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Dietary Soy Has Both Beneficial and Potentially Adverse Cardiovascular Effects: A Placebo-Controlled Study in Men and Postmenopausal Women*

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ABSTRACT

To address the cardiovascular effects of dietary soy containing phytoestrogens, we measured blood pressure (BP), lipids, vascular function (systemic arterial compliance and pulse wave velocity), and endothelial function (flow-mediated vasodilation) in a randomized, double-blind trial.

Two hundred thirteen healthy subjects (108 men and 105 postmenopausal women), 50–75 yr old, received either soy protein isolate (40 g soy protein, 118 mg isoflavones) or casein placebo for 3 months.

There were 34 withdrawals (16%), with 179 subjects (96 men and 83 women) completing the protocol. After intervention in the soy group, compared with casein placebo, urinary phytoestrogens increased, accompanied by a significant fall in BP reflected by the BP model ($P < 0.01$) encompassing mean change (±SEM) in systolic (-7.5 ± 1.2 *vs.* -3.6 ± 1.1 mm Hg, $P < 0.05$), diastolic (-4.3 ± 0.8 *vs.* -1.9 ± 0.7 mm Hg, $P < 0.05$), and mean BP (-5.5 ± 1 *vs.* -0.9 ± 1 mm Hg, $P < 0.008$). In the lipid model, soy induced greater changes, compared with placebo ($P < 0.001$). On individual analysis, significant contributors included a reduction in the low- to high-density lipoprotein ratio (-0.33 ± 0.1 *vs.* 0.04 ± 0.1 mmol/L, $P < 0.05$) and triglycerides (-0.2 ± 0.05 *vs.* -0.01 ± 0.05 mol/L, $P < 0.05$) and an increase in Lp(a) lipoprotein (± 95% confidence interval) [42 (range, 17–67) *vs.* 4 (range, −22–31) mg/L, $P < 0.05$], whereas total, low-density lipoprotein, and high-density lipoprotein cholesterol improved in both groups; but no treatment effect was demonstrated. The arterial functional model demonstrated no difference between groups; although again, overall function improved in both groups. On individual analysis, peripheral PWV (reflecting peripheral vascular resistance) improved with soy ($P < 0.01$), whereas flow-mediated vasodilation (reflecting endothelial function) declined (in males only), compared with casein placebo ($P < 0.02$). No effect of treatment on the hypothalamic-pituitary-gonadal axis was noted in males or females.

In normotensive men and postmenopausal women, soy improved BP and lipids but, overall, did not improve vascular function. Potential adverse effects were noted, with a decline in endothelial function (in males only) and an increase in Lp(a). Further research in hypertensive and hyperlipidemic populations is needed. (*J Clin Endocrinol Metab* **86:** 3053–3060, 2001)

PHYTOESTROGENS ARE UBIQUITOUS, nonsteroidal, plant-derived compounds reported to exhibit both estrogen agonist and antagonist activities (1). The soybean is a rich source of the isoflavone phytoestrogens genistein and daidzein (2), ligands for both estrogen receptors ERα and ERβ (3), with greater affinity demonstrated for ERβ. ERβ has been shown to be the primary estrogen receptor in the vessel wall and is up-regulated in response to vascular injury (4, 5). High soy diets are associated with low cardiovascular disease risk (6). Interventional studies, primarily focusing on the effects of adding soy protein supplements to existing diets, indicate that potential cardiovascular benefits may be partially attributable to lipoprotein effects (7).

Intact soy protein supplementation lowered total and low-density lipoprotein (LDL) cholesterol and triglycerides in some, but not all, studies (7), whereas high-density lipoprotein (HDL) cholesterol was unchanged (7). The effects on Lp(a) lipoprotein are yet to be resolved. Furthermore, it is not known whether reported lipoprotein effects are attributable to the soy isoflavones or to other nonphytoestrogen components.

Preliminary studies, in women only, suggest that phytoestrogen supplementation reduces diastolic blood pressure (BP) (8) and has direct effects on the vessel wall (9). The vasculoprotective effects of soy resemble those of exogenous estrogen (4, 10). Estrogen improves large artery function in postmenopausal women (11–13) and male-to-female transexuals (14). Hence, we hypothesize that dietary soy may influence large artery function via biological estrogenic mechanisms.

Endothelial dysfunction, an early marker of vascular disease, can be assessed *in vivo* with flow-mediated vasodilation (FMD) (15). Brachial artery FMD correlates with coronary endothelial function (16) and with cardiovascular risk factors (15, 17–19). It deteriorates after the menopause (19) and improves with estrogen therapy (20). It is not yet established whether soy can similarly influence endothelial function.

