See corresponding editorial on page 927.

Effect of soy protein from differently processed products on cardiovascular disease risk factors and vascular endothelial function in hypercholesterolemic subjects[1–4]

Nirupa R Matthan, Susan M Jalbert, Lynne M Ausman, Jeffrey T Kuvin, Richard H Karas, and Alice H Lichtenstein

ABSTRACT

Background: The magnitude of the effect of soy protein on lipoprotein concentrations is variable. This discordance is likely attributable to the various forms of soy protein used and to unrecognized shifts in dietary fatty acid, cholesterol, and fiber.

Objective: The objective was to evaluate the effect of soybean processing as well as soy consumption relative to animal protein, independent of alterations in major dietary variables, on cardiovascular disease risk factors and vascular endothelial function.

Design: Twenty-eight hypercholesterolemic subjects (LDL cholesterol ≥3.36 mmol/L) aged >50 y consumed each of 4 diets for 6-wk periods according to a randomized crossover design. The diets [55% of energy as carbohydrate, 30% of energy as fat, and 15% of energy as protein—7.5% of energy as experimental protein (37.5 g/d)] were designed to contain products made from either whole soybeans, soyflour, or soymilk and were compared with a diet containing an equivalent amount of animal protein (meat, chicken, and dairy products). The cholesterol, fiber, and fatty acid profiles of the diets were equalized. All food and drink were provided, and body weight was maintained throughout the study.

Results: No significant differences in blood pressure, vascular endothelial function, or total cholesterol, VLDL-cholesterol, triacylglycerol, apolipoprotein B, or C-reactive protein concentrations were observed between the diets. Consumption of the soymilk diet resulted in a modest decrease (4%) in LDL-cholesterol concentrations compared with the animal-protein and soyflour diets ($P < 0.05$) and higher HDL-cholesterol (1%) and apolipoprotein A-I (2%) concentrations compared with the soybean and soyflour diets ($P < 0.05$).

Conclusions: The results suggest that the consumption of differently processed soy-based products and different types of protein (animal and soy) has little clinical effect on cardiovascular disease risk factors, including peripheral endothelial function, when other major dietary variables are held constant. *Am J Clin Nutr* 2007; 85:960–6.

KEY WORDS Soy protein, lipids, lipoproteins, apolipoproteins, cholesterol, endothelial function

INTRODUCTION

Considerable interest has been focused on the role of soy protein and isoflavones in improving plasma lipoprotein profiles and subsequent cardiovascular disease (CVD) risk. This interest has stemmed from epidemiologic observations (1–4), data from early metabolic studies (5–10), and the results of a meta-analysis (11) showing that diets enriched in soy protein significantly reduced plasma cholesterol concentrations by 3–30% when compared with diets containing animal protein. In 1999, on the basis of the evidence from the available literature, the Food and Drug Administration approved a food label health claim of a reduced risk of heart disease from the consumption of 4 daily servings of foods containing ≥6.25 g soy protein or a total daily intake of 25 g/d (12).

Results from subsequent studies of soy protein and CVD risk have not been completely supportive of the original work, showing either a small or no plasma cholesterol reduction in normo- or hypercholesterolemic subjects (13–28). Interestingly, an early meta-analysis (11) concluded that the reduction in plasma cholesterol observed was not dependent on the amount of soy protein (8–124 g/d), but was strongly correlated with an individual's initial plasma cholesterol concentration. It is difficult to reconcile the earlier more pronounced effect of soy protein on plasma lipid concentrations with the subsequent data. One possible explanation is that a putative component of the soybean rather than of soy protein itself may be involved in the hypocholesterolemic effect and was unaccounted for in earlier studies. One such component studied that initially showed favorable effects on CVD risk factors was soybean isoflavones (4, 29, 30). However, results from more recent work do not support this hypothesis (28, 31, 32).

There are a variety of ways to process soybeans, and a large number of products are made from the different components.

[1] From the Cardiovascular Nutrition Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA (NRM, SMJ, LMA, and AHL), and the Department of Medicine, Division of Cardiology, Tufts–New England Medical Center Hospitals, Tufts University School of Medicine, Boston, MA (JTK and RHK).

[2] Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the US Department of Agriculture.

[3] Supported by grant HL 58008 (to NRM, SJM, LMA, and AHL) from the National Institutes of Health and the US Department of Agriculture, under agreement no. 58-1950-4-401.

[4] Address reprint requests to NR Matthan, Cardiovascular Nutrition Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington Street, Boston, MA 02111. E-mail: nirupa.matthan@tufts.edu.

Received September 5, 2006.
Accepted for publication November 1, 2006.

TABLE 1
Baseline characteristics of the study participants[1]

Variables	Subjects (n = 26 F, 2 M)
Age (y)	65 ± 6
Weight (kg)	71 ± 12
BMI (kg/m^2)	27 ± 3
Serum lipid and lipoproteins (mmol/L)	
Total cholesterol	6.14 ± 0.54
VLDL cholesterol	0.65 ± 0.31
LDL cholesterol	3.99 ± 0.52
HDL cholesterol	1.50 ± 0.34
Triacylglycerol	1.42 ± 0.68

[1] All values are $\bar{x}$ ± SD. To convert values for cholesterol and triacylglycerol to mg/dL, multiply by 38.67 and 88.54, respectively.

