

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

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Context: Isolated soy protein reduces plasma concentrations of total and low-density lipoprotein (LDL) cholesterol.

Objective: To identify the agent(s) responsible for the cholesterol-lowering effect of soy in mildly hypercholesterolemic volunteers: isoflavones isolated together with soy protein or soy protein itself.

Design: Double-blind randomized parallel trial.

Setting: Single-center study.

Participants: A total of 156 healthy men and women with LDL cholesterol levels between 3.62 mmol/L (140 mg/dL) and 5.17 mmol/L (200 mg/dL) after instruction in a National Cholesterol Education Program Step I diet and recruited by advertisement from the community.

Intervention: One of 5 daily diets (25 g of casein [for isoflavone-free comparison] or 25 g of isolated soy protein containing 3, 27, 37, or 62 mg of isoflavones).

Main Outcome Measures: Change and percent change from baseline in plasma concentrations of triglycerides and total, LDL, and high-density lipoprotein cholesterol after 9 weeks.

Results: Compared with casein, isolated soy protein with 62 mg of isoflavones lowered total and LDL cholesterol levels by 4% ($P = .04$) and 6% ($P = .01$), respectively. In patients with LDL cholesterol levels in the top half of the population studied (>4.24 mmol/L [>164 mg/dL]), comparable reductions were 9% ($P < .001$) and 10% ($P = .001$), respectively; in this group, isolated soy protein with 37 mg of isoflavones reduced total ($P = .007$) and LDL ($P = .02$) cholesterol levels by 8%, and there was a dose-response effect of increasing amounts of isoflavones on total and LDL cholesterol levels. Plasma concentrations of triglycerides and high-density lipoprotein cholesterol were unaffected. Ethanol-extracted isolated soy protein containing 3 mg of isoflavones did not significantly reduce plasma concentrations of total or LDL cholesterol.

Conclusions: Naturally occurring isoflavones isolated with soy protein reduce the plasma concentrations of total and LDL cholesterol without affecting concentrations of triglycerides or high-density lipoprotein cholesterol in mildly hypercholesterolemic volunteers consuming a National Cholesterol Education Program Step I diet. Ethanol-extracted isolated soy protein did not significantly reduce plasma concentrations of total or LDL cholesterol.

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CARDIOVASCULAR morbidity and mortality rates are lower in inhabitants of the Pacific Rim than in Western countries,¹⁻³ and some of the reduced risk is likely attributable to differences in diet. One of the more dramatic dietary differences is the high consumption of soybean products in the Pacific Rim compared with Western countries, which has been postulated to decrease risk of coronary heart disease.⁴⁻⁶ There is good rationale for this: soy is not only an excellent source of protein,⁷ but also contains high concentrations of isoflavones^{8,9} (micronutrient substances shown in nonhuman primates to have many of the properties of mammalian estrogen, including the

lowering of cholesterol levels¹⁰), reducing the risk of atherosclerosis,¹¹ and improving vascular function.¹²

A recent meta-analysis of 31 studies of the effects of soy protein on blood cholesterol levels in humans showed significant reduction in plasma concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides,¹³ as well as a small but nonsignificant increase in plasma concentrations of high-density lipoprotein (HDL) cholesterol. The authors of this meta-analysis identified 2 important aspects of the studies they analyzed. First, participants in the various studies had a wide range of baseline cholesterol levels. In fact, many of the studies were carried out with partici-

PARTICIPANTS AND METHODS

We conducted a 9-week randomized trial in 156 moderately hypercholesterolemic men and women to compare 25 g/d of protein from casein with 25 g/d of isolated soy protein for their effects on plasma concentrations of lipids and lipoproteins. The isolated soy protein contained isoflavones at 3 different levels (27 mg, 37 mg, and 62 mg) or consisted of ethanol-extracted isolated soy protein containing 3 mg of isoflavones. All isoflavone quantities are expressed as their aglycone equivalents. Thus, there were 5 groups of participants and 31 individuals per group.

This trial randomized men and women aged 20 to 70 years with LDL cholesterol levels between 3.62 mmol/L (140 mg/dL) and 5.17 mmol/L (200 mg/dL) after instruction in an NCEP Step I low-fat, low-cholesterol diet consisting of 30% of energy as fat (polyunsaturated-monounsaturated-saturated fat ratio, 1:1:1) and 300 mg of cholesterol daily.²¹ Exclusion criteria included current use of lipid-altering medications, history of cardiovascular disease, current history of diabetes or blood glucose levels higher than 6.94 mmol/L (125 mg/dL), other (uncorrected) secondary causes of hypercholesterolemia, excessive alcohol consumption, triglyceride concentrations greater than 4.52 mmol/L (>400 mg/dL) LDL, cholesterol levels higher than 5.17 mmol/L (>200 mg/dL), use of oral contraceptives or estrogen replacement, and usual consumption of a high soy protein diet.

