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Both Soybean and Kudzu Phytoestrogens Modify Favorably the Blood Lipoprotein Profile in Ovariectomized and Castrated Hamsters

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The present study compared the hypolipidemic activity of kudzu phytoestrogens with that of soybean phytoestrogen in estrogen- and androgen-deficient hamsters. In the first experiment, ovariectomized hamsters ($n = 37$) were randomly divided into four groups ($n = 9-10$ each group). The first group was the control group, whereas the second group had the time-releasing estradiol-17β subcutaneous (pellet) implants as a positive control. The third and fourth groups were orally administered soybean or kudzu phytoestrogen extracts (30 mg/kg of body weight) per day. In the second experiments, the first group of male hamsters ($n = 9$) received a sham operation, whereas the other three groups of male hamsters ($n = 9$ each) were castrated. The castrated control group received orally distilled water, whereas the second and third castrated groups were orally given 30 mg/kg soybean or kudzu phytoestrogen extracts. The results for the first experiment showed that the ovariectomized hamsters orally given soybean and kudzu phytoestrogen extracts had significantly decreased serum total cholesterol (TC) and non-high-density lipoprotein cholesterol (non-HDL-C) with HDL cholesterol (HDL-C) being unaffected. The data from the second experiment demonstrated that administration of soybean but not kudzu phytoestrogen extracts decreased significantly serum TC. However, administration of kudzu phytoestrogens caused redistribution of cholesterol among lipoproteins, leading to a significant decrease in the ratio of non-HDL-C to HDL-C. It was concluded that both soybean and kudzu phytoestrogens could modify favorably lipoprotein profiles in ovariectomized and castrated hamsters.

KEYWORDS: Cholesterol; gegen; kudzu; phytoestrogens; soybean

INTRODUCTION

Soybean phytoestrogens as a health supplement are becoming popular worldwide. Phytoestrogens are named for a group of isoflavones found in plants that are structurally and functionally similar to 17β-estradiol. Growing evidence has shown that consumption of phytoestrogens may prevent certain cancers (*1*), reduce the risk of osteoporosis (*2*), have a beneficial role in chronic renal disease (*3*), lower plasma cholesterol (*4*), exhibit an antiatherosclerotic activity (*5*), and decrease the risk of coronary heart disease (*6*). However, consumption of soybean phytoestrogens in a large quantity has been associated with potential developmental and reproductive toxicity (*7*).

The risk of cardiovascular disease (CVD) drastically increases among women who reach the age of menopause due to hypercholesterolemia induced by estrogen deficiency (*8*). The use of phytoestrogen supplement has received considerable attention for reducing osteoporosis and CVD risks of postmenopausal women (*6*). The phytoestrogen supplements available on the market in China are mainly derived from two plants, namely, soybean (*Glycine max*) and kudzu roots (*Pueraria lobata*); the latter is also called gegen in Chinese. It is known that the amount and type of phytoestrogens in soybean seeds are different from those in other plants. In this regard, soybean phytoestrogens consist mainly of genistein, daizein, and their glycosides, whereas kudzu roots contain mainly puerarin and its derivatives along with a little genistein and daizein (*9*, *10*).

Soybean phytoestrogens have proven to prevent ovariectomy-induced atherosclerotic lesions in hamsters, an animal model for postmenopausal hyperlipidemia (*11*). The present study was carried out to further compare the hypolipidemic activity of kudzu phytoestrogens with that of soybean phytoestrogens. In addition, elderly men develop gradually partial androgen deficiency over several decades and also consume phytoestrogens from either diet or health supplements. In light of this, the present work also studied whether phytoestrogens might have

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Table 1. Composition of Soybean and Kudzu Phytoestrogen Extracts (Percent Dry Weight)

	soybean	kudzu
daidzin	10.02	8.83
glycitin	2.84	0
genistin	14.25	1.42
6″-*O*-malonyldaidzin	9.61	0
6″-*O*-malonylglycitin	2.00	0
6″-*O*-malonylgenistin	16.85	0
daidzein	0.07	1.22
genistein	0.04	1.86
puerarin	0	51.12
3′-hydroxypueratin	0	0.34
unknown	44.32	35.21

a hypocholesterolemic activity in castration-induced androgen-deficient hamsters.

