Soy protein with isoflavones has favorable effects on endothelial function that are independent of lipid and antioxidant effects in healthy postmenopausal women[1–3]

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ABSTRACT

Background: Controversy exists about the ability of soy protein and isoflavones to modulate vascular reactivity and biochemical cardiovascular disease risk markers in healthy, normolipidemic postmenopausal women.

Objective: The objective was to investigate whether the consumption of soy protein with isoflavones would result in improved vascular reactivity and decreased biochemical markers of endothelial dysfunction and inflammation, independent of enhanced lipid and antioxidant effects.

Design: Healthy postmenopausal women ($n = 28$) were enrolled in a randomized, double-blind, crossover study, and they consumed 25 g of 3 protein products/d for 6 wk each, with intervening washout periods. The products were isolated soy protein with isoflavones, ethanol-washed isolated soy protein with trace isoflavones, and total milk protein, which supplied 107, 2, and 0 mg total isoflavone (aglycone) units/d, respectively. We studied vascular function by using brachial artery reactivity values, plasma concentrations of vasoactive factors, endothelial inflammatory markers, and plasma isoflavone concentrations. The resistance of whole plasma and isolated LDL to copper-mediated oxidation was measured by conjugated diene formation.

Results: Postocclusion peak flow velocity of the brachial artery was significantly ($P = 0.03$) lower after treatment with isolated soy protein with isoflavones, which is consistent with a vasodilatory response, than after treatment with total milk protein. Plasma isoflavones and metabolites were significantly ($P < 0.01$) higher after treatment with isolated soy protein with isoflavones. There were no significant changes in biochemical cardiovascular disease risk markers or conjugated diene formation between the 3 dietary groups.

Conclusion: Daily consumption of soy protein with isoflavones can result in positive vascular effects that are independent of lipid and antioxidant effects in healthy postmenopausal women. *Am J Clin Nutr* 2003;78:123–30.

KEY WORDS Soy, isoflavone, cardiovascular disease, endothelial function, lipid oxidation, postmenopausal women

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of hospitalization and death for menopausal women in the United States and other developed nations (1). The incidence of CVD among postmenopausal women has been directly related to the loss of endogenous estrogen protection (2). Estrogen replacement therapy or estrogen-and-progestin hormone replacement therapy (HRT) has favorable effects on blood lipids and lipoprotein concentrations, antioxidant protection, endothelial function, and vascular reactivity (2, 3). Yet despite the positive cellular effects of estrogen therapy on markers of CVD, the clinical cardiovascular health benefits of HRT for postmenopausal women remain controversial, as shown by recent clinical trial findings (4–6). Thus, cardioprotective nutritional, lifestyle, and pharmaceutical treatments such as selective estrogen receptor modulators, which offer postmenopausal women an alternative to traditional HRT, are of increasing interest.

Soy is a rich source of the polyphenolic isoflavones genistein and daidzein. Isoflavones are structurally similar to estradiol and have a high binding affinity for the primary estrogen receptor in the vascular wall, estrogen receptor β, relative to that for estrogen receptor α (7–9). Genistein, the predominate isoflavone, contributes to a decrease in the oxidative susceptibility of LDL in vitro and ex vivo (10–12) and has also been shown to have other cellular activities that may influence vascular tissue metabolism, such as inhibition of tyrosine kinase activity, decreased smooth muscle cell proliferation, and nitric oxide–dependent relaxation (13–15).

Animal data show that isoflavones improve vascular reactivity in a manner similar to that of estradiol and that they may interact with estradiol to enhance endothelial function (16–18). However, the effects of soy and isoflavones on vascular reactivity in menopausal women are less clear. Five studies investigated this question in postmenopausal women by using total isoflavones in a range of 54–118 mg/d (19–23). Two of these studies reported positive changes: one study found significant improvements in systemic arterial compliance (19), and the other, more recent study

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TABLE 1
Nutrient content of study proteins[1]

	TMP	Soy−	Soy+
Protein (g)	25	24	25
Fat (g)	0.2	1	1
Total isoflavones (mg)[2]	—	2	107
Genistein	—	1	55
Daidzein	—	0.5	47
Glycitein	—	0.5	5

[1] Values given are for 1 packet of study product (36.5 g) rounded to the nearest whole number. TMP, total milk protein; Soy−, ethanol-washed isolated soy protein with trace isoflavones; Soy+, isolated soy protein with isoflavones.
[2] Aglycone units.

reported a significant increase in flow-mediated vasodilation of the brachial artery, which was associated with changes in biochemical measures of endothelial metabolites (20). Of the other 3 studies, 1 reported significantly decreased blood pressure with peripheral vasodilation (23), and 2 reported no significant changes in endothelial function (21, 22).