To investigate the cardiovascular effects of soy, we have conducted a double-blind, randomized, placebo-controlled study of 3 months of addition of dietary soy protein sup-

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plementation in healthy normotensive men and postmenopausal women. No other dietary changes were made during the study. Endpoints included lipid parameters, BP, arterial compliance, and endothelial function measured by noninvasive Doppler and tonometry ultrasound techniques.

Materials and Methods

Study design

Two hundred thirteen healthy participants (108 men and 105 postmenopausal women) participated in a 3-month randomized, double-blind, placebo-controlled design. Participants (50–75 yr old) were recruited from community advertisements. They had not consumed antibiotics, soy products, or supplements (for 3 months), nor had they taken estrogen therapy (for 12 months) before entry. Postmenopausal status was defined as: 12 months of amenorrhea, and FSH more than 20 IU/L. Exclusion criteria included: moderate-to-severe menopausal symptoms, smoking (last 10 yr), diabetes, alcohol consumption more than 30 g/day, hypertension, abnormal uterine bleeding, cervical cytology or mammogram, and coexistent major illness. The Monash Medical Center Human Research and Ethics Committee approved the study, and all participants gave written informed consent. Randomization was performed independently using computer-generated random numbers, with 105 participants allocated to soy, (50 women and 55 men) and 108 to casein placebo (55 women and 53 men).

Participants were screened for dietary phytoestrogen intake at baseline based on history, dietary questionnaire, and urinary phytoestrogen excretion. Supplements were presented in identical unmarked powder sachets. They were consumed twice daily after mixing into beverage form and were taken in addition to the usual diet, with no other changes in daily dietary intake. The soy isolate powder was prepared by dehulling and defatting soybeans and then blending for 45 min. The product was then tested before packaging to ensure content uniformity for protein and isoflavones and was provided in a single batch, by Protein Technologies International (St. Louis, MO) (AB1.2 HG 70CA 29, lot A161–7). Each sachet contained 28 g powdered soy protein isolate, of which 71% was protein. This product was tested for isoflavone content using high-performance liquid chromatography (HPLC) with ultraviolet detection and was determined to contain 2.11 mg total isoflavones per g of protein. This was composed specifically of genestein 1.35, daidzein 0.66, and glycitein 0.09 (expressed as basic compound + respective glycosides, in milligrams per g of product). Overall, this provided 40 g soy protein per day, with a total of 118 mg isoflavones daily. This intake was selected because it provides a level of phytoestrogens between that of Pacific Rim nations (25–45 mg) and some areas in Japan (200 mg).

A baseline medical assessment was conducted, and a National Heart Foundation of Australia cardiovascular risk factor questionnaire was administered at both baseline and after 3 months (21). Exercise information was based on specific physical activities, not including household chores.

Dietary adherence was assessed by measurement of spot urine phytoestrogen concentrations at baseline and at the end of 3 months of therapy. Urine phytoestrogens were normalized for creatinine (Cr), a standard methodology applied in previous work both from our group and that of other international groups involved in human interventional studies using phytoestrogens (22, 23). Height, weight, waist-hip ratio, heart rate, and BPs were measured. Six brachial arterial BP readings were recorded, over 15 min, on a Dinamap (CRITIKON 1846 SX; Johnson and Johnson, Sydney, Australia), in a recumbent position, after 10 min of rest. The first reading was disregarded, the average of the subsequent readings being recorded.

Phytoestrogen assays

Urinary phytoestrogen excretion was analyzed using HPLC. The principal analytes were daidzein and genistein, as previously described (24). Phytoestrogen isolation was by reverse-phase HPLC (Shimadzu system LC 10A), following a method developed by Eldridge (25). Detection was by dual-wavelength ultraviolet absorbance (250 nm for daidzein, 262nm for genistein) and quantification by comparing the area under the curve with reference standards, genistein (Sigma, St. Louis, MO) and daidzein (ICN Biomedicals, Inc., Aurora, CO). Samples for each subject were processed in one batch.

