Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women[1–3]

Kerry E Wangen, Alison M Duncan, Xia Xu, and Mindy S Kurzer

ABSTRACT

Background: Soy-protein consumption is known to reduce plasma total and LDL cholesterol concentrations. However, the responsible soy component or components and the magnitude of effects in normocholesterolemic and mildly hypercholesterolemic subjects are unclear.

Objective: The present study examined the effects of soy isoflavone consumption on plasma concentrations of triacylglycerol, apolipoprotein (apo) A-I, apo B, lipoprotein(a), and total, LDL, and HDL cholesterol and on LDL peak particle diameter in normocholesterolemic and mildly hypercholesterolemic postmenopausal women.

Design: In a randomized crossover trial, fasting plasma samples were obtained from 18 postmenopausal women throughout three 93-d periods of daily isolated soy protein (ISP) consumption providing an average of 7.1 ± 1.1 (control), 65 ± 11 (low isoflavone), or 132 ± 22 (high isoflavone) mg isoflavones/d.

Results: Compared with values measured during the control diet, the plasma LDL cholesterol concentration was 6.5% lower ($P < 0.02$) during the high-isoflavone diet and the ratio of LDL to HDL cholesterol was 8.5% and 7.7% lower during the low- and high-isoflavone diets, respectively ($P < 0.02$). Isoflavone consumption did not significantly affect plasma concentrations of total or HDL cholesterol, triacylglycerol, apo A-I, apo B, or lipoprotein(a) or the LDL peak particle diameter.

Conclusions: Consumption of isoflavones as a constituent of ISP resulted in small but significant improvements in the lipid profile in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. Although the effects were small, it is possible that isoflavones may contribute to a lower risk of coronary heart disease if consumed over many years in conjunction with other lipid-lowering strategies. *Am J Clin Nutr* 2001;73:225–31.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death for both men and women in the United States, accounting for ≈42% of all deaths in 1995 (1). The 1998 estimated direct and indirect costs of CVD were >$274 billion in the United States (1). Significant research efforts focusing on the prevention and treatment of this disease have identified elevated plasma cholesterol as a primary risk factor for CVD.

Within the past 25 y, numerous studies have reported inverse associations between soy protein intake and plasma cholesterol concentrations. This association is particularly evident in hypercholesterolemic men and women (2, 3) but is less consistently evident in normocholesterolemic individuals (3–8). At this time, the active component or components and mechanisms of action remain unclear (9–15), although the role of phytoestrogenic isoflavones has recently gained attention.

Several studies comparing isoflavone-rich soy diets with isoflavone-free soy diets have been performed in experimental animals and humans. Studies in nonhuman primates found that consumption of isoflavone-rich diets resulted in decreased total and LDL-cholesterol concentrations (16–18), increased (16, 18) or unchanged (16, 17) HDL-cholesterol concentrations, increased (16) or unchanged (16–18) triacylglycerol concentrations, increased or unchanged apolipoprotein (apo) A-I concentrations (16), unchanged apo B concentrations (16), decreased (16) or unchanged (16, 17) lipoprotein(a) concentrations, and decreased (16) or unchanged (16, 17) LDL molecular weight. A recent study in hypercholesterolemic human subjects consuming isoflavone-rich or isoflavone-depleted soy protein found that the isoflavone-rich soy protein decreased total and LDL cholesterol concentrations; however, the reduction was significant only in a subgroup with the highest baseline LDL cholesterol concentrations (19). A recent study of premenopausal women in our laboratory showed that LDL

cholesterol concentrations and ratios of total to HDL cholesterol and of LDL to HDL cholesterol were lower after consumption of isoflavone-rich soy protein than after consumption of an isoflavone-depleted soy protein (20). Three studies that examined the effects of purified isoflavone capsules compared with placebo in normocholesterolemic and hypercholesterolemic subjects found no significant effects of isoflavone intake on concentrations of plasma lipids (21–23) or lipoprotein(a) (21). At this time, there are no data on the effects of soy, with or without isoflavones, on plasma lipid concentrations in normocholesterolemic and mildly hypercholesterolemic postmenopausal women.