Although alcohol extraction and acid precipitation reduce the isoflavone content of the soy protein, other processing methods can alter soybean fiber, fat, sugars, phytic acid, and saponin content (33, 34). Consequently, the marked discordance in the data may be related to dissimilar forms of soy protein used in different studies. The aim of the present investigation was to assess the effects of differently processed forms of soybeans (whole soybean and products made thereof), of products derived from soyflour (textured soy protein), and of products made from soymilk (tofu and yogurt) between one another and relative to animal protein, independent of potential additional changes in the fatty acid, cholesterol, and fiber contents of the diet on CVD risk factors, including lipoprotein, apolipoprotein, and inflammatory markers in moderately hypercholesterolemic subjects. We also monitored peripheral vascular endothelial function as a surrogate marker of atherosclerosis and an indicator of vascular health and reactivity.

SUBJECTS AND METHODS

Subjects

Subjects who were ≥50 y of age with LDL-cholesterol concentrations ≥3.36 mmol/L at the time of screening, free of chronic illness, and not taking medications known to affect lipid metabolism (lipid-lowering drugs, β-blockers, fish-oil capsules, *cis*-retinoic acid, ascorbic acid, vitamin E, diuretics, and hormones) were recruited for this study from the greater Boston area. Subjects reporting soy allergies, reporting the consumption of ≥2 alcoholic drinks/d, or who smoked were excluded from participation. All women were postmenopausal.

Twenty-one subjects initially recruited did not complete the study because of the following reasons: time constraints (n = 9), change in medical status (n = 3), noncompliance (n = 3), and gastrointestinal issues with diet foods (n = 6). With the exception of 2 subjects, all others terminated participation during phase 1 and were replaced. This high dropout rate was likely related to the long study duration (24-wk controlled diet period) and the relatively high level of daily soy protein (average intake: 37.5 g/d). Twenty-eight subjects (26 women and 2 men) completed the study and were included in the final statistical analysis. The characteristics of these study subjects (n = 28) at the time of screening are depicted in **Table 1**. The protocol was approved by the Human Investigation Review Committee of the Tufts University–New England Medical Center. All subjects gave written informed consents.

Experimental design

The subjects were provided with each of 4 diets for 6-wk periods, separated by 2- to 4-wk intervening periods, according to a randomized crossover design. All food and drink were prepared by our metabolic research unit (MRU) and were provided to the subjects who reported to the MRU a minimum of 3 times/wk, at which time they were weighed, had their blood pressure recorded, and consumed one meal on site. The remaining food was packaged for takeout. The subjects were not allowed to supplement their diet with additional food or drink, with the exception of noncaloric beverages. The Harris-Benedict equation was used to calculate initial energy requirements of the subjects, and adjustments were made when necessary to maintain body weight.

Diets

The animal-protein diet was designed to be consistent with current dietary recommendations for CVD prevention (35) and provided ≈55% of energy as carbohydrate, 15% of energy as protein, and 30% of energy as fat (7% of energy as saturated fat, 10–15% of energy as monounsaturated fat, and 10% of energy as polyunsaturated fat), 80 mg cholesterol/1000 kcal, and 12-15 g fiber/1000 kcal. For the soy protein–based diets, 50% of the protein was contributed by soy-based foods. This was accomplished by displacing animal protein–containing products (meats, chicken, egg, and dairy products) with products made with whole soybean, soyflour, or soymilk. The whole-soybean phase contained whole organic soybeans (Westbrae Natural, Hain Food Group Inc, Uniondale, NY), soynuts (Solnuts Inc, Hudson, IA), defatted soyflakes (Cargill Inc, Cedar Rapids, IA), soya granules (Fearn Natural Foods, Mequon, WI), and soynut butter (Health Trip Co., Concord, MA). The soyflour phase contained soy nutlettes (Dixie USA Inc, Tomball, TX), whole-grain soyflour (Arrowhead Mills Inc, Hereford, TX), and textured soy-protein products (Chicken-Not, Beef-Not, Turkey-Not; Dixie USA Inc, Houston, TX). The soymilk phase contained tofu (Nasoya-Vitasoy USA Inc, San Francisco, CA), plain soy yogurt (Silk-Whitewave Food Co, Broomfield, CO), and plain soymilk (Silk-Whitewave Food Co). The nutrient profiles of the soy-based diets were balanced for carbohydrate, protein, fatty acid profile, and dietary cholesterol through the addition of butter, cream, and egg yolks so that the levels were equivalent to the animal protein–based diet. The soy products used to formulate the menus were chosen only from those commercially available in local grocery stores, supermarkets, and health food stores and had soy as the main ingredient. Provided in **Table 2** are the macronutrient, fatty acid, cholesterol, fiber, oxalate, folate, and phytate contents (Covance Laboratories, Madison, WI); total isoflavone content, as aglycones (University of Iowa, Ames, IA); and total plant sterol concentrations (Cardiovascular Nutrition Laboratory, Tufts University, Boston, MA) of the diets. A 3-d rotating menu cycle was used during each phase, a sample of which is provided in **Appendix A**.

