The effect of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolemic postmenopausal women[1–3]

Christopher D Gardner, Kathryn A Newell, Rise Cherin, and William L Haskell

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ABSTRACT

Background: Clinical trial data and the results of a meta-analysis suggest a hypocholesterolemic effect of soy protein. The effect may be partially attributable to the isoflavones in soy. Few studies have examined the separate effects of soy protein and isoflavones.

Objective: The objective of this study was to determine the effect of soy protein and isoflavones on plasma lipid concentrations in postmenopausal, moderately hypercholesterolemic women.

Design: This was a randomized, double-blind, placebo-controlled clinical trial with 3 treatment groups. After a 4-wk run-in phase during which the women consumed a milk protein supplement, the subjects were randomly assigned to 12 wk of dietary protein supplementation (42 g/d) with either a milk protein (Milk group) or 1 of 2 soy proteins containing either trace amounts of isoflavones (Soy– group) or 80 mg aglycone isoflavones (Soy+ group).

Results: LDL-cholesterol concentrations decreased more in the Soy+ group (n = 31) than in the Soy– group (n = 33) (0.38 compared with 0.09 mmol/L; P = 0.005), but neither of these changes was significantly different from the 0.26-mmol/L decrease observed in the Milk group (n = 30). The results for total cholesterol were similar to those for LDL cholesterol. There were no significant differences in HDL-cholesterol or triacylglycerol concentrations between the 3 groups.

Conclusions: The difference in total- and LDL-cholesterol lowering between the 2 soy-protein supplements suggests an effect attributable to the isoflavone-containing fraction. However, the unexpected LDL-cholesterol lowering observed in the Milk group, and the fact that there was no significant difference between either soy group and the Milk group, suggests that changes may have been due to other factors related to participation in the study. *Am J Clin Nutr* 2001;73:728–35.

KEY WORDS Soy protein, isoflavones, LDL cholesterol, lipids, postmenopausal women, diet, sex hormones

INTRODUCTION

The importance of diet in the management of plasma lipids is well established. The primary emphasis has traditionally been on limiting intakes of saturated fat and cholesterol (1, 2). In addition, the inclusion of several plant-based dietary factors may have benefits in lipid management, such as soy protein, fiber, plant sterols, and garlic (3–6). In a meta-analysis of the effects of soy protein on serum lipid concentrations, it was speculated that the reported LDL-cholesterol-lowering effect was partially attributable to the phytoestrogen content (3).

The predominant phytoestrogens found in soy are the isoflavones genistein and daidzein. These isoflavones have a common diphenolic structure that resembles the potent synthetic estrogens diethylstilbesterol and hexestrol (7). The results of several other studies support the plausibility of an effect of isoflavones on plasma lipids (8–12). The authors of the soy protein meta-analysis suggested that isoflavones may be responsible for "up to 60% of the hypocholesterolemic activity of soy proteins" (3). A limited number of human clinical trials examined the effect of isoflavone intake on plasma lipids. Crouse et al (13) reported a significant LDL-cholesterol-lowering effect of soy protein with isoflavones that was not found with a soy protein with only trace amounts of isoflavones. Other investigators used various study designs to distinguish the lipid-lowering effects of isoflavones themselves from the combination of isoflavones and protein but did not attribute a significant effect specifically to the isoflavones in either humans (14–16) or monkeys (17). Because of the limited data available, it is unclear whether soy-derived isoflavones have a clinically relevant and beneficial effect on plasma lipid management in humans.

Soy-protein products can be prepared in a variety of ways. One preparation method involves ethanol extraction, which removes all but trace amounts of phytoestrogen. This procedure has made it possible to design 2 soy-protein products with identical macronutrient contents but different phytoestrogen contents. The purpose of this clinical trial was to contrast the effect on plasma lipid concentrations of 3 protein supplements—milk

[1] From the Stanford Center for Research in Disease Prevention and the Department of Medicine, Stanford University Medical School, Stanford, CA.

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[3] Address reprint requests to CD Gardner, Stanford Center for Research in Disease Prevention, 730 Welch Road, Suite B, Palo Alto, CA 94304-1583. E-mail: cgardner@stanford.edu.