This project was approved by the Clinical Research Practices Committee of the Wake Forest University School of Medicine. Participants who provided informed consent were screened and observed in the General Clinical Research Center of the Wake Forest University School of Medicine. They underwent 5 prerandomization visits. Following initial screening, eligible participants all completed a 7-day food record that was used to guide individualized group instruction in an NCEP Step I diet.²¹ Following 1 month on this diet, a

qualifying lipid profile was obtained. Those eligible on the basis of this qualifying lipid profile (LDL cholesterol level between 3.62 mmol/L [140 mg/dL] and 5.17 mmol/L [200 mg/dL]) were provided with sufficient servings of casein beverage for 1 month prior to randomization and instructed to substitute the casein beverage for dietary protein. Eligibility was contingent on 80% compliance with this 25-g casein beverage (casein run-in). Thus, all participants were consuming a beverage containing 25 g of casein protein at the time of randomization. In the final 2 weeks of this run-in period, replicate baseline lipid profiles were obtained on each eligible participant after an overnight fast. Eligible participants all completed a modified food frequency questionnaire at baseline (the semiquantitative food frequency component of the National Cancer Institute Health History and Habits Questionnaire [HHHQ])^{22,23} expanded to include tofu, soy milk, and tofu mixed dishes). Eligibility history and physical examination and blood tests (including thyrotropin levels) were obtained the week prior to randomization.

Individuals were randomized into 1 of the 5 groups identified above using stratified variable block size randomization stratifying by sex and smoking status. Randomization lists were prepared using SAS PROC PLAN (SAS Language, version 6, 1990; Cary, NC) and participants were randomized using a computer program that entered and verified eligibility of participants prior to providing a blinded group code known only to the study statistician. Thereafter, individuals were seen at weeks 4, 8, and 9 for clinical follow-up; adherence was checked by counting returned containers of beverage and evaluation of plasma concentrations of lipids and lipoproteins. We also quantified lipoprotein(a) [Lp(a)] at baseline and study end in a subset of participants (see below). Participants also completed the HHHQ at the end of the study, the results of which were analyzed by the associated software package (HHHQ-DIETSYS Analysis Software, version 3.0, 1993; National Cancer Institute, Bethesda, Md).

Continued on next page

pants with familial hypercholesterolemia.¹⁴⁻²⁰ Second, and perhaps of greater importance, the content of isoflavones varied in the particular soy products administered in these studies. The present protocol was designed to quantify the effects of isolated soy protein containing defined levels of isoflavones on plasma concentrations of lipids and lipoproteins to determine the relative importance of soy protein vis-à-vis the constituent isoflavones for reducing levels of cholesterol in a population selected for only moderately elevated cholesterol levels after counseling on a National Cholesterol Education Program (NCEP) Step I diet.

RESULTS

Over 40 000 letters were mailed to residents of Forsyth County, North Carolina, and 1268 individuals were evaluated for eligibility for the trial in the outpatient clinic of the Wake Forest University School of Medicine General Clinical Research Center. About 60% were ineligible on the basis of initial screening and plasma lipid and lipoprotein concentrations outside the eligibility window. Approximately 30% of the otherwise eligible individuals re-

sponded to counseling in an NCEP Step I diet with reduction of their LDL cholesterol levels and were subsequently ineligible. The remainder of the ineligible individuals had previously undiscovered hypothyroidism, noncompliance to run-in casein, clinical conditions that developed during the course of the screening period, etc. Twelve participants did not complete the study: 3 in the casein group, 4 in the 3-mg isoflavone group, and 5 in the 27-mg isoflavone group. All but 4 of these were lost to follow-up because of work conflicts or discontinuation of the study. Of the 4, two dropped out of the casein group because of development of urticaria (possibly related to casein) and exacerbation of asthma (not related to casein); 1 dropped out of the 3-mg isoflavone group because of indigestion (possibly related to soy protein), and 1 dropped out of the 27-mg isoflavone group because of development of chest pain and an abnormal exercise tolerance test (not believed to be due to the soy protein). All participants in the 62-mg isoflavone group completed the study without incident.

Demographic characteristics of the participants in the study are given in **Table 1**. Overall, 38 postmenopausal women, 24 premenopausal women, and 94 men were randomized; the average age was 52 years; and 12% were cur-

The isolated soy protein-containing beverages were provided by Protein Technologies International (St Louis, Mo). All of the isoflavone-containing products were obtained from naturally occurring soy cultivars that were aqueously processed after defatting with hexane to contain 62 mg, 37 mg, and 27 mg of isoflavones. Isolated soy protein was also processed with aqueous ethanol to achieve a virtually isoflavone-free product (3 mg of isoflavones). Calcium caseinate was obtained from New Zealand Milk Products (Wellington, New Zealand) and processed into beverages by Protein Technologies International. Subjects were instructed to consume the entire dose of soy protein as a single serving containing 25 g of protein each day. All beverage containers were identical, and neither the staff nor the patients were informed of the nature of the treatment allocation during the course of the study.

