Effect of soy protein varying in isoflavone content on serum lipids in healthy young men[1–3]

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ABSTRACT

Background: Previous research supports a role for soy protein in reducing serum lipids; however, few studies involved healthy male subjects or focused on soy isoflavones (or did both).

Objective: The objective was to ascertain the effects of soy protein varying in isoflavone content on serum lipids in healthy young men.

Design: Thirty-five males ($\bar{x}$ ± SD age: 27.9 ± 5.7 y) consumed milk protein isolate (MPI), low-isoflavone soy protein isolate (low-iso SPI; 1.64 ± 0.19 mg aglycone isoflavones/d), and high-isoflavone SPI (high-iso SPI; 61.7 ± 7.4 mg aglycone isoflavones/d) for 57 d each, separated by 4-wk washout periods, in a randomized crossover design. Blood samples were collected at the beginning and end of each treatment period, and total, LDL, and HDL cholesterol; triacylglycerols; apolipoprotein (apo) B; apo A-I; and C-reactive protein (CRP) were measured in serum. Twenty-four-hour urine samples were collected for 3 consecutive days at the end of each treatment period and analyzed for isoflavones.

Results: Urinary isoflavones were significantly greater with consumption of the high-iso SPI than with that of the low-iso SPI or MPI. The differences between the 3 treatments with respect to individual serum lipids were not significant, but the ratios of total to HDL cholesterol, LDL to HDL cholesterol, and apo B to apo A-I were significantly lower with both SPI treatments than with MPI treatment.

Conclusion: Soy protein, regardless of isoflavone content, modulates serum lipid ratios in a direction beneficial for cardiovascular disease risk in healthy young men. *Am J Clin Nutr* 2006;83:244–51.

KEY WORDS Soy protein, isoflavones, healthy men, serum lipids, lipid ratios, C-reactive protein

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death among North Americans (1), and thus research has focused on strategies for its prevention. Diet has received considerable attention as a CVD prevention strategy, and studies have linked the varied CVD incidences in different countries to dietary differences (2). The advance of research into dietary components that may relate to CVD has included a focus on soy and its constituent protein and isoflavones (3, 4).

A large body of evidence has established a role for soy in CVD risk reduction (5), particularly through the modulation of serum lipids (6). In epidemiologic studies, the consumption of soy, soy isoflavones, or both has been inversely related to circulating total (7, 8) or LDL (8) cholesterol and triacylglycerols (9) and positively related to HDL cholesterol (10). Human studies have also provided support, as indicated by a meta-analysis reporting that an average of 47 g soy protein/d resulted in reductions of 9.3% in circulating total cholesterol (TC), 12.9% in LDL cholesterol, and 10.5% in triacylglycerols (11). This evidence contributed to the Food and Drug Association (FDA) approval of a soy and heart disease health claim for foods (12).

Although the FDA-approved soy health claim is for soy protein (12), interest remains in other components of soy, particularly isoflavones. A role for soy isoflavones in the reduction of circulating lipids is supported by animal studies that compared the effects of high- and low-isoflavone diets. Studies in nonhuman primates found significant reductions in plasma LDL and VLDL cholesterol (13) and triacylglycerols (13) and in the ratio of total to HDL cholesterol (13, 14) and significant increases in HDL cholesterol (13, 14). Studies in rodents found significant reductions in circulating TC (15–17), LDL cholesterol (16), and triacylglycerols (18). Human studies focusing on the isoflavone component of soy protein have been less consistent. Some have attributed reductions in circulating TC (19) and LDL cholesterol (19–21) and increases in HDL cholesterol (22) to isoflavones, whereas others have been unable to attribute reductions in serum TC (23), LDL cholesterol (24), triacylglycerols (24, 25), total:HDL (23, 24), and LDL:HDL (20, 23, 24) to isoflavones.

Most studies investigating the effects of soy on serum lipids were conducted in hypercholesterolemic subjects (11, 19), largely because of the positive relation between baseline cholesterol and effect magnitude (11). To maximize the CVD-prevention potential of soy, it is also important to study healthy, normolipidemic subjects, as supported by data from a prospective study in young men that found a significant inverse association between serum lipid changes within normal endogenous ranges and future CVD incidence (26). Studies that evaluated the effects of soy protein, isoflavones, or both on lipids in healthy normocholesterolemic subjects produced inconsistent results;

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TABLE 1
Daily isoflavone and protein contribution of study treatment powders[1]