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TABLE 1
Contents of daily supplements (combined total of 2 packets/d)[1]

	Milk			
	Run-in phase	Study phase	Soy−	Soy+
Isoflavone (mg aglycone/d)				
Genistein	1	1	2	52
Daidzein	1	1	1	25
Glycetein	0	0	0	4
Total	2	2	3	80
Energy (kJ)[2]	1200	1200	1200	1200
Macronutrient (g/d)				
Protein	42	42	42	42
Carbohydrate	30	30	30	30
Fat	0	0	0	0
Calcium (mg/d)	500	500	500	500

[1] Analyzed by Protein Technologies International, St Louis.
[2] To convert to kcal, divide by 4.184.

protein, soy protein with only trace amounts of isoflavones, and soy protein with naturally occurring amounts of isoflavones—among postmenopausal, hypercholesterolemic women. The study was designed to test whether the reported LDL-cholesterol-lowering effect of soy-protein supplements (3) was attributable to the isoflavone-containing fraction. The milk protein supplement was included in the study design for 2 purposes: *1*) to control for other factors related to study participation that might affect plasma lipid concentrations when 42 g protein/d is incorporated into a fat- and cholesterol-free supplement for 12 wk, and *2*) to try to reproduce earlier and somewhat inconsistent findings that soy protein per se, relative to milk protein, has an effect on LDL-cholesterol concentrations (18–25).

SUBJECTS AND METHODS

Subjects

Postmenopausal women were recruited from the local community, primarily through radio and newspaper advertisements, letters, and flyers. Women were invited to be screened for plasma cholesterol concentrations if they were postmenopausal (≥1 y since their last menstrual cycle), were aged <80 y, and had a body mass index (BMI; in kg/m^2) of 20–31. Women were not eligible if they were smokers, had been taking hormone replacements or lipid-lowering medication during the previous 3 mo, had a history of cardiovascular disease or diabetes, or had had breast, endometrial, or ovarian cancer in the previous 10 y. Of the 705 women screened by telephone, 235 were eligible and willing to come in for cholesterol testing. Of those 235 women, 145 met the fasting plasma lipid inclusion criteria of having an LDL-cholesterol concentration of 3.37–4.92 mmol/L (130–190 mg/dL) and a triacylglycerol concentration <2.82 mmol/L (<250 mg/dL). Of those 145 women, 115 agreed to provide their written consent and enroll in the study. The protocol was approved by the appropriate university internal review board, and the study was carried out according to the guidelines of the Declaration of Helsinki.

Design

The study was a randomized, double-blind, placebo-controlled, 16-wk clinical trial. After a 4-wk run-in phase during which the women consumed a milk protein supplement, the subjects were randomly assigned to 12 wk of dietary protein supplementation (42 g/d) with either a milk protein (Milk group) or 1 of 2 soy proteins containing either ethanol-extracted isolated soy protein with trace amounts of isoflavone (Soy– group) or an isolated soy protein containing 80 mg aglycone isoflavones (Soy+ group). A staggered and continuous enrollment of participants (≈10/mo) was conducted over a period of ≈1 y, from June 1997 to April 1998. There were a total of 11 clinic visits, including 2 visits during the 4-wk run-in phase and 9 visits after random assignment to 1 of 3 treatments. Randomization was performed in blocks of 30 participants. Participants, investigators, study staff, and laboratory technicians were blinded to treatment assignments until the conclusion of the trial.

Protein supplements

Dietary supplements containing a mixture of protein, carbohydrate, and calcium in powder form (Shaklee Corporation, Hayward, CA) were provided in sealed packets, each containing one-half of the daily dose (21 g protein/packet × 2 packets/d). Protein Technologies International (St Louis) analyzed the nutrient and isoflavone contents of the supplements (**Table 1**). The composition of the supplements was assessed at 3 time points—before enrollment of the first participant, midway through the study, and after completion of the study. These analyses showed a stable composition of isoflavone concentrations throughout the study (data not shown). In addition to the protein, each of the 4 different preparations included 30 g carbohydrate and was fortified with 500 mg calcium phosphate.

During each bimonthly clinic visit, participants were given a box containing a 2-wk supply of protein packets with instructions to consume 2 packets/d, preferably with meals in the morning and the evening. It was suggested that the supplement be mixed with 240 mL (8 oz) of any liquid of the subject's choice, including juice, water, and soup. Shakers were provided for mixing purposes. All supplements were formulated to be identical in taste, color, and odor.

Diet

Participants were instructed by a registered dietitian to make dietary adjustments to incorporate the protein supplements into their diet without causing weight gain or changes in protein or energy intake [ie, to reduce the typical daily intake of protein by ≈40 g and of energy by ≈1200 kJ (≈300 kcal)]. The women were also instructed to avoid intake of all foods containing soy or flax, and this requirement was reinforced at clinic visits and monitored during the study by review of food records.

