Soybean Isoflavones Improve Cardiovascular Risk Factors without Affecting the Reproductive System of Peripubertal Rhesus Monkeys[1,2]

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ABSTRACT Although the beneficial effects of dietary soybean protein compared with animal proteins on plasma lipids, lipoproteins and atherosclerosis have been known for about 50 years, it has been uncertain whether these effects are due to its amino acid concentrations or other components in soybeans. To assess the effect of soybean protein's alcohol-extractable components (including the isoflavonic phytoestrogens genistein and daidzein) on plasma lipid and lipoprotein concentrations and to establish its lack of effect on the reproductive system, we fed 27 peripubertal male and female rhesus monkeys moderately atherogenic diets in which the source of dietary protein was a soy isolate (20% by weight), either containing phytoestrogens (also termed isoflavones) or with the phytoestrogens removed by alcohol extraction. The study was a crossover design with each period lasting for 6 mo. The phytoestrogen-intact soy protein (compared with the alcohol-extracted soy protein) had favorable effects on plasma lipid and lipoprotein concentrations, specifically by significantly reducing LDL + VLDL cholesterol concentrations in both males and females (~30–40% lower), significantly increasing high density lipoprotein cholesterol (HDLC) concentrations for females (~15% higher) and significantly lowering total plasma cholesterol (TPC):HDLC ratios (~20% lower for males and 50% lower for females). The phytoestrogens had no adverse effects on the reproductive systems of either the males or females, as evaluated by reproductive hormone concentrations and organ weights at necropsy. Thus, the isoflavones in soy protein improve cardiovascular disease risk factors without apparent deleterious effects on the reproductive system of peripubertal rhesus monkeys. J. Nutr. 126: 43–50, 1996.

INDEXING KEY WORDS:

- *rhesus monkeys* • *soybean protein*
- *cardiovascular disease* • *reproductive organs*
- *isoflavones* • *phytoestrogens*

The antiatherogenic effect of dietary soy protein in animals compared with casein has been recognized since the 1940s (Meeker and Kesten 1941), but the components of the soy responsible for this effect have not been determined. During the 1970s and early 1980s the amino acid composition was investigated for its effect on plasma lipids and role in atherosclerosis prevention (Balogun et al. 1982, Huff et al. 1977, Huff and Carroll 1980, Kritchevsky 1979, Kritchevsky et al. 1982, Nagata et al. 1982). Generally, soybean protein amino acid reconstitutions were not as effective in lowering plasma cholesterol as the intact protein, but casein amino acid reconstitutions induced a similar effect on cholesterolemia as the intact protein (Huff et al. 1977, Huff and Carroll 1980). Other attributes of soy have been hypothesized as responsible for the lipid-lowering effects (including saponins, protein digestibility and amino acid phosphorylation) but with no conclusive evidence (Forsythe et al. 1986, Potter 1995).

More than 300 plants have been determined to have estrogenic activity (Bradbury and White 1954, Farnsworth et al. 1975). The phytoestrogens vary greatly in both their estrogenic potency and physiologic effects.

[1] Presented at the First International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, February 20–23, 1994, Mesa, AZ (Anthony, M. S., Clarkson, T. B., Weddle, D. L., Wolfe, M. S. (1995) Effects of soy protein phytoestrogens on cardiovascular risk factors in rhesus monkeys. J. Nutr. 125: 803–804) and at the 67th Scientific Sessions of the American Heart Association, November 14–17, 1994, Dallas, TX (Anthony, M. S., Clarkson, T. B., Hughes, C. L., Jr. (1994) Plant and mammalian estrogen effects on plasma lipids of female monkeys. Circulation 90: I-235).

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Manuscript received 4 April 1995. Initial review completed 9 May 1995. Revision accepted 16 August 1995.

The two primary estrogenic isoflavones in soybeans are genistein and daidzein and their conjugates (aglycones and glucosides) (Eldridge 1982). Both these phytoestrogens bind to estrogen receptors and have relative potencies on biologic activity in a human endometrial adenocarcinoma cell line (Ishikawa-Var I) of 0.084 for genistein and 0.013 for daidzein compared with estradiol set at 100 (Markiewicz et al. 1993). Setchell (1985) was the first to speculate that compounds in soy with estrogenic activity, termed phytoestrogens, might be responsible for the plasma cholesterol-lowering effect of soy but presented no experimental evidence.

Genistein's effects appear to be tissue-specific, with estrogen agonist effects on plasma lipid concentrations, plasma lipoprotein distributions (Anthony et al. 1994, Balmir et al. 1995) and preservation of bone mass (Anderson et al. 1995) that are similar in magnitude to mammalian estrogens, but without estrogenic effects (i.e., endometrial proliferation) on the uterus at these same doses. Because of the abundant evidence for a cardioprotective effect of mammalian estrogens (Stampfer and Colditz 1991), the estrogenic activity of the phytoestrogens in soy and lipid-lowering effects of dietary soy, we compared the effects of diets containing isolated soy protein with or without its isoflavones on the reproductive system and cardiovascular risk factors in peripubertal rhesus monkeys.