Biochemical analyses

Three times during the sixth week of each of the 4 diet periods, blood samples were collected after a 14-h fast into EDTA-containing tubes. Plasma was separated by centrifugation at

TABLE 2
Composition of the 4 experimental diets[1]

Variables	Animal protein	Soybean	Soyflour	Soymilk
	% of energy			
Carbohydrate	54.5	57.0	58.0	55.8
Protein	16.9	14.9	14.9	15.8
Soy protein	—	7.5	6.8	7.3
Arginine:lysine	0.8	1.1	1.2	1.1
Total fat[2]	28.4	28.0	27.2	28.6
SFA	7.0	7.3	6.7	6.9
12:0	0.4	0.5	0.4	0.4
14:0	0.6	0.7	0.6	0.6
16:0	3.9	4.1	3.6	3.8
18:0	1.7	1.5	1.4	1.5
MUFA	10.3	9.5	9.9	9.7
16:1	0.2	0.1	0.2	0.1
18:1	9.1	9.1	9.7	9.6
PUFA	9.9	10.0	9.2	10.6
18:2n−6	7.9	8.9	8.0	9.2
18:3n−3	1.3	1.4	1.4	1.6
20:4n−6	0.1	0.1	<0.1	<0.1
Cholesterol (mg/1000 kcal)	83.3	77.3	76.5	76.0
Fiber (g/1000 kcal)	14.2	16.4	13.8	13.0
Isoflavones (mg/1000 kcal)	10.4	66.0	55.4	50.8
Daidzein	0.6	15.4	14.3	15.3
Genistein	9.8	47.9	36.2	32.9
Glycitein	0.0	2.8	4.8	2.5
Total plant sterols (mg/1000 kcal)[3]	150.8	151.6	170.7	173.3
Oxalates (g/1000 kcal)[3]	0.09	0.09	0.11	0.08
Folates (mg/1000 kcal)[3]	0.11	0.18	0.28	0.19
Phytates (%)[3]	<0.2	<0.2	<0.2	<0.2

[1] Mean of 2 values determined by chemical analysis; each value reflects the amount provided in a 3-d rotating menu per dietary phase.
[2] The difference between total fat (obtained by Soxhlet extraction) and sum of fatty acids (determined by gas chromatography) is attributable to the nonsaponifiable fraction of the lipid-soluble components.
[3] Single determination by chemical analysis.

1800 × *g* at 4 °C for 20 min. Total, HDL, and LDL cholesterol; triacylglycerol; and high-sensitivity C-reactive protein (CRP) concentrations were analyzed with a Hitachi 911 automated analyzer with the use of Roche Diagnostics reagents (Indianapolis, IN), and apolipoprotein (apo) A-I, and apo B were analyzed with Wako Diagnostics (Richmond, VA) reagents. VLDL cholesterol was calculated as the difference between total cholesterol and LDL plus HDL cholesterol. HDL and the subfractions were determined after selective precipitation with the use of a modified dextran sulfate–magnesium chloride method, and the cholesterol component was determined as previously described (22). Cholesterol assays were standardized through the Lipid Standardization Program of the Centers for Disease Control, Atlanta, GA.

Vascular endothelial function measurement

Peripheral vascular endothelial function was assessed with high-resolution ultrasound of the brachial artery during reactive hyperemia by standard methodology (36). Endothelial function measurements were performed at the end of each dietary phase by the same operator. Briefly, subjects were asked to lie down in a quiet, temperature-controlled, darkened room, and pulse rate and blood pressure were checked. Baseline measurements of the brachial artery in its longitudinal dimension were taken. Forearm ischemia was induced with a suprasystolic pressure cuff around the upper portion of the arm. After exactly 5 min of occlusion, reactive hyperemia ensued and the peak brachial artery diameter was measured between 45 and 75 s. Percentage flow-mediated dilation (FMD) was defined as the difference between the maximal brachial artery diameter during reactive hyperemia and the baseline brachial artery diameter divided by the baseline brachial artery diameter (%FMD = maximal brachial artery diameter − baseline brachial artery diameter/baseline brachial artery diameter).

Statistical analyses

The 3 values obtained per subject per variable were averaged, and this mean value was used in subsequent statistical analysis. Before the statistical testing was done, data were checked for normality, and appropriate transformations were performed when necessary (PROC UNIVARIATE; SAS version 9.1; SAS Institute Inc, Cary, NC). Variables log transformed included triacylglycerol, CRP, and total, VLDL-, HDL_2-, and HDL_3-cholesterol concentrations. An analysis of variance (PROC GLM) with main effect of diet and subject as repeated measures was carried out for each outcome measured, which was followed by a Tukey's honestly significant difference type of adjustment for the pairwise comparisons between each of the 4 treatment groups. Vascular endothelial function measurements were analyzed by using PROC GLM on rank-transformed data. Untransformed data are presented in the text and tables as means ± SD.

RESULTS

No significant differences in fasting plasma total, VLDL-cholesterol, triacylglycerol, apo B, or CRP concentrations were observed between the 4 diets (**Table 3**). LDL-cholesterol concentrations were lower after consumption of the soymilk diet than after consumption of the animal protein and soyflour diets ($P < 0.05$). HDL-cholesterol concentrations were higher after consumption of the animal-protein diet compared with the soyflour diet and higher after the soymilk diet compared with both the soybean and soyflour diets ($P < 0.05$ for all). These differences were attributable to changes in both the HDL_2 and HDL_3 subfractions. Apo A-I concentrations followed a pattern similar to that of HDL cholesterol, ie, no significant difference was observed between the animal-protein and soymilk diets, and concentrations were higher with the soymilk than with the soybean and soyflour diets ($P < 0.05$). The ratio of total cholesterol to HDL cholesterol was most favorable during the soymilk phase. No significant effect of the dietary perturbations in systolic or diastolic blood pressure or in peripheral endothelial function assessed by brachial artery FMD (Table 3) was observed. Stratifying and analyzing the data on the basis of LDL-cholesterol concentrations above (<3.80 mmol/L, $n = 15$) and below (≥3.80 mmol/L, $n = 13$) the median value and did not alter the conclusions (data not shown). Likewise, analysis of the data without the 2 male subjects did not change the conclusions.