LIPIDS AND LIPOPROTEINS

Plasma for quantification of concentrations of lipids and lipoproteins was obtained from fasting participants after they rested quietly for 5 to 10 minutes. Tubes for the phlebotomies contained crystalline EDTA. Quantification was performed in the Centers for Disease Control Standardized Laboratory at the Wake Forest University School of Medicine.²⁴ The LDL cholesterol levels were estimated from total and HDL cholesterol and triglyceride concentrations according to the Friedewald formula.²⁵

LIPOPROTEIN(a)

Lipoprotein(a) was measured in the casein control group and in the 3-mg and 62-mg isoflavone-containing soy groups with the Macra enzyme-linked immunoassay developed by Terumo and now available from Strategic Diagnostics Inc, Newark, Del. This enzyme-linked immunoassay uses a solid-phase monoclonal capture antibody directed at the apolipoprotein (A) portion of Lp(a) and a secondary

polyclonal apolipoprotein (A) antibody conjugated to horseradish peroxidase. The assay procedure was performed as described by the manufacturer using 10 L of plasma not previously thawed. Intra-assay and interassay percent coefficients of variation were each less than 7.0%. Samples were assayed in duplicate, and Lp(a) concentrations lower than 0.008 g/L (<0.8 mg/dL) (lower detection limit of the assay) were considered to be 0.008 g/L (0.8 mg/dL), whereas those higher than 0.6 g/L (>60 mg/dL) were diluted and repeated.

STATISTICAL ANALYSIS

The randomized clinical trial was designed to enroll 30 participants per group to evaluate 25 (assuming dropouts) and have 95% power to detect a 6% relative change in LDL cholesterol levels between groups for pairwise group comparisons at the 5% two-sided level of significance. Comparisons between treatment groups were based on analysis of covariance general linear models. Plasma concentrations of total, LDL, and HDL cholesterol and triglycerides were measured twice at weeks -1 and 0 prior to randomization and twice after randomization at weeks 8 and 9. The averages of the 2 baseline measures were used as covariates, and the averages of the 2 postrandomization values were used as the dependent variables. The primary comparison was between the isolated soy protein containing 62 mg of isoflavones and casein. We also compared the isolated soy protein containing 37 mg, 27 mg, and 3 mg (ethanol extracted) of isoflavones with casein. In addition, a trend test for the effect of dose was performed for the 4 doses of isoflavones using the actual dose (3, 27, 37, and 62 mg) as a continuous covariate. The trial was planned with a priori subgroup analyses by sex and overall with sex as an additive factor in the model. Analyses were also conducted separately for individuals testing above and below the median LDL cholesterol value. All group comparisons were performed by intent-to-treat analysis.

rent smokers. Mean \pm SD plasma concentration of total cholesterol was 6.26 ± 0.70 mmol/L (242 ± 27 mg/dL); of triglycerides, 1.72 ± 0.68 mmol/L (153 ± 60 mg/dL); of LDL cholesterol, 4.29 ± 0.62 mmol/L (166 ± 24 mg/dL); of HDL cholesterol, 1.16 ± 0.26 mmol/L (45 ± 10 mg/dL), and of Lp(a), 0.18 ± 0.17 g/L (18 ± 17 mg/dL).

Weight was stable throughout the trial, and at the beginning of the trial mean weight was 79 ± 14 kg, and body mass index (BMI or Quetelet index), calculated as weight in kilograms divided by the square of the height in meters: $\text{weight (kg)}/[\text{height (m)}]^2$, 26 ± 3 kg/m². There were no significant differences between groups in weight at baseline or the end of the trial.

Diet was stable and comparable between groups throughout the trial. Participants consumed, as a percent of daily energy, 15% protein, 56% carbohydrates, and 32% fat divided into 9% saturated fat, 12% monounsaturated fat, and 12% polyunsaturated fat.

Blood pressure in participants consuming the isolated soy protein containing 62 mg of isoflavones was similar at the end of the study to that of those consuming casein, and there was no trend for lower blood pressure with increasing isoflavone intake for the group as a whole or for men. However, among women randomized to iso-

lated soy protein, there was a statistically significant trend for diastolic (but not systolic) blood pressure to be lower at the end of the study by about 3 mm Hg in the groups consuming higher isoflavone amounts ($P < .04$ for trend).