Further investigation into the effects of soy protein with isoflavones in postmenopausal women is warranted to clarify issues of efficacy with regard to vascular function. The aim of the present study was to obtain information as to the additional benefits that soy protein with and without isoflavones may bring to an already healthy diet, by determining whether soy improved endothelial function, CVD risk markers, and antioxidant defense in healthy, free-living postmenopausal women who maintained their usual diets and use of vitamin and mineral supplements. This question is particularly relevant, given the increasing promotion and use of soy products and supplements in this population segment and in light of the still unanswered questions about potential cardiovascular benefits.

SUBJECTS AND METHODS

Subjects

Healthy postmenopausal women were recruited through advertisements in the local newspaper and postings throughout the University of California, Davis, Medical Center. The Institutional Review Board at the University of California, Davis, approved the protocol, and all participants provided written informed consent before the start of the study. Inclusion criteria for participants included menopausal status, as defined by the absence of menstrual bleeding in the past 12 mo and follicle-stimulating hormone concentrations of ≥23 IU/L. Exclusion criteria included the following: HRT use in the past 6 mo, hyperlipidemia [total cholesterol: ≥5.17 mmol/L (or 200 mg/dL)], medications to treat high blood pressure or hypercholesterolemia, gastrointestinal abnormalities affecting dietary intake, a previous heart attack or diagnosis of heart disease, hepatic or renal disease, diabetes, cancer, history of thrombosis or blood vessel abnormalities, history of migraine headaches, allergy to soy or milk protein, and a body mass index (in kg/m^2) > 30. A brief medical history questionnaire was completed by all participants to establish a state of general good health.

Study design

Participants were assigned to 1 of 3 treatment schemes in a randomized, double-blind, crossover design, such that all subjects ultimately underwent all 3 treatment regimens. Each scheme outlined the order of consumption of the 3 protein supplements: isolated soy protein with naturally occurring isoflavones (Soy+), ethanol-washed isolated soy protein with trace amounts of isoflavones (Soy−), and total milk protein (TMP), which contained 107.67, 1.82, and 0 mg of total isoflavones (aglycone units), respectively, in a daily dose of 25 g protein. This allowed for comparison of the effects due to isoflavones themselves with those due to the protein matrix. The macronutrient, vitamin, and mineral contents of the 3 protein powders were equal. Details of the isoflavone and nutrient contents of the study products are shown in **Table 1**. DuPont Protein Technologies (St Louis) supplied the protein products used in the study.

The study consisted of three 6-wk intervention periods separated by 4-wk washout periods. Participants completed a total of 4 clinic visits (at weeks 0, 6, 16, and 26) at which they met with a nutritionist, weight and height were recorded, blood samples were obtained, and flow-mediated dilation was measured. Fasting (12-h) blood samples were collected at each clinic visit. Plasma samples were immediately centrifuged, and serum samples were incubated at room temperature for 15 min before centrifugation (833 × *g* for 15 min at 4 °C). All samples were divided into aliquots and stored at −80 °C until they were analyzed.

A 6-wk supply of the assigned protein was provided in measured individual packets at weeks 0, 6, and 16. Any unused packets of protein were returned by the participants, and the quantity returned provided a gross measurement of adherence to the intervention. The participants mixed the protein powder with their choice of beverage and consumed the full serving daily. Instructions were given on how to include the daily protein in their normal diet without increasing their usual protein or energy intake.

Participants were advised to maintain their usual dietary habits consistently throughout the study and were instructed to abstain from all additional soy foods, soy- or phytoestrogen-fortified products, and known phytoestrogen-rich herbal supplements. Each participant's vitamin and mineral supplement use was recorded at the first clinic visit (week 0), and participants were instructed to make no changes in the doses used during the study period. They were advised to abstain from alcoholic beverages and phytochemical-rich foods (including, tea, flaxseed products, and chocolate), which may alter endothelial function, throughout the study and in particular for 24 h before each clinic visit.

Participants were instructed on how to keep accurate 3-d food records. Records were kept for 3 alternating days, including one weekend day, during the week before the clinic visit at weeks 6, 16, and 26. Completed food records were reviewed for adequate details and adherence to guidelines. Dietary intake was analyzed with the use of NUTRITIONIST V software, version 2 (First Data Bank, San Bruno, CA).

