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[1–4]

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ABSTRACT

Background: Studies have shown that soy protein reduces some atherogenic lipid and lipoprotein concentrations, although lipoprotein(a) concentrations may be increased. The dose response of soy protein has not been established; neither has its effect on plasma total homocysteine.

Objective: Our objective was to evaluate the effect of 2 doses of soy protein on lipid, lipoprotein, and homocysteine concentrations.

Design: Four to 24 wk after being instructed to consume a lipid-lowering diet, 130 men and women with LDL-cholesterol concentrations ≥ 4 mmol/L were studied during a parallel group trial in which 4 interventions were assigned randomly. Thirty grams isolated soy protein (ISP) and 10 g cotyledon fiber or 50 g ISP and 16.6 g cotyledon fiber or equivalent doses of casein and cellulose were consumed daily as a beverage for 16 wk.

Results: When the 2 groups who consumed ISP were compared with the 2 groups who consumed casein, the differences in the net changes from baseline to week 16 in the concentrations of LDL cholesterol and plasma total homocysteine were −0.26 mmol/L (95% CI: −0.43, −0.09 mmol/L; $P = 0.01$) and −0.8 μmol/L (−1.4, −0.2 μmol/L; $P = 0.005$), respectively. The effect of the ISP dose was not significant. There were no significant differences between the 2 ISP and the 2 casein groups in changes in lipoprotein(a), HDL-cholesterol, or triacylglycerol concentrations.

Conclusions: Adding 30–50 g soy protein/d to a lipid-lowering diet significantly reduced LDL-cholesterol concentrations without increasing lipoprotein(a) concentrations. Plasma total homocysteine concentrations also decreased, suggesting a novel, possibly antiatherosclerotic effect. *Am J Clin Nutr* 2002;76:78–84.

KEY WORDS Soy protein, casein, heart disease, diet, LDL cholesterol, apolipoprotein B, lipoprotein(a), homocysteine

INTRODUCTION

In a meta-analysis published in 1995, Anderson et al (1) concluded that the consumption of soy protein decreases total and LDL-cholesterol concentrations by 9% and 13%, respectively. Subsequent studies showed smaller effects of ≈2–7% or no effect of soy protein, isolated soy protein (ISP), or tofu on total, LDL-, or non-HDL-cholesterol concentrations (2–10). Adverse effects of soy protein on HDL-cholesterol, triacylglycerol, and lipoprotein(a) [Lp(a)] concentrations have also been reported (6, 8, 11).

Controversial results in soy protein studies can be attributed to variations in study design, including the choice of product and the length of intervention (4). Soy products composed of different amounts of soy protein and isoflavones have been consumed for varying lengths of time. The average dose of soy protein in studies included in the meta-analysis was 47 g/d (1). In more than one-third of the studies, ≤31 g soy protein/d was consumed, and some of those studies showed reductions in LDL-cholesterol concentrations. A recent study showed reductions of ≈2–3% in total and non-HDL-cholesterol concentrations in men who consumed ≥20 g soy protein/d. However, there was no dose response in groups who received 30, 40, or 50 g soy protein/d (9). In 3 other studies, there were either no effects or inconsistent effects of consuming 40–60 g soy protein/d (2, 3, 10). In studies that included only men, effects appeared after ≤4 wk (9), whereas Baum et al (3) reported that among postmenopausal women, reductions in non-HDL-cholesterol concentrations were observed only after 24 wk. Moreover, although LDL receptor messenger RNA concentrations were significantly lower in the casein group than in the soy group after 12 wk, there were no differences in non-HDL-cholesterol concentrations at that time (3). A subsequent trial, powered to detect a 6% difference in LDL-cholesterol concentrations, showed that 25 g soy protein/d produced the expected reduction in LDL-cholesterol concentrations (5). Although this study suggested that soy protein extracted with alcohol, which removes most of the isoflavones, has a minimal effect on lipid concentrations, other studies have shown no effect of isolated isoflavones on lipid concentrations (12, 13). Furthermore, the hypocholesterolemic effects of soy have been reported with products in which isoflavones were removed (14). Thus, major issues related to the effects of soy protein on serum lipid and lipoprotein concentrations remain unresolved (15).

