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Soy proteins and isoflavones reduce interleukin-6 but not serum lipids in older women: a randomized controlled trial^{☆,☆☆}

Kelsey M. Mangano^{a,b,1}, Heather L. Hutchins-Wiese^{c,d,1}, Anne M. Kenny^{c,*}, Stephen J. Walsh^e, Robin H. Abourizk^f, Richard S. Bruno^{a,g}, Rosanne Lipcius^f, Pamela Fall^h, Alison Kleppinger^c, Lisa Kenyon-Pesce^c, Karen M. Prestwood^c, and Jane E. Kerstetter^f

^aDepartment of Nutritional Sciences, University of Connecticut, Storrs, CT

^bInstitute for Aging Research, Hebrew SeniorLife, Harvard Medical School, Boston, MA

^cCenter on Aging, MC-5215, University of Connecticut Health Center, Farmington, CT

^dDepartment of Dietetics and Human Nutrition, Eastern Michigan University, Ypsilanti, MI

^eDepartment of Nursing, University of Connecticut, Storrs, CT

^fDepartment of Allied Health Sciences, University of Connecticut, Storrs, CT

^gDepartment of Human Nutrition, The Ohio State University, Columbus, OH

^hClinical Research Center, MC-3805, University of Connecticut Health Center, Farmington, CT

Abstract

Soy foods contain several components, notably, isoflavones and amino acids, that may improve cardiovascular health. We evaluated the long-term effect of soy protein and/or soy isoflavones supplementation on serum lipids and inflammatory markers using a 1-year randomized, double-blind, placebo-control, clinical trial in 131 healthy ambulatory women older than 60 years. We hypothesized that soy protein, in combination with isoflavones, would have the largest positive effect on coronary heart disease risk factors (serum lipids and inflammatory markers) compared with either intervention alone and that, within groups receiving isoflavones, equol producers would have more positive effects on coronary heart disease risk factors than nonequol producers. After a 1-month baseline period, participants were randomized into 1 of 4 intervention groups: soy protein (18 g/d) and isoflavone tablets (105 mg/d isoflavone aglycone equivalents), soy protein and placebo tablets, control protein and isoflavone tablets, or control protein and placebo tablets. *T* Tests were used to assess differences between equol and nonequol producers. Ninety-seven women completed the trial. Consumption of protein powder and isoflavone tablets did not differ among groups, and compliance with study powder and tablets was 79% and 90%, respectively. After 1 year, in the entire population, there were either no or little effects on serum lipids and inflammatory markers, regardless of treatment group. Equol producers, when analyzed separately,

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*Corresponding author. Center on Aging, University of Connecticut Health Center, Farmington, CT 06030. Tel.: +1 860 679 4928. Kenny@uchc.edu (A.M. Kenny).

¹These authors share co-first authorship as they provided equal contributions to the manuscript.

had significant improvements in total cholesterol/high-density lipoprotein and low-density lipoprotein/high-density lipoprotein ratios (-5.9% , $P = .02$; -7.2% , $P = .04$ respectively). Soy protein and isoflavone (either alone or together) did not impact serum lipids or inflammatory markers. Therefore, they should not be considered an effective intervention to prevent cardiovascular disease because of lipid modification in healthy late postmenopausal women lacking the ability to produce equol.

Keywords

Soy protein; Soy isoflavones; Serum lipids; Postmenopausal women; Equol producer; Cardiovascular health

1. Introduction

Coronary heart disease (CHD) is the most common and serious form of cardiovascular disease (CVD) and remains the leading cause of death in the United States [1]. Women have low rates of CHD until menopause when risk and incidence escalate. The loss of circulating estrogen with menopause can impact CHD risk factors by altering lipoprotein profiles [2]. The use of natural hormone replacement, namely, soy isoflavones, has increased in popularity, especially in light of hormone replacement therapy's adverse effects.

Most soy-derived food is a source of isoflavones, predominantly the β -glycosides, daidzein, and genistein. These compounds are phytoestrogens, which bind to estrogen receptors. Because the chemical structure of daidzein and genistein has 2 benzene rings linked through a heterocyclic pyrane C ring, it is similar to that of 17β -estradiol, the circulating estrogen [3]. On the basis of structure alone, it is not surprising that isoflavones bind to estrogen receptors; however, their actions do not necessarily mimic estrogen and are more similar to selective estrogen receptor modulators [4].

