavone compounds were detected by a Waters 991 photo-diode array ultraviolet spectrophotometric detection system (Waters Corp., Milford, MA, USA). Identification of the individual isoflavones was made by comparative chromatographic HPLC and ultraviolet spectral

analyses with published data [26, 27] and their amounts expressed as microgram or milligram genistein equivalents based on authentic standards (Sigma Chemical Co., St. Louis, MO, USA).

Purification of the soyasaponin B_b (study 2)

Novasoy 400® (ADM) and a prototypical "soy molasses" fraction (Central Soya, Fort Wayne, IN, USA) were used for the large scale purification of the approximately 5 g soyasaponin B_b needed for a feeding trial in the *pcy* mice (study 2). The starting material from the ADM and Central Soya products appeared as a yellowish-orange, free-flowing powder, containing 5.3% soyasaponin B_b as determined by HPLC-ELSD. The purification procedure involved solubilization of the starting material (either the Novasoy 400® or the soy molasses) in acidified aqueous EtOH, followed by the group separation of the soyasaponin- and isoflavone-containing fractions by a scale up of the analytical low-pressure liquid chromatography, described in the previous section. The eluate from this step was concentrated in vacuo at 40°C, redissolved in aqueous alcohol (50% to 80% EtOH) and passed through an anion exchange column, packed with QAE Sephadex A-25 (Amersham Pharmacia Biotech) in the formate form. The materials absorbed by the column, including the soyasaponin B_b, were recovered by washing the column with acidified aqueous 80% EtOH (containing 5% formic acid). Further separation of soyasaponin B_b from soyasaponins B_a and B_c was accomplished by preparative hydrophobic interaction chromatography using a hexadecyltrimethylammonium-substituted Sepharose Fast Flow Q gel (Amersham Pharmacia Biotech) prepared and eluted according to Collins et al [26] with aqueous 45% EtOH. The fraction containing the soyasaponin B_b had a purity greater than 97% as determined by HPLC-ELSD analyses. The residual coloration of this fraction and other impurities were removed by preparative liquid chromatography on Sephadex LH-20 (Amersham Pharmacia Biotech). HPLC analyses of this product showed a single, well-defined peak eluting 17 minutes into the run. The soyasaponin was then recrystallized from hot aqueous EtOH to give colorless, tasteless, fine needle–shaped crystals used to supplement the casein-based diet. Co-chromatography (TLC-HPLC) and mass spectroscopy confirmed that this material was 3-O-(α-L-rhamnosyl-1→2-β-D-galactosyl-1→2-D-glucuronosyl)-olean-12-en-3β,22β, 24-triol (soyasaponin B_b). The structure of the soyasaponin B_b is shown in Figure 1.

Characterization and quantitation of the soyasaponin

Authentic standards for the soyasaponins are not currently available from commercial or private sources; therefore, identification of the individual soyasaponins was done using the previously described method of Collins et al [26].

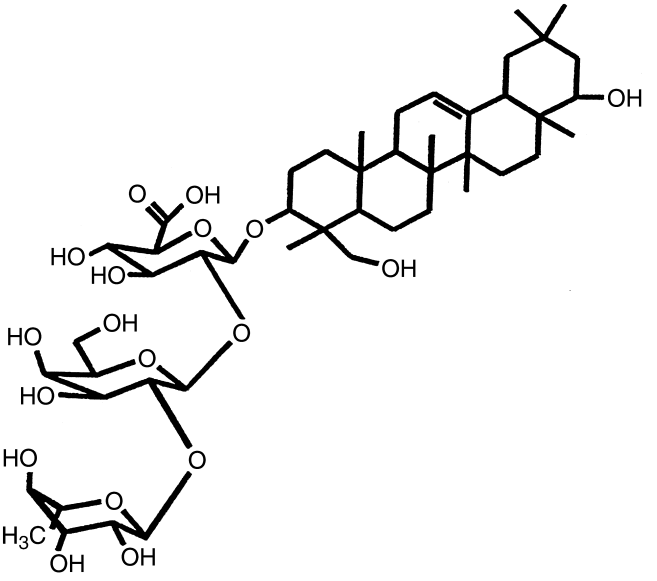

Fig. 1. Structure of soyasaponin B_b (3-O-(α-L-rhamnosyl-1→2-β-D-galactosyl-1→2-D-glucuronosyl-olean-12-en-3β,22β, 24-triol) used to supplement the casein-based diet in study 2.

Feeding trials in the *pcy* mouse

Conditions for the housing and care of the *pcy* mice have been described previously [2]. All procedures were in accordance with the Canadian Council for Animal Care Guidelines and were approved by the Animal Care Committee at the University of Guelph.

Sixty-day-old male *pcy* mice were randomly assigned to treatment groups and fed ad libitum the diets shown in Table 1. Body weight was measured weekly and the daily feed consumption over a 2-week period, midway in the trial, was recorded. After 90 days, the nonfasted mice were euthanized by exposure to CO_2 as described by Tomobe et al [2]. The kidney and liver tissues were quickly removed and stored at −80°C until measurements of tissue water content and cyst volume (quantitative morphometry) in study 1 and tissue water content in study 2 could be made [9]. Organ water content was determined by measuring the difference between tissue wet weight and its weight after freeze-drying [2, 28]. Plasma, obtained by centrifugation of the heparinized cardiac blood, was used for standard measurements of total protein, urea, and creatinine levels. Soy protein products enriched in soyasaponins are reportedly hypocholesterolemic in the hamster [29] and plasma cholesterol levels were measured in studies 1 and 2 to determine if a similar effect occurred in the *pcy* mouse. Comparisons were made between effects found in animals fed the casein-based (control) diet and the groups receiving the dietary treatments using a one-way ANOVA (analysis of variance) and the significance of these differences obtained by the least significant difference (LSD) test [30]. The data obtained in studies 1 and 2 were considered separately.