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In the present study, we examined the long-term (16 wk) dose-response effect of consuming 30 or 50 g ISP with high fixed amounts of isoflavones/d on lipid and lipoprotein concentrations. Because the concentrations of homocysteine, fibrinogen, and some fat-soluble vitamins have been shown to influence the risk of atherosclerosis (16–18), we asked whether the consumption of soy protein compared with that of casein affects these risk factors.

SUBJECTS AND METHODS

Subjects

Subjects were recruited at 2 centers in Oslo by newspaper announcement or from patient lists. Men aged 30–70 y and postmenopausal women aged 45–70 y with total cholesterol (TC) concentrations of 5.8–7.9 mmol/L and triacylglycerol concentrations <4.5 mmol/L were eligible to participate in the study. Postmenopausal status was assumed if no vaginal bleeding had occurred during the 6 mo before the study and if serum follicle stimulating hormone and estradiol concentrations were ≥26 IU/L and ≤0.2 nmol/L, respectively. The exclusion criteria were significant cardiovascular, renal, hepatic, gastrointestinal, and endocrine disease, including diabetes mellitus type I or type II treated with drugs; familial hypercholesterolemia; obesity [body mass index (in kg/m^2) ≥30]; and uncontrolled hypertension (blood pressure >160/100 mm Hg). Subjects who planned to lose weight during the study, who consumed more than 28 units alcohol/wk, or who had taken cholesterol-lowering statins, n−3 fatty acid supplements, or other lipid-lowering drugs within 8 wk of the start of the study and women who received hormone replacement therapy within 6 mo of the start of the study were also excluded. The study protocol was approved by the Regional Ethics Committee for Medical Research. Informed consent forms were signed by all subjects before the start of the study.

Of the 192 subjects screened, 33 were subsequently ineligible or withdrew consent before randomization. Thus, 159 subjects (134 men and 25 women) were randomly assigned to 1 of 4 treatment groups. Of these, 19 withdrew or were withdrawn from the analysis for various reasons: 2 were lost to follow-up, 9 withdrew consent or were unable to comply with the study protocol, 1 developed angina pectoris, 1 complained of dizziness, and 6 complained of gastrointestinal problems (eg, diarrhea, constipation, nausea, and vomiting). One withdrawal was from the 30-g ISP group, 4 withdrawals were from the 30-g casein group, 6 withdrawals were from the 50-g ISP group, and 8 withdrawals were from the 50-g casein group. An additional 10 subjects were excluded from the statistical analyses: 2 subjects, both in the 50-g casein group, gained or lost >5.0 kg body weight during the study, and 8 subjects, of whom 4 were from the 30-g ISP group, 3 from the 50-g ISP group, and 1 from the 50-g casein group, failed to consume ≥70% of the assigned protein dose. In summary, the rates of withdrawal from the 30-g ISP, 30-g casein, 50-g ISP, and 50-g casein groups were 13%, 10%, 23%, and 28%, respectively, leaving 108 men and 22 women. One-way multiple analysis of variance showed no differences in any of the baseline characteristics between the subjects who were excluded or did not complete the study (n = 29) and the rest of the subjects (n = 130) (data not shown).

Diet

All subjects were asked to consume a cholesterol-lowering diet similar to the American Heart Association Step I diet before and after randomization. Thus, the recommended intakes were <30% of energy from total fat, <10% of energy from saturated fat, and <300 mg dietary cholesterol/d. Instruction on fat intake was reinforced with a food exhibit that indicated the amounts of saturated fat in ordinary foods. After dietary instruction, subjects were required to complete a 3-d weighed dietary record. The 3 d were randomly chosen and included 2 weekdays and a Saturday or a Sunday. Subjects received instruction on the cumulative weighing technique, including how to use a digital dietary scale. All food and beverages consumed were described and weighed. The use of household measures as a substitute for weighing was acceptable when it was impossible to weigh the foods. The weighed dietary record was repeated 12 wk after randomization. Data from the dietary records were calculated with the use of the software program MAT PÅ DATA (version 3.0; 19) on the basis of the Norwegian food table (20). The weight and nutrient composition of foods and recipes that were not part of the program were calculated and added. Vitamin supplements were not included in the analyses. Overall, 14% of the subjects took a multivitamin; this proportion did not differ between the 4 groups (data not shown). One subject in each of the ISP groups reported taking vitamin E supplements, and one subject in the 30-g ISP group took a vitamin B-6 supplement (only reported in the baseline dietary record).