Hormone, lipid, and lipoprotein assays

Fasting morning blood samples were collected at baseline and at 3 months, for testosterone (in males), estradiol (in females), gonadotropins, and lipid profiles and were centrifuged at 2500 $\times$ *g* for 12 min. Plasma was stored at -80^{O} C and thawed immediately before analysis. Gonadotropins were measured by RIA performed on an automated MEIA using the AxSYM Immunoassay Analyzer (Abbott Laboratories Diagnostics Division, North Chicago, IL). Standardization was against the WHO 2nd International Reference Preparation (78/549) and (80/552) for FSH and LH, respectively. Total testosterone and estradiol were measured by standard automated immunoassay ACS180 (Bayer, Melbourne, Australia). Total cholesterol and triglycerides were measured using enzymatic reagents (DADE Diagnostics, Brisbane, Australia); HDL cholesterol was measured by homogeneous assay techniques (HDLC-Plus, DADE Diagnostics) adapted to a DADE Dimension RXL chemistry analyzer (DADE Diagnostics). LDL cholesterol was calculated using the Friedewald equation [LDL cholesterol = (total cholesterol − HDL cholesterol) − (triglycerides $\times$ 0.20)], adapted to S.I. units. Lp(a) lipoprotein was measured by an immunonephelometric assay on a nephlometric Analyzer (Behring, Marburg, Germany) using antiserum (DAKO Corp., Glostrup, Denmark).

Vascular parameters

All vascular parameters were measured by an experienced research assistant (D. Kotsopoulos). Studies were performed after an 8-h fast (including avoidance of caffeine-containing drinks), in a dark, quiet, air-conditioned clinical laboratory, after 10 min of rest in the supine position.

Total systemic arterial compliance (SAC). Noninvasive determination of blood flow and pressure waveforms were applied to determine SAC, as previously described (26). Aortic volumetric blood flow was measured from a hand-held 3.5-MHz continuous-wave Doppler flow velocimeter (Multidoplex M.D.1, Huntleigh Technology, Cardiff, UK) at the suprasternal notch. Simultaneous driving pressure was ascertained by applanation tonometry with a pressure transducer (Millar Mikro-tip, Millar Instruments Houston, TX) over the carotid artery, with pressures calibrated against Dinamap brachial artery pressures (CRITIKON 1846 SX). Given that SAC varies with changes in BP, we corrected for this by standardizing SAC values at the mean BP level for each individual. Compliance, over the total systemic arterial tree, was calculated by the following formula according to the method of Liu *et al.* (27): SAC = Ad $\div$ R(Ps − Pd), where Ad is the area under the BP diastolic decay curve from end-systole to end-diastole; Ps is end-systolic BP; Pd is end-diastolic BP (carotid); and R is total peripheral resistance derived from BP and aortic root blood flow.

Pulse wave velocity (PWV). PWV was determined from recorded pressure waveforms over both the aorto-femoral (AF) and the femoro-dorsalis (FD) arterial segments (26). Pulse transit time was defined as the time between the foot of simultaneously recorded pressure waves, occurring at the end of diastole and the beginning of systole, averaged over 10 cardiac cycles. Velocity was derived from computer-generated pulse transit times and measured distances between the two recording sites, as previously described (26). PWV was calculated based on the formula: PWV = D $\div$ Δt (m/sec), where D is distance and Δt is the time interval.

Brachial artery FMD. The brachial artery diameter was measured from B-mode ultrasound images captured on a Diasonics DRF-400 machine using a 10-MHz transducer, while an ECG trace was simultaneously recorded. Longitudinal scanning identified the clearest image of the brachial artery above the elbow, with continuous scanning held for 30 sec before and 4 min after ischemia, induced via a pneumatic tourniquet inflated (around the upper arm) to 40 mm Hg above systolic pressure, for 4 min. Vessel diameter was measured during systole and diastole and averaged over 5 cardiac cycles. FMD was determined as the percentage change, from baseline to 60 sec post ischemia (the point of maximal dilation) (26).

Repeatability. A repeatability study of vascular parameters was completed concurrently by our group (26). Bland-Altman plots showed satisfactory repeatability for SAC, PWV(AF), and PWV(FD), with repeatability coefficients of 9.2%, 3.2%, and 5.0%, respectively (26). FMD curves were not different between visits, with maximal diameter changes observed 60 sec post ischemia. Based on the sample size in this study, changes of 4% in SAC, 3% in PWV(AF), 2% in PWV(FD), and 10% in FMD were detectable, less than noted with estrogen therapy (11, 20). Also, a repeatability substudy was completed, before randomization, in 31 of the study participants who had 2 consecutive baseline evaluations. This demonstrated no significant differences in arterial compliance or PWV between visits.

Statistical analysis

The values for Lp(a) lipoprotein, urinary phytoestrogens, and SAC were positively skewed. This was corrected by log transformation, and the data was summarized as geometric means with 95% confidence intervals (CI). The other variables were summarized as arithmetic means ± 1 SE. Data from all participants who completed the study protocol were included in the analyses. The levels of significance was accepted at $P \leq 0.05$. Statistical calculations were performed using the SPSS, Inc. statistical package, version 9 (SPSS, Inc., Chicago, IL).