Table 1. Composition of the experimental diets

Diet ingredient	Casein	SPI	+ SEAE	+ Novasoy 400®	+ Soyasaponin B_b
Casein, vitamin-free *g/100 g diet*	15.0	—	15.0	15.0	15.0
Soy protein isolate *g/100 g diet*	—	15.0	—	—	—
Choline chloride[a] *g/100 g diet*	0.2	0.2	0.2	0.2	0.2
Vitamin/D L-methionine mix[a] *g/100 g diet*	1.0	1.0	1.0	1.0	1.0
Mineral mix[a] *g/100 g diet*	5.5	5.5	5.5	5.5	5.5
Cellulose *g/100 g diet*	2.45	2.45	1.95	0.5	2.27
Antioxidant[a] *g/100 g diet*	0.05	0.05	0.05	0.05	0.05
Corn oil *g/100 g diet*	15.0	15.0	15.0	15.0	15.0
Sucrose *g/100 g diet*	8.0	8.0	8.0	8.0	8.0
Cornstarch *g/100 g diet*	52.8	52.8	52.8	52.8	52.8
SEAE *g/100 g diet*	—	—	0.5	—	—
Novasoy 400® *g/100 g diet*	—	—	—	2.0	—
Soyasaponin B_b *g/100 g diet*	—	—	—	—	0.2

Abbreviations are: SPI, soy protein isolate; SEAE, saponin-enriched alcohol extract. Preparation of the SEAE is described in the **Materials and Methods** section.
[a] Antioxidants, vitamin and mineral mixes were added as previously described [2]

Three dietary treatments (Table 1) were used to assess the effect of the SEAE on cyst content in study 1. These included a control (casein-based) diet, a diet in which SPI replaced the casein, or the casein-based diet supplemented with the SEAE. In study 2, the mice were fed the unsupplemented control diet or this diet was supplemented with either a soyasaponin- and isoflavone-enriched soy product (Novasoy 400®) or the 99.5% pure soyasaponin powder. The amount of soyasaponin B_b used to supplement the casein-based diet was based on the soyasaponin B_b content of the SEAE used in study 1 (~181 mg/100 g diet rounded off to 0.1% of the diet). The Novasoy 400® contained 45.5 g isoflavones, 6.9 g total saponins, and 5.3 g soyasaponin B_b/100 g material and was added to the casein-based diet at 2% of the total diet (Fig. 2). All diets contained the same amounts of protein (150 g/kg) and had a similar physiologic fuel value (19.8 MJ/kg).

RESULTS

Chemical analyses of the SEAE and SPI supplements (study 1)

Qualitative TLC and HPLC analyses of the SEAE showed that it contained four components giving a positive color reaction as well as some early eluting peaks representing the isoflavones (vide infra). As shown in Table 2, the total saponin content of this SEAE supplement was 208.7 mg soyasaponin B/ g extract, the major portion of which was soyasaponin B_b (181.0 mg/g extract or 86% of the saponin weight of the SEAE extract) with smaller amounts of soyasaponins B_a and B_c. As well, the SEAE contained small amounts (4.8 mg/g extract) of two isoflavone glycosides, daidzin and genistin.

TLC and HPLC-ELSD analyses of the SPI showed a complex mixture of at least 11 compounds, of which soyasaponin B_b, identified by co-chromatography with an authentic sample, was a major component (Table 2). Based on the soyasaponin B_b standard, the total soyasaponin concentration in the SPI was estimated as 3.4 mg/g with soyasaponin B_b (3.0 mg/g) accounting for 88.1% of the total. Ireland, Dziedzic, and Kearsley [15] have reported that the total soyasaponin content of SPI was 7.6 mg/g, of which the group B soyasaponins represented 72.5% or 5.5 mg/g SPI. Their estimate is slightly larger than the amounts of the SPI soyasaponins found in the present experiment and may reflect the effects of growing conditions or differences in the procedures used to prepare the SPI. Small amounts of daidzin and genistin (totaling ~2.5 mg/g) were also present in the SPI (Table 2).

Soyasaponin B_a intake was similar in the SEAE and SPI-fed groups (less than 0.1 ± 0.0 mg/day), whereas intakes of the soyasaponins B_b and B_c in the SEAE-fed group were more than twice that amount consumed by the SPI-fed mice. The average daily isoflavone intake for a 2-week period was estimated as 1.2 ± 0.0 mg and 0.1 ± 0 mg for the SPI- and SEAE-fed groups, respectively (Table 2).

Effects of the SPI and SEAE-supplemented diets on body and tissue weight, cyst volume, and kidney water content

Body weight was lowest in the SEAE-fed mice during the first 9 weeks of the feeding trial (Fig. 3A); thereafter, it became similar among the three groups, as shown by the values for final body weight in Table 3. The 2-week feed intake did not differ among the control, SPI, and SEAE groups (Table 3)

Feeding the SEAE supplement was associated with the lowest kidney weight, compared to the casein-fed (control) group; relative to body weight, kidney weights from the SEAE-fed mice were ~75% those of the casein-fed animals. Total kidney weight, as grams/animal and 100 g body weight, did not differ between the casein and SPI-fed groups (Table 3). Liver weight (grams/animal) was similar among the three groups; expressed as relative