The primary purpose of this study was to assess the effects of soy isoflavones, when consumed in the form of isolated soy protein (ISP) powder, on plasma concentrations of triacylglycerol, apo A-I, apo B, lipoprotein(a), and total, LDL, and HDL cholesterol and on LDL peak particle diameter in healthy normocholesterolemic and mildly hypercholesterolemic postmenopausal women. In a randomized crossover design, 18 free-living subjects consumed each of 3 soy protein isolates that differed only in isoflavone content.

SUBJECTS AND METHODS

Study design

This study was part of a larger study investigating the effects of soy isoflavone consumption on reproductive hormones in postmenopausal women (24). The study protocol was approved by the University of Minnesota Institutional Review Board Human Subjects Committee and all subjects gave their written, informed consent. A randomized crossover design was used; the protocol consisted of three 93-d diet periods separated by 26-d washout periods. The subjects were free-living and consumed their habitual diets supplemented with 1 of 3 ISP powders during each diet period. The subjects were blinded as to which ISP they were receiving.

Subjects

Healthy postmenopausal subjects between 45 and 70 y of age were selected after they completed a telephone questionnaire, an interview, and a health screening. The exclusion criteria included the following: consumption of a strict vegetarian, high-fiber, high-soy, or low-fat diet; regular vitamin and mineral supplementation exceeding the recommended dietary allowances; athleticism; cigarette smoking; antibiotic or hormone use within the past 6 mo; menstrual bleeding within the past 12 mo; hysterectomy or oophorectomy; follicle-stimulating-hormone concentration <25 IU/L; history of chronic disorders, including endocrine or gynecologic diseases; regular use of medication (eg, aspirin) known to interfere with the study endpoints; body mass index (BMI; in kg/m^2) <18 or >35; weight changes of >4.5 kg within the previous year; and inability to abstain from alcoholic beverages during the study. Twenty-three women were enrolled in the study.

Experimental diet

The subjects consumed their habitual diets. We asked them to follow detailed instructions to minimize phytoestrogen consumption by avoiding soy, flaxseed, and sprouts and by limiting legume consumption to one serving per week. Subjects were also required to avoid alcoholic beverages during the intervention periods. The subjects' usual diets were supplemented with each

of 3 ISP beverage powders (Supro Brand Isolated Soy Protein; Protein Technologies International, St Louis).

The nutrient and isoflavone composition of the 3 ISP powders was described previously (24). The ISP powders had similar macronutrient compositions but different concentrations of isoflavones (0.12, 1.00, and 2.01 mg total isoflavones/g protein for the control, low-isoflavone, and high-isoflavone ISPs, respectively). The 3 ISPs provided 0.11 ± 0.01 (control), 1.00 ± 0.01 (low isoflavone), and 2.00 ± 0.02 (high isoflavone) mg total isoflavones/kg body wt/d (7.1 ± 1.1, 65 ± 11, and 132 ± 22 mg isoflavones/d, respectively), expressed as aglycone units. The isoflavone content was evaluated with HPLC (Patricia Murphy, Food Science and Human Nutrition Department, Iowa State University, Ames, IA) as described previously (25). The ISP used as the control diet was intended to be isoflavone-free; however, it contained a very low concentration of isoflavones as a result of incomplete alcohol extraction.

The ISP beverage powders were provided in daily dosage packets and were kept refrigerated until the day of consumption. The amount of ISP powder consumed per day averaged 85 g and provided an average of 1.46 MJ (348 kcal), 63 g protein, 21 g carbohydrate, 1.9 g fat, and no cholesterol or fiber. To confirm consumption of the ISP, we assessed urinary phytoestrogen excretion by analyzing three 24-h urine samples per subject per month.

Study procedures

Blood samples were obtained on day 1 of the study when subjects were fasting (baseline) and on days 36–38, 64–66, and 92–94 of each diet period at the same time ±30 min. Blood was collected into evacuated tubes containing EDTA. Within 30 min of blood collection, plasma was separated and sodium azide and aprotinin were added to concentrations of 1 g/L and 1 mg/L, respectively. Plasma samples were divided into aliquots and frozen at −70°C until analyzed.

Body weight was measured biweekly while subjects were fasting. Skinfold-thickness measurements were taken at triceps, biceps, suprailiac, and subscapular sites on the subject's nondominant side on day 1 of the study and at the end of each diet period. All skinfold-thickness measurements, obtained by the same registered dietitian, were taken twice to the nearest 0.1 mm with a skinfold caliper (Cambridge Scientific Instruments Ltd, Cambridge, MD). Body density was calculated from the sum of the 4 skinfold-thickness measurements by using an age- and sex-specific equation. A predictive equation was used to determine the percentage body fat (26). To assess changes in dietary intake, 3-d food records were obtained at the health screening (prestudy food records) and on days 35–37, 63–65, and 91–93 of each diet period.