	MPI treatment powder	Low-iso SPI treatment powder[2]	High-iso SPI treatment powder[3]
Isoflavones (mg)	—	1.64 ± 0.19	61.7 ± 7.35
Isoflavones (mg/kg body wt)	0.00 ± 0.00	0.02 ± 0.001	0.75 ± 0.01
Protein (g)	32.1 ± 4.60	31.6 ± 3.60	32.0 ± 3.95

[1] All values are $\bar{x}$ ± SD. $n = 35$. MPI, milk protein isolate; Low-iso SPI, low isoflavone soy protein isolate; High-iso SPI, high isoflavone SPI. Isoflavone concentrations were determined by using HPLC at an independent laboratory. The values are the sums of the individual isomers of each isoflavone (genistein, daidzein, and glycitein) normalized for their molecular-weight differences and presented as total aglycone isoflavones.

[2] The average percentage distribution of isoflavones was 78.9% genistein, 12.7% daidzein, and 8.4% glycitein.

[3] The average percentage distribution of isoflavones was 53.3% genistein, 35.6% daidzein, and 11.1% glycitein.

some showed significant decreases in LDL cholesterol (20, 21, 27) or increases in HDL cholesterol (10, 22), and others showed no significant effects (28–30). Most research into the effects of soy protein, isoflavones, or both on lipids also focused on female subjects. Although some studies have included both men and women (19, 23, 25, 31–33), few have included only men (24, 34–36).

Although substantial evidence supports a role for soy protein in the reduction of lipids, few studies involving healthy subjects have focused on the isoflavone component of soy, and even fewer studies have focused on men. The purpose of the current study was to ascertain the effects of soy protein and isoflavones on serum lipids in healthy young men. This study also evaluated C-reactive protein (CRP), an inflammatory biomarker linked to CVD risk (37).

SUBJECTS AND METHODS

Study design

As described previously (38), the study consisted of three 57-d treatment periods separated by 28-d washout periods. It used a randomized crossover design in which the subjects were blinded to the order of treatment.

Subjects

Healthy young men were recruited from the local community. Inclusion criteria included healthy males between the ages of 20 and 40 y and with a body mass index (BMI; in kg/m^2) of 19–29. Exclusionary criteria included diagnosis with a disease or serious medical condition, regular medication use, antibiotic use within the last 3 mo, smoking, vasectomy, recreational drug use, soy or milk protein allergy, vegan diet, body weight change of >5 kg within the last 6 mo, elite athletes, and intention to gain or lose weight within the following year.

After recruitment, subjects attended a study orientation session and were provided with a study handbook that outlined all aspects of the study. After the study orientation session, subjects provided written informed consent. The study protocol was approved by the Human Research Ethics Board of the University of Guelph.

Study diet and treatment powders

Subjects supplemented their habitual diets with 3 protein powders including milk protein isolate (MPI), ethanol-extracted low-isoflavone soy protein isolate (low-iso SPI), and high-isoflavone soy protein isolate (high-iso SPI) (The Solae Company, St Louis, MO). The study treatment powders were analyzed in duplicate for isoflavone content with the use of HPLC at the laboratory of Patricia Murphy (Iowa State University, Ames, IA).

Treatment powders were provided to subjects on an individual basis according to their body weight. Specifically, the amount of the high-iso SPI was calculated to provide an isoflavone dose in aglycone equivalents of 0.75 mg · kg body wt^{-1} · d^{-1}, and the amounts of the low-iso SPI and MPI were calculated to provide protein in an amount similar to that provided by the high-iso SPI. The average daily dietary isoflavone and protein contributions of each study treatment powder are shown in **Table 1**. The average daily energy, carbohydrate, fat, and calcium contributions of the protein powders were 236.1 kcal, 23.1 g, 1.48 g, and 1269 mg, respectively.

Subjects received specific instructions to minimize their background consumption of phytoestrogens by avoiding soy and soy products, flaxseed, beans and legumes, whole grains, and high-fiber foods; to limit their consumption of milk and calcium-fortified beverages to compensate for the high-calcium content of the study protein powders; to limit their alcohol intake to < 7 drinks/wk (≤2 drinks per sitting); to avoid green tea; and to avoid all dietary supplements.

Data collection

Study visits occurred every 2 wk, on days 1, 15, 29, 43, and 57 of each treatment period; the subjects presented at the Human Nutraceutical Research Unit at the University of Guelph. Fasting body weight was measured to the nearest 0.1 kg before the study and at each visit by using a digital scale while the subjects were wearing light clothing but no shoes. Height was measured before the study by using a metric measuring tape while the subjects, who were not wearing shoes, stood with their heels against the wall.