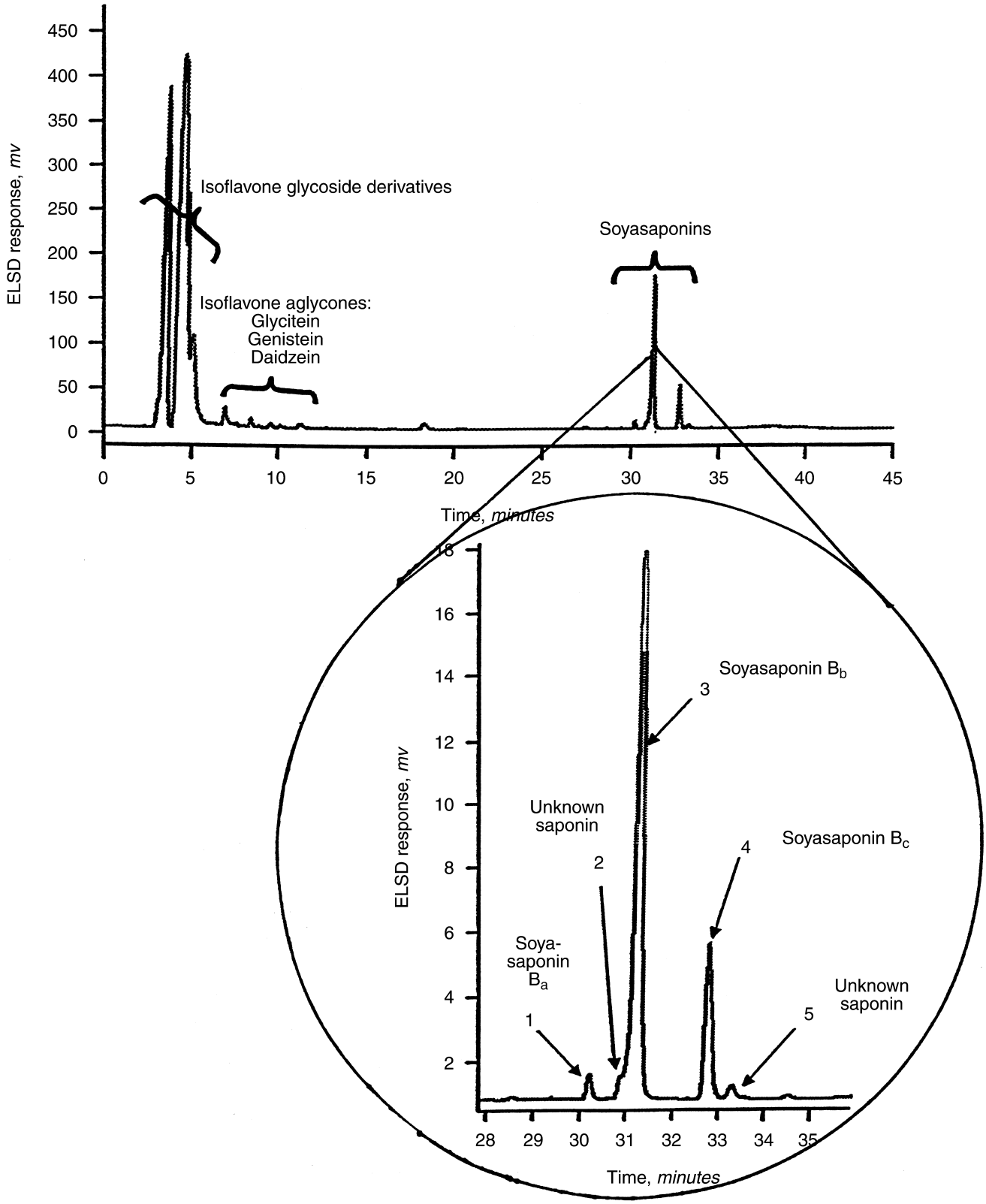

Fig. 2. High-performance liquid chromatography and evaporative light scattering detector (HPLC-ESLD) profile of Novasoy 400® [10 μL injection of 25 mg/mL Novasoy 400® in 50% aqueous 80% ethanol (EtOH)]. As shown in the top trace, Novasoy 400® contains a fairly large proportion of isoflavone glycoside derivatives and small amounts of the corresponding aglycones eluting during the first 12 minutes of the run; the soyasaponins eluted during 30 to 32 minutes of the run. The elution pattern for the individual soyasaponins is shown in a magnification of the saponin zone (encircled insert) and shows that soyasaponin B_b is the major soyasaponin present, followed by lesser amounts of soyasaponin B_c and B_a, and two unidentified saponins. Quantitation was carried out using a standard curve of soyasaponin B_b and an integration curve based on the quadratic equation $y = 1.109x^2 + 12.38x$ ($R^2 = 0.996$) where y is expressed in mv · sec^{-1} · 10^{-5} and x is expressed in mg/mL soyasaponin B_b equivalents.