MATERIALS AND METHODS

Preparation of Phytoestrogens from Soybean Seed and Kudzu Root. Soybean seed was purchased from a local market in Hong Kong. and kudzu root powder was obtained from a drug store in ShenZhen, Guangdong Province, China. In brief, the dry soybean and kudzu root powders (3 kg) were extracted with 18 L of 70% ethanol three times at 60 °C. Ethanol was evaporated under vacuum in a rotary evaporator. The extracts were then dissolved in 2 L of distilled water and partitioned with chloroform in a ratio of 1:1 three times. The chloroform was removed and the remaining aqueous solution containing the phytoestrogens freeze-dried and saved for diet preparation.

HPLC Analysis of Phytoestrogens. The dried extract (10 mg) was dissolved into a 10 mL test tube containing 4 mL of methanol, 2 mL of 0.1 N HCl, and 0.2 mL of flavone (2.5 mg/mL) as an internal standard. The solution was filtered through a 0.45 μm poly(tetrafluoroethylene) filter (Alltech Associates Inc., Deerfield, IL), and an aliquot was then transferred into a 1.5 mL sample vial and then subjected to HPLC analysis. Both soybean and kudzu phytoestrogen extracts were analyzed using a Shimadzu HPLC equipped with a ternary pump delivery system and a diode array detector. In brief, 10 μL of the sample was loaded onto a C18 column (Hypersil ODS, 4.6 × 250 mm, 5 μM, Alltech) via a Rheodyne valve (20 μL capacity; Cotati, CA). The diode array detector was set from 200 to 400 nm, and the eluting components were monitored at 260 nm. The analysis of individual phytoestrogens was carried out according to the method of Wang and Murphy (*12*) with some modifications (*13*). The mobile phase consisted of 1% acetic acid in water (v/v) (solvent A) and acetonitrile (solvent B). After injection of the sample, solvent B was increased from 10 to 20% in 40 min and then increased from 20 to 100% in the next 30 min. The flow rate was maintained at 0.8 mL/min. Each phytoestrogen was determined by comparing the retention time with that of authentic standard. Genistein, glycitein, and daidzein with purity >98% were obtained from Sigma Chemical Cp. (St. Louis, MO). All of the standards for kudzu phytoestrogens were provided by the Institute of Material Medical, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China. It was found that soybean phytoestrogen extract contained 16.85% 6″-*O*-malonylgenistin, 14.25% genistin, 10.02% daidzin, 9.61% 6″-*O*-malonyldaidzin, 2.84% glycitin, 2.00% 6″-*O*-malonylglycitin, 0.07% daidzein, and 0.04% genistein. Kudzu phytoestrogen extract had 51.12% pueratin, 8.83% daidzin, 1.42% genistin, 1.86% genistein, and 1.22% daidzein (**Table 1**).

Diets. A 0.1% choleserol diet was commercially purchased (PicoLab Rodent Diet20-Lab Diet, Glen Forrest Stockfeeds, Western Australia, Australia). According to the manufacturer, the diet contained (g/100 g) 61 carbohydrate, 19.1 protein, 12.3 fat, 4.5 crude fiber, 0.8% calcium, and 0.7 phosphorus; the remainder was a mixture of other minerals and vitamins. We have analyzed the fatty acid composition and found (in % total fat) the following: myristic acid, 1.4; palmitic acid, 24.7; palmitoleic acid, 1.4; stearic acid, 13.7; oleic acid, 34.7; linoleic acid, 20.2; and α-linolenic acid, 1.9.

Animals. Golden Syrian female hamsters ($n = 37$, aged 3 months, 160−170 g) were housed in an animal room at 25 °C with a 12:12 h light−dark cycle. In the first experiment, all of the female hamsters were ovariectomized and allowed a 7 day recovery from the operation before the experiment was started. They were all allowed free access to the diet and tap water. The ovariectomized hamsters were randomly divided into four groups ($n = 9-10$ each group). The first group was the control group, whereas the second group had the time-releasing estradiol-17β subcutaneous (pellet) implants (Sigma Chemical Co.) as a positive control. The third group was orally administered soybean phytoestrogen extracts (30 mg/kg of body weight) per day, and the fourth group was administered kudzu phytoestrogen extract (30 mg/kg of body weight) per day.