At the onset of the study, the participants were instructed to take the full dose of the protein supplements. However, we had a higher than anticipated dropout rate early in the study because of adverse gastrointestinal responses to the acute dietary change. Therefore, gradual adaptation to the protein supplements was recommended for the latter four-fifths of participants. These participants began the 4-wk run-in phase by consuming one-half of the goal dose rather than the full dose. These participants were then encouraged to increase their intake to the full dose by week 3 of the run-in phase.

Data collection

Body weight was measured on a digital scale at each of the 11 clinic visits. The study participants were advised to maintain

their weight throughout the 4 mo. During any clinic visit at which a weight gain or loss of 1 kg was observed, participants were counseled to alter their energy intake accordingly.

Self-reported, written questionnaires were filled out at baseline (week −4), at randomization (week 0), early midstudy (week 4), and at the end of the study (week 12) and included information regarding general physical health, physical activity, smoking status, and menopausal symptoms. Participants also completed self-administered questionnaires at the end of the study regarding adherence and blinding. To determine compliance, the participants were asked to estimate how many protein packets they had missed or forgotten to take, on average, in a typical week. Compliance was also assessed by counting returned empty supplement packages at each bimonthly clinic visit. To determine whether the blinding procedures were effective, participants were asked to select 1 of 3 responses regarding the group to which they thought they had been assigned: 1, with phytoestrogen; 2, without phytoestrogen; or 3, no idea.

Each participant completed a 3-d food record at the same 4 time points at which they filled out the questionnaires. Nutrient analyses were conducted with use of FOOD PROCESSOR (version 7.2; ESHA, Salem, OR). A habitual physical activity questionnaire was completed at the same 4 time points by using a modified version of the College Alumni Physical Activity Questionnaire (26).

Fasting (>12 h) blood samples were collected during 6 of the clinic visits by venipuncture in EDTA-containing tubes, refrigerated immediately, and centrifuged at 1500 × *g* for 30 min at 4°C within 2 h. Each participant's plasma samples were stored in Wheaton vials (Wheaton, Millville, NJ) and frozen at –70°C until the participant had completed the protocol. At that time, a set of samples for that participant was thawed and analyzed in a single batch to minimize laboratory variability. Plasma concentrations of total cholesterol and triacylglycerol (with subtraction of a free glycerol blank) were measured by enzymatic procedures according to established methods of the Lipids Research Clinics (ABA 200 instrument; Abbott Laboratories, North Chicago, IL) (27, 28). HDL cholesterol was measured by dextran sulfate-magnesium precipitation (29) followed by enzymatic determination of cholesterol (27). LDL-cholesterol was calculated according to the method of Friedewald (30) unless the triacylglycerols were >4.52 mmol/L (>400 mg/dL), in which case the LDL-C value was considered missing data (3 LDL-cholesterol data points were excluded: 1 in the Milk group, 0 in the Soy– group, and 2 in the Soy+ group). The lipoprotein laboratory assays as monitored by the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, and the National Heart, Lung, and Blood Institute were consistently within specific limits. The laboratory precision for measuring total-cholesterol, HDL-cholesterol, and triacylglycerol were assessed monthly and all CVs were ≤2.8%.

Estrone, estradiol, and androstenedione were assessed by radioimmunoassay as described by Anderson and Goebelsmann (31). Follicle-stimulating hormone concentrations were assessed by using a standard kit from Diagnostics Products Corporation (Los Angeles).

Statistical analysis

For the purpose of sample size estimation, the a priori minimum clinically relevant difference between groups in the changes in LDL-cholesterol concentration over 12 wk was selected to be 10%. We then estimated the SD of changes in LDL-cholesterol concentration from previous studies to be 0.47 mmol/L and determined that a sample size of 30/group would provide ≥80% power to detect a significant difference in change in LDL-cholesterol concentration of 10%, using an α level of 0.05. Statistical tests were performed by using the general linear model approach of SAS (version 8.0; SAS Institute Inc, Cary, NC). Descriptive statistics expressed as means and SDs were determined for the baseline characteristics of the participants. Differences in baseline characteristics between the 3 groups were tested by analysis of variance (ANOVA) for continuous variables. When ANOVA findings were significant, pairwise differences were examined by using *t* tests. Baseline differences in categorical variables (eg, ethnicity and marital status) were tested by using chi-square analysis. Analysis of covariance (ANCOVA) was used to test between-group differences in changes (δ values) in plasma lipids measured at randomization (average of 2 measurements) and at the end of the study (average of 2 measurements). The lipid values were adjusted for concentrations at randomization and the pre-post changes in saturated fat and weight. Adjustment for the changes in saturated fat and weight made negligible contributions to the model, so the results presented are for the final model that included adjustments for only the lipid concentrations at randomization. The significant ANCOVA results were followed up with *t* tests for pairwise comparisons between the 3 groups. All statistical tests were two-tailed and used a significance level (α) of 0.05.