MATERIALS AND METHODS

Animals. Fourteen female and 13 male colony-born rhesus monkeys (*Macaca mulatta*) were started in this study. The females were between 1.3 and 2.1 y of age at the start of the study, and the males were between 1.3 and 4.1 y of age. A stratified randomization, based on age and total plasma cholesterol (TPC)[4] to high density lipoprotein cholesterol (HDLC) ratio, was used to allocate monkeys to treatment groups. All procedures involving animals were conducted in compliance with state and federal laws, standards of the U.S. Department of Health and Human Services and guidelines established by the Institutional Animal Care and Use Committee.

During the course of the study two males were lost to the study for causes unrelated to the study. One monkey that had been fed the Soy(+) diet died during the first period of the study. A second monkey that was fed the Soy(−) diet (see next paragraph for diet descriptions) died about a week after the crossover.

[4] Abbreviations used: ANCOVA, analysis of covariance; apo, apolipoprotein; DHEAS, dehydroepiandrosterone sulfate; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; Lp(a), lipoprotein (a); SHBG, sex hormone-binding globulin; TPC, total plasma cholesterol; VLDLC, very low density lipoprotein cholesterol.

Diets. All monkeys were fed a diet with casein and lactalbumin as the source of protein for three weeks before initiation of the experimental diets. This initial diet was similar in amounts of fat and cholesterol to the experimental diets. Two experimental diets were used: Soy(+), a moderately atherogenic diet containing isolated soy protein with the isoflavones intact; and Soy(−), the same diet composition but with alcohol-extracted isolated soy protein. All diets were prepared in our diet laboratory in 10-kg batches and kept frozen until needed. One day's worth of diet was thawed overnight at 4°C before feeding.

The composition of the experimental diets are in **Table 1**. The isolated soy proteins used for this study were provided by Protein Technologies International

TABLE 1

Diet compositions

Ingredient	Soy(−) diet	Soy(+) diet
	g/kg diet	*g/kg diet*
SUPRO 670-IF Soy Protein Isolate®, low phytoestrogen (90.7% protein)	200.0	—
SUPRO 670 Soy Protein Isolate®, high phytoestrogen (87.0% protein)	—	200.0
DL-Methionine	5.0	5.0
Dextrin	152.0	152.0
Sucrose	178.0	178.0
Wheat flour	118.0	118.0
Alphacel	67.4	67.4
Lard	120.0	120.0
Butter	44.0	44.0
Corn oil	35.0	35.0
Crystalline cholesterol	1.1	1.1
Complete vitamin mix[1]	25.0	25.0
Modified Ausman-Hayes mineral mix[2]	50.0	50.0
Calcium carbonate	2.5	2.5
Calcium phosphate, monobasic	2.0	2.0
Protein (*% of energy*)	18.5	17.7
Carbohydrate (*% of energy*)	40.9	40.6
Fat (*% of energy*)	40.6	41.7
Saturated (*% of energy*)	16.4	16.6
Monounsaturated (*% of energy*)	16.1	16.3
Polyunsaturated (*% of energy*)	8.1	8.8
Cholesterol (*mg/kg body weight*)	36.7	36.5
Isoflavones (*mg/kg body weight*)	0.97	9.41

[1] Provided (g/kg diet): retinyl palmitate, 0.045; DL-α-tocopheryl acetate, 0.0625; iso-inositol, 0.125; riboflavin, 0.025; menadione sodium bisulfite complex, 0.05625; para-aminobenzoic acid, 0.125; niacin, 0.1125; pyridoxine hydrochloride, 0.025; thiamine hydrochloride, 0.025; calcium pantothenate, 0.075; vitamin B-12 in mannitol, 0.03375; biotin, 0.0005; folic acid, 0.00225; ascorbic acid, 2.25; choline chloride, 1.875; cholecalciferol, 0.005; dextrose monohydrate, 20.15725.

[2] Provided (g/kg diet): K_2CO_3, 15.696; NaCl, 8.119; $MgSO_4(H_2O)_7$, 7.195; $FeSO_4$, 0.3934; $MnSO_4(H_2O)$, 0.0676; $ZnCl_2$, 0.0455; $CuSO_4$, 0.0145; KI, 0.0039; $(CH_3CO_2)_7Cr_3(OH)_2$, 0.0024; NaF, 0.0012; $Na_2SeO_3(H_2O)_5$, 0.0002; Dextrin, 18.463.