The percentage differences in LDL-cholesterol concentrations relative to the animal protein diet were −1.8%, 1.5%, and −3.9% and in HDL-cholesterol concentrations were −4.3%, −2.9%, and 0.7% after consumption of the soybean, soyflour, and soymilk diets, respectively. However, only the changes seen with the soymilk diet were statistically significant (**Figure 1**). No

TABLE 3
Cardiovascular disease risk factors at the end of each dietary phase[1]

Variables	Animal protein	Soybean	Soyflour	Soymilk
Total cholesterol (mmol/L)	5.84 ± 0.66	5.76 ± 0.76	5.80 ± 0.65	5.74 ± 0.76
VLDL cholesterol (mmol/L)[2]	0.67 ± 0.31	0.73 ± 0.40	0.71 ± 0.37	0.73 ± 0.45
LDL cholesterol (mmol/L)	3.64 ± 0.57[a]	3.55 ± 0.60[a,b]	3.64 ± 0.52[a]	3.48 ± 0.59[b]
HDL cholesterol (mmol/L)	1.53 ± 0.32[a,b]	1.47 ± 0.36[b,c]	1.46 ± 0.31[c]	1.54 ± 0.31[a]
HDL_2 cholesterol (mmol/L)[2]	0.59 ± 0.23[a]	0.55 ± 0.24[a,b]	0.52 ± 0.20[b]	0.57 ± 0.21[a]
HDL_3 cholesterol (mmol/L)[2]	0.95 ± 0.12[a,b]	0.92 ± 0.15[b]	0.94 ± 0.15[a,b]	0.96 ± 0.14[a]
Triacylglycerol (mmol/L)[2]	1.47 ± 0.68	1.60 ± 0.87	1.54 ± 0.80	1.59 ± 0.98
Apolipoprotein B (mg/dL)	92.46 ± 11.83	90.78 ± 11.24	92.75 ± 10.08	90.06 ± 12.05
Apolipoprotein A-I (mg/dL)	123.22 ± 14.59[a,b]	120.74 ± 15.65[b]	120.35 ± 12.94[b]	124.94 ± 14.75[a]
Total:HDL cholesterol	3.96 ± 0.87[a,b]	4.10 ± 0.98[a]	4.13 ± 0.90[a]	3.89 ± 0.92[b]
CRP (mg/dL)[2]	2.88 ± 3.23	3.89 ± 5.19	2.91 ± 3.01	2.44 ± 2.13
Systolic BP (mm Hg)	119 ± 11	121 ± 12	120 ± 10	120 ± 12
Diastolic BP (mm Hg)	73 ± 6	74 ± 6	74 ± 6	74 ± 6
FMD (%)[3]	11.76 ± 8.20	9.89 ± 7.08	11.08 ± 6.28	13.57 ± 9.30

[1] All values are $\bar{x}$ ± SD. FMD, flow-mediated dilation; BP, blood pressure. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (PROC GLM and Tukey's honestly significant difference test).
[2] Log_{10} normalized for analysis.
[3] The analysis of FMD data was carried out by using PROC GLM on rank-transformed data.

significant differences were seen in total cholesterol, triacylglycerol, and apo B concentrations (Figure 1) or in VLDL-cholesterol and CRP concentrations (data not shown).

To compare the effects of animal versus soy protein per se, data from the soybean, soyflour, and soymilk groups were combined to form the average Soy group and compared with the animal-protein diet. No significant effect of soy protein relative to animal protein was observed in plasma total, LDL-, HDL-cholesterol, triacylglycerol or total/HDL-cholesterol concentrations (**Figure 2**) or in the other CVD risk factors measured (data not shown).

DISCUSSION

Recent data suggest that the magnitude of the effect of soy protein on lipoprotein profiles is variable and less dramatic than originally reported (27, 28). This discrepancy may be due to the presence or absence of specific components associated with the soy-protein preparations or unaccounted shifts in the fatty acid, cholesterol, or fiber content of the diets used in earlier studies. The novel aspect of the present study was to directly compare the effect of soy protein derived from differently processed products, between each other and relative to animal protein, while simultaneously controlling for the macronutrient, fatty acid, cholesterol, and fiber contents of the diet. The results showed that the consumption of differently processed soy products or type of protein (animal protein compared with soy protein), when provided at approximately 1.5 times (37.5 g/d) the minimum amount on which the Food and Drug Administration–approved health claim was predicated had little clinical effect on CVD risk factors and vascular endothelial function.