In the second experiment, male hamsters ($n = 36$, aged 2.5 months, 140−150 g) were similarly maintained on the 0.1% cholesterol diet and allowed free access to tap water. Twenty-seven male hamsters were castrated; 9 hamsters received a sham operation. After a 7 day recovery, the castrated hamsters were randomly divided into three groups. The first group was the control receiving orally distilled water, whereas the second and third groups were orally given either a single dose of soybean phytoestrogen extract (30 mg/kg of body weight) or kudzu phytoestogen extract (30 mg/kg of body weight), respectively.

The body weight of all the hamsters was recorded once a week, and food consumption was recorded every 2 or 3 days. At the end of 6 weeks on oral phytoestrogen dosing, all of the hamsters were sacrificed after overnight fasting. The blood was collected via the abdominal aorta. After clotting, the blood was centrifuged at 3000 rpm for 10 min, and serum was then collected. The liver, heart, and kidney were also removed, rinsed with ice-cold saline, weighed, and stored at −80 °C for determination of cholesterol content.

Serum Lipids. Serum total cholesterol (TC) and triglycerides (TG) were determined using enzymatic kits (Sigma Chemical Co.). The concentration of high-density lipoprotein cholesterol (HDL-C) was measured after precipitation of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) with phosphotungstic acid and magnesium chloride, using a commercial kit (Sigma). Non-HDL cholesterol (non-HDL-C) was calculated as the difference between TC and HDL-C.

Determination of Cholesterol in Liver, Heart, and Kidney. Cholesterol content in organs was analyzed according to the method previously described by Chan et al. (*14*). In brief, the liver (100 mg), kidney (300 mg), and heart (300 mg) were used to determine the cholesterol level. In brief, the tissue sample and 1 mg of stigmastanol, as an internal standard, were homogenized in 15 mL of chloroform/methanol (2:1, v/v) and 3 mL of saline. The chloroform/methanol phase was removed and dried under a gentle nitrogen steam. After 1 h of mild hydrolysis with 5 mL of 1 N NaOH in 90% ethanol at 90 °C, 1 mL of water and 6 mL of cyclohexane were added for extraction of cholesterol. The cyclohexane phase was evaporated to dryness under nitrogen, and cholesterol was converted to its TMS ether derivative by a commercial TMS reagent (dry pyridine/hexamethyldisilazane/trichlorosilane, 9:3:1, v/v/v, Sil-A regent, Sigma). After 1 h at 60 °C, the mixture was removed under a gentle stream of nitrogen. The TMS ether derivative was dissolved in 600 μL of hexane, and after centrifugation, the hexane phase was transferred to a vial for gas−liquid chromatography (GLC) analysis. The TMS ether derivative was analyzed in a fused silica capillary column (SAC-5, 30 m × 0.25 mm, i.d.; Supelco, Inc., Bellefonte, PA) in a Shimadzu GC-14B GLC equipped with a flame ionization detector (Shimadzu, Kyoto, Japan). The column temperature was set at 285 °C and maintained for 20 min. Helium was used as carrier gas at a head pressure of 22 psi. The cholesterol was quantified according to the stigmastanol added.

Statistics. Data are expressed as mean ± standard deviation (SD). The group means were analyzed using one-way analysis of variance (ANOVA) on SigmaStat Advisory Statistical software (SigmaStat version 11.0, SPSS Inc., Chicago, IL), followed by using Fisher's least significant difference method to statistically evaluate significant differences among groups. P values of <0.05 are regarded as statistically significant.

Table 2. Effect of Estradiol Implants and Soybean and Kudzu Phytoestrogen Extracts (30 mg/kg of Body Weight) on Food Intake and Body and Relative Organ Weights in Ovariectomized Hamsters Fed a High-Cholesterol Diet[a]

	control	estradiol	soybean	gegen
food consumption (g/rat/day)	7.5 ± 0.2	7.0 ± 0.1	7.4 ± 0.2	6.9 ± 0.1
food efficiency ratio (g of body wt/100 g of diet)	7.7 ± 3.8 a	0.4 ± 1.8 c	4.2 ± 2.3 b	3.0 ± 2.4 bc
body wt (g)				
initial	162.8 ± 8.7	162.8 ± 13.0	163.9 ± 14.1	163.5 ± 15.1
final	188.8 ± 8.7 a	164.0 ± 9.4 b	178.0 ± 12.9 ac	172.7 ± 14.1 bc
relative organ wt (g/100 g of body wt)				
liver	4.3 ± 0.3 a	3.7 ± 0.3 b	4.0 ± 0.3 a	4.1 ± 0.3 a
kidney	0.8 ± 0.0 b	0.9 ± 0.1 a	0.8 ± 0.1 b	0.8 ± 0.1 b
heart	0.4 ± 0.0 b	0.4 ± 0.0 a	0.4 ± 0.0 b	0.4 ± 0.0 b

[a] Values are means ± SD, $n = 9$–10. Means in the same row with different letters differ significantly at $P < 0.05$.