Analytic methods

Plasma samples obtained on 3 consecutive days were pooled and, along with a baseline sample, were analyzed for total and HDL cholesterol and triacylglycerol. Plasma total cholesterol and triacylglycerol concentrations were determined with an enzymatic colorimetric method that was adapted for microtiter plates (27) with commercially available enzyme reagents (Cholesterol/HP and Triglyceride/GB; Boehringer-Mannheim Corporation, Indianapolis). HDL cholesterol was isolated by selectively precipitating apo B–containing lipoproteins with phosphotungstic acid (6.1 mmol/L) and magnesium chloride (20 mmol/L) (Fisher Scientific, Pittsburgh); the supernate was analyzed for cholesterol content with the plasma total cholesterol

TABLE 1
Subject characteristics at baseline[1]

	Value
Age (y)	56.9 ± 5.8
Time since menopause (y)	7.6 ± 4.7
Body weight (kg)	65.6 ± 10.2
BMI (kg/m^2)	25.2 ± 3.6
Percentage body fat (%)[2]	40.3 ± 5.6
Plasma lipid concentrations	
Total cholesterol	
(mmol/L)	5.55 ± 0.68
(mg/dL)	215 ± 26
HDL cholesterol	
(mmol/L)	1.35 ± 0.43
(mg/dL)	52 ± 17
LDL cholesterol	
(mmol/L)	3.53 ± 0.81
(mg/dL)	136 ± 31
Triacylglycerol	
(mmol/L)	1.46 ± 1.29
(mg/dL)	130 ± 114
Total:HDL cholesterol	4.6 ± 1.7
LDL:HDL cholesterol	2.9 ± 1.4

[1] $\bar{x}$ ± SD; n = 18.
[2] Determined from skinfold-thickness measurements.

enzymatic assay. LDL cholesterol was calculated with the Friedewald equation (28):

$$LDL \ cholesterol = total \ cholesterol - HDL \ cholesterol - (triacylglycerol/5) \qquad (1)$$

where each variable is expressed in mg/dL. (To convert to mmol/L, multiply the product by 0.02586.) All samples from each subject were analyzed in duplicate in the same daily batch with one in-house plasma control and a standard curve on each plate. For each assay, the plates were allowed to incubate for 60 min at room temperature before being read at 490 nm with an automated microplate reader. Intraassay variability was 0.6%, 0.6%, and 1.4% and interassay variability was 1.2%, 7.4%, and 2.8% for total cholesterol, HDL cholesterol, and triacylglycerol, respectively.

Plasma samples from baseline and day 94 of each diet period were analyzed for apo A-I, apo B, and lipoprotein(a) concentrations and LDL peak particle diameter. Plasma concentrations of apo A-I and apo B were measured with an immunoturbidimetric method with commercially available reagents (Auto Apo A-I and Auto Apo B; Bacton Assay Systems Inc, San Marcos, CA) (29, 30). Lipoprotein(a) concentrations were measured with an enzyme-linked immunosorbent assay (31) that uses a polyclonal capture antibody immunospecific to apo(a) and a peroxidase-conjugated polyclonal detection antibody with recognition of the entire lipoprotein(a) molecule (Donner Laboratory, Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, CA). LDL peak particle diameter was evaluated in whole plasma with a nondenaturing polyacrylamide gradient gel electrophoresis method described previously by Krauss and Burke (32). Intraassay variability was 0.8%, 0.7%, and 5.3% and interassay variability was 3.9%, 5.3%, and 8.1% for apo A-I, apo B, and lipoprotein(a) concentrations, respectively. Interassay variability for LDL peak particle diameter was 1.1%.

Food records were analyzed throughout the study by the same registered dietitian using NUTRITIONIST IV for WINDOWS,

version 4.0 (The Hearst Corporation, San Bruno, CA). For each 3-d food record, we calculated the average intakes of energy, protein, carbohydrate, fat, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, cholesterol, dietary fiber, and all known essential micronutrients.