Table 2. Soyasaponin and isoflavone concentrations of the soy protein isolate (SPI) and saponin-enriched alcohol extract (SEAE) (study 1) and total soyasaponin, soyasaponin B_b, and isoflavone intakes by *pcy* mice fed either Novasoy 400® or soyasaponin B_b (study 2)

Study 1	SPI	SEAE
Total soyasaponins *mg/g material*	3.4	208.7
Soyasaponin B_a	0.1	1.5
Soyasaponin B_b	3.0	181.0
Soyasaponin B_c	0.4	24.0
Unknown saponin(s)	None detected	2.1
Total soyasaponin intake *mg/day*	1.6 ± 0.1 (7)[a]	3.2 ± 0.1 (6)[a]
Soyasaponin B_a	<0.05	<0.05
Soyasaponin B_b	1.4 ± 0.4	2.8 ± 0.1
Soyasaponin B_c	0.2 ± 0.0	0.4 ± 0.0
Unknown saponin(s)	None detected	0.3 ± 0
Total isoflavones *mg/g material*	2.5	4.8
Daidzin	0.3	1.4
Genistin	0.9	3.4
Others	1.3	—
Total isoflavone intake *mg/day*	1.2 ± 0.0	0.1 ± 0.0
Study 2	**Novasoy 400® (9)[a]**	**Soyasaponin B_b (9)[a]**
Total soyasaponin intake *mg/day*	5.3 ± 0.2	5.9 ± 0.4
Soyasaponin B_b intake *mg/day*	4.1 ± 0.1	5.9 ± 0.4
Total isoflavone intake *mg/day*	35.1 ± 2.6	—

[a]Numbers in parentheses show number of animals/group

to body weight, weight of this tissue was lower in the SPI- and SEAE-fed groups than in the casein-fed group (Table 3).

Morphometric analyses showed a significantly lower mean cyst volume in the right kidneys from the SEAE-supplemented group, as compared to tissues from the casein-fed (control) group (Table 3). Cyst volume was also lower in the SPI-fed mice, although this difference did not achieve statistical significance (Table 3) Values for the kidney water content (as grams/left kidney) mirrored these changes in cyst volume, but there was no difference among the groups when water content was expressed as %. This similar % water content among the three groups suggests that the reduced cyst volume found in the SEAE- and SPI-fed groups could not be attributed to changes in cyst proportion The water content of the liver was similar among the unsupplemented and supplemented groups (Table 3).

Plasma creatinine and urea contents were lowest in the SEAE-supplemented group (51% and 63% of the amounts found in the casein-fed control group, respectively). These values were also decreased in the SPI-fed mice compared to the control group, although none of these reductions in plasma urea achieved statistical significance. Plasma total protein and cholesterol level did not differ among the three groups (Table 4).

Effects of the Novasoy 400® and the soyasaponin B_b on body and tissue weight and composition

Body weight was similar in the control and soyasaponin B_b-supplemented groups throughout the feeding trial; however, mice fed the Novasoy 400®–based diet showed a decrease from the initial body weight during the first week of the trial and remained the lowest among the three groups during the remainder of the feeding trial (Fig. 3B). Feed intake did not did not differ among the groups (Table 5). The estimated total soyasaponin intake in the Novasoy 400®–fed mice was 5.3 ± 0.2 mg/day, of which 4.1 ± 0.1 mg was soyasaponin B_b; mice fed this diet also consumed approximately 35.1 ± 2.6 mg isoflavone/day as daidzin and genistin. The amount of soyasaponin B_b consumed by the mice fed the pure soyasaponin was slightly higher than the amount eaten by the Novasoy 400®–supplemented group (5.9 ± 0.0 versus 4.1 ± 0.1 mg/day, respectively).

Supplementation with Novasoy 400® and soyasaponin B_b was associated with significantly lower kidney weight (as g/animal and g/100 g body weight) compared to the casein-fed control group. Absolute liver weight did not differ among the three groups (Table 5); however, liver weight, expressed relative to body size, was highest in the Novasoy 400®-fed mice, consistent with the lower body weight of this group. Also, kidney water content as g/left kidney was decreased in the Novasoy 400® and soyasaponin B_b-fed mice, compared to the control group, although there were no significant differences among these groups when the water content was expressed as a percent (Table 5). Likewise, liver water given as a percent did not differ among these groups (Table 5).

Plasma creatinine and urea levels were lower in the Novasoy 400® and the soyasaponin B_b–supplemented groups, compared to those levels found in mice fed the casein-based (control) diet (Table 6). Plasma cholesterol level in animals fed the Novasoy 400® was lower than that of the casein-fed group but not the soyasaponin B_b–supplemented group. This difference between the

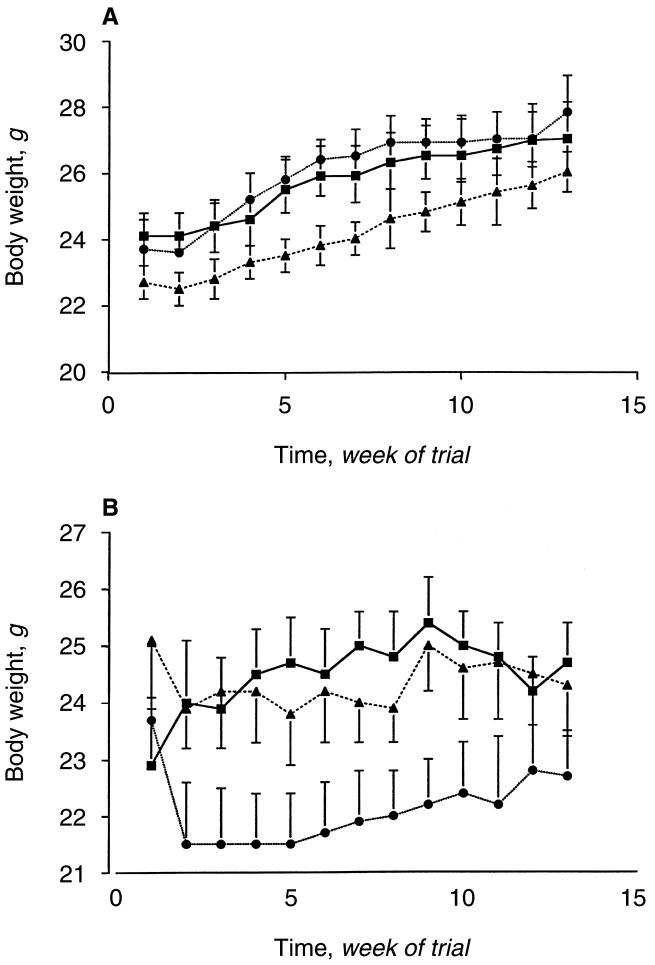

Fig. 3. Average weights. (*A*) Average body weight of *pcy* mice fed a casein-based diet (■), the soy protein isolate (SPI)-based diet (●), or the casein-based diet supplemented with soyasaponin-enriched alcohol extract (SEAE) (▲) (study 1). (*B*) Average body weight of *pcy* mice fed the casein-based diet unsupplemented (■), this diet supplemented with Novasoy 400® (●), or supplemented with soyasaponin B_b (▲) (study 2).