Equol is an isoflavone metabolite that is produced from daidzein by intestinal bacteria. Approximately 30% to 60% of individuals can produce equol from daidzein [5]. The ability of gut microflora to produce equol may result in additional health benefits [6]. For example, equol can act as an antioxidant by inhibiting superoxide radical production and enhancing nitric oxide production, thus modifying low-density lipoprotein (LDL) oxidation and CHD risk [7]. Equol production, however, is highly variable between individuals and can change interindividually [8].

Subanalyses of large intervention trials indicate that soy protein intake and time since menopause began affect CHD risk [9]. Late postmenopausal women (those at highest risk for CHD) have yet to be the focus of soy food investigations. In addition, most of the previous intervention studies have used either a combination of soy protein and isoflavones or isoflavones alone to examine CHD risk, making it impossible to differentiate the impact of the 2 components. The current clinical trial adds to previous findings by determining whether soy's effects on CHD risk markers are a result of the protein, the isoflavone, or an interaction of the 2. Therefore, the purpose of this nutrition intervention study, as a secondary outcome for analysis, was to assess the long-term (1-year) effects of soy protein

and isoflavone supplementation (separately and in combination) on cardiovascular risk factors in late postmenopausal women. We hypothesized that soy protein, in combination with isoflavones, would have the largest positive effect on CHD risk factors (serum lipids and inflammatory markers) compared with either intervention alone and that within groups receiving isoflavones, equol producers would have more positive effects on CHD risk factors than nonequol producers.

2. Methods and materials

2.1. Study overview

The study design was a 1-year, double-blind, randomized, placebo controlled, prospective, 2 × 2 factorial intervention trial in 131 postmenopausal women. Ninety-seven women completed the intervention. This cohort of women, not selected for lipid status, participated in this institutionally approved clinical trial; full details of the intervention were described previously [10]. At baseline, participants were assigned randomly to 1 of 4 treatment groups: soy protein + isoflavone tablets (SPI), soy protein + placebo tablets (SPP), control protein + isoflavone tablets (CPI), or control protein + placebo tablets (CPP). The primary outcome measure was serum lipids (total cholesterol [TC], LDL, high-density lipoprotein [HDL], and triglycerides [TGs]); the secondary outcome was lipid ratios (TC/HDL and LDL/HDL); and the tertiary outcome measure was inflammatory markers (high-sensitivity C-reactive protein [hsCRP] and interleukin [IL]-6). Subset analyses included a comparison of equol and nonequol producers on cardiovascular risk markers. The University of Connecticut Health Center Institutional Review Board approved the study, and all women provided written, informed consent before the screening evaluation.

2.2. Nutrition counseling and protocol

All participants received dietary counseling from a research dietitian and completed 4-d dietary records every 3 months at the University of Connecticut's General Clinical Research Center. Assessment of nutrient intakes and compliance were carefully monitored at each visit. Dietary records were reviewed with each participant for completeness and accuracy and analyzed with the Food Processor II Nutrient Analysis Program (ESHA Research Inc, Salem, OR, USA). Counseling to reduce fat and/or carbohydrate intake was provided as a way of decreasing energy intake, only if participants were gaining weight. Soy foods and nutritional or herbal supplements were prohibited for the duration of the study. At each 3-month visit, unused protein powders and tablets were returned, amount consumed was accounted for, and a new 3-month supply was dispensed.

2.3. Soy and placebo products

Control and soy protein were protein isolates (85%–90% protein; wt/wt) to minimize the volume ingested. The soy protein was an alcohol-washed, soy protein isolate containing 90% protein and negligible isoflavones (0.2 mg/g product; Pro Fam 930, 066-930). The soy protein and isoflavones were provided by Archer Daniels Midland, Co, Decatur, IL, USA. The control protein was a mix consisting of 50% sodium caseinate, 25% whey, and 25% egg white protein (Century Foods International, Sparta, WI, USA). The control protein constituted a mix of proteins to provide a balance of amino acids and better reflect the mix

of proteins humans habitually consume. The calculated amino acid composition of the soy and the control protein is provided in a previous publication [10]. Participants were instructed to incorporate the protein powder (20 g powder containing 18 g protein) into their commonly consumed beverages or foods. To achieve dietary protein maintenance from baseline, participants were counseled to reduce animal protein foods by approximately 3 oz/d (containing the equivalent protein as the study powder).