For the purpose of statistical analysis, the outcomes were defined as the arithmetic difference (change) between baseline observations and those made after 3 months of treatment. The outcome variables were divided into three sets: 1) descriptors of BP (SBP, DBP, and MBP); 2) descriptors of lipid profiles [total, LDL, HDL, and LDL/HDL cholesterol ratio; triglycerides; and Lp(a) lipoprotein]; and 3) descriptors of vascular function [SAC, PWV(AF), PWV(FD)]. The outcome variables within each set were entered as dependent variables in multivariate ANOVA (MANOVA). For all sets, the categorical, independent variables were: treatment group (soy or casein placebo), sex (male or female), and age, as well as their treatment/sex and treatment/age interactions. After initial MANOVA, univariate ANOVAs were completed for each of the independent variables. A process of backward elimination was then followed, in which the independent variables were successively eliminated if P was more than 0.05, from univariate analyses. In the case of descriptors of BP and vascular function, the final MANOVA model contained only treatment, with one exception (in the case of FMD, the treatment/sex interaction, on univariate analysis, gave $P = 0.02$). Therefore, separate analyses by sex were performed.

For the final MANOVA models, the subsequent univariate analyses involved testing multiple hypotheses, thus increasing the risk of type I error (false-positive inference). This was controlled by employing the Ryan-Holm step-down Bonferonni procedure (28). This technique was also applied when analyzing differences between groups at baseline.

Results

In total, 34 participants withdrew (19 active and 15 casein placebo), with 83 women and 96 men completing the study protocol. The 2 groups were well matched for baseline characteristics, including BP (Table 1), lipids (Table 2), vascular functional parameters (Table 3), age (61 ± 1 *vs.* 60 ± 1 yr), height (1.7 ± 0.01 *vs.* 1.7 ± 0.01 m), weight (72 ± 1 *vs.* 74 ± 1 kg), body mass index (25 ± 0.4 ± 26 ± 0.04 kg/m^2), waist-hip ratio (0.9 ± 0.01 *vs.* 0.9 ± 0.01), heart rate (61 ± 1 *vs.* 62 ± 1 beats/min), and exercise frequency (5.2 ± 0.5 *vs.* 5.2 ± 0.7 h/fortnight). We have previously reported that, based on a validated questionnaire, there was a 57% incidence of hot flashes of mild severity and a high incidence (80%) of very mild generalized menopausal symptoms in this older-community-dwelling population of postmenopausal women (29). These symptoms were similar in the active and placebo groups (29).

Side effects were similar in both groups (10 active, 7 placebo) and were primarily: intolerance of the supplements; or gastrointestinal effects, including constipation (3 active, 2 placebo), diarrhea (1 active, 2 placebo), weight gain (2 active), hot flashes (1 active), and unrelated personal reasons (2 active, 3 placebo). Two women were excluded from the active group because their FSH levels were not elevated.

Phytoestrogen levels

Baseline phytoestrogen excretion was similar in the two groups. After therapy, genistein increased 12-fold (15 ± 3 to 186 ± 23 ng/μmol Cr, $P < 0.001$) and daidzein increased 7-fold (44 ± 8 to 316 ± 37 ng/μmol Cr, $P < 0.001$) in the active group, whereas both genestein (10 ± 2 to 9 ± 2 ng/μmol Cr) and daidzein (23 ± 5 to 22 ± 5 ng/μmol Cr) remained unchanged in the casein placebo group.

Blood pressure and lipid parameters

Significantly greater falls in systolic, mean, and diastolic BP in the active (compared with the casein placebo) group were seen on both MANOVA ($P = 0.014$) and univariate ANOVA (Table 1). The multivariate model incorporating changes in total, LDL, HDL, and LDL/HDL cholesterol ratio; Lp(a) lipoprotein; and triglycerides (Table 2) demonstrated significantly greater changes in the active (compared with the casein placebo) group ($P = 0.006$). Only the improvements in triglycerides and LDL/HDL cholesterol ratio and the increase in Lp(a) lipoprotein contributed significantly to

TABLE 1. Blood pressure readings in the female, male, and combined soy and casein placebo groups: baseline (Bl), 3 months, and mean change