(St. Louis, MO). The soy protein with the phytoestrogens intact (SUPRO 670®) contained ~1.27 mg genistein and 0.42 mg daidzein per gram of soy protein. The alcohol-washed soy protein (SUPRO 670-IF®) contained only 0.121 mg genistein and 0.052 mg daidzein per gram of protein. The alcohol-washed soy protein contained 90.7% protein and only 0.7% fat compared with the unextracted protein, which was 87.0% protein and 4.0% fat. (Soy protein analyses were done by Ralston Analytical Laboratories, St. Louis, MO.) We analyzed the proteins by HPLC for β-sitosterol as an indicator of other sterols that may have been extracted and found no detectable amount of β-sitosterol in either protein. The isolated soy protein is carefully processed before ethanol extraction to reduce lactins, phytase and trypsin inhibitors, so the extracted and unextracted proteins were similar for components other than the phytoestrogens and lipid. As can be seen in Table 1, both diets contained 20% isolated soy protein by weight. The differences in the concentration of the protein in the product somewhat affected the percent of energy as protein, fat and carbohydrate. Five g/kg of DL-methionine was added to both diets to ensure the requirements of this essential amino acid were met.

Study design. The study was a crossover design with each period of treatment lasting for 6 mo after the 3-wk baseline period (**Fig. 1**). Plasma lipid concentrations (TPC, HDLC and triglycerides) and lipoprotein(a) [Lp(a)] concentrations were measured both at baseline and periodically during the treatment periods. Plasma apolipoprotein concentrations [apolipoprotein A-I (apo A-I), apolipoprotein A-2 (apo A-2) and apolipoprotein B (apo B)] and low density lipoprotein (LDL) molecular weight were measured only during the two periods of treatment.

Biochemical analyses. Blood was collected from the animals into evacuated tubes containing EDTA (final concentration 1 g/L) after food was withheld for 18 h. Total plasma cholesterol was measured by enzymatic techniques based on the methods of Allain et al. (1974). Plasma triglycerides were determined by the methods of Fossati and Principe (1982). HDLC concentrations were measured using the heparin-manganese precipitation procedure described in the Manual of Laboratory Operations of the Lipid Research Clinics Program (1974). Low density lipoprotein cholesterol plus very low density lipoprotein cholesterol (LDLC + VLDLC) was calculated as the difference between TPC and HDLC. All analyses were done on a COBAS FARA II autoanalyzer (Roche Diagnostic Systems, Montclair, NJ). The laboratory subscribes to the Centers for Disease Control (Atlanta, GA) Lipid Standardization Program.

During both treatment periods, LDL molecular weight and concentrations of apo A-I, apo A-2, apo B and Lp(a) were measured. Blood for these lipoprotein

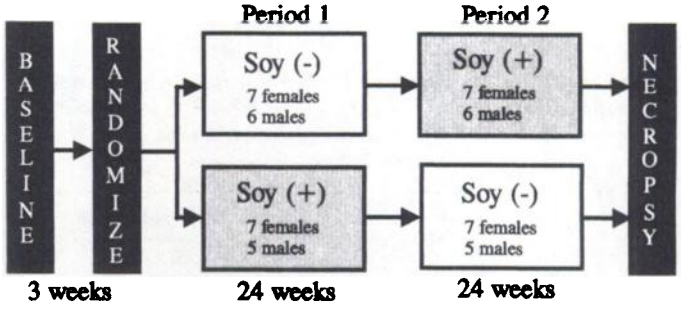

FIGURE 1 Schematic diagram of the crossover study design comparing diets containing soybean protein isolates with the isoflavones intact [Soy(+)] or with the isoflavones extracted with ethanol [Soy(−)] in peripubertal rhesus monkeys.

and apolipoprotein analyses was collected into evacuated tubes containing EDTA (1.5 g/L final concentration) and a protease inhibitor cocktail consisting of sodium azide (1 g/L final concentration), aprotinin (0.4 mg/L final concentration) and benzamidine (0.15 g/L final concentration). Food was withheld from the animals for 18 h before blood sample collection. The lipoprotein fraction ($d < 1.225$ kg/L) was isolated from whole plasma by ultracentrifugation in preparation for LDL molecular weight analysis. LDL molecular weight was calculated from the retention time of the peak of the LDL fraction after lipoprotein separation on 6% agarose by HPLC (Rudel et al. 1986). The HPLC method for LDL molecular weight determination was standardized against samples of known LDL molecular weight determined by gel filtration chromatography (Rudel et al. 1974) using radioiodinated LDL as the standard. Apo A-I (Koritnik and Rudel 1983) and apo B (Sorci-Thomas et al. 1989) were quantified by ELISA methods previously published. Apo A-2 and Lp(a) concentrations were measured using modifications of the ELISA for determining apo A-I that was developed at the Bowman Gray School of Medicine Lipoprotein Core Laboratory (Wagner et al. 1993). All samples were analyzed in duplicate and plasma pools were included with all assays.