Table 3. Effects of Soybean and Kudzu Phytoestrogen Extracts (30 mg/kg of Body Weight) on Food Intake and Body and Relative Organ Weights in Castrated Hamsters Fed a High-Cholesterol Diet[a]

	sham	control	soybean	gegen
food consumption (g/rat/day)	8.1 ± 0.8	8.7 ± 0.3	8.6 ± 0.3	9.1 ± 0.3
food efficiency ratio (g of body wt /100 g of diet)	7.0 ± 2.4	7.5 ± 2.4	9.2 ± 1.9	9.6 ± 2.4
body wt (g)				
initial	146.5 ± 6.0	149.9 ± 15.8	149.4 ± 11.1	150.2 ± 10.0
final	168.1 ± 13.3 b	177.5 ± 12.8 ab	180.0 ± 9.0 ab	184.7 ± 17.2 a
relative organ wt (g/100 g of body wt)				
liver	4.1 ± 0.3 b	4.4 ± 0.6 ab	4.4 ± 0.5 ab	4.6 ± 0.6 a
kidney	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
heart	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.1

[a] Values are means ± SD, $n = 9$. Means in the same row with different letters differ significantly at $P < 0.05$.

Table 4. Effects of Estradiol Implantation and Soybean and Kudzu Phytoestrogen Extracts (30 mg/kg of Body Weight) on Serum and Organ Cholesterol in Ovariectomized Hamsters Fed a High-Cholesterol Diet[a]

	control	eastradiol	soybean	gegen
serum cholesterol				
TC (mg/dL)	267.6 ± 33.1 a	189.4 ± 32.1 b	225.4 ± 24.0 b	215.0 ± 53.4 b
HDL-C (mg/dL)	102.0 ± 16.5	96.8 ± 12.0	103.7 ± 31.6	91.0 ± 14.1
HDL-C/TC	0.4 ± 0.1 b	0.5 ± 0.1 a	0.5 ± 0.2 ab	0.5 ± 0.1 ab
non-HDL-C (mg/dL)	165.6 ± 43.1 a	92.6 ± 34.5 b	121.7 ± 37.4 b	124.0 ± 43.3 b
non-HDL-C/HDL-C	0.61 ± 0.10 a	0.48 ± 0.11 b	0.54 ± 0.15 ab	0.55 ± 0.13 ab
triglyceride (mg/dL)	271.6 ± 52.4 a	109.0 ± 43.8 c	193.6 ± 54.4 b	250.4 ± 82.0 a
organ cholesterol (mg/g)				
liver	42.7 ± 4.2 ab	49.2 ± 6.4 a	41. 5 ± 7.9 b	38.5 ± 6.4 b
kidney	4.7 ± 0.3 a	4.5 ± 0.2 b	4.7 ± 0.2 a	4.6 ± 0.2 ab
heart	1.6 ± 0.1 a	1.6 ± 0.1 a	1.5 ± 0.1 b	1.6 ± 0.1 ab

[a] Values are means ± SD, $n = 9$–10. Means in the same row with different letters differ significantly at $P < 0.05$.

Animal Ethics. All protocols described in the present paper were approved by the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

RESULTS

Body Weight and Food Intake. Food intake was expressed as the diet each hamster consumed each day, whereas the food efficiency ratio was defined as the body weight gained by each hamster that consumed every 100 g of diet. Food consumption did not differ among the experimental groups in four ovariectomized hamsters (**Table 2**). Subcutaneous estradiol implants and oral administration of soybean and kudzu phytoestrogen extracts decreased significantly the food efficiency ratio in ovariectomized hamsters (**Table 2**). The group receiving estradiol implants had the lowest final body weight, whereas the control had the highest final body weight among the four groups. The two groups orally administered soybean and kudzu phytoestrogen extracts had lower final body weights, but only the latter was significantly smaller compared with the control group (**Table 2**). The estradiol treatment group had a smaller liver but a bigger kidney or heart compared with the other three groups (**Table 2**).