Blood pressure (mm Hg)	Men		Women		Combined		
	Bl	3 months	Bl	3 months	Bl	3 months	Mean change Bl to 3 months
Mean BP (mm Hg)							
Active	95 ± 1	91 ± 2	90 ± 2	82 ± 2	93 ± 1	87 ± 1	−5.5 ± 1.0[a]
Casein placebo	93 ± 1	92 ± 2	88 ± 2	87 ± 1	91 ± 1	89 ± 1	−1.3 ± 0.9
Systolic BP (mm Hg)							
Active	133 ± 2	127 ± 2	127 ± 3	118 ± 2	130 ± 2	123 ± 2	−7.5 ± 1.2[b]
Casein placebo	131 ± 2	127 ± 2	125 ± 2	122 ± 2	128 ± 2	125 ± 2	−3.6 ± 1.1
Diastolic BP (mm Hg)							
Active	79 ± 1	75 ± 1	73 ± 1	67 ± 1	76 ± 1	72 ± 1	−4.3 ± 0.8[b]
Casein placebo	78 ± 1	76 ± 1	73 ± 2	71 ± 1	76 ± 2	73 ± 1	−1.9 ± 0.7

MANOVA of the blood pressure model, incorporating all three parameters ($P = 0.014$).

[a] $P < 0.01$, [b] $P < 0.05$ on ANOVA comparing mean change in the soy and casein placebo groups, corrected by Ryan Holmes Stepdown Bonferroni procedure.

TABLE 2. Lipid profiles in the female, male, and combined soy and casein placebo groups: Bl, 3 months, and mean change

Lipid parameters	Men		Women		Combined		
	Bl	3 months	Bl	3 months	Bl	3 months	Mean change Bl to 3 months
Total cholesterol (mmol/L)							
Active	5.7 ± 0.1	5.3 ± 0.1	6.1 ± 0.2	5.4 ± 0.2	5.9 ± 0.1	5.3 ± 0.1	−0.55 ± 0.09
Casein placebo	5.8 ± 0.1	5.5 ± 0.1	6.0 ± 0.1	5.4 ± 0.1	5.9 ± 0.1	5.5 ± 0.1	−0.4 ± 0.09
LDL cholesterol (mmol/L)							
Active	3.8 ± 0.1	3.5 ± 0.1	4.0 ± 0.1	3.4 ± 0.1	3.9 ± 0.1	3.5 ± 0.1	−0.42 ± 0.07
Casein placebo	3.9 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.4 ± 0.1	3.8 ± 0.1	3.6 ± 0.1	−0.28 ± 0.07
HDL cholesterol (mmol/L)							
Active	1.3 ± 0.06	1.3 ± 0.05	1.6 ± 0.08	1.5 ± 0.06	1.44 ± 0.1	1.40 ± 0.04	−0.04 ± 0.03
Casein placebo	1.3 ± 0.05	1.3 ± 0.07	1.8 ± 0.08	1.5 ± 0.07	1.51 ± 0.1	1.40 ± 0.05	−0.11 ± 0.04
LDL/HDL ratio							
Active	3.4 ± 0.2	3.0 ± 0.2	2.7 ± 0.2	2.4 ± 0.2	3.1 ± 0.2	2.7 ± 0.1	−0.33 ± 0.09[a]
Casein placebo	3.3 ± 0.2	3.1 ± 0.1	2.3 ± 0.2	2.5 ± 0.2	2.8 ± 0.2	2.8 ± 0.1	−0.04 ± 0.08
Triglycerides (mmol/L)							
Active	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	1.0 ± 0.07	−0.19 ± 0.05[a]
Casein placebo	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.07	−0.01 ± 0.05
Lipoprotein (a) (mg/L)							
Active	190 (133–246)	214 (143–285)	410 (249–572)	474 (288–661)	286 (207–365)	328 (235–421)	42 (17–67)[a]
Casein placebo	273 (176–369)	294 (180–407)	417 (257–577)	402 (246–559)	341 (251–433)	346 (252–440)	4 (−22–30)

MANOVA of the lipid model, incorporating all six parameters ($P = 0.001$).

[a] $P < 0.05$ on ANOVA, comparing mean change in the soy and casein placebo groups, corrected by Ryan Holmes Stepdown Bonferroni procedure.

the multivariate model. Changes in these parameters remained significant (P always less than 0.05) on corrected ANOVA in the soy group, compared with the casein placebo group (Table 2). Total, LDL, and HDL cholesterol improved in both groups; yet, no effect of treatment status was noted (Table 2). No significant change in body weight was observed in either group (0.03 ± 0.03 *vs.* 0.15 ± 0.02 kg, $P = 0.6$).

Vascular compliance and endothelial function

Table 3 demonstrates mean (95% CI) SAC and mean (± SEM) PWV [PWV(AF) and PWV(FD)]. Soy supplementation had no significant effect on arterial compliance as assessed by MANOVA incorporating SAC, PWV(AF), and PWV(FD) ($P = 0.1$). On univariate ANOVA, only PWV(FD) improved significantly, compared with casein placebo ($P = 0.02$). Correction of SAC for BP changes did not alter these observations. There was no significant effect of treatment on resting brachial artery diameter in either sex. Brachial artery FMD was significantly reduced during soy treatment in men ($P < 0.05$) but was unaffected in women (Fig. 1).