Hormone assays were done at the Yerkes Regional Primate Research Center Endocrinology Laboratory. Estradiol concentrations were determined by a commercially available RIA kit utilizing a double-antibody technique (Diagnostics Products, Los Angeles, CA). Testosterone, dehydroepiandrosterone sulfate (DHEAS) and free thyroxine concentrations were quantitated by RIA with commercially available kits using antibody-coated tubes (Coat-A-Count®, Diagnostic Products). Sex hormone-binding globulin (SHBG) was determined by a saturation assay with dihydrotestosterone using a dextran-coated charcoal separation technique to remove endogenous steroids (Koritnik and Marschke 1986).

Other measurements. Systolic and diastolic blood pressure measurements were done on sedated subjects

TABLE 2

Effects of diets containing soybean protein isolates with isoflavones intact (+) or ethanol-extracted (−) on plasma lipid and lipoprotein concentrations in peripubertal rhesus monkeys[1]

	Soy (−)	Soy (+)	*P* value[2]
Total plasma cholesterol, *mmol/L*			
Males	8.25 ± 0.62	6.47 ± 0.70	0.008
Females	8.74 ± 0.72	6.36 ± 0.41	0.009
LDL + VLDL cholesterol, *mmol/L*			
Males	5.92 ± 0.65	4.14 ± 0.75	0.006
Females	6.83 ± 0.80	4.16 ± 0.36	0.003
HDL cholesterol, *mmol/L*			
Males	2.30 ± 0.10	2.33 ± 0.10	0.78
Females	1.91 ± 0.18	2.20 ± 0.13	0.05
Plasma triglycerides, *mmol/L*			
Males	0.25 ± 0.01	0.28 ± 0.01	0.02
Females	0.29 ± 0.03	0.29 ± 0.02	0.88
Total:HDL cholesterol ratio, *mmol/mmol*			
Males	3.77 ± 0.42	2.92 ± 0.48	0.004
Females	5.87 ± 1.01	3.01 ± 0.21	0.005

[1] Values are means ± SEM. For males, $n = 11$; for females, $n = 14$.
[2] Analysis by paired *t* test.

by a standardized protocol (Corbett et al. 1981) using a Dinamap Portable Adult, Pediatric and Neonatal Vital Signs Monitor (Model 8100, Critikon, Tampa, FL). Body weight was monitored when the animals were sedated for collection of other samples.

At the end of the study the monkeys were killed with an overdose of sodium pentobarbital (100 mg/kg administered intravenously), a method consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. Uterine, prostatic and testicular weights were measured immediately after dissection and removal of these tissues. Average testicular weight was calculated for each male and used for subsequent analysis.

Statistical analyses. All analyses were done separately for each gender using BMDP Statistical Software (Version 7.0; Los Angeles, CA). All variables measured during Periods 1 and 2 were analyzed for main effects of treatment and period and the interaction. If there were no significant period x treatment interactions ($P > 0.10$), the means for each animal for each treatment were compared by paired *t* test (Hills and Armitage 1979). Reproductive system organ weight data were analyzed by ANCOVA using the group assignment at necropsy as their classification and covarying for age and body weight at necropsy. An alpha level of 0.05 was used to determine statistical significance, unless otherwise specified.

RESULTS

Lipids, lipoproteins and apolipoproteins. Lipid and lipoprotein data are summarized in **Table 2**. Because there were no significant period × treatment interactions for any of the lipid and lipoprotein variables for males or females, all data analyses were done as paired *t* tests. LDLC + VLDLC concentrations were significantly lower in both males and females during Soy(+) treatment compared with Soy(−). In monkeys with such low triglyceride concentrations, this LDL + VLDL cholesterol was primarily in the LDL subfraction. The reduction was ~30% for the males and ~40% for the females. HDLC concentrations were unchanged for the males. However, the females had significantly greater HDLC concentrations (~15% higher) when they were fed the Soy(+) diet. For the males there was a significantly improved TPC:HDLC ratio when they consumed the Soy(+) diet. TPC:HDLC ratios were also significantly lower for the females when they consumed the Soy(+) diet. There was a small but significant increase in plasma triglycerides for the males with Soy(+) treatment. In the females plasma triglyceride concentrations were not affected by the diets.