No difference in food consumption and food efficiency was observed among the sham and three castrated hamster groups (**Table 3**). With regard to the final body weight, it was found that the kudzu group had a final body weight significantly greater than that of the sham group. Similarly, the kudzu group had a larger liver than the sham group.

Serum TC, HDL-C, TG, and Non-HDL-C/HDL-C. Estradiol implant and administration of soybean and kudzu phytoestrogen extract groups had significantly decreased serum TC and non-HDL-C, but they showed no significant effect on HDL-C compared with the control ovariectomized hamsters (**Table 4**). The estradiol implant group had a significantly decreased ratio of non-HDL-C/HDL-C compared with the control. Oral administration of soybean and kudzu phytoestroten extracts tended to reduce the ratio of non-HDL-C/HDL-C but, statistically, no difference was observed between the control and ovariectomized hamsters orally given soybean or kudzu phytoestrogen extracts. Administration of soybean phytoestrogen

Table 5. Effects of Soybean and Kudzu Phytoestrogen Extracts (30 mg/kg of Body Weight) on Serum and Organ Cholesterol Levels in Castrated Hamsters Fed a High-Cholesterol Diet[a]

	sham	control	soybean	gegen
serum cholesterol				
TC (mg/dL)	161.7 ± 19.8 c	257.3 ± 40.2 a	207.6 ± 42.4 b	241.9 ± 37.1 ab
HDL-C (mg/dL)	94.0 ± 19.5 b	130.1 ± 23.0 ac	120.2 ± 32.7 bc	153.4 ± 30.0 a
HDL-C/TC	0.6 ± 0.1 ab	0.5 ± 0.1 b	0.6 ± 0.1 ab	0.6 ± 0.1 a
non-HDL-C (mg/dL)	67.7 ± 20.2	127.2 ± 25.9 a	87.4 ± 16.5 b	88.5 ± 29.4 b
non-HDL-C/HDL-C	0.77 ± 0.34 ab	0.99 ± 0.19 a	0.76 ± 0.18 ab	0.60 ± 0.25 b
triglyceride (mg/dL)	197.7 ± 85.8	345.1 ± 137.7	219.5 ± 83.9 b	243.1 ± 42.2
organ cholesterol (mg/g)				
liver	47.9 ± 12.7 a	47.7 ± 9.3 a	27.8 ± 9.2 b	38.3 ± 10.9 b
kidney	4.6 ± 0.3 a	4.7 ± 0.4 a	4.5 ± 0.5 a	4.1 ± 0.3 b
heart	1.5 ± 0.2	1.5 ± 0.1	1.7 ± 0.3	1.6 ± 0.2

[a] Values are means ± SD, $n = 9$. Means in the same row with different letters differ significantly at $P < 0.05$.

extract and estradiol implants significantly lowered serum TG level, but administration of kudzu phytoestrogen extract had no significant effect compared with the control.

Castration increased serum TC, HDL-C, non-HDL-C, and TG when the castrated control group was compared with the sham group (**Table 5**). Administration of soybean but not kudzu phytoestrogen extracts decreased significantly serum TC compared with the control. Compared with the soybean phytoestrogen group, the kudzu phytoestrogen group had a higher HDL-C level (**Table 5**). Both soybean and kudzu phytoestrogen groups had non-HDL-C and non-HDL-C/LDL-C lower than those of the control hamsters. Administration of both soybean and kudzu phytoestrogen extracts reduced significantly the serum TG level compared with the control (**Table 5**).

Liver, Heart, and Kidney Cholesterol. The hepatic cholesterol level in ovariectomized hamsters given either soybean or kudzu phytoestrogen extract was similar to that in the ovariectomized control group, but it was significantly lower than that in the estradiol-treated group (**Table 4**). The renal cholesterol level in ovariectomized hamsters orally administered soybean and kudzu phytoestrogen extracts was similar to that of the control, but that in the estradiol-treated group was significantly lower than that in the control and the soybean phytoestrogen-treated groups (**Table 4**). The heart cholesterol level in the soybean phytoestrogen-treated group but not in the kudzu phytoestrogen-treated group was significantly lower than that in the control and estradiol-treated groups (**Table 4**).