RESULTS

Of the 115 women who entered the study, 21 withdrew before study completion. Fifteen women withdrew during the 4-wk run-in phase but before randomization. Of the 6 women who withdrew after randomization, 3 reported gastrointestinal discomfort (bloating and constipation), 1 from each of the 3 treatment groups. One woman from the Soy+ group discontinued participation and could not be located for follow-up. Another woman from the Soy+ group dropped out because of an increased number of hot flashes. One woman from the Milk group discontinued participation because of too much stress in her life. Statistical analyses were performed on data from the 94 participants that completed the full protocol.

Baseline characteristics assessed at week 0 (randomization) indicated that the 3 groups were comparable in terms of sociodemographics, age at menopause, diet, plasma lipid concentrations, and sex hormone concentrations. Exceptions to group comparability at randomization included a higher average age and a higher number of years since menopause in the Soy+ group and a lower percentage of married women in the Soy– group than in the Soy+ group (**Table 2**). Overall, these women were well educated, and most were white, non-Hispanic, and married.

Body weight, diet, and physical activity

Body weight remained stable in each of the 3 treatment groups throughout the study. Weight changes during the 4-wk run-in phase were negligible. The average weight changes over the 12 wk from randomization to the end of the study were 0.5 ± 1.5, 0.6 ± 1.2, and –0.1 ± 0.9 kg in the Milk, Soy–, and Soy+ groups, respectively, and were not significantly different.

Dietary intake was assessed at 4 time points during the study. According to the design of the study, the participants were

TABLE 2
Baseline characteristics of the subjects by study group[1]

	Milk (n = 30)	Soy− (n = 33)	Soy+ (n = 31)
Age (y)	57.7 ± 6.0	58.4 ± 7.2	62.6 ± 7.3[2]
Age at menopause (y)[3]	48.1 ± 4.9	49.7 ± 3.7	48.4 ± 5.8
Education (y)	16.1 ± 2.3	15.7 ± 2.5	15.7 ± 1.8
Ethnicity, non-Hispanic white (%)	73	69	87
Married (%)	60	44[4]	77
Body mass index (kg/m^2)	27.1 ± 4.8	25.4 ± 3.6	25.6 ± 4.4
Dietary intake			
Energy (kJ)[5]	7575 ± 1510	7070 ± 1675	7070 ± 1590
Protein (% of energy)[5]	16.7 ± 3.5	17.3 ± 4.2	15.9 ± 2.9
Carbohydrate (% of energy)[5]	51.0 ± 9.0	51.5 ± 10.4	54.0 ± 7.7
Calcium (mg/d)[5]	765 ± 305	766 ± 271	674 ± 246
Fat (% of energy)	30.9 ± 8.0	30.0 ± 8.2	29.3 ± 6.6
Saturated fat (% of energy)	9.3 ± 2.8	9.4 ± 3.3	8.0 ± 2.9
Cholesterol (mg/d)	215 ± 122	271 ± 192	182 ± 120
Vitamin C (mg/d)	146 ± 85	139 ± 70	160 ± 63
Fiber (g/d)	22.0 ± 8.6	20.3 ± 6.9	22.1 ± 6.4
Iron (mg/d)	14.4 ± 4.6	13.7 ± 3.8	13.2 ± 3.4
Vitamin E (IU/d)	11 ± 5	10 ± 4	11 ± 3
Folate (μg/d)	313 ± 198	293 ± 136	292 ± 121
Lipids (mmol/L)			
Total cholesterol	6.2 ± 0.7	6.2 ± 0.9	6.1 ± 0.6
LDL cholesterol	4.2 ± 0.6	4.2 ± 0.8	4.0 ± 0.7
HDL cholesterol	1.5 ± 0.4	1.4 ± 0.3	1.5 ± 0.4
Triacylglycerols	1.2 ± 0.6	1.2 ± 0.5	1.3 ± 0.7
Hormones			
Androstenedione (nmol/L)	2.3 ± 0.7	2.3 ± 0.8	2.4 ± 1.1
Estrone (pmol/L)	200 ± 48	204 ± 52	215 ± 67
Estradiol (pmol/L)	29 ± 15	26 ± 14	28 ± 18
Follicle stimulating hormone (IU/L)	63 ± 25	74 ± 30	62 ± 24