The results of this intervention are consistent with the findings of 2 recent comprehensive reviews on soy and measures of cardiovascular health (28, 32). Both reports concluded that there was a significant, albeit modest, effect of soy protein with isoflavones when consumed at relatively high levels compared with milk or other proteins on LDL-cholesterol (−3% to −5%) but not on HDL-cholesterol or triacylglycerol concentrations. The evidence also favored soy protein rather than soy isoflavones as the responsible nutrient. However, the authors of these reports could not rule out the possibility of other soybean components as the active factor. We addressed this issue by comparing the effect of differently processed forms of soybean. Although the major

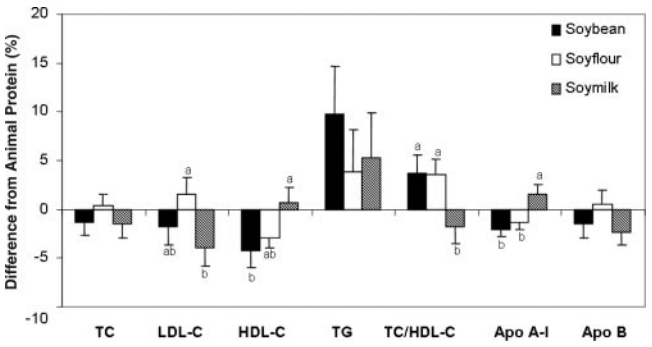

FIGURE 1. Mean (±SE) percentage difference in lipid, lipoprotein, and apolipoprotein profiles relative to the animal-protein diet. For each variable, bars with different lowercase letters are significantly different, $P < 0.05$ (PROC GLM and Tukey's honestly significant difference test). TC, total cholesterol; C, cholesterol; apo, apolipoprotein; TG, triacylglycerol.

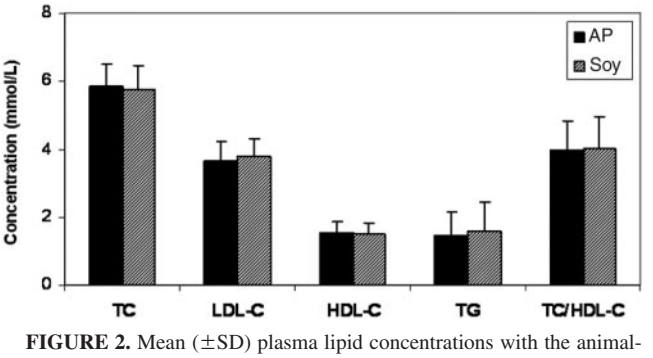

FIGURE 2. Mean (±SD) plasma lipid concentrations with the animal-protein (AP) and soy-protein (Soy) diets. The average response to the 3 Soy diets was compared with the response to the AP diet in a one-factor ANOVA at $P < 0.05$ (PROC GLM and Tukey's honestly significant difference test); no significant differences between groups were observed. TC, total cholesterol; C, cholesterol; TG, triacylglycerol.

dietary variables in our diets were equalized, isoflavone and trace components—such as plant sterols, folates, phytates, and oxalates—that could be altered by the processing method of the soy product and that have been shown or hypothesized to influence cholesterol concentrations were not equalized. Interestingly, processing appeared to have had little effect because the analysis of the final diets showed similar amounts of these components between diets (Table 2), which suggests that these components are not major contributing factors in the purported hypocholesterolemic effect of soy.

Our study also assessed the effect of soy consumption on other CVD risk factors: CRP concentrations, blood pressure, and vascular endothelial function. No significant differences were observed between diets. This is consistent with published studies evaluating the effect of soy consumption on CRP concentrations (18, 37, 38) and, with one exception (39), most studies on blood pressure (17, 18, 21, 24, 37). Similarly, most of the studies that assessed vascular endothelial function, either by increased brachial artery diameter (mm), FMD (%), flow (mL/min), or decreased peak flow velocity (cm/s), showed no effect or a slight improvement (18, 23, 40–43). Any beneficial effect was frequently attributed to the isoflavone component of the diet (23, 40, 41).

The reason for the more favorable plasma lipoprotein profile after consumption of the soymilk and not the soybean- and soyflour-enriched diets is unclear. Although no comparable studies were found in the literature, previous studies of soymilk have reported an average 5–8% reduction in plasma total cholesterol in normocholesterolemic subjects who consumed 350 mL soymilk/d (14.5 g soy protein) (43) and in hypercholesterolemic subjects who consumed 1 L soymilk/d (41.1 g soy protein) compared with cow milk (44). However, because of the lack of a control group fed an equivalent amount of protein (43), and dissimilar carbohydrate, unsaturated fat, fiber, and cholesterol contents of the diets (44), the hypocholesterolemic effect observed in these studies cannot be solely attributed to the soymilk supplementation.

It has been hypothesized that the specific amino acid profile of the soy protein may be the active agent found in soy (45). Data from animal experiments (46, 47) have shown that the amino acids lysine and methionine tended to be hypercholesterolemic, whereas arginine was hypocholesterolemic, which suggests that the higher arginine to lysine and methionine amino acid profile of soy protein might explain, at least in part, its hypocholesterolemic effects. In the present study, despite the use of a variety of soy products, the amino acid profiles of the 3 soy-based diets were similar (ratios of arginine to lysine of 0.8, 1.1, 1.2, and 1.1 for the animal-protein, soybean, soyflour, and soymilk diets, respectively). Whether the incorporation of even higher amounts of the soy-based products would have sufficiently altered the amino acid profile to elicit a greater hypocholesterolemic response than observed is unknown. More recent studies (48, 49) have shown that the 7S globulin protein present in soy can upregulate LDL receptors in vitro. The authors suggest that this mechanism might explain the discordance in results because the concentration of the 7S globulin differs among soy varieties and, consequently, in products prepared thereof. Insufficient data exist to draw conclusions regarding this postulated mechanism in the present study.