Study design

Instruction to consume the Step I diet was given a minimum of 4 and a maximum of 24 wk before baseline. After this, baseline blood samples were drawn, and subjects whose LDL-cholesterol concentration was still ≥4 mmol/L were randomly assigned to 1 of the 4 treatment groups for 16 wk. All subjects were randomly assigned in February or March of the same year to minimize seasonal changes in lipid concentrations. The 4 groups continued to consume the cholesterol-lowering diet and were asked to consume one packet of ISP (FXP HO159 IP Non-GM; Protein Technologies International, St Louis) with high fixed isoflavones (3.7 mg total isoflavone aglycone units/g protein) and cotyledon fiber (15 g protein and 5 g fiber or 25 g protein and 8.3 g fiber) or one packet of casein and cellulose (15 g protein and 5 g fiber or 25 g protein and 8.3 g fiber) a day at breakfast and one packet a day at the evening meal, in the place of 1 or 2 pieces of bread with spread. The test products were incorporated into ready-to-mix beverages. Throughout the study, all subjects were asked to maintain their body weight. Subjects and investigators were blinded to the source of test product during the course of the trial, but because of the different sizes of the boxes containing the test products, the study coordinator and dietitian was not blinded to whether low or high doses were assigned.

After randomization, clinic visits were conducted every 4 wk. Body weight was measured by using a digital scale, and vital signs and changes in symptoms and concomitant medications were recorded. At each clinic visit study protein was delivered, and any leftover packets from the previous visit were collected to determine compliance.

Laboratory analyses

Blood sampling was done at each visit in the morning after the subjects had fasted for 10–12 h. Samples were analyzed at a central laboratory. Serum total cholesterol and triacylglycerol concentrations were measured enzymatically, and HDL cholesterol

TABLE 1
Baseline characteristics of the subjects[1]

	Treatment group			
	30 g ISP (n = 27 M, 7 F)	30 g Casein (n = 29 M, 7 F)	50 g ISP (n = 26 M, 5 F)	50 g Casein (n = 26 M, 3 F)
Age (y)	51.4 ± 9.8	52.3 ± 9.7	54.1 ± 7.2	52.0 ± 7.3
BMI (kg/m^2)				
Men	25.7 ± 2.4	25.6 ± 2.3	25.5 ± 2.0	25.3 ± 2.1
Women	24.4 ± 1.6	23.7 ± 2.6	25.0 ± 2.3	24.5 ± 0.4
Systolic BP (mm Hg)	135 ± 13	135 ± 14	135 ± 17	133 ± 14
Diastolic BP (mm Hg)	86 ± 7	85 ± 7	85 ± 9	85 ± 8
Total cholesterol (mmol/L)	6.9 ± 0.5	7.0 ± 0.6	6.9 ± 0.6	7.0 ± 0.5
LDL cholesterol (mmol/L)	4.9 ± 0.5	4.9 ± 0.6	4.8 ± 0.5	5.0 ± 0.5
HDL cholesterol (mmol/L)	1.5 ± 0.4	1.4 ± 0.4	1.5 ± 0.3	1.4 ± 0.4
Triacylglycerols (mmol/L)	1.3 ± 0.5	1.6 ± 0.7	1.4 ± 0.8	1.4 ± 0.5

[1] $\bar{x}$ ± SD. ISP, isolated soy protein; BP, blood pressure. There were no significant differences between the groups.

was quantified directly with the use of polyethylene glycol–modified enzymes (all with commercial kits from Roche Diagnostics, Mannheim, Germany). The concentrations of LDL cholesterol were calculated according to the formula of Friedewald et al (21). Serum apolipoprotein B (apo B) and Lp(a) concentrations were both quantified immunoturbidimetrically with the use of an automated analyzer (Roche Diagnostics). The interassay CVs were as follows: total cholesterol, <2.2%; HDL cholesterol, <6.0%; triacylglycerols, <2.2%; apo B, <2.0%; Lp(a), <5.0%. For analysis of plasma total homocysteine, blood samples collected in EDTA-coated tubes were immediately placed on ice, and the plasma was separated within 30 min. Total plasma homocysteine was measured with the use of HPLC and fluorescence detection at the Department of Clinical Chemistry, Haukeland Hospital, Bergen, Norway (22). The precision (between-day CV) of the assay was <3%. Serum concentrations of α-tocopherol were measured with the use of HPLC and fluorescence detection (Agilent Technologies, Palo Alto, CA) (between-day CV: <5%). Plasma fibrinogen was measured with the use of an automated clotting assay (between-day CV: <4%), and serum concentrations of folate were measured with the use of an automated immunoassay system (Auto-Delfia; Wallac, Helsinki) (between-day CV: 8.2%). Serum concentrations of vitamin B-12 were measured with the use of an automated immunoassay system (Automated Chemiluminescence System; Bayer Corporation, East Walpole, MA) (between-day CV: 4.4%).