Data analysis

Statistical analyses were performed with the STATISTICAL ANALYSIS SYSTEM, version 6.12 (SAS Institute Inc, Cary, NC). We performed a repeated-measures analysis of variance (ANOVA) on each anthropometric and food-record endpoint with subject and diet controlled for. To allow for adaptation to each diet, plasma lipid concentrations in the day 36–38 plasma pools (the first time point in each diet period) were excluded from the statistical analyses. To evaluate the effects of isoflavone consumption on concentrations of triacylglycerol and total, LDL, and HDL cholesterol, repeated-measures ANOVAs were performed with subject, diet, and time of collection (days 64–66 compared with days 92–94) controlled for. No significant interactions between diet and time of collection were seen.

To evaluate the effects of isoflavone consumption on concentrations of apo A-I, apo B, and lipoprotein(a) and on LDL peak particle diameter, repeated-measures ANOVAs were performed with subject and diet controlled for. After the ANOVA, Tukey's procedure was performed on all endpoints to adjust the significance level for multiple comparisons. We used unpaired t tests to compare baseline concentrations with concentrations during each of the 3 diets for LDL peak particle diameter and concentrations of triacylglycerol, apo A-I, apo B, lipoprotein(a), and total, LDL, and HDL cholesterol; a Bonferroni correction was applied to P values to adjust for multiple comparisons. The results are expressed as means ± SDs; in the event of missing data, least-squares means ± SE are presented to account for the imbalance. $P < 0.05$ was considered significant.

RESULTS

Subject characteristics and dietary data

Twenty-three women were enrolled in the study. Four subjects dropped out during the first diet period because they had difficulties complying with the study protocol. One subject was excluded from all analyses when her plasma hormone concentrations showed that she was not postmenopausal. The baseline characteristics of the 18 subjects who completed the study are summarized in **Table 1**. As described previously, there were no significant changes during the study in body weight (control diet, 66.2 ± 10.7 kg; low-isoflavone diet, 65.4 ± 11.0 kg; and high-isoflavone diet, 66.3 ± 11.0 kg), BMI, or percentage body fat (24).

The dietary data are shown in **Table 2**. There were no significant differences among the 3 diets in the contents of energy, cholesterol, dietary fiber, or any macronutrients or micronutrients. However, there were significant differences between food records collected during the study and the prestudy food records. The latter reflected a significantly lower consumption of protein (69 ± 3 g; P = 0.0001) and a significantly higher consumption of carbohydrate (262 ± 8 g; P = 0.005), total fat (54 ± 3 g; P = 0.03), saturated fatty acids (19 ± 1 g; P = 0.01), polyunsaturated fatty acids (10 ± 1 g; P = 0.02), cholesterol (233 ± 21 mg; P = 0.001), and dietary fiber (18 ± 1 g; P = 0.0001) when compared with food records collected during the study. There was no

TABLE 2
Daily energy, macronutrient, cholesterol, and fiber intakes[1]

	Diet		
	Control (*n* = 18)	Low isoflavone (*n* = 17)	High isoflavone (*n* = 18)
Energy			
(MJ)	7.53 ± 0.15	7.34 ± 0.16	7.46 ± 0.15
(kcal)	1799 ± 36.4	1755 ± 38.0	1783 ± 36.4
Protein (g)	112 ± 2	114 ± 2	114 ± 2
Carbohydrate (g)	243 ± 6	232 ± 6	233 ± 6
Total fat (g)	45.9 ± 2.1	45.3 ± 2.1	47.7 ± 2.1
SFA (g)	14.3 ± 0.9	14.4 ± 0.9	15.1 ± 0.9
PUFA (g)	8.1 ± 0.5	7.7 ± 0.5	8.1 ± 0.5
MUFA (g)	13.2 ± 0.6	13.3 ± 0.6	13.9 ± 0.6
Cholesterol (mg)	154 ± 10	155 ± 11	149 ± 10
Dietary fiber (g)	13.4 ± 0.6	13.1 ± 0.7	12.9 ± 0.6

[1] Least-squares $\bar{x}$ ± SE calculated from three 3-d food records per subject; these data include contributions from the soy powder. There were no significant differences between diets by ANOVA. SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

significant difference in energy intake between prestudy food records (7.41 ± 0.23 MJ, or 1772 ± 55 kcal) and food records collected during the study. However, during the study the subjects consumed significantly higher amounts of riboflavin, vitamin B-12, vitamin D, calcium, folate, iron, magnesium, phosphorus, and pantothenic acid and significantly lower amounts of niacin, biotin, vitamin K, vitamin B-6, copper, selenium, and chromium than before the study, as indicated by the prestudy food records ($P < 0.01$ for all, data not shown).