Body composition was measured on days 1 and 57 of each treatment period with the use of bioelectrical impedance analysis (BIA; BodyStat 1500, BodyStat, Tampa, FL). The subject rested in a prone position, and 2 electrodes were attached to the right hand (one over the knuckles and one over the wrist bone) and 2 electrodes were attached to the right foot (one over the ankle bone and one over the base of the middle toe). To ensure hydration and therefore promote a more accurate reading, subjects were instructed to consume 2–4 glasses of water in the 12 h before the BIA measurement.

To monitor dietary intake throughout the study, subjects completed 3-d food records once before the study and on days 1–3, 26–28, and 54–56 of each treatment period. Subjects were provided with food record forms, labeled with the dates on which

they were to be completed, that included spaces in which subjects were to record the time when the food or beverage was consumed, the amount consumed, and information such as the commercial brand and the method of cooking. Subjects were encouraged to provide as much detail as possible, including attaching food labels and recipes.

Blood samples were collected from subjects on days 1 and 57 of each treatment period. Subjects were instructed to avoid all food and drink (except water) for 12 h and to refrain from alcohol and medication for 72 h before each blood draw. Blood was drawn into evacuated tubes, left at room temperature for 30 min, and centrifuged at 1200 × *g* and 4 °C for 15 min. Serum was then aliquoted into cryovials and stored at −80 °C until it was analyzed.

Twenty-four-hour urine collections were performed on each day of days 54–56 of each treatment period. Subjects collected their urine into 3-L opaque plastic bottles (VWR International, Mississauga, Canada) containing 3 g ascorbic acid as a preservative. Subjects were also provided with a 1-L wide-mouth bottle (Nalgene, Rochester, NY), a lunch-size cooler bag, and an ice pack and were given instructions to transfer their urine to the 3-L container and to keep the container refrigerated at all times. Complete 24-h urine collections were gently mixed, aliquoted into 15-mL conical-tip polypropylene tubes (Sarstedt, Montreal, Canada), and stored at −20 °C until they were analyzed.

Analytic methods

Three-day food records were analyzed for energy, nutrient, and dietary fiber intakes by using NUTRIBASE IV CLINICAL EDITION software (version 2001; Cybersoft, Phoenix, AZ). From each 3-d food record, average intakes of energy; protein; carbohydrate; fat; saturated, monounsaturated, and polyunsaturated fatty acids; dietary fiber; cholesterol; and calcium were calculated.

Serum samples were analyzed for TC, HDL cholesterol, and triacylclygerols by using an autoanalyzer (Synchron CX systems; Beckman Coulter, Mississauga, Canada) that measured absorbance at 520 nm. LDL cholesterol was calculated by using the equation of Friedewald et al (39). Serum samples were analyzed for apolipoprotein (apo) B and apo A-I by endpoint nephelometry (40) with the use of a Behring Nephelometer 100 Analyzer (Dade Behring Inc, Mississauga, Canada). Finally, serum samples were analyzed for CRP by using endpoint nephelometry with a high-sensitivity CRP reagent on a Nephelometer 100 Analyzer.

All samples from each subject were analyzed in the same batch, and control samples were included in every run to estimate interassay variability. Interassay variability was 1.61% for TC, 2.88% for HDL cholesterol, 2.80% for triacylglycerols, 2.15% for apo B, 1.15% for apo A-I, and 1.75% for CRP.

Twenty-four-hour urine samples were analyzed for creatinine by using an enzymatic ultraviolet method (Randox Laboratories Canada Ltd, Mississauga, Canada) on a Roche Hitachi 911 autoanalyzer with an interassay variability of 1.36%. Aliquots from every consecutive 3-d urine collection were thawed and proportionally combined to create a pooled sample, which was analyzed for isoflavones (genistein and daidzein) and isoflavone metabolites (equol and *O*-desmethylangolensin) by using gas chromatography–mass spectrometry as described previously (41). Intraassay and interassay variability was 4.20% and 11.1%, respectively, for genistein, 4.27% and 9.74% for daidzein, 5.30% and 16.2% for *O*-desmethylangolensin, and 4.04% and 16.0% for equol.