Table 2 shows the plasma concentrations of lipids and lipoproteins in the group as a whole at baseline and follow-up, and in the individual groups at the end of the study after adjustment for baseline values of the entire group of 156 participants. Overall, compared with casein, consumption of isolated soy protein containing 62 mg of isoflavones was associated with reductions in plasma concentrations of total and LDL cholesterol of 4% (6.24 vs 5.99 mmol/L [241.2 vs 231.7 mg/dL], a reduction of 0.25 mmol/L [9.5 mg/dL]; 95% confidence interval [CI], -0.49 to -0.0003 mmol/L [-18.9 to -0.1 mg/dL]) and 6% (4.26 vs 3.99 mmol/L [164.7 vs 154.5 mg/dL], a reduction of 0.27 mmol/L [10.2 mg/dL]; 95% CI, -0.47 to -0.06 mmol/L [-18.1 to -2.3 mg/dL]). These reductions occurred in both men and women but were of borderline significance in the subgroups ($P = .14$ and $P = .06$, respectively). No effect was seen on plasma concentrations of triglycerides or HDL cholesterol in the group as a whole.

To evaluate the relative strength of the cholesterol-lowering effect of isolated soy protein in participants with

Table 1. Baseline Participant Characteristics*

Characteristic	Men (n = 94)	Women		Total (N = 156)
		Premenopausal (n = 24)	Postmenopausal (n = 38)	
White/nonwhite, No.	83/11	23/1	34/4	140/16
Age, y	50 ± 11	44 ± 5	63 ± 5	52 ± 11
Weight, kg	86 ± 12	71 ± 9	66 ± 8	79 ± 14
BMI, kg/m ²	27 ± 3	26 ± 3	25 ± 3	26 ± 3
Current smoking, %	11	25	11	13
Blood pressure, mm Hg				
Systolic	126 ± 13	122 ± 16	133 ± 14	127 ± 14
Diastolic	72 ± 8	70 ± 10	69 ± 9	71 ± 9
Total cholesterol, mmol/L (mg/dL)	6.05 ± 0.65 (234 ± 25)	6.67 ± 0.65 (258 ± 25)	6.47 ± 0.59 (250 ± 23)	6.26 ± .70 (242 ± 27)
Triglycerides, mmol/L (mg/dL)	1.73 ± 0.69 (153 ± 61)	1.76 ± 0.65 (156 ± 58)	1.73 ± 0.68 (153 ± 60)	1.73 ± 0.68 (153 ± 60)
LDL cholesterol, mmol/L (mg/dL)	4.19 ± 0.59 (162 ± 23)	4.58 ± 0.70 (177 ± 27)	4.34 ± 0.62 (168 ± 24)	4.29 ± 0.62 (166 ± 24)
HDL cholesterol, mmol/L (mg/dL)	1.06 ± 0.21 (41 ± 8)	1.29 ± 0.26 (50 ± 10)	1.32 ± 0.23 (51 ± 9)	1.16 ± 0.26 (45 ± 10)
Lipoprotein(a), g/L (mg/dL)†	0.18 ± 0.18 (18 ± 18)	0.16 ± 0.14 (16 ± 14)	0.18 ± 0.17 (18 ± 17)	0.18 ± 0.17 (18 ± 17)
Dietary intake, % of energy‡				
Protein	15 ± 3	15 ± 3	15 ± 3	15 ± 3
Carbohydrate	55 ± 10	52 ± 7	57 ± 8	55 ± 9
Saturated fat	9 ± 3	9 ± 2	8 ± 3	9 ± 3
Monounsaturated fat	11 ± 4	14 ± 4	11 ± 4	12 ± 4
Polyunsaturated fat	11 ± 4	11 ± 3	12 ± 5	11 ± 4
Cholesterol	223 ± 104	162 ± 75	150 ± 87	193 ± 94

*Unless otherwise indicated, values are mean ± SD. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index.

†Lipoprotein(a) measured in 61 men and 13 premenopausal and 26 postmenopausal women.

‡Dietary intake records were available for 81 men and 20 premenopausal and 37 postmenopausal women.

Table 2. Lipid and Lipoprotein Concentrations at Baseline and Follow-up in Participants Consuming 25 g of Soy Protein Containing Variable Amounts of Isoflavones (Iflav) or 25 g of Casein*