Daily, participants ingested 3 identical study tablets that contained soy isoflavones or placebo (kindly provided by Archer Daniels Midland, Co). Each soy isoflavone tablet contained 35 mg isoflavone aglycone equivalents (from primarily genistein, glycitein, and daidzein and their β -glycosides). The aglycone equivalent value was determined by multiplying the total isoflavones by 0.61. The placebo tablets contained a blend of 10-diatomaceous earth maltodextrin (90%) and caramel color (10%) to match the color of the isoflavones; both components were food grade. Tablet composition was reported previously [10].

2.4. Anthropometric and medical history assessment

Standing height was measured in centimeters using a standing stadiometer. Weight was measured to the nearest 0.5 kg using a Detecto manual physician's scale. Height and weight were used to calculate the participant's body mass index (BMI). Medical history was taken by written questionnaire and reviewed during the baseline study visit. Four-day food records were obtained and analyzed using Nutritionist Pro (ESHA version 10.1).

2.5. Biochemical assessment

After the participants fasted for 10 to 12 hours, blood samples were collected between 0700 and 1000 hours and divided into 0.5-mL aliquots and stored at -70°C .

2.5.1. Serum markers—Serum TC, LDL, HDL, and TG were measured using the Synchron@ System with kits nos. 467858, 467825, 969706, and 650207, respectively. Serum hsCRP and IL-6 were measured by the Immulite 1000 analyzer using a solid-phase chemiluminescent immunometric assay (Siemens Healthcare Diagnostics, Los Angeles, CA, USA); the interassay coefficients of variance were 4.5% and 6.8%, respectively. Manufacturer instructions were followed for all kits.

2.5.2. Serum isoflavones and equol—We developed a high-sensitivity high-performance liquid chromatography binary gradient separation method coupled with Coularray electrochemical detection (capable of sensitivity to ~ 20 nmol/L) to measure serum isoflavones (daidzein, genistein, and equol) from free-living individuals. A binary gradient approach effectively resolved interfering unknown compounds in the serum from parent isoflavones and the metabolite equol. In brief, the samples were separated at 1 mL/min on a C₁₈ Luna [2,11], 250 $\times$ 4.6 mm, 5 μm (Phenomenex, Torrance, CA, USA), using 25 mM potassium phosphate buffer (pH 2.7) as mobile phase A and methanol/ acetonitrile/mobile phase A (50:30:20) as mobile phase B. The gradient was delivered as follows: 50% B to 65% B from 0 to 20 minutes, a linear gradient to 75% B from 20 to 30 minutes, and a linear gradient to 100% from 30 to 35 minutes. Initial conditions were

returned more than 2 minutes, and the system was allowed to equilibrate at 50% B for 12 minutes before subsequent injection. Analytes were detected using potential settings of 325, 450, 575, and 700 mV and were quantitated on their dominant channel. Serum isoflavone concentrations were calculated using area ratios for standards and the internal standard. Under these conditions, each analyte had excellent linearity.

Equol is an isoflavone metabolite produced from intestinal microflora that appears in the blood in limited quantities (<100 nmol/L). In brief, 500 μ L of serum was buffered with 100 mM ammonium acetate (pH 4.6), mixed with 50 μ L of internal standard (4-hydroxybenzophenone; 110 pmol/ μ L), and subjected to enzymatic hydrolysis (overnight, 37°C) using 200 U β -glucuronidase and 15 U sulfatase prepared in 100 μ L of ammonium acetate (pH 4.6). After incubation, proteins were precipitated using acetonitrile (1 mL), the sample was delipidated using hexane (3 mL), and the isoflavones were extracted with methyl tert-butyl ether. Extracts were dried under nitrogen gas, reconstituted in mobile phase A, and injected on the high-performance liquid chromatography system. Equol producers were defined as those individuals who had a 12-month serum concentration of S-equol 20 nmol/L (5 μ g/L), according to the method of Setchell and Cole [12]. Analyte recovery was greater than 95% for the isoflavones and equol, and the intra-assay and interassay coefficients of variance were less than 8% for all analytes.