Statistical analysis

Examination of all data with the use of box plots and residual error plots showed that the urinary isoflavone, serum triacylglycerol, and serum CRP data were not normally distributed and required log transformation before statistical analysis to comply with the normality and equal variance assumptions of the statistical analyses. To ensure that the washout periods between treatments were sufficient, repeated-measures analysis of variance (ANOVA) was performed on the day 1 values for serum lipids, lipid ratios (ie, total:HDL, LDL:HDL, and apo B:apo A-I), and CRP.

To evaluate the effect of treatment on serum lipids, lipid ratios, and CRP, repeated-measures ANOVA was performed on the calculated change from day 1 to day 57, with control for subject, treatment order, and treatment, and Tukey's test for multiple comparisons was then conducted. Further analysis of the effect of treatment on serum lipids, lipid ratios, and CRP was performed by including equol excretor status as a covariate in the model and by testing for an interaction between equol excretor status and treatment. The effects of treatment on anthropometric, food record, and urinary isoflavone data were ascertained by using repeated-measures ANOVA after control for subject, treatment order, and treatment and then Tukey's test for multiple comparisons.

All data that were log transformed were exponentiated back to the natural scale. SAS software (version 8.2; SAS Institute Inc, Cary, NC) was used for all statistical analyses, and $P \leq 0.05$ was considered significant.

RESULTS

Subject dropouts and exclusions

Over the course of the study, 4 subjects dropped out (3 subjects because of job relocation and 1 subject because of a dislike of the treatment powder), and 4 subjects were excluded (2 subjects because of initiation of antibiotics, 1 subject because of initiation of antidepressants, and 1 subject because of examination of urinary isoflavone data that led to concerns about the subject's compliance with the study dietary soy restrictions). Thirty-five subjects completed the study and were included in the final statistical analysis.

Subject characteristics

Baseline characteristics of the 35 healthy young men are shown in **Table 2**. During the study, there were no significant effects of treatment or treatment order on anthropometric measurements, including body weight, BMI, and percentage body fat (data not shown).

Energy, macronutrient, dietary fiber, and calcium intakes

Energy, macronutrient, dietary fiber, and calcium intakes before and during the study are shown in **Table 3**. The consumption of energy; protein; carbohydrate; total fat; saturated, monounsaturated, and polyunsaturated FAs; dietary fiber; cholesterol; and calcium did not differ significantly between the treatment periods. However, comparison of prestudy and study food records found that subjects consumed significantly more protein

TABLE 2
Subject characteristics at baseline[1]

	Value
Age (y)	27.9 ± 5.7
Body weight (kg)	82.5 ± 9.5
Height (m)	1.81 ± 0.07
BMI (kg/m^2)	25.4 ± 3.0
Body fat (%)	16.4 ± 4.6
Total cholesterol (mmol/L)	4.50 ± 1.25
LDL cholesterol (mmol/L)	2.74 ± 1.06
HDL cholesterol (mmol/L)	1.07 ± 0.17
Triacylglycerols (mmol/L)	1.45 ± 1.07

[1] All values are $\bar{x}$ ± SD. $n = 35$.

($P = 0.0005$), more calcium ($P < 0.001$), and less fat ($P = 0.015$) during the study than before the study.

Urinary isoflavone excretion

Urinary excretion of genistein and daidzein was significantly greater with the consumption of the high-iso SPI than with that of the low-iso SPI and the MPI (**Figure 1**; $P < 0.0001$ for all comparisons). Urinary excretion of equol and *O*-desmethylangolensin also were significantly greater with the consumption of the high-iso SPI than with that of the low-iso SPI or the MPI ($P < 0.0001$ for all comparisons; data not shown). As reported previously (38), further analysis of the variability in urinary equol excretion within the high-iso SPI treatment found that 34% subjects ($n = 12$) could be categorized as equol excretors (urinary equol > 1000 nmol/24 h) (42).

Serum lipids and C-reactive protein

Serum lipid concentrations for each treatment are shown as means in **Table 4** and as percentage changes from baseline in **Figure 2**. The day 1 concentrations of TC, LDL cholesterol, HDL cholesterol, non-HDL cholesterol, triacylglycerols, apo B, apo A-I, and CRP did not differ significantly among the 3 treatments, which provided evidence that the washout periods between treatments were sufficient. Further analysis indicated that 3 treatments did not differ significantly for any serum lipid,