Hormone profiles

Baseline levels were similar in the active and casein placebo groups for FSH and LH and serum testosterone (Table 4). Serum estrogen levels were less than 20 mmol/L (because of the assay sensitivity constraints); and therefore, average levels were not given. After 3 months of therapy, there were no significant changes in any hormonal levels in males or females in either group (Table 4).

Discussion

Three months of soy protein supplementation in normotensive subjects significantly reduced systolic, diastolic, and mean BP and also lowered triglycerides and LDL/HDL cholesterol ratio in men and postmenopausal women but did not affect arterial compliance. The hypothalamic-pituitary-gonadal axis was unaffected by treatment in both males and females. Not all effects of soy treatment were beneficial, given that Lp(a) lipoprotein rose by 15% and a reduction in mean brachial artery FMD was noted in males.

In the current study, though baseline levels of isoflavone excretion were similarly low in both groups at baseline, 3months of soy dietary supplementation resulted in significant increases in urinary genistein (12-fold) and daidzein (7-fold) levels, whereas no change was noted with casein placebo. Despite some variability, demonstrated with equivalent phytoestrogen intakes (30, 31), urinary phytoestrogen excretion is considered a reliable reflection of dietary phytoestrogen intake.

We have observed a reduction in systolic, diastolic, and mean BP in a healthy normotensive population, a novel and potentially important effect of soy. Dietary soy supplementation resulted in placebo-adjusted falls of 4.2 mm Hg in mean, 2.4 mm Hg in systolic, and 3.9 mm Hg in diastolic pressure. These changes are greater than those noted in the DASH study, in normotensive individuals, with dietary intervention alone (32). Our findings are consistent with the 4.9 mm Hg reduction in diastolic BP noted in a study of soy therapy in 51 normotensive women (8). However, in that study, differences in ion concentrations between active and placebo supplements may have contributed to the BP effects (8). In contrast, Hodgson *et al.* reported that 8 weeks of concentrated phytoestrogens (55 mg/day), in tablet form, did not alter supine, erect, or ambulatory BP in normotensive subjects (33). Potentially, this discrepancy is attributable to several factors, including comparatively lower phytoestrogen doses (33), the necessity for phytoestrogens to be ingested in combination with other soy components, or alternatively, the fact that hypotensive effects of soy may not be mediated by estrogenic mechanisms. In the present study, the observation that distal PWV [PWV(FD)], which reflects

TABLE 3. Vascular parameters in the female, male, and combined soy and casein placebo groups: Bl, 3 months, and mean change

Vascular parameters	Men		Women		Combined	
	Bl	3 months	Bl	3 months	3 months	Mean change Bl to 3 months
SAC (units/mm Hg)						
Active	0.54 (0.47–0.61)	0.60 (0.53–0.68)	0.52 (0.46–0.58)	0.54 (0.47–0.61)	0.57 (0.52–0.63)	0.04 (−0.003–0.08)
Casein placebo	0.62 (0.51–0.73)	0.62 (0.55–0.68)	0.50 (0.43–0.56)	0.52 (0.45–0.57)	0.57 (0.52–0.61)	0.01 (−0.06–0.08)
PWV (AF) (m/sec)						
Active	10.5 ± 0.3	10.2 ± 0.3	9.6 ± 0.3	8.8 ± 0.3	9.6 ± 0.2	−0.41 ± 0.14
Casein placebo	10.1 ± 0.3	9.7 ± 0.4	9.2 ± 0.3	9.0 ± 0.3	9.4 ± 0.2	−0.30 ± 0.12
PWV (FD) (m/sec)						
Active	11.7 ± 0.2	10.8 ± 0.2	10.3 ± 0.2	9.8 ± 0.3	10.3 ± 0.2	−0.7 ± 0.20[a]
Casein placebo	10.9 ± 0.2	10.9 ± 0.2	10.3 ± 0.2	10.1 ± 0.2	10.5 ± 0.2	−0.12 ± 0.15

MANOVA of the vascular compliance model, incorporating all three parameters ($P = 0.1$).
[a] $P < 0.05$ on corrected ANOVA, comparing mean change in the soy and casein placebo groups, corrected by Ryan Holmes Stepdown Bonferroni procedure.

FIG. 1. Brachial artery FMD (±SEM), reflecting endothelial function in the soy *vs.* casein placebo groups, in males and females.

vasoconstriction in peripheral resistance vessels, improved significantly with soy therapy is consistent with the observed fall in BP.