2.6. Statistical analyses

All analyses were conducted with SPSS version 14.0 (SPSS Inc, Chicago, IL, USA). A *P* value less than .05 was considered statistically significant, unless otherwise noted. Baseline and clinical characteristics are reported as means $\pm$ SD or n (%), stratified by treatment group. Baseline characteristic between group differences were tested using 1-way analysis of variance (ANOVA). Percent changes in outcome measures (serum lipid and inflammatory markers) over the 12-month intervention period were calculated. An outlier was observed for percent change in hsCRP (1344% change). The data were verified, and this outlier was changed to the next highest value + 1% (466% change). Two-way ANOVA was used to evaluate between group differences in percent change of primary outcome variables (TC, LDL, HDL, and TG), secondary outcome variables (lipid ratios), and tertiary outcome variables (inflammatory markers). To account for multiple testing, Bonferroni correction was used within primary, secondary, and tertiary outcome groups. Therefore, primary outcome measures (serum lipids) were tested at the 1.25% significance level, and secondary and tertiary outcomes (lipid ratios and inflammatory markers, respectively) were tested at the 2.5% significance level. Among those receiving isoflavones, independent *t* tests were used to evaluate changes in outcome variables (serum lipids, lipid ratios, and inflammatory markers) between equol and nonequol producers.

3. Results

3.1. Baseline characteristics

Of 1510 advertisement respondents, 767 qualified for the study based on the initial telephone screen. The profile of the study recruitment, treatment allocation, and retention were reported previously [10]. Baseline characteristics (age, anthropometric measures,

lifestyle, dietary intake, serum lipids, and inflammatory markers) are presented in Table 1. Total fat and saturated fat intake were significantly different between treatment groups at baseline (Table 1); however, there were no differences between treatment groups in percent change for 12 months (total fat $P = .34$; saturated fat $P = .19$). Levels of IL-6 were significantly different between groups at baseline ($P = .03$). Serum lipids or lipid ratios were not different among treatment groups at baseline (Table 1). Based on the National Cholesterol Education Program ATPIII guidelines, in this generally healthy sample of postmenopausal women, baseline TC and LDL cholesterol were categorized as borderline high and HDL cholesterol and TGs categorized as normal [13].

3.2. Intervention compliance

Consumption of protein powder and isoflavone tablets did not differ between groups. On average, participants consumed 16.1 ± 0.4 g soy (or control) protein supplement daily (14.3 ± 3.6 g protein and 79% compliance). Isoflavone pill intake was 2.7 ± 0.35 pills/d (containing 94 ± 12 mg aglycone equivalents), which reflected 90% compliance. As anticipated, genistein and daidzein were present in serum from women supplemented with isoflavones at month 12 (Table 2). Fifty-one women received isoflavones tablets; all but 3 had measurable concentrations of serum isoflavones. Twenty-five women were equal producers (49%). In the 46 women who received placebo tablets, 4 had measurable concentrations of serum isoflavones.

3.3. Dietary intake and anthropometric assessment at month 12

Energy and nutrient intakes during the intervention period (average of four 4-day diet records; Table 3) did not differ between groups. On average, participants gained 0.5 kg during the 1-year intervention; the percentage of weight change did not differ between treatment groups ($P = .45$).

3.4. Serum lipids and inflammatory markers

After 1 year of dietary intervention, no significant interactions of soy protein and isoflavones were observed on serum lipids, lipid ratios, or inflammatory markers ($P = .07$ –.99). Main effects models examining the effects of soy protein and isoflavones on outcome measures are presented in Table 4. Overall, HDL cholesterol was significantly reduced by 3% in the soy protein groups compared with placebo protein ($P = .04$); however, this difference was not statistically significant after the Bonferroni correction for multiple comparisons. No other effects on serum lipids were observed with either soy protein or isoflavone interventions ($P = .30$ –.79). A significant decline in percent change from baseline in IL-6 was observed in the soy protein group compared with the control protein group ($P = .007$) and remained significant with Bonferroni correction for multiple comparisons. The addition of baseline saturated fat intake as a covariate did not alter results from the main effects models (percent change in IL-6 remained significantly different between groups, $P = .01$).

3.5. Equol production, serum lipids, and inflammatory markers

By evaluating the women who received the isoflavones, we tested the hypothesis that equol producers would demonstrate more positive CVD outcomes than the nonequol producers. However, no significant differences in percent change in serum lipids and inflammatory markers were observed between equol and nonequol producers ($P = .11-.66$). Equol producers showed a small but statistically significant improvement from baseline to month 12 in their serum lipid ratios (Fig.).