[1] $\bar{x}$ ± SD. To convert kJ to kcal, divide by 4.184. To convert mmol/L to mg/dL, divide by 0.0259 for cholesterol and by 0.0113 for triacylglycerols.
[2] Significantly different from the other 2 groups, $P = 0.01$.
[3] Data were missing for 7, 4, and 5 subjects in the Milk, Soy−, and Soy+ groups, respectively.
[4] Significantly different from the Soy+ group, $P < 0.05$ (chi-square test = 6.9).
[5] Nutrients that were components of the dietary supplements.

expected to make substantial reductions in energy and protein intakes to accommodate the supplements during the run-in phase. No specific advice was given to alter intakes of carbohydrate or calcium, the other 2 nutrients provided by the supplements. As estimated from the 3-d food records, at the end of the 4-wk run-in phase the average participant had significantly reduced energy, protein, carbohydrate, and calcium intakes (**Table 3**). After addition of the contributions of the supplement to intakes of energy and these nutrients, the net dietary changes in energy and carbohydrate were not significant, but the total protein and calcium intakes were both significantly higher at the end of the run-in phase than at baseline.

Changes in the intake of several nutrients not provided by the supplement were observed between baseline and randomization for the group as a whole, although there were no significant differences between groups during this run-in phase. These nutrient changes were indirect consequences of the subjects being advised to reduce their intakes of high-protein foods and to consume ≈1200 kJ/d (≈300 kcal/d) from a fat-free and fiber-free supplement. The significant changes included decreases in total fat, saturated fat, cholesterol, fiber, and iron intakes and an increase in vitamin C intake (Table 3). All of the significant changes in dietary intake noted above were observed during the run-in phase. There were no significant changes in intake of any of these nutrients between randomization and the end of the study, either between groups or in the overall combined study population. Therefore, the run-in phase was successful in allowing for dietary changes, to accommodate the daily dietary supplement, to stabilize before randomization.

Baseline activity levels were similar among the 3 groups and there were no significant changes in activity in any treatment group over the course of the study (data not shown). Compliance was high in all 3 groups and not significantly different between groups. All 3 groups consumed ≥90% of their supplements over the 12-wk phase from randomization to the end of the study. This is likely an underestimate because some of the women, on some visits, forgot to return their empty packets. According to self-rating by questionnaire, participants in all 3 groups reported ≥95% compliance. Forty-five percent, 35%, and 34% of the Milk, Soy−, and Soy+ groups, respectively, correctly identified their assignments (NS), indicating the success of the blinding.

Plasma lipids and hormones

Plasma lipid concentrations at randomization, early midstudy, and the end of the study are presented in **Table 4**. A significant difference between groups was found in 12-wk changes in total cholesterol and LDL cholesterol. Follow-up pairwise comparisons indicated that the only significant pairwise difference was the change between the Soy– and Soy+ groups. For total cholesterol the difference was –0.02 compared with –0.27 mmol/L, and

TABLE 3
Dietary changes (adaptation) during the run-in phase of the study for all participants combined[1]

	Change from baseline to randomization	*P*
Contained in supplement		
Energy (kJ)[2]		
Without supplementation	−57 ± 97[3]	<0.0001
With supplementation	11 ± 97	0.3
Protein (g/d)		
Without supplementation	−14.4 ± 23.4	<0.0001
With supplementation	27.6 ± 23.4	<0.0001
Carbohydrate (g/d)		
Without supplementation	−17.9 ± 60.6	0.006
With supplementation	12.1 ± 60.6	0.06
Calcium (mg/d)		
Without supplementation	−152 ± 275	<0.0001
With supplementation	348 ± 275	<0.0001
Not contained in supplement		
Total fat (% of energy)[4]	−6.5 ± 6.9	0.03
Saturated fat (% of energy)[4]	−2.3 ± 1.1	<0.0001
Cholesterol (mg/d)	−58 ± 132	<0.0001
Vitamin C (mg/d)	25 ± 97	0.01
Fiber (g/d)	−2.7 ± 7.9	0.001
Iron (mg/d)	−2.2 ± 4.8	<0.0001
Vitamin E (IU/d)	−1.1 ± 5.7	0.06
Folate (μg/d)	−19 ± 173	0.3