Effects of soy isoflavone consumption on plasma lipid concentrations

Plasma lipid data are shown in **Tables 3** and **4**. Compared with values measured during the control diet, the LDL-cholesterol concentration was 6.5% lower during the high-isoflavone diet ($P = 0.02$) and was nearly significantly lower during the low-isoflavone diet ($P = 0.07$). Compared with values measured during the control diet, the ratio of LDL to HDL cholesterol was 8.5% lower during the low-isoflavone diet ($P = 0.01$) and 7.7% lower during the high-isoflavone diet ($P = 0.02$). There were no significant effects of isoflavone consumption on concentrations of total or HDL cholesterol, triacylglycerol, apo A-I, apo B, or lipoprotein(a) or LDL peak particle diameter. However, compared with values measured during the control diet, the total cholesterol concentration and the ratio of total to HDL cholesterol tended to be lower during the high-isoflavone diet ($P = 0.17$ and $P = 0.19$, respectively) and the LDL-cholesterol concentration and the ratio of total to HDL cholesterol tended to be lower during the low-isoflavone diet ($P = 0.07$ and $P = 0.11$, respectively).

Although complicated by differences in nutrient intake, comparisons between the soy-free baseline diet and the low-isoflavone and high-isoflavone diets allow for assessment of the combined effects of isoflavones and other soy components and are thus similar to comparisons made by other researchers who used a soy-free control diet. The baseline lipid concentrations and their ratios are shown in Table 1. The additional endpoints evaluated at baseline were apo A-I (1.17 ± 0.02 g/L), apo B (1.08 ± 0.03 g/L), lipoprotein(a) (0.91 ± 0.04 μmol/L; 25 ± 1 mg/dL), and LDL peak particle diameter (26.8 ± 0.1 nm).

During the low- and high-isoflavone diets, concentrations were significantly reduced from baseline for total cholesterol ($P = 0.0004$ for both diets), LDL cholesterol ($P = 0.0006$ and $P = 0.0003$, respectively), and apo B ($P = 0.002$ and $P = 0.02$, respectively). During the low- and high-isoflavone diets, LDL:HDL cholesterol decreased ($P = 0.0009$ and $P = 0.002$, respectively) and total:HDL cholesterol decreased ($P = 0.002$ for both diets) compared with baseline values.

Comparisons between the baseline diet and the control diet presumably reflect the effects of the nonisoflavone components of soy. Plasma total cholesterol ($P = 0.0006$), LDL cholesterol ($P = 0.02$), apo B ($P = 0.048$), apo A-I ($P = 0.03$), and LDL:HDL cholesterol ($P = 0.03$) decreased during the control diet compared with baseline values.

TABLE 3
Plasma lipid concentrations[1]

	Diet			P^2
	Control (*n* = 18)	Low isoflavone (*n* = 17)	High isoflavone (*n* = 18)	
Total cholesterol				
(mmol/L)	5.09 ± 0.06	4.99 ± 0.07	4.93 ± 0.06	0.19
(mg/dL)	197 ± 2	193 ± 3	191 ± 2	
HDL cholesterol				
(mmol/L)	1.34 ± 0.02	1.38 ± 0.02	1.36 ± 0.02	0.56
(mg/dL)	52.0 ± 0.9	53.3 ± 0.9	52.7 ± 0.9	
LDL cholesterol				
(mmol/L)	3.22 ± 0.05[a]	3.05 ± 0.05[a,b]	3.01 ± 0.05[b]	0.01
(mg/dL)	124 ± 2	118 ± 2	116 ± 2	
Triacylglycerol				
(mmol/L)	1.16 ± 0.08	1.22 ± 0.08	1.22 ± 0.08	0.79
(mg/dL)	103 ± 7	108 ± 7	108 ± 7	
Total:HDL cholesterol	4.19 ± 0.07	3.98 ± 0.07	4.02 ± 0.07	0.09
LDL:HDL cholesterol	2.72 ± 0.05[a]	2.49 ± 0.06[b]	2.51 ± 0.05[b]	0.007

[1] Least-squares $\bar{x}$ ± SE calculated from two 3-d plama pools per subject. Values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] Overall ANOVA of the comparisons between the 3 diets.