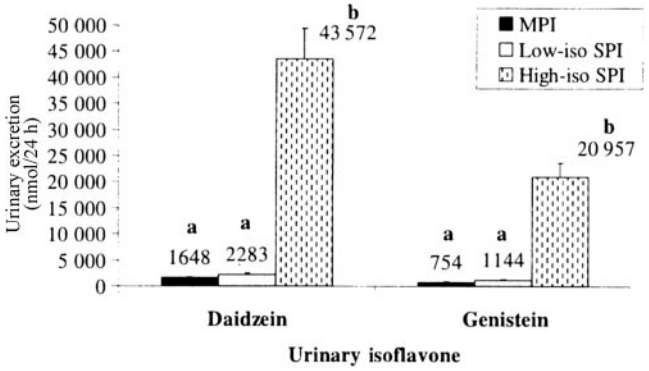

FIGURE 1. Geometric mean (+SE) urinary isoflavone excretion. $n = 35$ within each treatment. MPI, milk protein isolate; Low-iso SPI, low-isoflavone soy protein isolate; High-iso SPI, high-isoflavone SPI. Values within an isoflavone with different superscript letters are significantly different, $P < 0.0001$ (repeated-measures ANOVA followed by Tukey's test).

including TC, LDL cholesterol, HDL cholesterol, non-HDL cholesterol, triacylglycerols, apo B, and apo A-I (Table 4 and Figure 2), or for serum CRP (Table 4).

When ratios of serum lipids were evaluated, results showed that total:HDL, LDL:HDL, and apo B:apo A-I were significantly lower with consumption of the low-iso SPI ($P = 0.031$, 0.006, and 0.011, respectively) and the high-iso SPI ($P = 0.054$, 0.012, and 0.005, respectively) than with that of the MPI (Table 4 and **Figure 3**).

Inclusion of equol excretor status as a covariate in the statistical model did not change the study results, except those for LDL cholesterol, which were significantly lower with consumption of the low- ($P = 0.035$) and high-iso SPI ($P = 0.041$) than with that of the MPI (data not shown). The interactions between equol excretor status and treatment were not significant for any endpoints, and thereby a separate evaluation of treatment effects within equol excretors and nonexcretors was precluded.

DISCUSSION

The purpose of this study was to ascertain the effects of the consumption of soy protein and soy isoflavones on serum lipids

TABLE 3
Energy, macronutrient, dietary fiber, cholesterol, and calcium intakes[1]

	Before study[2]	MPI[3] treatment powder	Low-iso SPI[3] treatment powder	High-iso SPI[3] treatment powder
Energy (kcal)	2647 ± 97.4	2564 ± 58.1	2536 ± 58.1	2587 ± 58.1
Protein (g)	105.7 ± 4.91[a]	123.5 ± 2.78[b]	122.8 ± 2.78[b]	125.6 ± 2.78[b]
Carbohydrate (g)	339.2 ± 13.7	334.9 ± 8.31	320.1 ± 8.31	326.9 ± 8.31
Fat (g)	96.4 ± 4.79[a]	81.2 ± 3.00[b]	84.9 ± 3.00[b]	86.3 ± 3.00[b]
SFA (g)	27.9 ± 1.82	25.6 ± 1.13	25.1 ± 1.13	25.5 ± 1.13
MUFA (g)	12.9 ± 1.38	11.7 ± 0.80	11.3 ± 0.80	13.0 ± 0.80
PUFA (g)	6.70 ± 0.67	5.55 ± 0.41	6.12 ± 0.41	6.53 ± 0.41
Dietary fiber (g)	14.9 ± 0.78	13.7 ± 0.46	13.9 ± 0.46	13.4 ± 0.46
Cholesterol (mg)	292.0 ± 22.5	268.7 ± 12.9	265.9 ± 12.9	291.4 ± 12.9
Calcium (mg)	770.6 ± 94.0[a]	1905 ± 54.5[b]	1856 ± 54.5[b]	2004 ± 54.5[b]

[1] All values are least-squares $\bar{x}$ ± SE. $n = 35$. Values in a row with different letter superscripts are significantly different, $P \leq 0.05$ (repeated-measures ANOVA followed by Tukey's test). MPI, milk protein isolate; Low-iso SPI, low-isoflavone soy protein isolate; High-iso SPI, high-isoflavone SPI; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

[2] Values are based on the results of one 3-d food record completed before the study.

[3] Values are based on the average results of three 3-d food records completed on days 1–3, 26–28, and 54–56; these data include the contributions from the study treatment protein powders.