Novasoy 400® and the soyasaponin B_b–fed mice suggests that soyasaponin B_b on its own may not hypocholesterolemic or that it may require factors, such as other soyasaponins and the isoflavones, for this property. Total plasma protein levels did not differ among the three dietary groups.

DISCUSSION

We have reported that kidney size was reduced in *pcy* mice fed diets supplemented with an extract (SEAE) containing soyasaponins, an isoflavone-enriched extract (Novasoy 400®) as well as pure soyasaponin B_b. Total kidney weight of mice fed the SEAE, Novasoy 400®, and soyasaponin B_b–supplemented diet was approximately 23% to 27% lower than observed in *pcy* mice fed a casein-based diet. The kidney to body weight ratio

Table 3. Body and tissue weight, cyst volume, and kidney water content in *pcy* mice fed either the casein or soy protein isolate (SPI)-based diets and the casein-based diet + saponin-enriched alcohol extract (SEAE) (study 1)

Diet measurement	Casein (8)	SPI (7)	SEAE (6)
Final body weight *g*	27.0 ± 1.2[a]	27.8 ± 1.1	26.0 ± 0.7
Two-week food intake *g/day*	3.2 ± 0.1	3.1 ± 0.1	3.2 ± 0.2
Total kidney weight *g/animal*	1.6 ± 0.1	1.5 ± 0.1	1.2 ± 0.1[b]
Range *g*	(1.4–2.1)	(1.1–2.0)	(1.0–1.4)
Total kidney weight *g/100 g body weight*	5.8 ± 0.4	5.6 ± 0.4	4.5 ± 0.3[c]
Liver weight *g/animal*	1.2 ± 0.2	1.1 ± 0.1	1.0 ± 0.1
Liver weight *g/100 g body weight*	4.3 ± 0.1	4.0 ± 0.1[b]	4.0 ± 0.1[b]
Cyst volume *mL/right kidney*	0.68 ± 0.05	0.57 ± 0.07	0.41 ± 0.05[b]
Kidney water content *g/left kidney*	0.73 ± 0.05	0.62 ± 0.05	0.55 ± 0.03[b]
Kidney water content %	89.6 ± 1.6	85.5 ± 2.3	88.4 ± 1.0

[a]Values are given as mean ± SEM for the number of animals shown in the parentheses; [b]$P < 0.01$; [c]$P < 0.001$, significantly different from the casein-fed animals.

Table 4. Plasma chemisteries in *pcy* mice fed either the casein- or soy protein isolate (SPI)-based diet or the casein-based diet + saponin-enriched alcohol extract (SEAE) (study 1)

Diet measurement	Casein (8)	SPI (7)	SEAE (6)
Plasma creatinine *μmol/L*	35.3 ± 6.0[a]	21.5 ± 5.5[c]	18.0±6.0[d]
Plasma urea *mmol/L*	10.2 ± 1.4	7.9 ± 2.7	6.4 ± 1.2[b]
Plasma cholesterol *mmol/L*	3.5 ± 0.4	4.6 ± 0.3	3.3 ± 0.2
Plasma total protein *g/L*	40.2 ± 3.6	46.4 ± 1.4	41.7 ± 2.5

[a]Data are given as mean ± SEM for the number of mice shown in the parentheses
[b]$P < 0.05$; [c]$P < 0.01$; [d]$P < 0.001$, significantly different from the control group

Table 5. Body and tissue weight and water and dry matter contents in *pcy* mice fed the casein-based diet or this diet supplemented with either Novasoy 400® or soyasaponin B_b (study 2)

Diet measurement	Casein (9)	Novasoy 400® (9)	Soyasaponin B_b (9)
Final body weight *g*	25.2 ± 0.9[a]	23.3 ± 0.8[c]	25.1 ± 0.1
Two-week food intake *g/day*	3.4 ± 0.9	3.9 ± 0.9	3.3 ± 0.5
Total kidney weight *g/animal*	1.8 ± 0.1	1.3 ± 0.1[c]	1.3 ± 0.1[c]
Total kidney weight *g/100 g body weight*	7.0 ± 0.3	5.4 ± 0.3[c]	5.2 ± 0.3[d]
Liver weight *g/animal*	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0
Liver weight *g/100 g body weight*	4.6 ± 0.1	5.6 ± 0.4[c]	4.6 ± 0.1
Liver water %	69.6 ± 0.5	70.5 ± 0.4	69.4 ± 0.4
Kidney water content *g/left kidney*	0.82 ± 0.03	0.63 ± 0.07[b]	0.61 ± 0.04[d]
Kidney water content %	89.0 ± 0.5	88.1 ± 0.8	88.2 ± 0.5
Kidney dry matter *mg/left kidney*	87.2 ± 4.5	81.8 ± 6.5	82.3 ± 4.2