A limitation of the present work was the use of a high amount of protein (7.5% of total protein intake) from a single source of soy and its products, in contrast with the more likely real life situation in which multiple animal and vegetable protein sources are habitually consumed. However, this approach maximized our ability to detect any potential beneficial effect were one present. Another limitation is that we did not directly test the effect of substituting each category of soy products for animal protein–based products without controlling for the multiple shifts in associated dietary variables. To do that would not have allowed us to address our primary hypothesis, partitioning out the independent effect of the soy-protein source, per se, on CVD risk factors. For similar reasons we did not attempt to obtain isolated subfractions of individual soybean components to assess the effect of each on the outcome measures. A strength of this study was that soy protein from differently processed but readily available products, and not from one type of soy protein isolate or specialized preparation of soy protein, was assessed relative to each other and animal protein. Another strength of the study was that moderately hypercholesterolemic subjects served as study subjects, the population most likely to benefit from alterations in the diet to improve CVD risk factors.

In conclusion, relatively high intakes of differently processed forms of soy protein derived from commonly available soy products appear to have little effect on CVD risk factors, either between each other or when substituted for animal protein. Nonetheless, the use of foods containing soy protein to displace animal protein, which would result in reductions in saturated fat and cholesterol intakes, could be beneficial to overall CVD risk reduction.

We are indebted to the Metabolic Research Unit staff (the study volunteer recruiters, the nurses for their expert care of the study subjects, and the dietitians and kitchen staff for their meticulous planning and preparation of the study foods for each dietary phase), to Kathleen Sliney and Paula Heggericks for technical assistance with the vascular endothelial function measurements, and to the study subjects without whom this investigation would not have been possible.

AHL (principal investigator) and NRM (project coordinator) designed, implemented, and oversaw all aspects of this study. NRM wrote the initial draft of the manuscript. AHL, SMJ, LMA, JTK, and RHK contributed to critically reviewing the manuscript. SMJ was responsible for the biochemical analysis. JTK and RHK were responsible for supervising the vascular endothelial measurements. LMA was responsible for the statistical analysis. None of the authors had any conflicts of interest.

REFERENCES

1. Ho SC, Woo J, Leung SS, Sham AL, Lam TH, Janus ED. Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population. J Nutr 2000;130:2590–3.
2. Nagata C, Takatsuka N, Kurisu Y, Shimizu H. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. J Nutr 1998;128:209–13.
3. Keys A. Seven Countries: a multivariate analysis of death and coronary heart disease. Cambridge, MA: Harvard University Press, 1980.
4. Merz-Demlow BE, Duncan AM, Wangen KE, et al. Soy isoflavones improve plasma lipids in normocholesterolemic premenopausal women. Am J Clin Nutr 2000;71:1462–9.
5. Sirtori CR, Agradi E, Conti F, Mantero O, Gatti E. Soybean-protein diet in the treatment of type-II hyperlipoproteinaemia. Lancet 1977;1:275–7.
6. Carroll KK, Giovannetti PM, Huff MW, Moase O, Roberts DC, Wolfe BM. Hypocholesterolemic effect of substituting soybean protein for animal protein in the diet of healthy young women. Am J Clin Nutr 1978;31:1312–21.
7. Sirtori CR, Gatti E, Mantero O, et al. Clinical experience with the soybean protein diet in the treatment of hypercholesterolemia. Am J Clin Nutr 1979;32:1645–58.
8. Descovich GC, Ceredi C, Gaddi A, et al. Multicentre study of soybean