Statistical analysis

We chose subjects with elevated LDL-cholesterol concentrations to potentially maximize the effect of soy protein, in accordance with previous data. The trial was designed to have 90% power to detect a 10% relative change in total cholesterol concentrations (assuming an SD of the reduction to be 10%) between groups for pairwise group comparisons at the 5% two-tailed level of significance. The required number of subjects per group was 26; to allow for dropouts, 40 subjects were randomly assigned to each group.

To test whether there were any significant differences between the 4 groups at baseline, we used a one-way multiple analysis of variance test. The means of 3 measurements of serum lipids taken at weeks −2, −1, and 0 (ie, before randomization) were calculated and were used as the baseline values. The results when only the week 0 value was used were not different from the results when the mean of the 3 values was used (data not shown). Because previous data did not consistently indicate dose-response effects of soy protein on lipid concentrations, we were interested in the changes in both of the soy protein and casein groups. Changes in values over time for each variable were analyzed by a two-factor repeated-measures analysis of variance to determine the effects of time, treatment, and dose and the interactions between the effects of time, treatment, and dose. If a significant interaction between time and treatment or dose (or both) was found, we used an estimate of the difference in the change from baseline to week 16 (with a 95% CI) between the groups as a measure of the effect of treatment and dose. Spearman's correlation coefficient was used to calculate the correlation between plasma total homocysteine concentrations and concentrations of vitamin B-12 and folate. Statistical analyses were performed with the use of SAS software (version 8.1; SAS Institute, Inc, Cary, NC). Significance for all analyses was set at $P < 0.05$.

RESULTS

The baseline characteristics of the subjects are summarized in **Table 1**. The fasting serum lipid profiles were consistent with the criteria for participation in the study. The nutrient intakes of the subjects at baseline and at week 12 are shown in **Table 2**. Nutrient intakes were not significantly different between the 4 groups. For each of the 4 groups, there was a small but significant ($P < 0.01$) decrease in reported energy intake between baseline and week 12. Subjects from all groups consumed ≈18% of daily energy as protein, 50% as carbohydrate, and 28% as fat (10% as saturated, 10% as monounsaturated, and 5% as polyunsaturated). The median change in body weight between baseline and the end of the study was 0 kg (range: −4.9 to 5.0 kg) in the groups who consumed 30 g ISP, 30 g casein, or 50 g ISP and 0.5 kg (range: −4.0 to 3.5 kg) in the group who consumed 50 g casein. The median compliance with study protein was 95%, 97%, 93%, and 92% in the 30-g ISP, 30-g casein, 50-g ISP, and 50-g casein groups, respectively.

Lipid and lipoprotein concentrations measured at baseline and at subsequent visits are shown in **Table 3**. The difference in the change in serum TC concentration from baseline to week 16 between the 2 ISP and the 2 casein groups was −0.24 mmol/L (95% CI: −0.43, −0.04 mmol/L; $P = 0.01$ for the interaction between time and treatment). This difference was because of a

TABLE 2
Nutrient intakes of the subjects at baseline and at week 12, calculated from 3-d weighed-food diaries[1]