[a]Values are given as mean ± SEM for the number of animals shown in parentheses
[b]$P < 0.05$; [c]$P < 0.01$; [d]$P < 0.001$, significantly different from the corresponding casein-fed group

Table 6. Plasma chemisteries in *pcy* mice fed a casein-based diet or this diet supplemented with either Novasoy 400® or soyasaponin B_b (study 2)

Diet measurement	Casein (9)	Casein + Novasoy 400® (9)	Casein + soyasaponin B_b (9)
Plasma creatinine *μmol/L*	26.0 ± 2.8[a]	19.0 ± 2.5[b]	17.5 ± 1.8[c]
Plasma urea *mmol/L*	3.7 ± 0.4	2.1 ± 0.3[c]	2.8 ± 0.2[b]
Plasma cholesterol *mmol/L*	5.3 ± 0.2	4.3 ± 0.2[c]	4.8 ± 0.3
Plasma total protein *g/L*	56.0 ± 1.9	56.0 ± 0.8	54.3 ± 1.1

[a]Values are mean ± SEM for number of animals in parentheses
[b]$P < 0.05$; [c]$P < 0.01$, significantly different from the corresponding casein-fed group

showed the same progression, suggesting that the decreased kidney weight in the SEAE, Novasoy 400®, and soyasaponin B_b-supplemented groups did not depend on other, nonspecific changes in growth. Our findings indicate that cyst development in the *pcy* mouse kidney could be attenuated by feeding soyasaponin- enriched soy products such as SEAE or soyasaponin B_b; these products are nearly isoflavone-free, suggesting that the isoflavones play a relatively minor role in the suppression of cyst growth.

Studies in other animal models of PKD, including the Han:SPRD rat [32, 33] the CD-1 *pcy/pcy* mouse [10], the *kat/kat*^{2J} mouse [34], and the BDF-1-*pcy* hybrid mouse [35], have shown that increased organ and cyst size and water content are associated with age and increasing severity of the disease. Morphometric measurements of cyst volume in the right kidney from the casein-fed, SPI-fed, and the SEAE-supplemented groups in study 1 indicated that cyst development was least in the SEAE-supplemented group, increased slightly, although not significantly, in the SPI-fed group, and was greatest in the casein-fed mice. This reduction in kidney weight was associated with a reduced water content in the soy protein–fed groups, as compared to the casein-fed animals (Table 3). Cyst volume measurements were not made in the Novasoy 400®– and soyasaponin B_b–supplemented groups in study 2, although the kidney water content was markedly reduced in these groups compared to the casein-fed mice (Table 5). These findings support our previous observation that the cyst fluid in the *pcy* mouse kidney is mainly water and gives further support to the suggestion that kidney water content is a good indicator of cyst development [28, 31]. Dry kidney weight was similar in all groups, indicating that the aqueous component of this tissue did not change, as reported in the *kat/kat*^{2J} [34] and in the C57BL/6J-*cpk* mouse [28].

Plasma creatinine and urea levels were reduced in the Novasoy 400®, soyasaponin B_b, SPI, and SEAE-fed groups, compared to the casein-fed group (Tables 4 and 6). For plasma creatinine, these reductions ranged from 27% in the Novasoy 400®–fed group to 33%, 39%, and 49% found in the soyasaponin B_b-, SPI-, and the SEAE-fed groups, respectively. Decreased plasma creatinine levels have been reported in *pcy* mice and in Han:SPRD rats fed diets in which soy protein replaced the casein as the protein source [10, 33]. Elevated plasma creatinine levels have been reported in other animal models of PKD and associated with enhanced cyst development and the onset of proteinuria and/or azotemia, indicative of a compromised renal function [34, 36, 37]. In the present study, changes in plasma urea contents were less remarkable than those observed for the plasma creatinine levels and ranged from 29%, 38%, and 24% reductions in the SPI-, SEAE-, and soyasaponin B_b/Novasoy 400®–fed groups, respectively. Significant reductions in the creatinine and urea levels have been observed in mouse and rat models of PKD fed soy protein–based diets [2, 9], as well as during a moderate protein restriction [38]. Urea is reportedly toxic to the kidney in a number of species [39, 40] and reduced serum levels of this end product of protein metabolism have been associated with preservation of renal function and attenuation of those inflammatory reactions found in the interstitium of the cystic kidney [9, 35].

The mechanism by which soyasaponin B_b controls cyst growth in the *pcy* mouse kidney has not been elucidated. Ex vitro studies in bovine aortic and tracheal smooth muscle cells have shown that the group B soyasaponins and the structurally similar analog, dehydrosoyasaponin I (DHS-I), can extend the open probability time of the high conductance, outwardly rectifiying potassium channels (or maxi-K+ channels) [41, 42]. This response to DHS-I and the soyasaponins B is apparently unique to these channels, found throughout the nephron where they may act as cell volume regulators [43, 44]. It is possible that increasing the open probability of these maxi-K+ channels serves to limit the amount of potassium accumulated within the cell/cyst cytoplasm and further solute secretion into the cell and/or cyst sac. Alternatively, the soyasaponins may destabilize membrane structure and disrupt permeability in a manner similar to that described for the cauloside C saponins [24].

This response of the *pcy* mouse kidney to the group B soyasaponins may be highly specific. In a previous study (unpublished), we found that supplementation of the casein-based diet with ginseng *(Panax notoginseng)* root containing saponins [45] had no effect on kidney size and cyst development when fed at the level of 5%

of the diet. This finding suggests that, unlike the soyasaponins, the ginseng-derived saponins may have little effect on cyst development in the *pcy* mouse model of PKD.