protein diet for outpatient hyper-cholesterolaemic patients. Lancet 1980; 2:709–12.
9. Shorey RL, Bazan B, Lo GS, Steinke FH. Determinants of hypocholesterolemic response to soy and animal protein-based diets. Am J Clin Nutr 1981;34:1769–78.
10. Goldberg AP, Lim A, Kolar JB, Grundhauser JJ, Steinke FH, Schonfeld G. Soybean protein independently lowers plasma cholesterol levels in primary hypercholesterolemia. Atherosclerosis 1982;43:355–68.
11. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333: 276–82.
12. Food and Drug Administration. Food labeling: health claims; soy protein and coronary heart disease; final Rule. Fed Reg 1999;64:57700–33.
13. Baum JA, Teng H, Erdman JW Jr, et al. Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. Am J Clin Nutr 1998;68:545–51.
14. Wong WW, Smith EO, Stuff JE, Hachey DL, Heird WC, Pownell HJ. Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. Am J Clin Nutr 1998;68 (suppl):1385S–9S.
15. Crouse JR III, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch Intern Med 1999;159:2070–6.
16. Teixeira SR, Potter SM, Weigel R, Hannum S, Erdman JW Jr, Hasler CM. Effects of feeding 4 levels of soy protein for 3 and 6 wk on blood lipids and apolipoproteins in moderately hypercholesterolemic men. Am J Clin Nutr 2000;71:1077–84.
17. Vigna GB, Pansini F, Bonaccorsi G, et al. Plasma lipoproteins in soy-treated postmenopausal women: a double-blind, placebo-controlled trial. Nutr Metab Cardiovasc Dis 2000;10:315–22.
18. Teede HJ, Dalais FS, Kotsopoulos D, Liang YL, Davis S, McGrath BP. Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. J Clin Endocrinol Metab 2001;86:3053–60.
19. Van Horn L, Liu K, Gerber J, et al. Oats and soy in lipid-lowering diets for women with hypercholesterolemia: is there synergy? J Am Diet Assoc 2001;101:1319–25.
20. Lichtenstein AH, Jalbert SM, Adlercreutz H, et al. Lipoprotein response to diets high in soy or animal protein with and without isoflavones in moderately hypercholesterolemic subjects. Arterioscler Thromb Vasc Biol 2002;22:1852–8.
21. Puska P, Korpelainen V, Hoie LH, Skovlund E, Lahti T, Smerud KT. Soy in hypercholesterolaemia: a double-blind, placebo-controlled trial. Eur J Clin Nutr 2002;56:352–7.
22. Tonstad S, Smerud K, Hoie L. A comparison of the effects of 2 doses of soy protein or casein on serum lipids, serum lipoproteins, and plasma total homocysteine in hypercholesterolemic subjects. Am J Clin Nutr 2002;76:78–84.
23. Steinberg FM, Guthrie NL, Villablanca AC, Kumar K, Murray MJ. Soy protein with isoflavones has favorable effects on endothelial function that are independent of lipid and antioxidant effects in healthy postmenopausal women. Am J Clin Nutr 2003;78:123–30.
24. Kreijkamp-Kaspers S, Kok L, Grobbee DE, et al. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. J Am Med Assoc 2004;292:65–74.
25. Engelman HM, Alekel DL, Hanson LN, Kanthasamy AG, Reddy MB. Blood lipid and oxidative stress responses to soy protein with isoflavones and phytic acid in postmenopausal women. Am J Clin Nutr 2005;81: 590–6.
26. Roughead ZK, Hunt JR, Johnson L, Badger TM, Lykken GI. Controlled substitution of soy protein for meat protein: effects on calcium retention, bone, and cardiovascular health indices in postmenopausal women. J Clin Endocrinol Metab 2005;90:181–9.
27. Dewell A, Hollenbeck PLW, Hollenbeck CB. Clinical review: a critical evaluation of the role of soy protein and isoflavone supplementation in the control of plasma cholesterol concentrations. J Endocrinol Metab 2006;91:772–80.
28. Sacks FM, Lichtenstein AH, Van Horn L, et al. Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for Professionals From the Nutrition Committee. Circulation 2006;113:1034–44.
29. Wangen KE, Duncan AM, Xu X, Kurzer MS. Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. Am J Clin Nutr 2001;73:225–31.
30. Gardner CD, Newell KA, Cherin R, Haskell W. The effect of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolemic postmenopausal women. Am J Clin Nutr 2001; 73:728–35.
31. Weggemans RM, Trautwein EA. Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: a meta-analysis. Eur J Clin Nutr 2003;57:940–56.
32. Balk E, Chung M, Chew P, et al. Effects of soy on health outcomes. Evid Rep Technol Assess (Summ) 2005;126:1–8.
33. Oakenfull D. Soy protein, saponins and plasma cholesterol. J Nutr 2001; 131:2971–2.
34. Potter SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J Nutr 1995;125(suppl):606S–11S.
35. National Cholesterol Education Program. Executive Summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285: 2486–97.
36. Kuvin JT, Patel AR, Sliney KA, et al. Peripheral vascular endothelial function testing as a noninvasive indicator of coronary artery disease. J Am Coll Cardiol 2001;38:1843–9.
37. Jenkins DJ, Kendall CW, Jackson CJ, et al. Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am J Clin Nutr. 2002;76:365–72.
38. Nikander E, Metsa-Heikkila M, Tiitinen A, Ylikorkala O. Evidence of a lack of effect of a phytoestrogen regimen on the levels of C-reactive protein, E-selectin, and nitrate in postmenopausal women. J Clin Endocrinol Metab 2003;88:5180–5.
39. Rivas M, Garay RP, Escanero JF, Cia PJ, Cia P, Alda JO. Soy milk lowers blood pressure in men and women with mild to moderate essential hypertension. J Nutr 2002;132:1900–2.
40. Cuevas AM, Irribarra VL, Castillo OA, Yanez MD, Germain AM. Isolated soy protein improves endothelial function in postmenopausal hypercholesterolemic women. Eur J Clin Nutr 2003;57:889–94.
41. Nestel PJ, Yamashita T, Sasahara T, et al. Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. Arterioscler Thromb Vasc Biol 1997;17:3392–8.
42. Simons LA, von Konigsmark M, Simons J, Celermajer DS. Phytoestrogens do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women. Am J Cardiol 2000;85:1297–301.
43. Takatsuka N, Nagata C, Kurisu Y, Inaba S, Kawakami N, Shimizu H. Hypocholesterolemic effect of soymilk supplementation with usual diet in premenopausal normolipidemic Japanese women. Prev Med 2000; 31:308–14.
44. Bricarello LP, Kasinski N, Bertolami MC, et al. Comparison between the effects of soy milk and non-fat cow milk on lipid profile and lipid peroxidation in patients with primary hypercholesterolemia. Nutrition 2004;20:200–4.
45. Erdman JW Jr. AHA Science Advisory: soy protein and cardiovascular disease: a statement for healthcare professionals from the Nutrition Committee of the AHA. Circulation 2000;102:2555–9.
46. Kurowska EM, Carroll KK. Hypercholesterolemic responses in rabbits to selected groups of dietary essential amino acids. J Nutr 1994;124: 364–70.
47. Tasker T, Potter SM. Influence of dietary proteins and amino acid variation on plasma lipids, HMG-CoA reductase activity, and reduced glutathione concentrations in inbred versus outbred gerbils. J Nutr Biochem 1993;4:458–62.
48. Duranti M, Lovati MR, Dani V, et al. The alpha' subunit from soybean 7S globulin lowers plasma lipids and upregulates liver beta-VLDL receptors in rats fed a hypercholesterolemic diet. J Nutr 2004;134:1334–9.
49. Lovati MR, Manzoni C, Gianazza E, et al. Soy protein peptides regulate cholesterol homeostasis in Hep G2 cells. J Nutr 2000;130:2543–9.