Characteristic	25 g of Soy				
	25 g of Casein (n = 31)	3 mg of Iflav (n = 28)	27 mg of Iflav (n = 27)	37 mg of Iflav (n = 30)	62 mg of Iflav (n = 30)
Total cholesterol, mmol/L (mg/dL)					
Baseline	6.21 ± 0.62 (240 ± 24)	6.18 ± 0.70 (239 ± 27)	6.41 ± 0.75 (248 ± 29)	6.08 ± 0.70 (235 ± 27)	6.28 ± 0.67 (243 ± 26)
Follow-up	6.23 ± 0.70 (241 ± 27)	6.10 ± 0.65 (236 ± 25)	6.21 ± 0.78 (240 ± 30)	5.97 ± 0.57 (231 ± 22)	6.03 ± 0.52 (233 ± 20)
Adjusted follow-up	6.26 (242)	6.13 (237)	6.08 (235)	6.08 (235)	6.00 (232)†
LDL cholesterol, mmol/L (mg/dL)					
Baseline	4.27 ± 0.54 (165 ± 21)	4.24 ± 0.59 (164 ± 23)	4.40 ± 0.62 (170 ± 24)	4.16 ± 0.59 (161 ± 23)	4.29 ± 0.65 (166 ± 25)
Follow-up	4.27 ± 0.59 (165 ± 23)	4.14 ± 0.57 (160 ± 22)	4.27 ± 0.65 (165 ± 25)	4.03 ± 0.49 (156 ± 19)	4.03 ± 0.44 (156 ± 17)
Adjusted follow-up	4.27 (165)	4.16 (161)	4.16 (161)	4.09 (158)	4.01 (155)†
Triglycerides, g/L (mg/dL)					
Baseline	1.73 ± 0.70 (153 ± 62)	1.63 ± 0.68 (144 ± 60)	1.87 ± 0.71 (166 ± 63)	1.69 ± 0.62 (150 ± 55)	1.73 ± 0.67 (153 ± 59)
Follow-up	1.89 ± 0.84 (167 ± 74)	1.72 ± 0.65 (152 ± 58)	1.74 ± 0.79 (154 ± 70)	1.64 ± 0.63 (145 ± 56)	1.72 ± 0.75 (153 ± 66)
Adjusted follow-up	1.89 (167)	1.80 (159)	1.61 (143)	1.66 (147)	1.73 (153)
HDL cholesterol, mmol/L (mg/dL)					
Baseline	1.16 ± 0.23 (45 ± 9)	1.19 ± 0.28 (47 ± 11)	1.16 ± 0.26 (45 ± 10)	1.16 ± 0.23 (45 ± 9)	1.19 ± 0.23 (46 ± 9)
Follow-up	1.14 ± 0.23 (44 ± 9)	1.19 ± 0.28 (46 ± 11)	1.16 ± 0.23 (45 ± 9)	1.19 ± 0.21 (46 ± 8)	1.22 ± 0.28 (47 ± 11)
Adjusted follow-up	1.16 (45)	1.14 (44)	1.19 (46)	1.22 (47)	1.19 (46)

*Unless otherwise indicated, all data are given as mean ± SD. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein.

†P < .05 vs casein.

varying baseline cholesterol levels, we divided the overall group into 2 groups of high and low cholesterol at the median LDL cholesterol levels at baseline for the group as a whole (4.29 mmol/L [166 mg/dL]). The high LDL cholesterol group had a mean plasma LDL cholesterol concentration at baseline of 4.76 ± 0.49 mmol/L (184 ± 19 mg/dL).

Table 3 gives the baseline and follow-up plasma concentrations of lipids and lipoproteins in individuals in the high LDL and low LDL cholesterol groups. In those

participants in the high LDL cholesterol group, isolated soy protein containing 62 mg of isoflavones reduced plasma concentrations of total and LDL cholesterol by 9% (6.73 vs 6.12 mmol/L [260.3 vs 236.6 mg/dL], a reduction of 0.61 mmol/L [23.7 mg/dL]; 95% CI, -0.94 to -0.28 mmol/L [-36.3 to -10.7 mg/dL] and 10% (4.69 vs 4.21 mmol/L [181.1 vs 162.7 mg/dL], a reduction of 0.48 mmol/L [18.4 mg/dL]; 95% CI -0.74 to -0.21 mmol/L [-28.6 to -8.2 mg/dL]), respectively, compared with ca-

Table 3. Lipid and Lipoprotein Concentrations at Baseline and Follow-up in High and Low LDL Cholesterol Groups (Divided at the Median for LDL) Among Participants Consuming 25 g of Soy Protein Containing Variable Amounts of Isoflavones (Iflav) or 25 g of Casein*