3.6. Adverse events and participant safety

No significant differences in endometrial thickness at baseline or 1 year after the intervention were observed between groups. One woman had an endometrial thickness greater than 6 mm. Other complaints included gastrointestinal disturbance ($n = 10$), differences in mammogram or breast tenderness ($n = 2$), new-onset cardiac symptoms ($n = 2$), increase in blood pressure ($n = 4$), respiratory infection ($n = 3$), and unrelated medical conditions ($n = 4$). The rates of adverse events did not differ between groups.

4. Discussion

The objective of this prospective intervention trial was to identify the potential cardiovascular benefit of incorporating soy protein and/or soy isoflavones into the diets of healthy, late postmenopausal women. After 1 year of intervention, in comparison with the control group, we observed little measurable improvements in serum lipids or inflammatory markers among women consuming soy protein or isoflavones, either alone or in combination. Therefore, we reject the first part of our hypothesis and accept the second part. The ability to synthesize equol from dietary isoflavone precursors was associated with an improvement in the ratio of serum lipids. The women in this study had generally good health with baseline serum lipid levels near National Cholesterol Education Program recommendations. Reported compliance to protein and isoflavone intervention corresponded with measures of serum isoflavone concentrations in study completers.

In recent years, long- and short-term soy intervention studies [14–16] have found little or no benefit to serum lipid profiles. Our results are consistent with that of others; changes to serum lipids are minimal, even when exposed to soy over a 1-year intervention. In addition, the 2×2 factorial design allowed for comparisons between soy protein and soy isoflavones. Neither soy protein nor isoflavones alone demonstrated meaningful effects on serum lipids or inflammatory markers in older, postmenopausal women.

It is probable that the effects that soy protein or isoflavones have on the cardiovascular system could be caused by mechanisms other than improvements in the cholesterol profile. Kreijkamp-Kasper and colleagues [17] found no benefit after a yearlong intervention comparing soy and milk protein on blood pressure in postmenopausal women. The Women's Isoflavone Soy Health study evaluated carotid artery intima-media thickness and found no benefit with long-term (2.7-year) intake of soy protein containing 91 mg aglycone isoflavones [9]. A subgroup analysis of women less than 5 years postmenopausal found a lower progression rate compared with placebo [9], thus suggesting that late postmenopausal

women, as we have reported herein, demonstrate little to no cardiovascular health benefits from soy.

Soy may have anti-inflammatory activities that may reduce the risk of CVD. Soy isoflavones have anti-inflammatory properties in cytokine activated endothelial cells via inhibition of monocyte adhesion [18], therefore limiting early events in atherogenic progression. The evaluation of inflammatory markers in our investigation revealed that, among individuals in the control protein group, there was an increase in IL-6 levels, whereas the soy protein prevented a rise in IL-6. No other effects on change in IL-6 or hsCRP during the intervention were observed. Previous investigations that supplemented healthy postmenopausal women with soy protein or isoflavones [16,19,20] found no change in inflammatory markers. Postmenopausal women with elevated C-reactive protein may see a reduction in inflammation with soy isoflavone intake [21]. Declines in inflammatory markers have also been reported when evaluating special, high-risk populations, including those with diabetic nephropathy [22], ischemic stroke [23], and metabolic syndrome [24]; thus suggesting a possible benefit for high-risk cardiac populations. Aside from anti-inflammatory processes, soy may improve CVD health by acting as an antioxidant, providing a low-fat protein food, or by affecting the vascular walls themselves [25].

The ability of some individuals to produce equol from the soy isoflavone, daidzein, has been hypothesized to be responsible for some of the health benefits of soy. Approximately 30% to 60% of humans have the intestinal flora required to produce equol, [5], and equol production may be associated with a reduced risk of chronic diseases [5]. We hypothesized that individuals who produce equol would have greater cardioprotection compared with nonequol producers. Although comparison of equol producers with nonequol producers did not alter serum lipids or inflammatory markers, serum lipid ratios (TC/HDL and LDL/HDL) were significantly lower in equol producers. Whether this statistical difference is large enough to be biologically important is unknown.