The most consistently demonstrated beneficial effect of soy has been on lipids (7). Federal Drug Administration approval for consumer information labeling of soy foods has given in the United States, stating that "included in the daily diet, they may reduce the risk of heart disease". This is based primarily on the lipid benefits. A metaanalysis of 38 published controlled human clinical trials, of an average of 47g daily soy protein consumption (primarily in subjects with hyperlipidemia), noted significant reductions in total [9.3% decrease, 95% CI (0.35–0.85 mmol/L)] and LDL cholesterol [12.9% decrease, 95% CI (0.30–0.82 mmol/L)] and triglycerides [10.5% decrease, 95% CI (0.003–0.29 mmol/L)], with the hypocholesterolemic effect significantly related to pretreatment cholesterol levels (7).

In the present study, we observed improvements in the overall lipid profile, the LDL/HDL cholesterol ratio, and triglycerides in both men and postmenopausal women (Table 2). The fall in triglycerides is encouraging, because there is increasing epidemiological evidence that elevated triglycerides independently increase vascular risk and emerging interventional data that suggest that reduction in triglyceride levels reduces vascular risk (34). However, the significance of the improvement in the LDL/HDL ratio is more difficult to interpret, with epidemiological data consistently suggesting that it is a predictor of vascular risk; yet, it has not been shown to be an independent factor in intervention studies. In the current study, total and LDL cholesterol levels fell in both groups; yet, despite greater falls with soy, the differences between groups were not significant. We would suggest that this is potentially attributable to the normolipidemic population studied, with only small changes in lipid levels expected. The fall in cholesterol levels in the placebo group is consistent with the effects of involvement in clinical intervention trials. Dietary casein protein has been noted to increase cholesterol levels (35). However, given the observed improvements in lipid profiles in both groups, this effect of casein is unlikely to have contributed to the differences observed between the soy and casein groups. As in previous literature, HDL cholesterol did not change with soy therapy (7). The mechanism of soy effects on lipids remains unresolved; however, the response pattern does not parallel that

TABLE 4. Hormone levels at Bl, 3 months, and mean change

Hormone levels	Male baseline	Male 3 months	Male mean change Bl to 3 months	Female baseline	Female 3 months	Female mean change Bl to 3 months
Follicle-stimulating hormone (mmol/L)						
Active	11.1 ± 1.4	13.8 ± 1.6	2.7 ± 1.5	97.4 ± 6	98.6 ± 6	1.1 ± 3.4
Casein placebo	9.2 ± 0.8	13.9 ± 2.7	4.7 ± 2.7	112.7 ± 6	111.2 ± 6	−2.6 ± 3.2
Lutenizing hormone (mmol/L)						
Active	5.8 ± 0.5	5.2 ± 0.4	−0.5 ± 0.4	34.2 ± 2	31.8 ± 2	−2.6 ± 1.3
Casein placebo	5.0 ± 0.8	4.8 ± 0.7	−0.2 ± 1.1	35.9 ± 2	33.3 ± 2	−2.6 ± 1.1
Testosterone (mmol/L)						
Active	16.8 ± 0.9	15.1 ± 0.8	−1.5 ± 0.9			
Casein placebo	15.8 ± 0.8	14.7 ± 0.8	−1.0 ± 0.6			

of estrogen. Also, isolated phytoestrogen supplements do not seem to effect lipid profiles (36). Potentially, the saponins, polyunsaturated oils, or vegetable proteins in soy (either individually or in a combined way) may contribute to the beneficial reduction in triglycerides.

The rise in Lp(a) observed here with dietary soy supplementation is consistent with the results of a previous report of an unblinded cross-over study in nine normal men (37). This is potentially an adverse effect because, although Lp(a) is recognized as a risk factor for cardiovascular disease, it may be a cofactor rather than an independent risk factor (38). The pathophysiological significance of this, the most complex and polymorphic of the lipoprotein particles, remains unclear (38, 39). In contrast to the observed increase in Lp(a) with dietary soy, HRT has been reported to significantly reduce Lp(a) levels (40).

Both estrogen and phytoestrogens seem to have direct effects on the vessel wall. Animal data have shown similar dose-dependent vasculoprotective effects in animal carotid injury models, with both ligands equally inhibiting replication and migration of smooth muscle cells *in vitro* (4). In female monkeys, estrogen treatment significantly reduced aortic cholesteryl ester content, with a similar trend ($P = 0.14$) noted with soy protein, compared with casein (41). Also improved acetylcholine-mediated coronary artery vasodilation has been documented with estrogen (42), intact soy, and iv genistein administration (43). In male monkeys, the effects may be more complex because, although reduced atherosclerosis has been noted with intact soy (44), improved acetylcholine-mediated coronary artery vasodilation was not observed (43).