Characteristic	25 g of Casein	25 g of Soy			
		3 mg of Iflav	27 mg of Iflav	37 mg of Iflav	62 mg of Iflav
High Group					
No. of subjects	16	12	15	12	15
Total cholesterol, mmol/L (mg/dL)					
Baseline	6.67 ± 0.41 (258 ± 16)	6.75 ± 0.57 (261 ± 22)	6.83 ± 0.70 (264 ± 27)	6.72 ± 0.41 (260 ± 16)	6.75 ± 0.59 (261 ± 23)
Follow-up	6.67 ± 0.54 (258 ± 21)	6.52 ± 0.54 (252 ± 21)	6.47 ± 0.80 (250 ± 31)	6.21 ± 0.65 (240 ± 25)	6.13 ± 0.54 (237 ± 21)
Adjusted follow-up	6.72 (260)	6.52 (252)	6.41 (248)	6.23 (241)†	6.13 (237)†§
LDL cholesterol, mmol/L (mg/dL)					
Baseline	4.71 ± 0.31 (182 ± 12)	4.78 ± 0.47 (185 ± 18)	4.81 ± 0.52 (186 ± 20)	4.71 ± 0.49 (182 ± 19)	4.78 ± 0.54 (185 ± 21)
Follow-up	4.65 ± 0.47 (180 ± 18)	4.53 ± 0.44 (175 ± 17)	4.50 ± 0.62 (174 ± 24)	4.27 ± 0.57 (165 ± 22)	4.22 ± 0.47 (163 ± 18)
Adjusted follow-up	4.68 (181)	4.50 (174)	4.47 (173)	4.29 (166)†	4.22 (163)†§
Triglycerides, g/L (mg/dL)					
Baseline	1.59 ± 0.60 (141 ± 53)	1.76 ± 0.63 (156 ± 56)	1.83 ± 0.81 (162 ± 72)	1.66 ± 0.54 (147 ± 48)	1.65 ± 0.75 (146 ± 66)
Follow-up	1.93 ± 0.91 (171 ± 81)	1.93 ± 0.64 (171 ± 57)	1.65 ± 0.80 (146 ± 71)	1.64 ± 0.67 (145 ± 59)	1.57 ± 0.65 (139 ± 58)
Adjusted follow-up	2.02 (179)	1.87 (166)	1.54 (136)†	1.66 (147)	1.61 (143)†
HDL cholesterol, mmol/L (mg/dL)					
Baseline	1.24 ± 0.23 (48 ± 9)	1.16 ± 0.23 (45 ± 9)	1.16 ± 0.18 (45 ± 7)	1.24 ± 0.26 (48 ± 10)	1.19 ± 0.21 (46 ± 8)
Follow-up	1.19 ± 0.23 (46 ± 9)	1.11 ± 0.18 (43 ± 7)	1.22 ± 0.21 (47 ± 8)	1.19 ± 0.28 (46 ± 11)	1.16 ± 0.26 (45 ± 10)
Adjusted follow-up	1.16 (45)	1.14 (44)	1.24 (48)	1.16 (45)	1.16 (45)
Low Group					
No. of subjects	15	16	12	18	15
Total cholesterol, mmol/L (mg/dL)					
Baseline	5.72 ± 0.31 (221 ± 12)	5.77 ± 0.44 (223 ± 17)	5.92 ± 0.49 (229 ± 18)	5.66 ± 0.49 (219 ± 19)	5.84 ± 0.39 (226 ± 15)
Follow-up	5.74 ± 0.52 (222 ± 20)	5.79 ± 0.52 (224 ± 20)	5.87 ± 0.67 (227 ± 26)	5.82 ± 0.44 (225 ± 17)	5.95 ± 0.47 (230 ± 18)
Adjusted follow-up	5.77 (223)	5.79 (224)	5.77 (223)	5.84 (226)	5.87 (227)
LDL cholesterol, mmol/L (mg/dL)					
Baseline	3.77 ± 0.21 (146 ± 8)	3.83 ± 0.23 (148 ± 9)	3.88 ± 0.23 (150 ± 9)	3.77 ± 0.23 (146 ± 9)	3.80 ± 0.23 (147 ± 9)
Follow-up	3.85 ± 0.41 (149 ± 16)	3.85 ± 0.52 (149 ± 20)	3.98 ± 0.57 (154 ± 22)	3.88 ± 0.36 (150 ± 14)	3.83 ± 0.31 (148 ± 12)
Adjusted follow-up	3.85 (149)	3.83 (148)	3.88 (150)	3.85 (149)	3.80 (147)
Triglycerides, g/L (mg/dL)					
Baseline	1.87 ± 0.78 (166 ± 69)	1.54 ± 0.72 (136 ± 64)	1.93 ± 0.56 (171 ± 50)	1.70 ± 0.68 (151 ± 60)	1.82 ± 0.59 (161 ± 52)
Follow-up	1.84 ± 0.76 (163 ± 68)	1.56 ± 0.64 (138 ± 57)	1.85 ± 0.80 (164 ± 71)	1.63 ± 0.63 (144 ± 56)	1.87 ± 0.81 (166 ± 72)
Adjusted follow-up	1.73 (153)	1.73 (153)	1.69 (150)	1.64 (145)	1.82 (161)
HDL cholesterol, mmol/L (mg/dL)					
Baseline	1.06 ± 0.21 (41 ± 8)	1.24 ± 0.31 (48 ± 12)	1.14 ± 0.34 (44 ± 13)	1.09 ± 0.18 (42 ± 7)	1.22 ± 0.26 (47 ± 10)
Follow-up	1.06 ± 0.21 (41 ± 8)	1.24 ± 0.31 (48 ± 12)	1.14 ± 0.28 (44 ± 11)	1.19 ± 0.18 (46 ± 7)	1.27 ± 0.28 (49 ± 11)
Adjusted follow-up	1.14 (44)	1.16 (45)	1.11 (43)	1.24 (48)	1.22 (47)§

*Unless otherwise indicated, all data are given as mean ± SD. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein.