TABLE 4
Plasma apolipoprotein (apo) A-I, apo B, and lipoprotein(a) concentrations and LDL peak particle diameter (PPD)[1]

	Diet		
	Control (n = 18)	Low isoflavone (n = 17)	High isoflavone (n = 18)
Apo A-I (g/L)	1.11 ± 0.02	1.14 ± 0.02	1.15 ± 0.02
Apo B (g/L)	0.98 ± 0.02	0.96 ± 0.02	0.98 ± 0.02
Lipoprotein(a)			
(μmol/L)	0.96 ± 0.03	0.97 ± 0.03	0.98 ± 0.03
(mg/dL)	26.8 ± 0.8	27.2 ± 0.9	27.4 ± 0.8
LDL-PPD (nm)	26.8 ± 0.1	26.8 ± 0.1	26.9 ± 0.1

[1] Least-squares $\bar{x}$ ± SE. There were no significant differences between the 3 diets by ANOVA.

DISCUSSION

The purpose of this study was to investigate the effects of soy isoflavones, when consumed in ISP powder, on plasma lipid concentrations in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. The dosages of isoflavones used in this study were chosen to fall within the range of previously reported intakes in typical Asian diets (33). However, data published in 1998 suggest that the typical isoflavone intake is far lower than previously reported (34), most likely between 20 and 50 mg isoflavones/d. Therefore, our low dosage was at the upper end of current estimates of typical isoflavone intake in Asia and our high dosage was significantly higher.

This study differs from previous studies in several ways. First, the isoflavones were part of an ISP powder and the soy protein was not completely substituted for the protein in the subjects' habitual diets. Second, the isoflavones were provided relative to individual body weights and not as a standardized dosage. Third, plasma lipid values represented the average of two 3-d plasma pools per subject per diet, thereby reducing intrasubject variability. Finally, we evaluated other plasma endpoints associated with CVD risk. Although low coronary artery disease risk in humans has been associated with favorable plasma lipid concentrations, low risk has also been linked to elevated concentrations of apo A-I (the major apolipoprotein in HDL), low concentrations of apo B (the major apolipoprotein of VLDL, LDL, and intermediate-density lipoproteins) and lipoprotein(a) (an LDL-like lipoprotein structurally similar to plasminogen), and elevated LDL peak particle diameter (35–37).

Compared with the control diet, the high-isoflavone diet reduced LDL cholesterol by ≈6% and there was a trend toward a similar reduction by the low-isoflavone diet (P = 0.07). In addition, the low-isoflavone and high-isoflavone diets reduced the ratio of LDL to HDL cholesterol by ≈8% compared with the control diet. These small effects were seen despite a limited statistical power that resulted from the small number of subjects. We found no significant effects of isoflavone consumption on concentrations of total or HDL cholesterol, triacylglycerol, apo A-I, apo B, or lipoprotein(a) or the LDL peak particle diameter, although we found trends toward lowered total cholesterol concentrations and decreased total:HDL cholesterol.

Our results are consistent with those of a recent study of hypercholesterolemic men and women that compared the effects of an isoflavone-depleted ISP with those of an ISP containing 3 concentrations of isoflavones (19). This study found progressively lower total and LDL cholesterol concentrations as

isoflavone intakes increased; however, the results were significant only for total cholesterol concentrations and only in the subset of subjects with the highest baseline LDL cholesterol concentrations. These results are also consistent with those of studies in nonhuman primates in which LDL cholesterol concentrations were lower after consumption of isoflavone-rich soy protein than after consumption of isoflavone-depleted soy protein (16–18). Although all of these studies showed that isoflavone intake was associated with decreases in total cholesterol and increases in HDL cholesterol concentrations, the changes were not significant in all studies (16, 17). The same group of investigators reported that in general, apo A-I, apo B, and lipoprotein(a) concentrations remained unchanged after isoflavone consumption (16, 17), although one study in female monkeys consuming very high doses of isoflavones showed increased apo A-I concentrations, decreased lipoprotein(a) concentrations, and decreased LDL molecular weight (16).