[1] $n = 94$.
[2] To convert to kcal, divide by 4.184.
[3] $\bar{x} \pm$ SD.
[4] Determined on the basis of the total energy intake, including the 1200 kJ/d provided by the supplement.

for LDL cholesterol the difference was –0.09 compared with –0.38 mmol/L, respectively. None of the changes in total or LDL cholesterol were significantly different between the Milk and either the Soy– or the Soy+ group. There were no significant differences in HDL cholesterol or triacylglycerol concentrations between any groups. The average 12-wk changes in plasma lipid concentrations for the group are presented in **Figure 1**.

In all 3 groups, an average reduction in LDL-cholesterol concentrations was observed during the run-in phase (**Figure 2**): –0.18, –0.31, and –0.17 mmol/L for the Milk, Soy–, and Soy+ groups, respectively. At that time, all participants were receiving the identical milk protein supplement. These decreases were an anticipated consequence of supplementing the diet and replacing high-protein food sources in the diet that were also sources of saturated fat and cholesterol. When the 16 wk of pre- and postrandomization changes in LDL-cholesterol concentrations were combined, the net changes were –0.45, –0.39, and –0.53 mmol/L for the Milk, Soy–, and Soy+ groups, respectively, and were not significantly different between any of the 3 groups.

Concentrations of 4 hormones were determined at weeks 0 and 12 for all participants. No significant between-group differences were found for 12-wk changes in estrone, estradiol, androstenedione, or follicle-stimulating hormone (data not shown).

DISCUSSION

In this randomized clinical trial, the only significant difference observed in changes in plasma lipid concentrations between the 3 study groups was a clinically relevant decrease in total- and LDL-cholesterol concentrations that was larger in the Soy+ than in the Soy– group. This difference suggests that the isoflavone-containing fraction of soy protein has a hypocholesterolemic effect. However, total- and LDL-cholesterol concentrations also decreased in the Milk group, and the decreases were not significantly different from those in either of the 2 soy groups. When the outcomes of all 3 study groups were taken into account, the presence or absence of the isoflavone-containing fraction of soy did not sufficiently explain the observed results. An alternative interpretation to be considered is that other factors related to study participation in all 3 groups caused the observed decrease in total- and LDL-cholesterol concentrations.

TABLE 4
Plasma lipid concentrations by study group at 3 time points during the study[1]

	Milk ($n = 30$)	Soy− ($n = 33$)	Soy+ ($n = 31$)	*P*[2]
		mmol/L		
Total cholesterol				
Randomization (week 0)	6.1 ± 0.6	5.9 ± 0.7	5.9 ± 0.6	
Early midstudy (week 4)	6.1 ± 0.8	5.9 ± 0.8	5.7 ± 0.6	0.03[3]
End of study (week 12)	5.9 ± 0.7	5.9 ± 0.9	5.7 ± 0.5	
LDL cholesterol				
Randomization (week 0)	4.0 ± 0.5	3.9 ± 0.6	3.9 ± 0.6	
Early midstudy (week 4)	4.0 ± 0.7	3.9 ± 0.7	3.6 ± 0.7	0.01[3]
End of study (week 12)	3.7 ± 0.6	3.8 ± 0.8	3.5 ± 0.5	
HDL cholesterol				
Randomization (week 0)	1.5 ± 0.4	1.4 ± 0.3	1.5 ± 0.3	
Early midstudy (week 4)	1.5 ± 0.4	1.4 ± 0.2	1.5 ± 0.3	1.0
End of study (week 12)	1.5 ± 0.4	1.5 ± 0.2	1.6 ± 0.3	
Triacylglycerols				
Randomization (week 0)	1.3 ± 0.7	1.3 ± 0.5	1.3 ± 0.8	
Early midstudy (week 4)	1.2 ± 0.7	1.4 ± 0.6	1.5 ± 0.9	0.3
End of study (week 12)	1.4 ± 1.0	1.3 ± 0.6	1.3 ± 0.7	

[1] $\bar{x} \pm$ SD. To convert mmol/L to mg/dL, divide by 0.0259 for cholesterol and by 0.0113 for triacylglycerols.
[2] Significance level of between-group differences in the 12-wk changes (randomization to end of study), with adjustment for week 0 lipid concentrations.
[3] Pairwise comparisons indicated that the only pairwise difference that was significant was in the Soy− group compared with the Soy+ group ($P = 0.03$ for total cholesterol, $P = 0.005$ for LDL cholesterol).