For the castrated hamsters, only soybean but not kudzu phytoestrogen extracts decreased significantly hepatic cholesterol level compared with the control and the sham groups (**Table 5**). The kudzu phytoestrogen-treated group had the least renal cholesterol compared with the other three groups (**Table 5**). Administration of both soybean and kudzu phytoestrogen extracts had no effect on the heart cholesterol level.

DISCUSSION

The present results demonstrated that oral administration of 30 mg of soybean and kudzu phytoestrogens per kilogram body weight for only 6 weeks was effective in lowering serum TC in hypercholesterolemic ovariectomized hamsters. This cholesterol-lowering activity of soybean and kudzu phytoestrogen was similar to that of estradiol implants. Soybean and kudzu phytoestrogen extracts could also significantly reduce the non-HDL-C level in ovariectomized hamsters, suggesting they possess a similar estrogenic-like activity in modifying the lipid profile. The majority of cholesterol in blood circulates is located in LDL and HDL particles. Epidemiological observations and clinical trials have consistently documented a positive relationship between LDL cholesterol concentrations and cardiovascular disease risk and a negative relationship between HDL cholesterol concentrations and CVD risk (*15*). Observational data suggest that the ratio of non-HDL-C to HDL-C is a better predictor of subsequent CVD than individual lipid concentration, especially in the presence of increased TG (*16*, *17*). In the present study, this ratio could be statistically decreased only by estradiol treatment but not by soybean and kudzu phytoestrogen extracts. Although the results on hamsters cannot be transferred thoroughly to those on humans, the present study suggests that both soybean and kudzu phytoestrogens possess weak estrogenic activity and modulate favorably blood lipids to prevent CVD in postmenopausal women.

Several mechanisms by which soybean phytoestrogens reduce blood cholesterol levels have been proposed. First, similar to that of estradiol, the cholesterol-lowering effect of phytoestrogens is most likely mediated by its activation of the LDL receptor (*18*). Second, soybean phytoestrogens up-regulate the sterol regulatory binding protein 2 (SREBP-2), which in turn produces an increase in surface LDL-receptor expression (*19*). Third, hypocholesterolemic activity associated genistein and daidzein have been reported to link with their inhibitory effect on apolipoprotein B secretion and assemble apo-B-containing lipoproteins (*20*). Last, soybean phytoestrogens have been shown to lower blood lipids by their action on peroxisome proliferator activated receptors (PPARs) (*21*). It is noteworthy to point out these studies have focused mainly on soybean phytoestrogens, namely, genistein and daidzein, and investigations on phytoestrogens from other sources are limited. To our knowledge, no study to date has been carried out to compare the hypocholesterolemic activity of soybean phytoestrogens with ones from other plants. It is known that that phytoestrogens in soybean are mainly genistein, daidzein, and their glycosides, whereas those in kudzu roots are mainly pueratin and its derivatives. The present study demonstrated clearly that kudzu phytoestrogens were equally effective in reducing serum TC and LDL-C levels, but they were weaker than soybean phytoestrogens in lowering serum TG in estrogen-deficient hamsters (**Table 4**).

Phytoestrogens are part of the human diets. Many commercial phytoestrogen products are widely consumed not only by women but also by men. Unlike the menopausal women who have a sharp decrease in estrogen, men develop gradually a partial androgen deficiency over several decades. The present study confirmed that castration led to elevation of serum TC, HDL, and non-HDL-C (**Table 5**). The present results are in agreement with the report of Moorjani et al. (*22*), who found that orchiectomy in humans caused hypercholesterolemia. The

present work is the first to study the effect of soybean and kudzu phytoestrogens on lipoprotein profiles in castrated animals, finding that only soybean phytoestrogens were able to reduce serum TC in castrated hamsters, whereas those from kudzu roots had no significant effect (**Table 5**). It appeared that soybean phytoestrogens caused a slight reduction in HDL-C but a marked reduction in non-HDL-C. Most interesting was that kudzu phytoestrogens caused an increase in HDL-C (not significant) and a marked decrease in non-HDL-C, leading to a significant reduction in the ratio of non-HDL-C to HDL-C, although they had no effect on serum TC (**Table 5**). The present data suggested the hypocholesterolemic mechanism for soybean phytoestrogens was not completely similar to that for kudzu phytoestrogens. This may be attributed to a difference in phytoestrogen composition between soybean and kudzu root. The former contains mainly genistein, daidzein, and their derivatives, whereas the latter consists of mainly pueratin and its derivatives.