This study had inherent limitations. We experienced a dropout rate of 25%. However, similar studies where soy proteins were fed long term experienced similar attrition rates [14,26]. Owing to the large variability in the lipid outcome measures, we had reduced power to detect effect sizes of 1.7% to 2.8% change between groups. The presence or absence of heart disease was not an inclusion/exclusion criterion; therefore, benefits to a subpopulation, as seen in those with higher baseline cholesterol [27–29], may be missed in our mixed population. Lastly, a recent finding stating that hypercholesterolemic middle-aged men and women experienced beneficial effects on LDL cholesterol from short-term soy diet interventions with an added benefit of maintaining HDL cholesterol in equol producers compared with nonproducers [30] suggests that younger, high-risk individuals may benefit from soy and equol production, and we did miss this earlier window of opportunity for lipid changes.

Older women are rarely studied in long-term nutrition intervention trials. That this population is at high risk for heart disease is a study strength. Additional strengths include the 1-year intervention period and the 2 × 2 study design that allowed for the separation of the potential effect of soy protein from soy isoflavones. The women in this study were

healthy but exhibited characteristics typical of an older adult population (eg, mean BMI overweight, approximately 15%–20% smokers, and typical report of chronic disease) [31], thus suggesting that the results may be similar to the general population.

The addition of dietary soy protein and/or isoflavones, without other dietary changes, did not affect serum lipids or inflammatory markers over a 1-year period in healthy late postmenopausal women, although lipid ratios improved 5% to 7% in individuals that produced equol from daidzein. These data suggest that soy supplementation will not play a major clinical role in serum lipid management, especially among those lacking the ability to produce equol. If soy protein or isoflavones provide cardiovascular benefits, it would most likely be via a mechanism other than lipid alteration. Future studies should consider enrolling individuals on the basis of their ability to produce equol to better define the potential benefits of soy on lipids.

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Abbreviations

ANOVA	analysis of variance
BMI	body mass index
CHD	coronary heart disease
CVD	cardiovascular disease
HDL	high-density lipoprotein
hsCRP	high-sensitivity C-reactive protein
IL-6	interleukin-6
LDL	low-density lipoprotein
TC	total cholesterol
TG	triglycerides

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Fig.

Values are percent change of ratios of lipids in serum between equol ($n = 25$) and nonequol ($n = 26$) producers in those who received isoflavone supplementation, as assessed by independent t test (error bars are $\pm$ SEM). The ratios of serum lipids in closed boxes are TC/HDL cholesterol, and those in the open boxes are LDL cholesterol/HDL cholesterol. Independent t test analyses were used to assess differences between equol and nonequol producers. $*P = .018$ for percent change in the TC/HDL ratio between equol and nonequol producers. $\#P = .043$ for percent change in the LDL/HDL ratio between equol and nonequol producers.