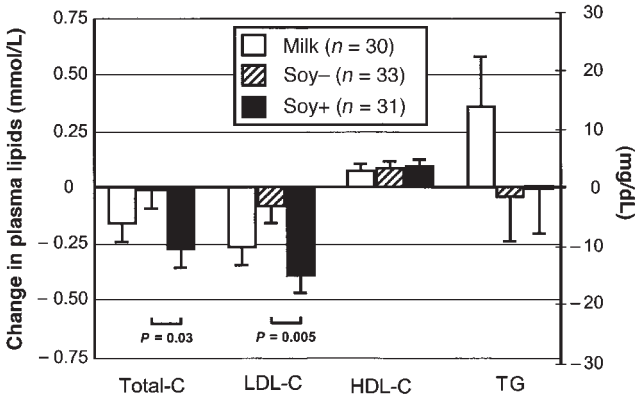

FIGURE 1. Mean (±SD) plasma lipid changes, adjusted for lipid concentrations at baseline, in subjects consuming milk protein (Milk group), soy protein containing trace amounts of aglycone isoflavones (Soy− group), or soy protein containing 80 mg aglycone isoflavones (Soy+ group). C, cholesterol; TG, triacylglycerol.

For the purpose of discussion, it is of interest to consider the combined pre- and postrandomization changes in plasma lipid concentrations observed in the study, focusing on LDL cholesterol. These 16-wk changes were clinically relevant in all 3 groups, but there were no significant between-group differences (range: –0.39 to –0.53 mmol/L). For each of the 3 study groups, the largest decreases in LDL-cholesterol concentrations occurred in different study phases: Soy– group during the run-in phase, –0.31 mmol/L; Soy+ group during the first 4 wk after randomization, –0.28 mmol/L; and Milk group during the final 8 wk of the protocol, –0.27 mmol/L (Figure 2). There were no between-group differences in body weight changes or diet in any of the 3 study phases described above that help to explain these observations. Decreases in saturated fat and cholesterol intakes were observed during the run-in phase for all 3 groups; however, the reported magnitude of changes in those nutrients would not be considered sufficient to explain the magnitude of observed decreases in LDL-cholesterol concentrations.

We are left with 2 possible interpretations of the data, without clear evidence to support one over the other. The first interpretation is that the difference in LDL-cholesterol concentration changes in the 12-wk randomization phase between the Soy– and the Soy+ groups indicates a hypocholesterolemic effect of the isoflavone-containing fraction of the soy-protein supplements. In this interpretation, the parallel decrease in LDL-cholesterol concentration observed in the Milk group, primarily in the last 8 wk, was due either to chance or to some undetected factor unique to the Milk group. A second interpretation is that the decreases in LDL-cholesterol concentrations observed in all 3 groups were due to factors to which all participants were exposed—the protein supplements, concomitant dietary adaptations, or other possible unmeasured factors—and not to the soy or the isoflavone content of the protein supplements.

These findings should be considered in the context of related clinical trials. In a 1995 meta-analysis of the effects of soy-protein intake on serum lipids, most of the selected 31 trials reported a net beneficial change in LDL-cholesterol concentration for soy protein relative to a control animal protein (3). The pooled effect for an average intake of 47 g soy protein/d was a change of –0.56 mmol/L (95% CI: 0.82, 0.29 mmol/L). However, as presented, 24 of the 31 individual trials had 95% CIs that included 0. Similar to this pattern, the decrease in LDL cholesterol among the Soy+ relative to the Milk group in our study produced a net decrease for the Soy+ group, but this was neither statistically significant nor clinically relevant.

The possibility that isoflavones are responsible for "up to 60% of the hypocholesterolemic activity of soy proteins" (3) has been examined with use of several approaches. Baum et al (14) tested the effects of soy protein containing 2 doses of isoflavone (56 and 90 mg/d) and a milk-based protein containing negligible isoflavones on serum lipids. Both soy groups had greater decreases in non-HDL-cholesterol concentrations at 24 wk than did the milk-based protein group, but there was no significant difference between the 2 soy groups. Nestel et al (15) also reported a lack of a hypocholesterolemic effect of isoflavones in a trial contrasting a daily tablet containing 80 mg isoflavones with a placebo. In a study of cynomolgus monkeys, intact soy protein significantly lowered LDL-cholesterol concentrations relative to casein-lactalbumin, but the LDL-cholesterol effect of an isoflavone rich extract added to casein-lactalbumin was not significantly different from that of the casein-lactalbumin alone (17). Sirtori et al (16) reported that the soy product they used reduced LDL-cholesterol concentrations significantly among hypercholesterolemic men but contained only trace amounts of isoflavones. Taken together, these studies do not support a hypocholesterolemic effect of isoflavones.