†P < .03 vs casein.

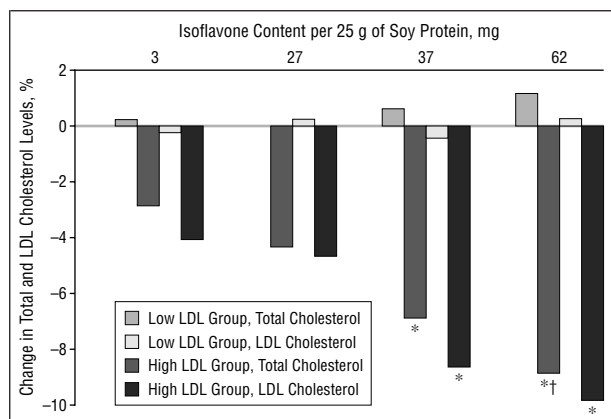
‡P < .05 vs 3 mg of isoflavone containing soy protein.

§P < .04 for trend.

sein (**Figure**). In the high LDL cholesterol group, both total and LDL cholesterol levels were also lowered by 8% by soy protein containing 37 mg of isoflavones. In these participants the isolated soy protein containing 62 mg of isoflavones also reduced plasma concentrations of total and LDL cholesterol by 6% compared with the soy protein containing 3 mg of isoflavones ($P = .04$ and $P = .06$ for total and LDL cholesterol levels, respectively). There was a statistically significant dose-response effect of isoflavones to lower total and LDL cholesterol levels ($P = .01$ for the trends in total and $P = .02$ for LDL cholesterol levels). Plasma concentrations of HDL cholesterol were not affected by soy protein containing any dose of isoflavones in this study. Plasma concentrations of triglycerides were significantly lower in the groups consuming soy protein containing 27 mg and 62 mg of isoflavones compared with casein and ethanol-extracted soy protein; however, this reflected increases in plasma concen-

trations of triglycerides in the groups that continued consumption of casein or that changed to ethanol-extracted soy protein after randomization rather than decreases in plasma concentrations of triglycerides in the groups consuming isolated soy protein containing higher levels of isoflavones. Isolated soy protein did not affect lipoprotein levels in participants whose LDL cholesterol at baseline was below the median LDL cholesterol level for the group as a whole. The ethanol-extracted isolated soy protein containing 3 mg of isoflavones and the preparation containing 27 mg of isoflavones had nonsignificant effects in reducing plasma concentrations of total and LDL cholesterol in the group as a whole and in the high LDL cholesterol subgroup. These preparations had no effect on HDL cholesterol levels or on plasma concentrations of triglycerides in any group studied.

Postmenopausal women who consumed the soy protein containing 62 mg of isoflavones had reductions of



Percent change in total and low-density lipoprotein (LDL) cholesterol compared with casein in low LDL and high LDL groups. An asterisk indicates $P < .03$ vs casein; a dagger, $P < .05$ vs soy protein containing 3 mg of isoflavones.

total and LDL cholesterol of 7% and 8%, respectively, compared with casein, that were of borderline significance ($P = .06$ and $P = .07$, respectively), but had no change in HDL cholesterol or triglyceride concentrations. There were no effects of isolated soy protein on any lipid or lipoprotein parameter in premenopausal women. There were no effects of isolated soy protein with or without isoflavones on plasma concentrations of Lp(a) in this study.

COMMENT

Asian populations have a low prevalence of chronic diseases (eg, breast and uterine cancer and cardiovascular disease) in countries of the Pacific Rim¹⁻⁶ and consume 30 to 50 times more soy protein than Western populations.²⁶ While the cholesterol-lowering properties of soy are widely accepted,¹³ the mechanism is not completely understood and has been postulated to be a result of the protein itself,²⁷ as well as other components including saponins, phytic acid, trypsin inhibitors, and fiber, although not all investigators agree.²⁸ The latter may have more potency to lower cholesterol levels when fed with casein than when fed with soy protein.²⁸

Isoflavones found in soybeans include genistein and daidzein.^{8,9} Differences in urinary excretion of isoflavones between Asian and American populations are striking (Asian, 2000-3000 nmol/d of genistein and daidzein; American, 30-40 nmol/d).²⁹ Isoflavones are particularly good candidates for the cardioprotective effects of soy because of their many chemical and biological similarities to mammalian estrogens. Comparable effects have been observed for estrogens in human beings and isoflavones in nonhuman primates for vascular endothelial function^{12,30} and lipid lowering.^{10,31} Isolated soy protein containing isoflavones reduces atherosclerosis in nonhuman primates,¹¹ and there is considerable evidence suggesting the same is true for estrogens in human beings.³²