Table 1

Baseline characteristics of study participants in the intervention groups

	SPI	SPP	CPI	CPP	P
Age (y)	73.0 ± 5.7	74.0 ± 6.2	72.3 ± 5.7	72.9 ± 6.1	.72
Height (cm)	157.7 ± 6.4	159.9 ± 6.1	159.6 ± 6.1	159.1 ± 5.2	.44
Weight (kg)	72.3 ± 14.8	70.5 ± 10.8	70.3 ± 12.2	71.8 ± 12.6	.9
BMI (kg/m ²)	29.3 ± 6.9	27.6 ± 4.3	27.8 ± 5.4	28.4 ± 4.8	.59
Smokers, n (%)					.63
Never	12 (40)	19 (61)	15 (50)	16 (52)	
Current ^a /Previous	18 (60)	12 (39)	15 (50)	15 (48)	
Drink alcohol, n (%)	22 (76)	21 (68)	21 (70)	13 (43)	.05
Time since last menses (y)	23.5 ± 9.9	24.3 ± 10.8	22.6 ± 9.3	22.8 ± 8.1	.91
Medical history, n (%)					
Heart disease	7 (23)	4 (13)	3 (10)	2 (6.5)	.24
Hypertension	9 (30)	11 (37)	10 (33)	15 (48)	.47
Nutrient intake ^b					
Energy (kcal/d)	1424 ± 337	1608 ± 468	1387 ± 408	1292 ± 408	.06
Protein (g/d)	63 ± 14	65 ± 17	68 ± 26	57 ± 22	.3
Carbohydrate (g/d)	177 ± 48	199 ± 67	171 ± 47	166 ± 55	.16
Total fat (g/d)	51 ± 15	62 ± 24	48 ± 19	46 ± 20	.02 *
SFA (g/d)	18 ± 6	21 ± 8	16 ± 5	15 ± 7	.02 *
MUFA(g/d)	16 ± 5	20 ± 10	16 ± 8	14 ± 7	.09
PUFA (g/d)	8 ± 2	11 ± 7	9 ± 5	9 ± 5	.14
Omega-3 PUFA (g/d)	1.0 ± 0.6	0.9 ± 0.3	1.1 ± 0.9	0.8 ± 0.7	.54
Omega-6 PUFA (g/d)	6.2 ± 2.1	9.1 ± 6.5	6.8 ± 3.7	6.9 ± 4.5	.13
Cholesterol (g/d)	219 ± 90	236 ± 113	205 ± 100	188 ± 120	.44
Fiber (g/d)	17 ± 5	18 ± 9	17 ± 7	18 ± 11	.88
Soluble fiber (g/d)	3 ± 1	3 ± 1	3 ± 2	3 ± 2	.82
Insoluble fiber (g/d)	5 ± 2	6 ± 3	5 ± 3	5 ± 4	.94
Alcohol (g/d)	6 ± 8	4 ± 8	5 ± 8	1 ± 2	.16
Caffeine (mg/d)	138 ± 151	112 ± 99	172 ± 171	100 ± 106	.27

	SPI	SPP	CPI	CPP	P
Serum lipids					
Cholesterol (mmol/L)					
Total (mmol/L)	5.45 ± 0.87	5.30 ± 0.74	5.30 ± 1.04	5.46 ± 1.29	.9
HDL (mmol/L)	1.39 ± 0.32	1.54 ± 0.40	1.39 ± 0.33	1.33 ± 0.33	.21
LDL (mmol/L)	3.50 ± 0.77	3.27 ± 0.61	3.36 ± 0.88	3.57 ± 1.13	.64
TG (mmol/L)	1.23 ± 0.62	1.07 ± 0.34	1.19 ± 0.55	1.23 ± 0.54	.71
Ratios					
TC/HDL	4.13 ± 1.20	3.59 ± 0.76	3.95 ± 0.93	4.28 ± 1.10	.12
LDL/HDL	2.68 ± 0.94	2.25 ± 0.66	2.51 ± 0.75	2.81 ± 0.90	.13
Inflammatory markers					
hsCRP (pg/mL)	5.24 ± 6.76	4.28 ± 4.55	3.09 ± 2.71	4.16 ± 5.45	.52
IL-6 (pg/mL)	2.76 ± 0.76	3.97 ± 2.65	3.13 ± 2.03	2.51 ± 0.74	.03 *

Data are presented as means ± SD or n (%). CPI, control protein + isoflavone tablets, n = 32; CPP, control protein + control tablets, n = 33; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SPI, soy protein + isoflavone tablets, n = 32; SPP, soy protein + control tablets, n = 34.

^aThree active smokers in the study.

^bNutrient intakes from food sources only, supplements not included.

* Significantly different between groups: $P < .05$ as assessed by 1-way ANOVA.

Table 2

Serum isoflavones and equol at month 12 in the intervention groups

Serum isoflavones ($\mu\text{mol/L}$)	SPI	SPP	CPI	CPP	P
Daidzein	1.18 $\pm$ 1.14	0.21 $\pm$ 0.56	0.87 $\pm$ 0.85	0.13 $\pm$ 0.60	<.001 *
Genistein	0.90 $\pm$ 0.98	0.03 $\pm$ 0.11	0.88 $\pm$ 0.90	0.01 $\pm$ 0.07	<.001 *
Equol ^a	0.15 $\pm$ 0.14	–	0.19 $\pm$ 0.24	–	.72

Data are presented as means $\pm$ SD. CPI, control protein + isoflavone tablets, n = 26; CPP, control protein + control tablets, n = 22; SPI, Soy protein + isoflavone tablets, n = 25; SPP, soy protein + control tablets, n = 24.

^aFrom 25 equol producers.

*. Significantly different between groups: $P < .05$ as assessed by ANOVA.