	Treatment group			
	30 g ISP (n = 34)	30 g Casein (n = 36)	50 g ISP (n = 31)	50 g Casein (n = 29)
Energy (MJ)				
Baseline	8.6 ± 3.1	8.5 ± 2.7	9.1 ± 3.5	8.7 ± 2.5
Week 12	7.6 ± 2.2[2]	7.0 ± 2.2[2]	7.4 ± 2.2[2]	7.0 ± 1.7[2]
Protein (% of energy)				
Baseline	17.9 ± 2.7	17.5 ± 3.2	19.0 ± 7.8	18.2 ± 4.0
Week 12	17.5 ± 3.1	18.0 ± 3.3	18.0 ± 3.7	17.0 ± 2.8
Carbohydrate (% of energy)				
Baseline	50.8 ± 8.6	49.9 ± 6.4	51.2 ± 8.8	49.3 ± 9.2
Week 12	47.3 ± 8.0	48.7 ± 6.9	50.0 ± 9.0	49.3 ± 8.3
Fat (% of energy)				
Baseline	26.6 ± 6.8	30.4 ± 5.8	26.4 ± 5.5	29.3 ± 8.0
Week 12	29.6 ± 7.4	30.8 ± 6.9	28.6 ± 7.5	30.8 ± 6.2
Saturated fat (% of energy)				
Baseline	9.4 ± 3.0	10.5 ± 2.7	9.1 ± 2.8	10.0 ± 3.1
Week 12	10.0 ± 3.0	10.4 ± 2.9	9.8 ± 3.9	11.1 ± 3.0
Monounsaturated fat (% of energy)				
Baseline	9.1 ± 2.8	11.2 ± 3.2	9.6 ± 2.6	10.6 ± 3.8
Week 12	10.2 ± 3.1	11.0 ± 3.5	10.9 ± 3.8	10.0 ± 2.1
Polyunsaturated fat (% of energy)				
Baseline	4.9 ± 1.4	5.6 ± 1.7	4.6 ± 1.7	5.3 ± 2.2
Week 12	6.0 ± 2.6	5.9 ± 2.1	4.8 ± 1.5	5.8 ± 2.5
Cholesterol (mg)				
Baseline	233 ± 107	209 ± 102	224 ± 111	231 ± 152
Week 12	199 ± 97	173 ± 76	206 ± 143	194 ± 113
Alcohol (g)				
Baseline	14.5 ± 15.8	7.1 ± 10.4	8.8 ± 10.6	9.4 ± 9.6
Week 12	13.8 ± 15.2	6.8 ± 9.5	8.1 ± 10.1	7.7 ± 10.6

[1] $\bar{x}$ ± SD. ISP, isolated soy protein. There were no significant differences in changes between the groups.
[2] Significantly different from baseline, $P < 0.01$.

difference in the change in LDL-cholesterol concentration from baseline to week 16 between the 2 ISP and the 2 casein groups of −0.26 mmol/L (95% CI: −0.43, −0.09 mmol/L; $P = 0.01$ for the interaction between time and treatment); however, in the case of both TC and LDL-cholesterol concentrations, the interaction between time and dose was not significant, indicating no effect of dose. Changes in the concentrations of HDL cholesterol, triacylglycerols, apo B, Lp(a), and α-tocopherol were not significantly different between the 2 ISP and the 2 casein groups (Table 3).

Baseline concentrations of plasma total homocysteine were inversely associated with serum vitamin B-12 concentrations (Spearman's $\rho = -0.20$, $P = 0.03$) and with serum folate concentrations (Spearman's $\rho = -0.42$, $P < 0.001$) as expected. As shown in **Table 4**, at the end of week 16 the difference in the change in plasma total homocysteine concentration from baseline between the 2 ISP and the 2 casein groups was −0.8 μmol/L (95% CI: −1.4, −0.2 μmol/L; $P = 0.005$ for the interaction between treatment and time). There were no significant differences in the changes in folate, vitamin B-12, or fibrinogen concentrations between the 2 ISP and the 2 casein groups (Table 4).

The results given above for the 130 compliant subjects were not significantly different from those obtained in the intention-to-treat analysis, in which all subjects who received treatment for ≥4 wk after randomization and who were examined at week 4 or later were included. In summary, in the intention-to-treat analysis, there was a significant difference in the decrease in total and LDL-cholesterol concentrations from baseline ($P = 0.03$ and 0.02, respectively) between the 2 ISP and the 2 casein groups. There was also a significant difference in the change in plasma total homocysteine concentrations from baseline (a decrease in the 2 ISP groups but an increase in the 2 casein groups, $P = 0.009$). There were no significant differences between the 2 ISP and the 2 casein groups in the change in HDL-cholesterol, triacylglycerol, or Lp(a) concentrations.