A meta-analysis of trials of soy effects on lipids substantiated the observation that soy protein reduced plasma concentrations of total and LDL cholesterol and triglycerides; however, this effect was influenced by the participants' baseline cholesterol concentrations.¹³ Our communication supports these conclusions. We noted a statistically significant reduction of total and LDL cho-

lesterol with isolated soy protein containing 62 mg of isoflavones in our overall population of moderately hypercholesterolemic participants, and the effects were observed in both men and postmenopausal women; however, the effects were not statistically significant in these subgroups, likely owing to restricted sample sizes. The impact of isolated soy protein containing isoflavones on plasma concentrations of lipids and lipoproteins was greatest in those in our population with baseline plasma concentrations of LDL cholesterol above the median.

Previous analyses have also noted that variability in the plasma cholesterol response from study to study might result from variable isoflavone content of soy preparations.¹³ Our study demonstrated that the cholesterol-lowering effect of isolated soy protein depended on its isoflavone content, and ethanol-extracted soy protein with only trace isoflavones as well as soy protein with only 27 mg of isoflavones had no significant effect on lipids and lipoproteins.

The effect of isolated soy protein containing isoflavones to reduce plasma concentrations of LDL cholesterol is slightly less than the effect of mammalian estrogen. However, in contrast to estrogen, isolated soy protein containing isoflavones had no triglyceride-raising effect. In fact, compared with casein, plasma concentrations of triglycerides were lower at the end of the study in the groups consuming isolated soy protein with isoflavones. However, interpretation of these data is difficult because the groups consuming casein and ethanol-extracted soy experienced an increase in plasma concentrations of triglycerides, whereas the groups consuming isoflavone containing isolated soy protein experienced little change in plasma concentration of triglycerides. There was neither a reduction of plasma concentrations of Lp(a) associated with consumption of isolated soy protein containing isoflavones in this study as has been noted in the past with estrogen administration to postmenopausal women,³³ nor an increase in concentration of Lp(a).

Our study does not allow us to define which of the many component isoflavones in isolated soy protein may lower cholesterol. The recent report of Nestel et al³⁴ suggests that some synergy between soy protein and natural isoflavones is necessary to reduce levels of cholesterol since a soy isoflavone extract provided without soy protein had no cholesterol-lowering effect.

Others have reported that soy protein containing only minimal concentrations of isoflavones reduced cholesterol.³⁵ Participants in those studies tended to have markedly elevated levels of LDL cholesterol, and the diets provided in those studies tended to be higher in protein than ours (20% vs 13% of energy). Nonetheless, we cannot exclude the possibility that some component in soy protein other than isoflavones is partly removed by the washing procedure and the ethanol extraction procedure and accounts for the greater cholesterol-lowering properties of the isolated soy protein containing 37 mg and 62 mg of isoflavones. Ethanol-extracted isolated soy protein did not lower cholesterol levels significantly, and there was a significant dose-response effect of isoflavone on LDL cholesterol in the group with higher LDL cholesterol at baseline. Our study cannot eliminate the possibility that isolated

soy protein per se (or the 3 mg of isoflavone remaining in the ethanol-extracted product) has cholesterol-lowering properties. It is also possible that the protein and the isoflavone have synergistic cholesterol-lowering effects.²⁸ The dose-response relationship raises the possibility that soy protein with higher amounts of isoflavone might have even greater cholesterol-lowering effects.

The effect of isolated soy protein containing isoflavones was additive to that of an NCEP Step I diet low in saturated fat (9% of energy). The magnitude of the effect (11% reduction of LDL cholesterol) was similar to that recently reported for the more fat-restrictive NCEP Step II diet³⁶; however, the isolated soy protein containing isoflavones did not increase the concentration of triglycerides (as described above) or lower HDL cholesterol levels as has been reported for the Step II diet.³⁶

We noted a trend for decreased diastolic blood pressure in women in this study with increasing isoflavone content of soy protein isolate. We have observed this effect previously in a study of postmenopausal women.³⁷

These results support the conclusion that isolated soy protein containing isoflavones lowers cholesterol beyond the reduction achieved with an NCEP Step I diet, particularly in individuals with elevated plasma concentrations of LDL cholesterol. Isolated soy protein containing isoflavones does not adversely affect plasma concentrations of triglycerides or HDL cholesterol. Ethanol-extracted isolated soy protein or soy protein containing 27 mg/d of isoflavones or less did not have a significant cholesterol-lowering effect in this study.

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