CONCLUSION

Our studies have shown a 25% reduction in kidney size and water content when a SEAE was used to supplement a casein-based diet (study 1) and reductions of 23% and 26% when this casein-based diet was supplemented with either Novasoy 400® or soyasaponin B_b, respectively. Replacement of the casein protein with SPI in study 1 was associated also with a slight decrease in kidney size that was smaller than that reported for the SPI-fed mice in our previous paper [2]. These results show that a reduced kidney size is a consistent finding in the SPI-fed mouse, although the extent of this change may be very variable.

Recently, attenuations of lesion incidence and severity were reported in kidneys from Imai hypertensive rats fed a semipurified alcohol extract of soy protein and enriched in the total soyasaponins [46]. These results confirm previous reports that cyst growth in PKD can be moderated by dietary means [2, 9, 10] and point to the therapeutic effectiveness of soybean. Results from the present study show that soyasaponin B_b, present in minute amounts in soy-derived products, may be an effective therapeutic agent for attenuation of cyst growth in the *pcy* mouse model of PKD. Future studies will determine the most effective therapeutic dose, the effects of gender and age at initiation of therapy for the maximal efficacy of this treatment.

ACKNOWLEDGMENTS

This work was supported by grants (to B.J.H.) from the Natural Sciences and Engineering Council of Canada (NSERC), the Polycystic Kidney Research Foundation, the Ontario Soybean Growers' Marketing Board, and by a grant from the Hannam Soy Utilization Fund, which was matched by Agriculture and Agri-Food, Canada.

The services of Dr. Malcolm Ogborn and Mr. Sanjay Sareen at the Children's Hospital of Winnipeg for the morphometric analyses and Dr. Bashir Sarr at ECORC for the preparation of the soyasaponin B_b are appreciated.

Reprint request to Bruce J. Holub, Ph.D., Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.
E-mail: bholub@uoguelph.ca

REFERENCES

1. Takahashi H, Calvet JP, Dittemore-Hoover D, *et al*: A hereditary model of slowly progressive polycystic kidney disease in the mouse. *J Am Soc Nephrol* 1:980–989, 1991
2. Tomobe K, Philbrick D, Ogborn MR, *et al*: Effect of dietary soy protein and genistein on disease progression in mice with polycystic kidney disease. *Am J Kidney Dis* 31:55–61, 1998
3. D'Amico G, Gentile MG, Manna G, *et al*: Effect of vegetarian soy diet on hyperlipidæmia in nephrotic syndrome. *Lancet* 339: 1131–1134, 1992
4. D'Amico G, Gentile MG: Influence of diet on lipid abnormalities in human renal disease. *Am J Kidney Dis* 22:151–157, 1993
5. Gentile MG, Fellin G, Cofano F, *et al*: Treatment of proteinuric patients with a vegetarian soy diet and fish oil. *Clin Nephrol* 40:315–320, 1993
6. Soroka N, Silverberg DS, Greemland M, *et al*: Comparison of a vegetable-based (soya) and an animal-based low-protein diet in predialysis chronic renal failure patients. *Nephron* 79:173–180, 1998
7. Williams AJ, Baker F, Walls J: Effect of varying quantity and quality of dietary protein intake in experimental renal disease in rats. *Nephron* 46:83–90, 1987
8. Howie AJ, Kizaki T, Beaman M, *et al*: Different types of segmental sclerosing glomerular lesions in six experimental models of proteinuria. *J Pathol* 157:141–151, 1989
9. Ogborn MR, Bankovic-Calic N, Shoesmith C, *et al*: Soy protein modification of rat polycystic kidney disease. *Am J Physiol 274* (Renal Physiol) 43:F541–F549, 1998
10. Aukema HM, Housini I, Rawling J: Dietary soy protein effects on inherited polycystic kidney disease are influenced by gender and protein level. *J Am Soc Nephrol* 10:300–308, 1999
11. Linassier C, Pierre M, LePecq JB, *et al*: Mechanism of action in N1H 3T3 cells of genisten: an inhibitor of the EGF repector tyrosine kinase activity. *Biochem Pharmacol* 39:187–193, 1989
12. Pugh JL, Sweeney WE, Avner ED: Tyrosine kinase activity of the EGF receptor in murine metanephritic organ culture. *Kidney Int* 47:774–781, 1995
13. Richards WG, Sweeney WE, Yoder BK, *et al*: Epidermal growth factor receptor activity mediates renal cyst formation in polycystic kidney disease. *J Clin Invest* 101:935–939, 1998
14. Sweeney WE Jr, Chen Y, Nakanishi K, *et al*: Treatment of polycystic kidney disease with a novel tyrosine kinase inhibitor. *Kidney Int* 37:33–40, 2000
15. Ireland PA, Dziedzic SZ, Kearsley MW: Saponin content of soya and some commercial soya products by means of high-performance liquid chromatography of the sapogenins. *J Sci Food Agri* 37:694–698, 1986
16. Price KR, Eagles J, Fenwick GR: Saponin composition of 13 varieties of legume seed using fast atom bombardment mass spectrometry. *J Sci Food Agri* 42:183–193, 1988
17. Taniyama T, Yoshikawa M, Kitagawa I: Saponin and Sapogenol. XLIV. Soyasaponin composition in soybeans of various origins and soyasaponin content in various organs of soybean. Structure of soyasaponin V from soybean hypocotyl. *Yakugaku Zasshi* 108:562–571, 1988
18. Shiraiwa M, Harada K, Okubo K: Composition and structure of "Group B" saponin in soybean seed. *Agric Biol Chem* 55:911–917, 1991
19. Tsukamoto C, Kikuchi A, Harada K, *et al*: Genetic and chemical polymorphisms of saponins in soybean seed. *Phytochemistry* 54:1351–1356, 1993
20. Bureau DP, Harris AM, Cho CY: The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 161:27–43, 1998
21. Price KR, Johnson IT, Fenwick GR: The chemistry and biological significance of saponins in foods and feeding stuffs. *CRC Crit Rev Food Sci Nutr* 26:27–123, 1987
22. Oakenfull D, Sidhu GS: Could saponins be a useful treatment for hypercholesterolæmia? *Eur J Clin Nutr* 44:79–88, 1990
23. Konoshima T: Anti-tumor-promoting activities of triterpenoid glycosides: Cancer chemoprevention by saponins, in *Saponins Used in Traditional and Modern Medicine,* edited by Waller GR, Yamasaki K, New York, Plenum Press, 1996, pp 87–100
24. Likhatskaya GN, Aminin DL, Agafonova IG, *et al*: The pH-dependent channels formed by cauloside A, in *Saponins Used in Traditional and Modern Medicine*, edited by Waller GR, Yamasaki K, New York, Plenum Press, 1996, pp 239–249
25. Kitagawa I, Saito M, Taniyama T, *et al*: Saponin and Sapogenol XXXVIII. Structure of Soyasaponin A_2, a bisdesmoside of soyasapogenol A, from soybean, the seeds of *Glycine max*. MERRILL. *Chem Pharm Bull* 33:598–608, 1985
26. Collins FW, Fielder DA, Sarr AB, *et al*: A process for the