LDL molecular weight, apolipoprotein and Lp(a) data are shown in **Table 3**. LDL molecular weight, a measure of LDL particle size, was significantly lower for the females during Soy(+) diet. LDL molecular

TABLE 3

Effects of diets containing soybean protein isolates with isoflavones intact (+) or ethanol-extracted (−) on LDL molecular weight, apolipoprotein concentrations, and lipoprotein(a) concentrations in peripubertal rhesus monkeys[1]

	Soy (−)	Soy (+)	*P* value[2]
LDL molecular weight, *g/μmol*			
Males	3.68 ± 0.15	3.42 ± 0.19	0.29
Females	3.60 ± 0.11	3.21 ± 0.12	0.03
Apolipoprotein B, *g/L*			
Males	1.49 ± 0.12	1.16 ± 0.13	0.09
Females	1.24 ± 0.11	0.99 ± 0.10	0.07
Apolipoprotein A-I, *g/L*			
Males	2.34 ± 0.19	2.75 ± 0.10	0.11
Females	2.19 ± 0.18	2.97 ± 0.18	0.004
Apolipoprotein A-2, *g/L*			
Males	0.20 ± 0.02	0.22 ± 0.01	0.48
Female	0.16 ± 0.01	0.19 ± 0.01	0.03
Lipoprotein(a), *g/L*			
Males	0.50 ± 0.03	0.46 ± 0.03	0.11
Females	0.39 ± 0.04	0.32 ± 0.03	0.003

[1] Values are means ± SEM. For males, $n = 11$; for females, $n = 14$.
[2] Analysis by paired *t* test.

weight was not significantly lower when males were fed the Soy(+) diet.

Lp(a) concentrations were significantly lower during Soy(+) treatment in females. Although the Lp(a) concentrations were lower for the males during Soy(+) treatment, the change was of borderline statistical significance. Plasma apolipoprotein concentrations were favorably affected by Soy(+) diet, although some of these changes were not significant. Apo A-I and apo A-2 concentrations were significantly higher during Soy(+) treatment for the females as expected, given the changes in HDLC concentrations. Apo B concentrations were lower with Soy(+) treatment for both males and females, again in keeping with the differences in LDLC + VLDLC, but these differences did not reach statistical significance for either gender.

Hormones and reproductive system. The hormone and reproductive system data are shown in **Table 4**. Soy(+) treatment had no significant effect on free thyroxine, SHBG or DHEAS for either gender. There were no effects of Soy(+) treatment on estradiol concentrations in the females or testosterone in the males. For the males there were no differences between the treatment groups for testicular or prostatic weight. There were also no differences for the females between treatment groups for uterine weight.

Blood pressure and body weight. Neither systolic nor diastolic blood pressures were affected by treatment for either gender (**Table 5**). Body weight increased for both groups and both genders during both periods, but there were no differences in weight gain between treatment groups for either gender. Growth rates for all animals were normal compared with growth curves generated from our colony (unpublished data) and higher than those published for other colonies (Cupp and Uemura 1981).

DISCUSSION

The phytoestrogen-containing soy protein had favorable effects on plasma lipid and lipoprotein concentrations. Specifically, phytoestrogen-containing soy reduced LDL + VLDL cholesterol concentrations by 30–40% in both males and females, increased HDLC concentrations by 15% for females and decreased TPC:HDLC ratios by 20% for males and 50% for females. It seems to us most likely that the beneficial effects of the Soy(+) diet are due to its phytoestrogens. However, we cannot exclude the possibility that the effect may be due in part to one or more of the other alcohol-extractable components of the soy isolate.

Among the alcohol-extractable substances, the saponins and phytosterols are the only ones for which there is a rational basis for a potential effect on plasma lipid and lipoprotein concentrations. We are influenced in our assumption that the effect is largely due to phytoestrogens for three primary reasons. First, in another ongoing study with ovariectomized monkeys we have seen both the lipoprotein effects and effects on bone biomarkers (unpublished data). Effects on bone biomarkers would not be expected from saponins. Second, we are influenced by the apparent lack of an effect of soy saponins on plasma cholesterol concentrations in the presence of soy protein (Calvert et

TABLE 4

Effects of diets containing soybean protein isolates with isoflavones intact (+) or ethanol-extracted (−) on hormone concentrations and reproductive system organ weights in peripubertal rhesus monkeys[1]

	Soy (−)	Soy (+)	*P* value[2]
Free thyroxine, *pmol/L*			
Males	8.75 ± 1.35	8.37 ± 1.13	0.79
Females	6.56 ± 0.82	7.46 ± 0.86	0.29
Sex hormone-binding globulin, *nmol/L*			
Males	65.9 ± 4.72	73.1 ± 3.90	0.12
Females	79.7 ± 3.97	76.2 ± 3.97	0.33
Dehydroepiandrosterone sulfate, *μmol/L*			
Males	0.97 ± 0.16	0.91 ± 0.15	0.61
Females	1.32 ± 0.19	1.28 ± 0.18	0.87
Estradiol, *pmol/L*	45.9 ± 9.25	36.3 ± 6.86	0.42
Testosterone, *nmol/L*	2.84 ± 1.17	1.04 ± 0.27	0.13
Reproductive system organ weights, *g*[3]			
Prostate weight	1.78 ± 0.110	1.63 ± 0.100	0.37
Average testicle weight	9.80 ± 2.048	6.73 ± 1.865	0.31
Uterus weight	1.07 ± 0.087	1.12 ± 0.087	0.72

[1] Values are means ± SEM. For males, $n = 11$; for females, $n = 14$.