Table 3

Nutrient intake at 12 months in the intervention groups for 97 valid completers

Nutrient intake ^a	SPI	SPP	CPI	CPP	P ^b
Energy (kcal/d)	1453 ± 258	1558 ± 279	1505 ± 325	1512 ± 300	.66
Protein (g/d)					
Food sources	65 ± 11	65 ± 12	65 ± 16	68 ± 13	.87
Supplement	15 ± 3	15 ± 4	14 ± 4	14 ± 4	.46
Total	81 ± 12	80 ± 13	79 ± 18	81 ± 14	.94
Carbohydrate (g/d)	187 ± 37	203 ± 45	191 ± 40	199 ± 58	.6
Total fat (g/d)	50 ± 19	53 ± 14	53 ± 16	50 ± 14	.84
SFA (g/d)	18 ± 6	18 ± 6	19 ± 7	17 ± 5	.86
MUFA (g/d)	15 ± 5	17 ± 6	17 ± 5	16 ± 5	.75
PUFA (g/d)	9 ± 5	9 ± 4	10 ± 3	9 ± 4	.93
Omega-3 PUFA (g/d)	0.9 ± 0.5	0.9 ± 0.4	1.1 ± 0.8	1.0 ± 0.5	.54
Omega-6 PUFA (g/d)	7 ± 5	7 ± 3	7 ± 2	7 ± 4	.97
Cholesterol (g/d)	212 ± 76	204 ± 72	199 ± 71	219 ± 56	.93
Fiber (g/d)	17 ± 6	17 ± 5	18 ± 5	18 ± 5	.95
Soluble fiber (g/d)	3 ± 1	2 ± 1	3 ± 1	3 ± 1	.26
Insoluble fiber (g/d)	6 ± 3	5 ± 2	6 ± 2	6 ± 3	.17
Alcohol (g/d)	3 ± 6	5 ± 8	5 ± 11	4 ± 8	.65
Caffeine (mg/d)	187 ± 182	141 ± 92	186 ± 100	137 ± 82	.32

Data are presented as means ± SD. Data obtained from quarterly 4-day diet records and averaged. CPI, control protein + isoflavone tablets, n = 26; CPP, control protein + control tablets, n = 22; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SPI, Soy protein + isoflavone tablets, n = 25; SPP, soy protein + control tablets, n = 24.

^aEnergy from food sources only, not supplement, unless otherwise noted.

^bData assessed by ANOVA; no significant differences between groups in nutrient intake.

Table 4
Cardiovascular characteristics among 97 valid completers in the intervention groups

	Soy protein + isoflavones (n = 25)	Soy protein + Placebo (n = 24)	Control protein + isoflavones (n = 26)	Control protein + placebo (n = 22)	P value (2-way ANOVA)	Main effects: soy protein	Main effects: isoflavone
Serum lipids							
Cholesterol							
Total	-2.95 ± 13.28	-2.23 ± 14.37	-0.37 ± 13.92	0.87 ± 11.20	.30		.72
HDL	-3.23 ± 12.32	-3.35 ± 14.93	4.66 ± 20.03	2.98 ± 16.72	.03 <i>NS</i>		.79
LDL	-3.70 ± 17.38	-1.72 ± 22.21	-1.09 ± 18.00	-0.41 ± 14.21	.60		.72
TG	13.96 ± 40.08	5.31 ± 47.71	-4.45 ± 26.69	17.51 ± 43.36	.64		.44
Serum lipid ratios							
TC/HDL	0.86 ± 12.52	3.32 ± 23.98	-3.28 ± 14.34	-0.32 ± 15.51	.26		.44
LDL/HDL	-0.01 ± 16.88	4.58 ± 35.15	-3.68 ± 19.12	-1.68 ± 16.89	.30		.49
Inflammatory markers							
hsCRP	28.25 ± 64.21	36.40 ± 95.68	71.49 ± 121.22	52.39 ± 118.55	.15		.80
IL-6	8.57 ± 23.33	-6.76 ± 36.95	33.25 ± 66.23	17.62 ± 35.92	.007 *		.09

Data are percent change from baseline to months 12 presented as means ± SD.

NS Nonsignificant at $P < .0125$ after Bonferroni correction for multiple comparisons.

* Significant at $P < .025$ after Bonferroni correction for multiple comparisons.