TABLE 4
Serum lipid, C-reactive protein, and lipid ratio concentrations[1]

	MPI treatment	Low-iso SPI treatment	High-iso SPI treatment
Total cholesterol (mmol/L)			
Day 1	4.55 ± 0.07	4.63 ± 0.07	4.61 ± 0.07
Day 57	4.55 ± 0.06	4.47 ± 0.06	4.40 ± 0.06
LDL cholesterol (mmol/L)[2]			
Day 1	2.74 ± 0.06	2.82 ± 0.06	2.76 ± 0.06
Day 57	2.86 ± 0.05	2.71 ± 0.05	2.65 ± 0.05
HDL cholesterol (mmol/L)			
Day 1	1.13 ± 0.02	1.11 ± 0.02	1.12 ± 0.02
Day 57	1.10 ± 0.02	1.15 ± 0.02	1.13 ± 0.02
Non-HDL cholesterol (mmol/L)			
Day 1	3.42 ± 0.06	3.52 ± 0.06	3.49 ± 0.06
Day 57	3.45 ± 0.05	3.33 ± 0.05	3.27 ± 0.05
Triacylglycerols (mmol/L)			
Day 1	1.51 ± 0.10	1.49 ± 0.10	1.60 ± 0.10
Day 57	1.30 ± 0.07	1.35 ± 0.07	1.39 ± 0.07
Apo B (g/L)			
Day 1	0.92 ± 0.02	0.94 ± 0.02	0.94 ± 0.02
Day 57	0.92 ± 0.01	0.88 ± 0.01	0.87 ± 0.01
Apo A–I (g/L)			
Day 1	1.50 ± 0.02	1.48 ± 0.02	1.48 ± 0.02
Day 57	1.45 ± 0.02	1.49 ± 0.02	1.48 ± 0.02
C-reactive protein (mg/L)			
Day 1	0.63 ± 0.06	0.62 ± 0.06	0.74 ± 0.07
Day 57	0.67 ± 0.06	0.57 ± 0.05	0.67 ± 0.06
Total:HDL			
Day 1	4.16 ± 0.06	4.26 ± 0.06	4.21 ± 0.06
Day 57	4.22 ± 0.06[a]	4.01 ± 0.06[b]	4.00 ± 0.06[b]
LDL:HDL			
Day 1	2.50 ± 0.05	2.60 ± 0.05	2.54 ± 0.05
Day 57	2.66 ± 0.05[a]	2.45 ± 0.05[b]	2.41 ± 0.05[b]
Apo B:apo A–I			
Day 1	0.62 ± 0.01	0.64 ± 0.01	0.64 ± 0.01
Day 57	0.64 ± 0.01[a]	0.60 ± 0.01[b]	0.60 ± 0.06[b]

[1] All values are least-squares $\bar{x}$ ± SE. $n = 35$. MPI, milk protein isolate; Low-iso SPI, low-isoflavone soy protein isolate; High-iso SPI, high-isoflavone SPI; Apo, apolipoprotein. Statistical analysis was performed on the change in endpoint values between study day 1 and study day 57. Values in the same row with different superscript letters are significantly different, $P \leq 0.05$ (repeated-measures ANOVA followed by Tukey's test).

[2] Inclusion of equol excretor status as a covariate in the statistical model altered the results for serum LDL cholesterol; specifically, serum LDL cholesterol was significantly lower with consumption of the low-iso SPI ($P = 0.035$) and high-iso SPI ($P = 0.041$) than with that of the MPI (data not shown).

and CRP in healthy young men. The current study adds to the existing literature on soy and CVD risk through its focus on subjects who have not been widely studied (ie, healthy young men), its analysis of a full range of serum lipids including lipid ratios and CRP, and its use of 3 treatments to allow for evaluation of the effects of both soy protein (comparison of SPI with MPI) and soy isoflavones (comparison of high-iso SPI with low-iso SPI).

The analysis of urinary isoflavones showed significantly higher excretion after consumption of the high-iso SPI than after that of the low-iso SPI and MPI, which provided evidence that subjects complied with consumption of the high-iso SPI. As reported previously (38), analysis of urinary equol showed that, during the high-iso SPI treatment, 34% of subjects excreted equol in amounts > 1000 nmol/24 h. When equol was included as a covariate, the serum LDL-cholesterol results became significant. Although this significant result did not occur for the other endpoints, its occurrence for serum LDL cholesterol underscores equol's previously identified (43) potential to influence the effects of soy protein and identifies the need for a focused investigation of this possibility.