Our results are somewhat different from those of 3 studies in human subjects given isoflavone tablets derived from subterranean clover or soy (21–23). In these studies, there were no significant effects on concentrations of triacylglycerol (21–23), lipoprotein(a) (21), or total, LDL, or HDL cholesterol (21–23). The lack of any significant findings in studies that used purified isoflavones suggests that soy isoflavones may require other components found in soy protein to exert their cholesterol-lowering effects.

Although the effects of isoflavones were the focus of the current study, the comparisons between baseline plasma lipid values and those measured during the low-isoflavone and high-isoflavone diets are similar, in terms of the study design and statistical tests, to comparisons performed in most previous studies. Concentrations of total and LDL cholesterol and apo B, total:HDL cholesterol, and LDL:HDL cholesterol decreased significantly from baseline by 10–15% after the low- and high-isoflavone diets. These results are consistent with those of studies in perimenopausal and postmenopausal women in whom soy consumption had no significant effect on triacylglycerol (19, 38–40), HDL cholesterol (19, 38, 40), or apo A-I (39) concentrations, but resulted in reductions in total cholesterol (19, 40) and LDL or non-HDL cholesterol (19, 39, 40) concentrations. On the other hand, there are also reports that soy consumption resulted in increases in HDL cholesterol (39) but no changes in total cholesterol (38, 39) or apo B (39) concentrations. Differences between the baseline and control-diet values may reflect the effects of nonisoflavone soy components. Total and LDL cholesterol, apo A-I, apo B, total:HDL cholesterol, and LDL:HDL cholesterol all decreased by 5–10% during the control diet, compared with baseline. It is likely that the dietary differences between baseline and the 3 diet periods contributed to these effects, although the Keys equation (41) predicts that differences in fat and cholesterol intakes between baseline and the 3 diets would account for only 50% of the observed change in total cholesterol concentrations.

In summary, this study is the first to show that soy isoflavones per se, when consumed as a constituent of ISP, lower LDL-cholesterol concentrations and the ratio of LDL to HDL cholesterol in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. Although the magnitude of the change in LDL-cholesterol concentrations was small, a decrease of the magnitude observed (0.2 mmol/L, or 8 mg/dL) could be associated with a 16% reduction in coronary artery disease risk (42).

Additional non-lipid-lowering effects of soy isoflavones (12, 13, 17, 18, 22) may contribute to a further reduction in risk. The current study also suggests that nonisoflavone soy components may exert beneficial effects on coronary artery disease risk factors in normocholesterolemic and mildly hypercholesterolemic postmenopausal women.