No beneficial effect of soy on arterial compliance or central PWV [PWV(AF)] was observed in the current study, despite a sample size adequate to detect improvements less than those noted with estrogen therapy. In contrast, two small studies, using lower doses of concentrated phytoestrogens rather than soy, reported improved SAC (9, 45). They involved both peri- and postmenopausal women and had no male subjects (9, 45). We have previously demonstrated a 22–39% greater compliance in healthy postmenopausal HRT users, compared with nonusers (11, 12, 13). Also, cessation of HRT results in an acute, significant (23%) fall in SAC (11). However, there are no reported long-term placebo-controlled studies of the effects of HRT on arterial function. Peripheral PWV [PWV(FD)] did improve with soy therapy, reflecting a reduction in the degree of vasoconstriction in peripheral vessels, most likely related to the observed changes in BP. However, the significance of isolated changes in this marker of vascular function, without accompanying changes in the more robust and predictive measure [PWV(AF)], must be interpreted with caution, with further study required.

Brachial FMD, mediated by nitric oxide release (46), has a 95% positive predictive value for coronary artery endothelial dysfunction (16). Impaired FMD is positively associated with cardiovascular risk factors (15, 17–19). Estrogen seems to improve endothelial function in normal healthy postmenopausal women (20, 47), in women with cardiovascular disease (47), and in male-to-female transexuals (14). An aim of the present study was to determine whether dietary soy had estrogen-like effects on endothelial function, as assessed by brachial artery FMD. It is recognized that this technique has its limitations, with less than ideal repeatability, significant operator dependence, and the influence of external factors such as exercise and food ingestion (15). In this study, a single experienced operator performed FMD under controlled conditions, with the study powered to detect a 10% change in FMD (26).

Compared with casein placebo, soy supplementation had no effect on FMD in healthy postmenopausal women, and it reduced FMD responses in males. A recent double-blind placebo-controlled cross-over study in 20 postmenopausal women has also noted no improvement of FMD; however, the intervention used was 80 mg isolated phytoestrogens in tablet form (36). The mechanism for this apparent deterioration in endothelial function in males is unclear, although previous studies have shown that acute estrogen administration did not improve FMD in human healthy males (48), nor did it induce endothelial dependent vasodilation in response to acetylcholine in the male monkey model (43).

The incidence of side effects was similar in both groups. Importantly, this is the first report on the impact of estrogenic soy components on the hypothalamic-pituitary-testicular axis in men and women. In men, lowered serum testosterone and altered sexual behavior have been demonstrated with herbal remedies containing phytoestrogens (49), although soy therapy in male monkeys did not affect testicular weights (44). Reassuringly, in males, we have demonstrated no impact of soy on gonadotropins or serum testosterone levels. In females, soy also did not affect the reproductive axis. This was expected, however, because (by definition) these women are menopausal and therefore in primary ovarian failure. Literature suggests that menopause is associated with feedback insensitivity to more potent estrogens; therefore, it was

expected that weakly biologically active phytoestrogens would not actively modulate this reproductive axis (50). The mechanisms of phytoestrogen action are varied and complex. At ERα and ERβ, phytoestrogens can act as pure agonists or antagonists or have partial or selective agonist/antagonist activity, further modified by variable affinity for the ER subtypes (3) and by coactivators and corepressors. This complexity significantly limits our understanding of the mechanisms and sites of action of phytoestrogens (1). It is clear, however, from both the current findings and those of animal studies (41), that the effects of soy containing phytoestrogens do not parallel those of estrogen, with respect to BP responses, lipoprotein changes, or effects on vascular function or on the hypothalamic-pituitary-gonadal axis.

Conclusion

Dietary soy protein supplement significantly reduced BP and improved LDL/HDL cholesterol ratio and triglycerides in healthy men and postmenopausal women, without improving indices of arterial function. Not all effects of soy treatment were beneficial. Lp(a) lipoprotein increased overall, and there was a reduction in mean brachial artery FMD in males. Soy consumption did not suppress the hypothalamic-pituitary-gonadal axis in males. Thus, it seems that the vascular, lipid, and hormonal effects of soy and estrogen differ. Further research is required to elucidate the active component(s) of soy and its mechanisms of action. The results of the present study suggest that soy may have a role to play in the prevention of cardiovascular disease, by improving both BP and lipid profiles; however, more research is needed, specifically in hyperlipidemic and hypertensive populations, before public health recommendations can be made.

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