[2] Analysis by paired *t* test for free thyroxine, sex hormone-binding globulin, dehydroepiandrosterone sulfate, estradiol, and testosterone. Analysis by analysis of covariance for reproductive system organ weights, covariates = age and body weight.

[3] Means adjusted for age and body weight as covariates in the ANCOVA model.

TABLE 5

Effects of diets containing soybean protein isolates with isoflavones intact (+) or ethanol-extracted (−) on blood pressure measurements in peripubertal rhesus monkeys[1]

	Soy (−)	Soy (+)	*P* value[2]
Systolic blood pressure, *mm Hg*			
Males	107 ± 3	103 ± 2	0.40
Females	99 ± 3	97 ± 2	0.51
Diastolic blood pressure, *mm Hg*			
Males	56 ± 2	55 ± 2	0.69
Females	54 ± 5	52 ± 2	0.69

[1] Values are means ± SEM. For males, $n = 11$; for females, $n = 14$.
[2] Analysis by paired *t* test.

al. 1981, Pathirana et al. 1981, Potter et al. 1993, Topping et al. 1980). Potter and colleagues (1993) found that soy proteins interact with the saponins to form insoluble complexes, and thus it is unlikely that saponins are responsible for the hypocholesterolemic actions of soy protein. Finally, we analyzed the soy protein isolates for β-sitosterol, as an indicator of the plant sterols that might be present, and there was none detectable in either the extracted or unextracted soy protein isolates, probably because the soy isolate has been processed rather extensively before the alcohol extraction.

The lack of an HDLC effect in males may be related to age and sexual maturation. All the monkeys were prepubertal at the start of the study, and some became sexually mature during the study. Changes in endogenous hormone concentrations may have affected the responsiveness of the individuals to phytoestrogens. Although the monkeys were randomized prestratifying for age in each gender, maturation rates do vary; thus, it is possible that imbalances developed between groups in sexual maturity and endogenous hormones as the trial progressed. With such small group sizes, such differences may have affected the comparisons and made actual effects difficult to detect.

Although the increase in plasma triglyceride concentrations with Soy(+) in the males is small, this finding is consistent with another ongoing trial in prepubertal cynomolgus monkeys (unpublished data). That the effect is more consistent in males than females may again be related to differences in endogenous hormones between the sexes.

LDL molecular weight is generally one of the best predictors of coronary artery atherosclerosis extent in macaques, with reduced coronary artery atherosclerosis associated with lower LDL molecular weight (Clarkson et al. 1990). In the current study we found significantly lower LDL molecular weight with Soy(+) treatment in the females. A difference of this magnitude in LDL molecular weight was associated with a 75% lower coronary artery lesion extent in another study with premenopausal females (Clarkson et al. 1990). Because of the crossover study design, we were unable to compare treatment effects on coronary artery atherosclerosis extent in the present study.

Higher Lp(a) concentrations have been associated with increased coronary heart disease (Dahlen et al. 1986). Concentrations appear to be genetically determined and generally unresponsive to environmental factors (e.g., lipid-lowering drugs, diet), but hormone replacement therapy has been shown to reduce Lp(a) concentrations (Soma et al. 1993). It is of interest that Lp(a) concentrations were reduced with Soy(+) treatment in females, again suggesting a beneficial effect.

Our results show that ethanol-soluble compounds, most likely phytoestrogens in soy, account for a substantial portion of the lipid-lowering effect of soy protein isolates. Numerous other components of soy protein have been investigated for their hypocholesterolemic properties (e.g., amino acid composition, lysine:arginine ratios), but only a small portion of soy's effect can be explained by these components. Although the study design does not permit us to evaluate any LDL-lowering effects of soy attributable to properties other than its alcohol-extractable components, LDLC + VLDLC concentrations were 30–40% lower when the Soy (+) diet was fed compared with the Soy(−) diet.

Soybean estrogens did not affect uterine, testicular or prostatic weights in this study. Although this study used a crossover design, the duration of treatment in the second period should have been long enough to eliminate any effects of the first treatment period. Given that the time course for response of these organs to hormones is on the order of weeks, the lack of an effect likely reflects only the most recent dietary exposure. Our data support an interpretation that soybean estrogens have tissue specificity in part because of their mixed estrogen agonist and antagonist properties.