The present study found that there was a decreasing trend in liver cholesterol level associated with oral administration of soybean and kudzu phytoestrogens (**Tables 4** and **5**). This effect appeared to be much stronger in castrated hamsters than in ovariectomized hamsters (**Table 5**). The underlying mechanism remains poorly understood. Perhaps phytoestrogens decreased the liver cholesterol level by inhibiting hepatic cholesterol synthesis. In this regard, genistein and daidzein have been shown to inhibit in vitro 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is a key enzyme involved in cholesterol synthesis (*23*−*25*). However, there is no study to date that has examined the effect of pueratin on HMG-CoA reductase.

Men have a greater risk of coronary heart disease than women, although it is controversial that androgen is proatherogenic whereas estrogen attenuates atherogenesis (*26*). The results in the literature concerning androgen and androgen substitution therapy on blood lipids in the adult and aging hypogonadal males were inconsistent (*27*, *28*). Our present results, if confirmed in humans, may have important clinical applications, because they suggest that soybean and kudzu phytoestrogens could modify favorably serum lipids in both aged men and postmenopausal women. It would be very interesting to evaluate a combination of androgen substitution and phytoestrogens on serum lipids in aging hypogonadal males.

ABBREVIATIONS USED

CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; VLDL, very-low-density lipoprotein.

LITERATURE CITED

(1) Omoni, A. O.; Aluko, R. E. Soybean foods and their benefits: potential mechanisms of action. *Nutr. Rev.* **2005**, 63, 272−283.

(2) Branca, F. Dietary phyto-oestrogens and bone health. *Proc. Nutr. Soc.* **2003**, *62*, 877−887.

(3) Ranich, T.; Bhathena, S. T.; Velasquez, M. T. Protective effects of dietary phytoestrogens in chronic renal disease. *J. Ren. Nutr.* **2001**, *11*, 183−193.

(4) Demonty, I.; Lamarche, B.; Jones, P. J. Role of isoflavones in the hypocholesterolemic effect of soy. *Nutr. Rev.* **2003**, *61*, 189−203.

(5) Arliss, R. M.; Biermann, C. A. Do soy isoflavones lower cholesterol, inhibit atherosclerosis, and play a role in cancer prevention? *Holist. Nurs. Pract.* **2002**, *16*, 40−48.

(6) Lissin, L. W.; Cokke, J. P. Phytoestrogens and cardiovascular health. *J. Am. Coll. Cardiol.* **2000**, *35*, 1403−1410.

(7) Chen, A.; Rogan, W. J. Isoflavones in soy infant formula: a review of evidence for endocrine and other activity in infants. *Annu. Rev. Nutr.* **2004**, *24*, 33−54.

(8) Derry, P. S. Hormones, menopause, and heart disease: making sense of the Women's Health Initiative. *Womens Health Issues* **2004**, *14*, 212−219.

(9) Ho, H. M.; Chen, R. Y.; Leung, L. K.; Chan, F. L.; Huang, Y.; Chen, Z. Y. Difference in flavonoid and isoflavone profile between soybean and soy leaf. *Biomed. Pharmacother.* **2002**, *56*, 289−295.

(10) Zhang, Y.; Xu, Q.; Zhang, X.; Chen, J.; Liang, X.; Kettrup, A. High-perormance liquid chromatography−tandem mass spectrometry for identification of isoflavones and description of the biotransformation of kudzu root. *Anal. Bioanal. Chem.* **2005**, *33*, 787−796.

(11) Lucas, E. A.; Lightfoot, S. A.; Hammond, L. J.; Devareddy, L.; Khalil, D. A.; Daggy, B. P.; Soung, D.; Arjmandi, B. H. Soy isoflavones prevent ovariectomy-induced atherosclerotic lesions in Golden Syrian hamster model of postmenopausal hyperlipidemia. *Menopause* **2003**, *10*, 314−321.