DISCUSSION

Studies have fairly consistently shown that soy protein decreases total and LDL-cholesterol concentrations (1). However, the mechanisms of action are uncertain, given the absence of a clear dose response (9, 12). We attempted to delineate the effects of 30-g and 50-g doses of ISP on lipid, lipoprotein, and plasma total homocysteine concentrations within the same study and with the use of matching casein control groups. Total and LDL-cholesterol concentrations decreased ≈3–4% more in the groups who consumed ISP than in those who consumed casein, and there were no significant differences in changes in Lp(a) concentrations between the ISP and casein groups. A novel finding was that plasma total homocysteine concentrations were lower in the groups who consumed ISP than in those who consumed casein.

The causes of variability in lipid responses to soy protein remain unknown (15). The difference in the change in serum TC concentrations between the 2 ISP and the 2 casein groups in the present study was −0.24 mmol/L, which was less than

TABLE 3
Serum concentrations of lipids, lipoproteins, and α-tocopherol at baseline and follow-up in subjects who consumed 2 doses of soy protein or casein[1]

	Treatment group				
	30 g ISP (n = 34)	30 g Casein (n = 36)	50 g ISP (n = 31)	50 g Casein (n = 29)	Difference in change from baseline[2] (30 + 50 g ISP − 30 + 50 g casein)
Total cholesterol (mmol/L)					
Baseline	6.86 ± 0.67[3]	6.84 ± 0.72	6.52 ± 0.63	6.90 ± 0.54	
Week 4	6.37 ± 0.60	6.68 ± 0.72	6.12 ± 0.84	6.53 ± 0.65	
Week 8	6.38 ± 0.72	6.59 ± 0.67	6.00 ± 0.86	6.43 ± 0.63	
Week 12	6.24 ± 0.70	6.64 ± 0.65	5.90 ± 0.76	6.58 ± 0.52	
Week 16	6.00 ± 0.55	6.29 ± 0.54	5.61 ± 0.71	6.13 ± 0.53	−0.24 (−0.43, −0.04)[4]
HDL cholesterol (mmol/L)					
Baseline	1.48 ± 0.42	1.26 ± 0.39	1.35 ± 0.33	1.28 ± 0.30	
Week 4	1.57 ± 0.40	1.34 ± 0.36	1.44 ± 0.31	1.41 ± 0.36	
Week 8	1.58 ± 0.44	1.31 ± 0.34	1.46 ± 0.35	1.35 ± 0.32	
Week 12	1.55 ± 0.45	1.37 ± 0.38	1.47 ± 0.36	1.38 ± 0.37	
Week 16	1.56 ± 0.42	1.33 ± 0.36	1.47 ± 0.33	1.33 ± 0.37	0.04 (−0.02, 0.10)
LDL cholesterol (mmol/L)					
Baseline	4.81 ± 0.54	4.88 ± 0.66	4.58 ± 0.50	4.96 ± 0.50	
Week 4	4.26 ± 0.57	4.62 ± 0.64	4.08 ± 0.72	4.47 ± 0.58	
Week 8	4.31 ± 0.69	4.62 ± 0.68	4.01 ± 0.69	4.48 ± 0.66	
Week 12	4.19 ± 0.54	4.61 ± 0.62	3.91 ± 0.57	4.57 ± 0.39	
Week 16	3.94 ± 0.45	4.33 ± 0.56	3.63 ± 0.53	4.18 ± 0.45	−0.26 (−0.43, −0.09)[4]
Triacylglycerols (mmol/L)					
Baseline	1.28 ± 0.38	1.57 ± 0.76	1.33 ± 0.70	1.46 ± 0.67	
Week 4	1.19 ± 0.47	1.57 ± 0.81	1.33 ± 0.64	1.45 ± 0.77	
Week 8	1.09 ± 0.40	1.47 ± 0.74	1.18 ± 0.73	1.33 ± 0.64	
Week 12	1.12 ± 0.43	1.46 ± 0.67	1.15 ± 0.58	1.39 ± 0.74	
Week 16	1.12 ± 0.45	1.40 ± 0.69	1.13 ± 0.62	1.36 ± 0.65	−0.04 (−0.22, 0.14)
Lp(a) (mg/L)					
Baseline	437 ± 410	405 ± 314	417 ± 498	367 ± 409	
Week 8	481 ± 451	438 ± 351	516 ± 603	412 ± 485	
Week 16	482 ± 455	443 ± 362	498 ± 580	489 ± 415	25 (−4, 54)
Apolipoprotein B (g/L)					
Baseline	1.32 ± 0.12	1.40 ± 0.17	1.28 ± 0.16	1.37 ± 0.13	
Week 8	1.24 ± 0.16	1.36 ± 0.17	1.19 ± 0.17	1.30 ± 0.17	
Week 16	1.25 ± 0.15	1.38 ± 0.17	1.17 ± 0.17	1.30 ± 0.12	−0.04 (−0.09, 0.00)
α-Tocopherol (μmol/L)					
Baseline	37.5 ± 6.3	40.5 ± 7.7	37.9 ± 11.5	37.5 ± 6.4	
Week 8	36.4 ± 7.2	37.9 ± 8.8	34.7 ± 8.1	35.2 ± 5.9	
Week 16	36.6 ± 6.6	40.8 ± 11.4	34.4 ± 8.8	36.8 ± 7.8	−2.5 (−5.4, 0.5)