The American Journal of Clinical Nutrition

APPENDIX A
Protocol menu for the 4 diets[1]

Dietary phase and meal	Cycle 1	Cycle 2	Cycle 3
Animal protein			
Breakfast	Cranberry juice	English muffin, jelly	Bran muffin
	Skim milk	Butter	Butter
	Oatmeal, raisins	Skim milk	Strawberry, banana shake
	Fruit mix	Cereal (Total, All Bran)	
Lunch	Lettuce, fat-free Italian dressing	Chicken fried rice	Tomato soup
	Wheat bread	Carrots	Wheat bread
	Tuna salad	Pear cobbler, nuts	Grilled turkey breast
	High-fiber cookies		Fat-free yogurt, plain; canned peach
Dinner	Turkey rice casserole	Cube salad (cucumber, tomato)	Beef tenderloin
	Carrots	Pasta with tomato sauce	Roasted potato
	Chocolate pudding	Parmesan cheese	Green beans
	Apple juice	Applesauce	Strawberries, frozen
	Fresh orange	Skim milk	Apple juice
Soybean			
Breakfast	Orange juice	Fresh grapefruit	Soy granule muffins
	Skim milk	White bread with butter	Butter
	Oatmeal, raisins	Skim milk	Strawberry banana shake
	Fruit mix	Toasted soy flake, cornflakes, raisins	
Lunch	Lettuce, fat-free Italian dressing	Soybean fried rice	Soybean chili
	White bread	Pear cobbler, soynuts	Pita bread
	Tuna salad	Roasted soybeans	Canned pears
	Baked soybeans		
	Soynut fudge balls		
Dinner	Soybean rice casserole	Cube salad (cucumber, tomato, soybean)	Soybean loaf
	Chocolate pudding	Pasta with tomato sauce	Green beans
	Apple juice	Parmesan cheese	Applesauce
	Fresh orange		Cranberry juice
Soyflour			
Breakfast	Orange juice	Fresh grapefruit	SoyFlour Nutlette muffins
	Skim milk	Skim milk	Butter
	Oatmeal, raisins	Cereal mix (Total, SoyFlour Nutlettes)	Strawberry, banana shake
	Fruit mix		
Lunch	Lettuce, raw carrots	"Chicken Not" fried rice	"Beef Not" stew
	Fat-free Italian dressing	Pear cobbler	White bread
	Pita bread "Chicken Not" salad		Canned pear
	Soyflour cookies		
Dinner	"Turkey Not" rice casserole	Cube salad (cucumber, tomato, corn)	Sweet and Sour "Chicken Not"
	Chocolate pudding	Pasta with meat sauce	White rice
	Fresh orange	Parmesan cheese	Soyflour cookie
		Applesauce	
Soymilk			
Breakfast	Cranberry juice	Strawberry tofu smoothie, soymilk	Tofu blueberry muffin, butter
	Soymilk	Cereal (Total, All Bran)	Soy yogurt, plain
	Oatmeal, raisins		Canned peach (heavy syrup)
	Fruit mix		All Bran cereal topping
Lunch	Lettuce, fat-free Italian dressing	Tofu, chicken fried rice	Tofu tomato soup
	Wheat bread	Pear cobbler	Wheat bread
	Tofu, egg salad		Grilled turkey breast
	High-fiber cookies		Mayonnaise
Dinner	Tofu, turkey rice casserole	Pasta	Sweet and sour tofu
	Soymilk chocolate pudding	Tofu tomato sauce	Brown rice
	Apple juice	Parmesan cheese	Tofu bread pudding
	Fresh orange	Tofu, broccoli, baked potato	

[1] Chicken Not, Beef Not, and Turkey Not (Dixie USA Inc, Houston, TX); Total cereal (General Mills, Minneapolis, MN); All Bran cereal (Kellogg's, Battle Creek, MI); SoyFlour Nutlettes (Dixie USA Inc, Tomball, TX).

Erratum

Popkin BM, Armstrong LE, Bray GM, Caballero B, Frei B, Willett WC. A new proposed guidance system for beverage consumption in the United States. Am J Clin Nutr 2006;83:529–42.

Page 533, left-hand column, fourth full paragraph: the sentence "Fortified soymilk is a good alternative for individuals who prefer not to consume cow milk, although consumers should be aware that soymilk cannot be legally fortified with vitamin D…" is erroneous. Currently, soy milk is legally fortified with vitamin D.

Erratum

Matthan NR, Jalbert SM, Ausman LM, Kuvin JT, Karas RH, Lichtenstein AH. Effect of soy protein from differently processed products on cardiovascular disease risk factors and vascular endothelial function in hypercholesterolemic subjects. Am J Clin Nutr 2007;85:960–6.

Page 961, right-hand column, next-to-last sentence in the second full paragraph: the total isoflavone content, as aglycones, was determined by Iowa State University (Ames, IA)—not its sister institution, the University of Iowa.