isolation, recovery, and purification of non-polar extractives. Patent application #PCT/CA99/0003, 1999
27. Fuzzati N, Pace R, Papeo G, *et al*: Identification of soyasaponins by liquid chromatography-thermospray spectroscopy. *J Chromatog* (Series A)7777:233–238, 1997
28. Gattone VH, II, Calvet JP, Cowley BD, Jr, *et al*: Autosomal recessive polycystic kidney disease in a murine model. A gross and microscopic description. *Lab Invest* 59:231–238, 1988
29. Gatchalian-Yee M, Arimura Y, Ochiai E, *et al*: Soy protein lowers serum cholesterol in hamsters: Effect of debittered undigested fraction. *Nutrition* 13:633–639, 1997
30. Snedecor GW, Cochran WG: One-way classification: analysis of variance, in *Statistical Methods*, Ames, The Iowa State University Press, 1967, pp 234–236
31. Towner RA, Yamaguchi T, Philbrick DJ, *et al*: In vivo proton magnetic resonance imaging and localized spectroscopic analysis of polycystic kidney disease in DBA/2FG-*pcy* mice. *Magn Reson Imaging* 9:429–434, 1991
32. Cowley BD, Jr, Gudapaty S, Kraybill A, *et al*: Autosomal-dominant polycystic kidney disease in the rat. *Kidney Int* 43:522–534, 1993
33. Ogborn MR, Nitschmann E, Weiler HA, *et al*: Modification of polycystic kidney disease and fatty acid status by soy protein diet. *Kidney Int* 57:159–166, 2000
34. Vogler C, Homan S, Pung A, *et al*: Clinical and pathologic findings in two new allelic murine models of polycystic kidney disease. *J Am Soc Nephrol* 10:2534–2539, 1999
35. Nagao S, Yamaguchi T, Kasahara M, *et al*: Effect of probucol in a murine model of slowly progressive polycystic kidney disease. *Am J Kidney Dis* 35:221–226, 2000
36. Gretz N: Progression of chronic renal failure in a rat strain with autosomal dominant polycystic kidney disease. *Nephron* 68:462–467, 1994
37. Kränzlin B, Schieren G, Gretz N: Azotemia and extrarenal manifestations in old female Han:SPRD (*cy/+)* rats. *Kidney Int* 51:1160–1169, 1997
38. Tomobe K, Philbrick D, Aukema HM, *et al*: Early protein restriction slows disease progression and lengthens survival in mice with polycystic kidney disease. *J Am Soc Nephrol* 5:1355–1360, 1994
39. Yancey PH, Clark ME, Hand SC, *et al*: Living with water stress: Evolution of osmolyte systems. *Science* 217:1214–1222, 1982
40. Peterson DP, Murphy KM, Ursino R, *et al*: Effects of dietary protein and salt on rat renal osmolytes: Covariation in urea and GPC contents. *Am J Physiol* 263:F594–F600, 1992
41. Kaczorowski GJ, Knaus H-G, Leonard RJ, *et al*: High conductance calcium-activated potassium channels: Structure, pharmacology, and function. *J Bioenerg Biomembr* 28:255–267, 1996
42. Giangiacomo KM, Kamassah A, Harris G, *et al*: Mechanism of maxi-K^+ channel activation by dehydrosoyasaponin-I. *J Gen Physiol* 112:485–501, 1998
43. Morita T, Hanaoka K, Morales MM, *et al*: Cloning and characterization of maxi-K^+ channel a subunit in rabbit kidney. *Am J Physiol* 273:F615–F624, 1997
44. LaTorre R, Oberhauser A, Labercan P, *et al*: Varieties of calcium-activated potassium channels. *Ann Rev Physiol* 51:385–399, 1989
45. Chen Q, Zheng Z, Yang S, *et al*: Pharmacology of the fibrous root of *Panax notoginseng. Zhong Tongbao* 45:682–686, 1987
46. Sakemi T, Ikeda Y, Shimazu K, *et al*: Attenuating effect of a semipurified alcohol extract of soy protein on glomerular injury in spontaneous hypercholesterolemic male Imai rats. *Am J Kidney Dis* 37:832–837, 2001