Serum TC; LDL, HDL, and non-HDL cholesterol; and triacylglycerols did not differ significantly between the treatments in the current study. The few previous studies that evaluated the effect of soy protein on circulating lipids in normolipidemic men produced inconsistent results. Wong et al (27) found that SPI consumption significantly decreased plasma LDL cholesterol in normolipidemic men, whereas 2 other, similar studies found that it did not significantly affect plasma TC (35, 36), LDL cholesterol (35, 36), or triacylglycerols (36) but did significantly increase HDL cholesterol (36). Previous investigations including normolipidemic men and women reported significant decreases from baseline in plasma TC and LDL cholesterol with the use of isoflavone-extracted and isoflavone-intact SPI and in triacylglycerols with the use of isoflavone-extracted SPI (25). When changes from baseline were evaluated in that study, the results

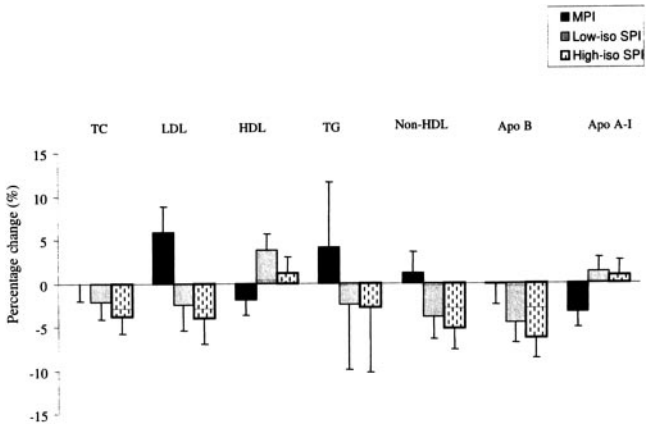

FIGURE 2. Least-squares mean (+SE) percentage changes in serum lipids. $n = 35$ within each treatment. MPI, milk protein isolate; Low-iso SPI, low-isoflavone soy protein isolate; High-iso SPI, high-isoflavone SPI; TC, total cholesterol; TG, triacylglycerols; Apo, apolipoprotein. The 3 treatments did not differ significantly for any serum lipids.

indicated that both isoflavone-extracted and isoflavone-intact SPI treatments significantly decreased plasma triacylglycerols relative to casein; however, the effects on total, LDL, and HDL cholesterol were not significant (25). A similarly designed study reported that, when compared with low-iso soy protein concentrate (SPC), high-iso SPC significantly increased plasma HDL cholesterol but did not affect TC, LDL cholesterol, or triacylglycerols (22).

Although not directly related to the current study, previous studies in hyperlipidemic men showed more consistent effects of SPI than did the current study, including significant decreases in plasma TC (34), LDL cholesterol (27), and non-HDL cholesterol (34) after SPI consumption. Studies including hyperlipidemic men and women also showed significant decreases in circulating TC (23, 33), LDL cholesterol (24, 32, 33), and triacylglycerols (24, 31) after consumption of SPI regardless of its isoflavone content, whereas other studies found no significant reductions in circulating lipids after SPI consumption (31). Studies of hyperlipidemic men and women also related reductions in circulating lipids to the isoflavone content of SPI, as shown by Crouse et al (19), who found significant decreases, compared with casein, in plasma TC and LDL cholesterol after the consumption of SPI containing 62 mg isoflavones but not after the consumption of that containing 37, 27, or 3 mg isoflavones.

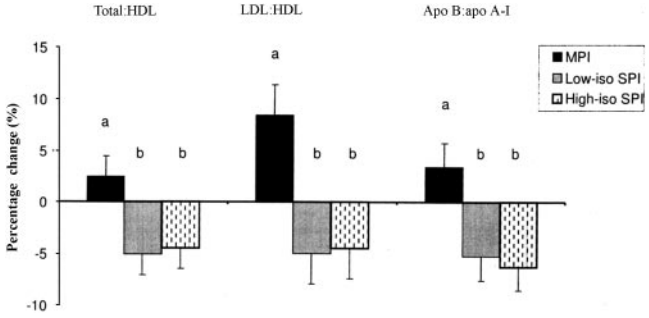

FIGURE 3. Least-squares mean (+SE) percentage changes in serum lipid ratios. $n = 35$ within each treatment. MPI, milk protein isolate; Low-iso SPI, low-isoflavone soy protein isolate; High-iso SPI, high-isoflavone SPI; TC:HDL, the ratio of total cholesterol to HDL cholesterol; LDL:HDL, the ratio of LDL to HDL cholesterol; Apo B:apo A-I, the ratio of apolipoprotein B to apolipoprotein A-I. Values within a serum lipid ratio with different superscript letters are significantly different, $P \leq 0.05$ (repeated-measures ANOVA followed by Tukey's test).