In contrast with the studies cited above, Crouse et al (13) reported a significant effect of isoflavones on LDL-cholesterol concentrations and a dose response by using isolated soy protein containing 3, 27, 37, or 62 mg isoflavones. Notably, ancillary analyses in this study showed that the effect was restricted to the subset of participants with LDL-cholesterol concentrations greater than the median concentration who had an average LDL-cholesterol concentration of 4.8 mmol/L. In agreement with these findings, 2 separate trials with rhesus (11) and cynomolgus (12) monkeys showed a greater LDL-cholesterol-lowering effect of isoflavone-rich soy supplements compared with supplements

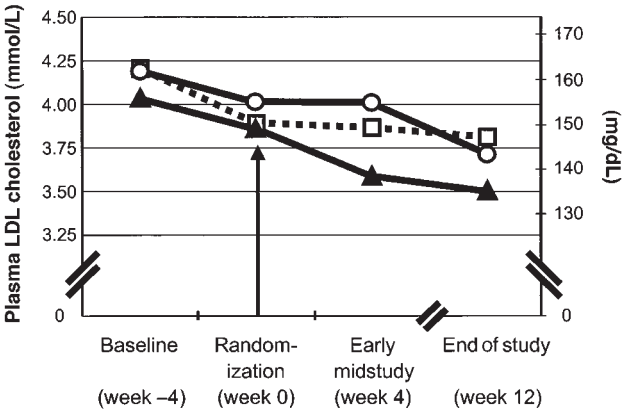

FIGURE 2. Plasma LDL-cholesterol concentrations at all 4 time points of data collection in subjects consuming milk protein (Milk group, ○; n = 30), soy protein containing trace amounts of aglycone isoflavones (Soy− group, □; n = 33), or soy protein containing 80 mg aglycone isoflavones (Soy+ group, ▲; n = 31).

with isoflavones that were mostly extracted. Notably, in both these studies the average "LDL-cholesterol + VLDL-cholesterol" concentrations of the monkeys were in a range that would be considered very high in humans (5.8–11.1 mmol/L). Therefore, if there is a hypocholesterolemic effect of soy isoflavones, the effect may be restricted to persons with relatively high LDL-cholesterol concentrations and to isoflavones found in intact soy-protein preparations (3, 15–17). The women in our study were considered to have moderately elevated LDL-cholesterol concentrations (3.4–4.9 mmol/L at screening), lower than the concentrations in the 3 trials noted above.

The hypothesis that soy isoflavones influence plasma lipids would be strengthened if there were a mechanistically plausible basis for such an effect. At this time, such supportive data do not appear to be available. Baum et al (14) reported an increase in mononuclear cell LDL receptor messenger RNA with soy intake relative to animal protein, but no differences were noted between the intact soy protein and a soy protein with reduced isoflavone content. Nogowski et al (32) reported an effect of genistein on lipid metabolism in rat liver and adipose tissue, but supraphysiologic concentrations were used to elicit the changes. In the absence of data showing a direct mechanistic effect of isoflavones on serum lipids, it has been suggested that the chemical and biological similarities of isoflavones with mammalian estrogens might lead to a beneficial lipid effect (9, 13, 33).

A variety of potential health benefits of soy and soy components are currently being examined by many investigators worldwide. Some of the components of soybeans that might confer these health benefits are fiber, unsaturated fat, folate, arginine, sterols, saponins, and tyrosine kinase inhibitors. There are the additional potential health effects of soy consumption related to foods that may be replaced when soy intake is increased (eg, animal protein sources that are also important sources of saturated fat). The results of the current study specifically address the possible effect of soy protein and isoflavone intake on plasma lipids. In this study, we observed a decrease in LDL-cholesterol concentrations in all 3 protein-supplemented groups. However, our results do not support a benefit of soy-protein supplements at the dose provided, with or without the alcohol extractable fraction of soy that contains isoflavones, relative to milk protein, on plasma lipids in healthy, moderately hypercholesterolemic, postmenopausal women. Future trials designed to specifically address the possibility that the effect of isoflavones on cholesterol concentrations is restricted to adults with higher concentrations than those observed in the women studied here may be warranted.

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