(12) Wang, H. J.; Murphy, P. A. Isoflavone composition of American and Japanese soybeans in Iowa: effect of variety, crops year and locations. *J. Agric. Food Chem.* **1994**, *42*, 1674−1677.

(13) Lee, C. H.; Yang, L.; Xu, J. Z.; Yeung, S. Y. V.; Huang, Y.; Chen, Z. Y. Relative antioxidant activity of soybean isoflvaones and their glycosides. *Food Chem.* **2005**, *90*, 735−741.

(14) Chan, P. T.; Fong, W. P.; Huang, Y.; Ho, W. K. K.; Chen, Z. Y. Jasmine green tea epicatehins are hypolipidemic in hamster (*Mesocricetus auratus*) fed a high fat diet. *J. Nutr.* **1999**, *129*, 1094−1101.

(15) Stein, O.; Stein, Y. Atheroprotective mechanisms of HDL. *Atherosclerosis* **1999**, *144*, 285−301.

(16) Castelil, W. P.; Abbott, R. D.; McNamara, P. M. Summary estimates of cholesterol used to predict coronary heart disease. *Circulation* **1983**, *67*, 730−734.

(17) Wakatsuki, A.; Sagara, Y. Lipoprotein metabolism in postmenopausal and oophorectomized women. *Obstet. Gynecol.* **1995**, *85*, 523−528.

(18) Owen, A. J.; Roach, P. D.; Abbey, M. Regulation of low-density lipoprotein receptor activity by estrogens and phytoestrogens in a HepG2 cell model. *Ann. Nutr. Metab.* **2004**, *48*, 269−275.

(19) Mullen, E.; Brown, R. M.; Osborne, T. F.; Shay, N. F. Soy isoflavones affect sterol regulatory element binding proteins (SREBPs) and SREBP-regulated genes in HepG2 cell. *J. Nutr.* **2004**, *134*, 2942−2947.

(20) Borradaile, N. M.; Dreu, L. E.; Wilcox, L. J.; Edwards, J. Y.; Huff, M. W. Soya phytoestrogens, genistein and daidzein, decrease apoliprotein B secretion from HepG2 cells through multiple mechanism. *Biochem. J.* **2002**, *366*, 531−539.

(21) Richetts, M. L.; Moore, D. D.; Banz, W. J.; Mezei, O.; Shay, N. F. Molecular mechanisms of action of the soybean isoflavones includes action of promiscuous nuclear receptor. A review. *J. Nutr. Biochem.* **2005**, *16*, 321−330.

(22) Moorjani, S.; Dupont, A.; Labrie, F.; Lupien, P. J.; Gagne, C.; Brun, D.; Giguere, M.; Belanger, A.; Cusan L. Changes in plasma lipoproteins during various androgen suppression therapies in men with prostatic carcinoma: effect of orchiectomy, estrogen, and combination treatment with luteining hormone-releasing hormone agonist and flutamide. *J. Clin. Endocrinol. Metab.* **1988**, *66*, 314−322.

(23) Sung, J. H.; Lee, S, J.; Park, K. H.; Moon, T. W. Isoflavones inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase in vitro. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 428−432.

(24) Sung, J. H.; Choi, S. J.; Lee, S. W.; Park, K. H.; Moon, T. W. Isoflavones found in Korean soybean paste as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 1051−1058.

(25) Duncan, R. E.; El-Sohemy, A.; Archer, M. C. Regulation of HMG-CoA reductase in MCF-7 cells by genistein, EPA, and DHA, alone and in combination with mevastatin. *Cancer Lett.* **2005**, *224*, 221−228.

(26) Wu, F. C. W.; von Eckardstein, A. Androgens and coronary heart disease. *Endocrine Rev.* **2003**, *24*, 183−217.
(27) Schleich, F.; Legros, J. J. Effect of androgen substitution on lipids profile in the adults and ageing hypogonadal male. *Eur. J. Endocrinol.* **2004**, *151*, 415−424.
(28) Hurley, B. F.; Seals, D. R.; Hagberg, J. M.; Goldberg, A. C.; Ostrove, S. M.; Holloszy, J. O.; Wiest, W. G.; Goldberg, A. P. High-density-lipoprotein cholesterol in bodybuilders vs power-lifters. Negative effects of androgen use. *JAMA−J. Am. Med. Assoc.* **1984**, *252*, 507−513.

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