As with the serum lipid results, the current study did not find any significant differences in serum apo B or apo A-I between the 3 treatments. The 2 previous studies of normolipidemic men that included apolipoproteins in their investigation of the effects of soy protein on lipids reported conflicting results: one found no significant changes in plasma apo B or apo A-I (27), and the other found no significant change in plasma apo B but a significant increase in apo A-I (22). Previous studies of hyperlipidemic men also reported inconsistent results: one study reported a significant decrease in plasma apo B (34), and other studies reported no significant changes in circulating apo B (23, 32, 33) or apo A-I (23, 34).

In contrast to the individual serum lipids and apolipoprotein results, the current study found that total:HDL, LDL:HDL, and apo B:apoA-I were significantly lower after both the low-iso and high-iso SPI treatments than after the MPI treatment. These results are comparable to those of a previous study of normolipidemic men that reported a significant reduction in plasma LDL:HDL after consumption of SPI (27) and with those of 2 previous studies of hyperlipidemic men that reported significant decreases in serum total:HDL (23, 24), LDL:HDL (23, 24), and apo B:apo A-I (23) after consumption of both low- and high-isoflavone SPI treatments. Finally, Teede et al (31) observed a significant decrease, relative to casein, in plasma LDL:HDL in hyperlipidemic men who consumed soy protein. There is evidence linking changes in lipid ratios to CVD risk. Specifically, elevated total:HDL was shown to predict cardiac events (44) and TC:HDL, HDL:LDL, and apo A-I:apo B are considered to be more accurate than are individual lipids for predicting coronary heart disease (45). In summary, the current study contributes to the soy and CVD literature by showing that SPI can induce changes in serum lipid ratios, which was not explored in many previous studies in men.

The current study did not find any significant differences in serum CRP between the 3 treatments. These results are consistent with previous studies that found no significant effects of a diet containing soy foods (46) or SPI (47, 48) on circulating CRP in postmenopausal women (47) or groups of men and postmenopausal women (46, 48). Circulating CRP is considered a CVD risk factor (49) and can be influenced by estrogens (50) and hormone replacement therapy (51), which provides a rationale for the investigation of its response to soy isoflavones. The limited number of studies that have evaluated effects of isoflavone-rich soy on circulating CRP make it challenging to draw conclusions, and circulating concentrations of CRP are low in healthy persons (49) (as was the case in the current study), which makes it difficult to observe effects. Related to the potential relation between soy and CRP is a study that showed an influence of CRP status on serum lipid response to a cholesterol-lowering diet supplemented with soy protein (48). Overall, continued research into the potential of soy isoflavones to influence CRP is needed.

It is noteworthy both that, in the current study, the significant effects of the SPI treatments on serum lipid ratios occurred regardless of isoflavone content and that the low- and high-iso SPI treatments did not differ significantly. Taken together, these observations support the notion that the lipid-lowering effect of soy is attributable to its constituent protein rather than to isoflavones. Also supportive is the considerable interest in the relevance of

soy protein's composition—particularly its bioactive peptide components—to its lipid-lowering effects (52, 53). It is also relevant that the consequences of the isoflavone-extraction process for the production of the low-iso SPI treatment are unknown, and thus it cannot be assumed that the nonisoflavone compositions of the low- and high-iso SPIs were similar. Further research is needed to evaluate the effects of processing techniques on the composition of soy protein and its influence on lipid lowering.

In summary, the current study evaluated the effects of soy protein and isoflavones in a soy protein matrix on serum lipids, apolipoproteins, and CRP in healthy young men. Results support the ability of soy protein to modulate the ratios of serum lipids in healthy young men in a beneficial direction for CVD risk, irrespective of the isoflavone content of the soy protein. The relevance of these results to CVD risk is supported by epidemiologic data in young men that document a significant inverse association between changes in serum lipids within normal endogenous ranges and the future risk of CVD (26).

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BLM and BLD coordinated the subject recruitment and data collection and assisted in the data and statistical analyses. BLM also coordinated the laboratory analyses, interpreted the results, and wrote the manuscript. BLD also assisted in the manuscript preparation. JLW performed the urinary isoflavone analyses and assisted in the manuscript preparation. AMD designed the study, supervised the subject recruitment and data collection, supervised the laboratory analyses, directed the data and statistical analyses, directed the interpretation of results, and directed the manuscript preparation. None of the authors had any personal or financial conflict of interest.

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