[1] ISP, isolated soy protein; Lp(a), lipoprotein(a).
[2] 95% CI in parentheses.
[3] $\bar{x}$ ± SD.
[4] P = 0.01 for the interaction between time and treatment.

the 95% CI (−0.85, −0.35 mmol/L) of the decrease in the meta-analysis conducted by Anderson et al (1). A number of well-controlled trials, as well as most of the studies included in the meta-analysis when considered individually (1, 15), failed to show differences in serum lipid concentrations between subjects who consumed soy and those who consumed milk protein within the time frame of our study (2, 3, 10, 23). Variability in responses was reported in persons who consumed liquid-formula diets containing soy protein or casein (24). However, the subjects in that study were normolipidemic and thus may have been less likely than hyperlipidemic subjects (1) to show decreases in serum cholesterol concentration.

Baum et al (3) found no change in serum TC concentrations in hypercholesterolemic women who consumed 40 g soy protein or casein, in contrast to previous and subsequent studies by the same group in hypercholesterolemic men (9). Consumption of soy protein by moderately hypercholesterolemic men also decreases non-HDL cholesterol (9). Subsequently, Gardner et al (10) failed to show a lipid-lowering effect of soy protein compared with milk protein in postmenopausal women. Sex did not affect the response to soy protein in the meta-analysis (1). In the present study, too few women were recruited to account for the effects of sex. Future trials should be planned to have adequate power to compare responses in men and women within the same study.

Despite adequate power, we were unable to show a dose-response effect of ISP compared with casein. In the meta-analysis, the effect of the amount of soy protein ingested on serum TC concentrations was estimated without accounting for the effects of the control diet (1). When the effects of the control diet were calculated, the dose of soy protein that was tested did not affect the response; nor was a dose-response effect shown in the study by Teixeira et al (9). Consuming amounts of soy protein >20–30 g/d is impracticable, and we noted that more subjects in the 50-g casein or ISP groups than in the 30-g casein or ISP groups were withdrawn from the study because of low compliance or side effects. Our findings thus confirm those of earlier studies that

TABLE 4
Plasma total homocysteine and serum fibrinogen, vitamin B-12, and folate concentrations at baseline and follow-up in subjects who consumed 2 doses of soy protein or casein[1]

	Treatment group				
	30 g ISP (n = 34)	30 g Casein (n = 36)	50 g ISP (n = 31)	50 g Casein (n = 29)	Difference in change from baseline[2] (30 + 50 g ISP − 30 + 50 g casein)
Plasma total homocysteine (μmol/L)					
Baseline	10.0 ± 2.2[3]	10.1 ± 2.2	10.6 ± 2.9	10.6 ± 2.5	
Week 8	10.7 ± 1.9	11.0 ± 2.6	10.8 ± 2.6	12.1 ± 3.5	
Week 16	9.8 ± 1.4	10.6 ± 2.5	10.4 ± 2.9	11.5 ± 2.7	−0.8 (−1.4, −0.2)[4]
Serum fibrinogen (g/L)					
Baseline	3.0 ± 0.4	3.1 ± 0.5	3.2 ± 0.5	3.1 ± 0.6	
Week 8	3.1 ± 0.4	3.2 ± 0.7	3.3 ± 0.4	3.2 ± 0.4	
Week 16	3.1 ± 0.4	3.3 ± 0.6	3.2 ± 0.4	3.3 ± 0.7	−0.1 (−0.3, 0.1)
Serum vitamin B-12 (nmol/L)					
Baseline	300 ± 55	320 ± 77	316 ± 89	308 ± 75	
Week 8	284 ± 59	325 ± 79	306 ± 79	305 ± 62	
Week 16	278 ± 60	314 ± 82	294 ± 74	302 ± 64	−15 (−31, 1)
Serum folate (nmol/L)					
Baseline	13.0 ± 5.7	14.1 ± 4.8	15.4 ± 8.1	14.8 ± 7.7	
Week 8	13.7 ± 5.0	13.3 ± 4.4	15.4 ± 7.6	12.8 ± 5.9	
Week 16	13.5 ± 4.7	13.5 ± 6.4	14.5 ± 7.4	11.7 ± 4.3	1.51 (−0.08, 3.09)