REFERENCES

1. American Heart Association. 1998 Heart and stroke statistical update. Dallas: American Heart Association, 1997.
2. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333:276–82.
3. Carroll KK. Review of clinical studies on cholesterol-lowering response to soy protein. J Am Diet Assoc 1991;91:820–7.
4. Duane WC. Effects of soybean protein and very low dietary cholesterol on serum lipids, biliary lipids, and fecal sterols in humans. Metabolism 1999;48:489–94.
5. Önning G, Åkesson B, Öste R, Lundquist I. Effects of consumption of oat milk, soya milk, or cow's milk on plasma lipids and antioxidative capacity in healthy subjects. Ann Nutr Metab 1998;42:211–20.
6. Nilausen K, Meinertz H. Variable lipemic response to dietary soy protein in healthy, normolipemic men. Am J Clin Nutr 1998;68(suppl): 1380S–4S.
7. Wong WW, Smith EO, Stuff JE, Hachey DL, Heird WC, Pownell HJ. Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. Am J Clin Nutr 1998;68(suppl): 1385S–9S.
8. Gooderham MJ, Adlercreutz H, Ojala ST, Wähälä K, Holub BJ. A soy protein isolate rich in genistein and daidzein and its effects on plasma isoflavone concentrations, platelet aggregation, blood lipids and fatty acid composition of plasma phospholipid in normal men. J Nutr 1996;126:2000–6.
9. Sirtori CR, Lovati MR, Manzoni C, Monetti M, Pazzucconi F, Gatti E. Soy and cholesterol reduction: clinical experience. J Nutr 1995; 125:598S–605S.
10. Carroll KK, Kurowska EM. Soy consumption and cholesterol reduction: review of animal and human studies. J Nutr 1995;125:594S–7S.
11. Potter SM. Soy protein and serum lipids. Curr Opin Lipidol 1996; 7:260–4.
12. Anthony MS, Clarkson TB, Williams JK. Effects of soy isoflavones on atherosclerosis: potential mechanisms. Am J Clin Nutr 1998; 68(suppl):1390S–3S.
13. Lichtenstein AH. Soy protein, isoflavones and cardiovascular disease risk. J Nutr 1998;128:1589–92.
14. Potter SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J Nutr 1995;125:606S–11S.
15. Potter SM. Soy protein and cardiovascular disease: the impact of bioactive components in soy. Nutr Rev 1998;56:231–5.
16. Anthony MS, Clarkson TB, Hughes CL, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 1996;126:43–50.
17. Honore EK, Williams JK, Anthony MS, Clarkson TB. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. Fertil Steril 1997;67:148–54.
18. Anthony MS, Clarkson TB, Bullock BC, Wagner JD. Soy protein versus soy phytoestrogens in the prevention of diet-induced coro-
19. nary artery atherosclerosis of male cynomolgus monkeys. Arterioscler Thromb Vasc Biol 1997;17:2524–31.
19. Crouse JR, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch Intern Med 1999;159:2070–6.
20. Merz-Demlow BE, Duncan AM, Wangen KE, et al. Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. Am J Clin Nutr 2000;71:1462–9.
21. Hodgson JM, Puddey IB, Beilin LJ, Mori TA, Croft KD. Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: a randomized controlled trial in humans. J Nutr 1998;128:728–32.
22. Nestel PJ, Yamashita T, Sasahara T, et al. Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. Arterioscler Thromb Vasc Biol 1997;17:3392–8.
23. Nestel PJ, Pomeroy S, Kay S, et al. Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. J Clin Endocrinol Metab 1999;84:895–8.
24. Duncan AM, Underhill KEW, Xu X, LaValleur J, Phipps WR, Kurzer MS. Modest hormonal effects of soy isoflavones in postmenopausal women. J Clin Endocrinol Metab 1999;84:3479–84.
25. Murphy PA, Song T, Buseman G, Barua K. Isoflavones in soy-based infant formulas. J Agric Food Chem 1997;45:4635–8.
26. Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77–97.
27. Carr TP, Andresen CJ, Rudel LL. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. Clin Biochem 1993;26:39–42.
28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
29. Rifai N, King ME. Immunoturbidimetric assays of apolipoproteins A, AI, AII, and B in serum. Clin Chem 1986;32:957–61.
30. Smith SJ, Cooper GR, Henderson LO, et al. An international collaborative study on standardization of apolipoproteins A-I and B. Clin Chem 1987;33:2240–9.
31. Tijssen P. Practice and theory of enzyme immunoassays. In: Burdon RH, van Knippenberg PH, eds. Laboratory techniques in biochemistry and molecular biology. Vol 15. Amsterdam: Elsevier Science Publishers BV, 1985:345–7.
32. Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982;23:97–104.
33. Coward L, Barnes NC, Setchell KDR, Barnes S. Genistein, daidzein, and their β-glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. J Agric Food Chem 1993;41:1961–7.
34. Nagata C, Takatsuka N, Kurisu Y, Shimizu H. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. J Nutr 1998;128:209–13.
35. Rader DJ, Hoeg JM, Brewer HB. Quantitation of plasma apolipoproteins in the primary and secondary prevention of coronary artery disease. Ann Intern Med 1994;120:1012–25.
36. Orth-Gomer K, Mittleman MA, Schenck-Gustafsson K, et al. Lipoprotein(a) as a determinant of heart disease in young women. Circulation 1997;95:329–34.
37. Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 1996;276:875–81.
38. Murkies AL, Lombard C, Strauss BJG, Wilcox G, Burger HG, Morton MS. Dietary flour supplementation decreases post-menopausal hot flushes: effect of soy and wheat. Maturitas 1995;21:189–95.
39. Baum JA, Teng H, Erdman JW Jr, et al. Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. Am J Clin Nutr 1998;68:545–51.

40. Washburn S, Burke GL, Morgan T, Anthony M. Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. Menopause 1999;6:7–13.

41. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet. Metabolism 1965;14:776–87.

42. O'Brien T, Nguyen TT. Lipids and lipoproteins in women. Mayo Clin Proc 1997;72:235–44.