The beneficial effects on lipid and lipoproteins of the unextracted soy protein isolate containing phytoestrogens would suggest protection against development of coronary artery atherosclerosis. However, further studies must be done to evaluate effects of soy protein containing phytoestrogenic isoflavones on coronary heart disease. Additional studies also must be done to determine whether genistein is the active component and to elucidate mechanisms of action, because there may be multiple mechanisms by which soy protein protects against coronary heart disease (Raines and Ross 1995, Wilcox and Blumenthal 1995). Studies should also evaluate the effects of the soybean estrogens on the reproductive system and endocrinologic measures throughout the life cycle, because there may be different effects of these compounds, de-

pending on the hormonal milieu (Clarkson et al. 1995). Cross-cultural and some experimental evidence suggests soy has beneficial effects on risk of chronic disease (Adlercreutz 1990). Thus, there may be great potential for improving human health with isoflavone-containing soy foods.

ACKNOWLEDGMENT

Protein Technologies (St. Louis, MO) provided the soy proteins used in this study.

LITERATURE CITED

Adlercreutz, H. (1990) Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scand. J. Clin. Lab. Invest. 50 (suppl. 201): 3–23.

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. & Fu, P. C. (1974) Enzymatic determination of total serum cholesterol. Clin. Chem. 20: 470–475.

Anderson, J. J., Ambrose, W. W. & Garner, S. C. (1995) Orally dosed genistein from soy and prevention of cancellous bone loss in two ovariectomized rat models. J. Nutr. 125: 799S (abs.).

Anthony, M. S., Clarkson, T. B. & Hughes, C. L. (1994) Plant and mammalian estrogen effects on plasma lipids of female monkeys. Circulation 90:I-235 (abs.).

Balogun, E. A., Balogun, O. O. & Odutuga, A. A. (1982) Arginine: lysine ratio as a contributory factor to the hypocholesterolemic effect of plant protein sources. International Research Communications System J. Med. Sci. 10: 643–644.

Balmir, F., Staack, R. F., Jeffery, E. H., & Potter, S. M. (1995) Soybean isoflavones influence serum lipid levels in rats. J. Nutr. 125: 805S (abs.).

Bradbury, R. B. & White, D.C. (1954) Oestrogens and related substances in plants. Vitamin Horm. 12: 207–233.

Calvert, G. D., Blight, L., Illman, R. J., Topping, D. L. & Potter, J. D. (1981) A trial of the effects of soya-bean flour and soybean saponins on plasma lipids, faecal bile acids and neutral sterols in hypercholesterolaemic men. Br. J. Nutr. 45: 277–281.

Clarkson, T. B., Anthony, M. S. & Hughes, C. L., Jr. (1995) Estrogenic soybean isoflavones and chronic disease: risks and benefits. Trends Endocrinol. Metab. 6: 11–16.

Clarkson, T. B., Shively, C. A., Morgan, T. M., Koritnik, D. R., Adams, M. R. & Kaplan, J. R. (1990) Oral contraceptives and coronary artery atherosclerosis of cynomolgus monkeys. Obstet. Gynecol. 75: 217–222.

Corbett, W. T., Schey, H. M., Lehner, N. D. M. & Greene, A. W. (1981) Standardized method for recording blood pressure in anaesthetized *Macaca fascicularis*. Lab. Anim. 15: 37–40.

Cupp, C. J. & Uemura, E. (1981) Body and organ weights in relation to age and sex in *Macaca mulatta*. J. Med. Primatol. 10: 110–123.

Dahlen, G. H., Guyton, J. R., Attar, M., Farmer, J. A., Kautz, J. A. & Gotto, A. M. (1986) Association of levels of lipoprotein Lp(a), plasma lipids, and other lipoproteins with coronary artery disease documented by angiography. Circulation 74: 758–765.

Eldridge, A. C. (1982) Determination of isoflavones in soybean flours, protein concentrates, and isolates. J. Agr. Food Chem. 30: 353–355.

Farnsworth, N. R., Bingel, A. S., Cordell, G. A., Crane, F. A. & Fong, H. H. S. (1975) Potential value of plants as sources of new antifertility agents II. J. Pharm. Sci. 64: 717–754.

Forsythe, W. A., Green, M. S. & Anderson, J. J. B. (1986) Dietary protein effects on cholesterol and lipoprotein concentrations: a review. J. Am. Coll. Nutr. 5: 533–549.

Fossati, P. & Principe, L. (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28: 2077–2080.

Hills, M. & Armitage, P. (1979) The two-period cross-over clinical trial. Br. J. Clin. Pharmacol. 8: 7–20.

Huff, M. W. & Carroll, K. K. (1980) Effects of dietary proteins and amino acid mixtures of plasma cholesterol levels in rabbits. J. Nutr. 110: 1676–1685.

Huff, M. W., Hamilton, R. M. G. & Carroll, K. K. (1977) Plasma cholesterol levels in rabbits fed low fat, cholesterol-free, semipurified diets: effects of dietary proteins, protein hydrolysates and amino acid mixtures. Atherosclerosis 28: 187–195.