[1] ISP, isolated soy protein.
[2] 95% CI in parentheses.
[3] $\bar{x}$ ± SD.
[4] P = 0.005 for the interaction between treatment and time.

suggest that larger amounts apparently do not confer any additional benefit on serum lipid and lipoprotein concentrations (1, 9).

The decreases in total and LDL-cholesterol concentrations in the casein groups were greater than expected, possibly because of seasonal or dietary adaptation. In many dietary studies, it has been difficult to show a dose response, often because changes in the intake of other dietary components occur when dietary supplements are consumed (25). Although the reported dietary intake in the present study does not explain the lack of additional cholesterol-lowering effects in the group who consumed 50 g ISP compared with the group who consumed 30 g ISP, self-reported dietary data have inherent limitations (25).

Our finding that there was no difference in the change in HDL-cholesterol and triacylglycerol concentrations from baseline between the 2 ISP and the 2 casein groups is consistent with those of most studies published since the meta-analysis (1), with the exception of Baum et al (3), who found an increase in HDL-cholesterol concentrations among postmenopausal women consuming soy protein. We were unable to show a difference in the change in apo B concentrations between the ISP and casein groups, although the P value (0.10) for the interaction between treatment and time was nearly significant, indicating a trend toward a decrease in the ISP groups. Teixeira et al (9) have explained reports of decreases or no change in apo B concentrations in different studies on the basis of different populations, sample sizes, or both.

An increase in Lp(a) concentration has been reported in subjects receiving liquid-formula diets based on soy protein (11). The component in soy protein that may mediate such an increase is unknown. Previous dietary supplement studies using 25–30-g amounts of soy protein found no increase in Lp(a) (5, 9). Thus, our finding of no difference in the change in Lp(a) concentrations from baseline between the soy and casein groups confirms the results of these previous studies and indicates that consumption of dietary supplements of 30–50 g ISP does not increase Lp(a) concentrations compared with consumption of casein.

Plasma total homocysteine concentration has been shown to be a potent independent indicator of atherosclerotic vascular risk (17). The results of a meta-analysis suggest that an elevation in the plasma total homocysteine concentration of 5 μmol/L is equivalent in risk to a 0.5-mmol/L increase in TC concentration (26). The difference in the change in plasma total homocysteine concentrations between the 2 ISP and the 2casein groups in the present study (−0.8 μmol/L) is within the 0.5–2.0-μmol/L range of differences usually found that have been attributed to lifestyle factors (27). In a study of patients with type II diabetes, the effects on homocysteine concentrations of a soy-based dietary supplement compared with those of casein were found to be similar to those in the present study (28).

An increase in the concentration of homocysteine could arise by inhibition of its 2 major breakdown pathways, both dependent on B vitamins (29). The remethylation pathway depends on folate and vitamin B-12. However, we found no significant parallel changes in folate or vitamin B-12 concentrations, although serum folate decreased by >20% in the 50-g casein group. A number of lifestyle factors, including a high dietary intake of methionine (17), are associated with elevated plasma total homocysteine concentrations. Whether differences in the amino acid compositions of casein, which is rich in lysine, and ISP account for these findings will require further study.

In conclusion, our data suggest favorable effects of 30–50 g soy protein compared with casein on TC, LDL-cholesterol, and plasma total homocysteine concentrations in hyperlipidemic subjects. Subjects who consumed 50 g soy protein/d were somewhat more likely to drop out of the study than were those who consumed only 30 g/d, indicating that the higher amount is impractical. Thus, our data support the dose of soy protein (25 g) that the US Food and Drug Administration allows to carry a health claim.

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