Koritnik, D. R. & Marschke, K. B. (1986) Sex steroid hormone binding globulin levels and free 17β-estradiol and testosterone in cynomolgus monkeys during different reproductive states. J. Steroid Biochem. 25: 135–141.

Koritnik, D. R. & Rudel, L. L. (1983) Measurement of apolipoprotein A-I concentration in nonhuman primate serum by enzyme-linked immunosorbent assay (ELISA). J. Lipid Res. 24: 1639–1645.

Kritchevsky, D. (1979) Vegetable protein and atherosclerosis. J. Am. Oil Chem. Soc. 56: 130–140.

Kritchevsky, D., Tepper, S. A., Czarnecki, S. & Klurfeld, D. M. (1982) Atherogenicity of animal and vegetable protein: Influence of the lysine to arginine ratio. Atherosclerosis 47: 429–431.

Manual of Laboratory Operations: Lipid Research Clinics Program, Vol. 1. Lipid and Lipoprotein Analysis (1974) National Heart, Lung and Blood Institute. DHEW Publ. No. (NIH)1974:75–268. National Institutes of Health, Bethesda, MD.

Markiewicz, L., Garey, J., Adlercreutz, H. & Gurpide, E. (1993) In vitro bioassays of non-steroidal phytoestrogens. J. Steroid Biochem. Mol. Biol. 45: 399–405.

Meeker, D. R. & Kesten, H. D. (1941) Effect of high protein diets on experimental atherosclerosis of rabbits. Arch. Pathol. 31: 147–162.

Nagata, Y., Ishiwaki, N. & Sugano, M. (1982) Studies on the mechanism of antihypercholesterolemic action of soy protein and soy protein-type amino acid mixtures in relation to the casein counterparts in rats. J. Nutr. 112: 1614–1625.

Pathirana, C., Gibney, M. J. & Taylor, T. G. (1981) The effect of dietary protein source and saponins on serum lipids and the excretion of bile acids and neutral sterols in rabbits. Br. J. Nutr. 46: 421–430.

Potter, S. M. (1995) Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J. Nutr. 125: 606S–611S.

Potter, S. M., Jimenez-Flores, R., Pollack, J., Lone, T. A. & Berber-Jiminez, M. D. (1993) Protein-saponin interaction and its influence on blood lipids. J. Agric. Food Chem. 41: 1287–1291.

Raines, E. W. & Ross, R. (1995) Biology of atherosclerotic plaque formation: possible role of growth factors in lesion development and the potential impact of soy. J. Nutr. 125: 624S–630S.

Rudel, L. L., Lee, J. A., Morris, M. D. & Felts, J. M. (1974) Characterization of plasma lipoproteins separated and purified by agarose column chromatography. Biochem. J. 139: 89–95.

Rudel, L. L., Marzetta, C. & Johnson, F. L. (1986) Separation and analysis of lipoproteins by gel filtration. Methods Enzymol. 129: 45–57.

Setchell, K. D. R. (1985) Naturally occurring non-steroidal estrogens of dietary origin, and discussion. In: Estrogens in the Environment. II. Influences on Development (McLachlan, J.A., ed.), pp. 69–85. Elsevier, New York, NY.

Soma, M. R., Osnago-Gadda, I., Paoletti, R., Fumagalli, R., Morrisett, J. D., Meschia, M. & Crosignani, P. (1993) The lowering of lipoprotein[a] induced by estrogen plus progesterone replacement therapy in postmenopausal women. Arch. Intern. Med. 153: 1462–1468.

Sorci-Thomas, M., Wilson, M. D., Johnson, F. L., Williams, D. L. & Rudel, L. L. (1989) Studies on the expression of genes encoding apolipoproteins B100 and B48 and the low density lipoprotein receptor in nonhuman primates. J. Biol. Chem. 264: 9039–9045.

Stampfer, M. J. & Colditz, G. A. (1991) Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. Prevent. Med. 20: 47–63.

Topping, D. L., Storer, G. B., Calvert, G. D., Illman, R. J., Oakenfull, D. G., Weller, R. A. (1980) Effects of dietary saponins on fecal bile acids and neutral sterols, plasma lipids, and lipoprotein turnover in the pig. Am. J. Clin. Nutr. 33: 783–786.

Wagner, J. D., Adams, M. R., Schwenke, D. C. & Clarkson, T. B. (1993) Oral contraceptive treatment decreases arterial low density lipoprotein degradation in female cynomolgus monkeys. Circ. Res. 72: 1300–1307.

Wilcox, J. N. & Blumenthal, B. F. (1995) Thrombotic mechanisms in atherosclerosis: potential impact of soy proteins. J. Nutr. 125: 631S–638S.