Brachial artery reactivity

Changes in vessel diameter after reactive hyperemia (endothelium-dependent vasodilation) and after the taking of sublingual nitroglycerin (endothelium-independent vasodilation) were measured according to previously described methods (24). Brachial artery ultrasound scans were performed after an overnight fast and with the subjects at rest after lying in the supine position for ≥10 min. Studies were performed by 2 experienced vascular ultrasound technicians with the use of a Sonos 5500 ultrasound machine (Hewlett-Packard, Palo Alto, CA) and a high-resolution (7.5-MHz) linear array transducer. The variability in measurements between the tests performed by the 2 technicians was <5%.

The left brachial artery was imaged above (3–7 cm) the antecubital fossa and arterial bifurcation with a vascular probe positioned at an angle of 60°. First, baseline brachial artery diameter and flow were measured. Then, a blood pressure cuff was inflated to suprasystolic pressure (≥30 mm Hg higher than the subject's systolic blood pressure), and the vessel was compressed for 5 min. To obtain the postocclusion data, the cuff was deflated and brachial artery diameter and flow were remeasured within 15 s after compression during reactive hyperemia. A second baseline measurement of brachial artery diameter and flow was taken after 10 min. Then, a sublingual nitroglycerin tablet (0.4 mg) was administered to each subject, and a postnitroglycerin measurement of left brachial artery flow velocity and diameter was made within 4 min. The mean of the 2 baseline measurements was defined as the resting vessel diameter. Peak flow velocity (PFV) was defined as blood flow in cm/s during reactive hyperemia. The change in PFV was calculated as the percentage of change in flow velocity after occlusion compared with the mean of the 2 baseline values. Flow-mediated dilation was calculated as the percentage of change in vessel diameter after occlusion compared with the mean of the 2 baseline values. In addition, the percentage of change in postnitroglycerin vessel diameter and flow was calculated and compared with the baseline and postocclusion values to evaluate the endothelium-dependent dilation in relation to the maximum dilation of the brachial artery as assessed after nitroglycerin administration.

Biochemical endothelial cell markers

To determine whether changes in brachial artery reactivity were coupled with changes in biochemical markers of endothelial function and vascular inflammation, we measured plasma concentrations of vasoactive factors and inflammatory markers. The counterregulatory vasoactive factor endothelin 1 (ET-1) and nitric oxide metabolites were of particular interest because of genistein's postulated nitric oxide–dependent effects on the vascular wall. Plasma total nitrite concentration was assayed with a colorimetric kit (Cayman Chemical, Ann Arbor, MI), which uses the reduction of nitrates to nitrites and the addition of Griess reagents (Cayman Chemical) for detection at 550 nm. Citrated plasma was ultrafiltrated through a 30-kDa molecular weight microfuge ultrafiltration device (Millipore, Bedford, MA) before assay to reduce background absorbance. Sensitivity of the assay was 2.5 mmol/L. Serum ET-1 was measured with the use of a QuantiGlo chemiluminescence immunoassay (R & D Systems, Minneapolis). The sensitivity of the assay was 0.16 pg/mL. Crossreactivity with endothelium 3 was 7.8%, that with endothelium 2 was 27.4%, and that with proendothelin was negligible.

Cellular adhesion molecules (CAMs) are among the many markers of vascular inflammation and are known to respond to hormonal manipulation. We sought to determine the effect of the soy diets on 2 such markers, soluble intercellular CAM-1 (sICAM-1) and soluble vascular CAM-1 (sVCAM-1), and on E-selectin. Soluble CAMs were measured in serum with the use of sandwich enzyme-linked immunoassay kits (R & D Systems). Serum samples were diluted 20-fold for measurement of sICAM-1 and E-selectin and 50-fold for measurement of sVCAM-1. The sensitivity of the assays was 0.35, 0.1, and 2.0 ng/mL, respectively, and there was no cross-reactivity with other adhesion molecules. Interassay and intraassay CVs for all assays were <10% as determined in human serum.

Follicle-stimulating hormone and estradiol

Menopausal status was verified by serum concentrations of follicle-stimulating hormone measured at the University of California, Davis, Medical Center clinical laboratory with the use of a two-site chemiluminometric immunoassay (Bayer Diagnostics, Medfield, MA). Plasma estradiol (E-2) and estrone (E-1) concentrations were measured at the Laboratory for Reproductive Biology, University of North Carolina, Chapel Hill, with the use of radioimmunoassay kits (Diagnostic Systems Laboratories, Inc, Webster, TX). The intraassay and interassay CVs for analysis of E-1 were 9.4% and 10.2%, and those for analysis of E-2 were 5.3% and 4.9%, respectively. The E-1 radioimmunoassay has cross-reactivity with E-2 of 1.25% and the E-2 radioimmunoassay has cross-reactivity with E-1 of 0.86%. The cross-reactivity of both the E-1 and E-2 radioimmunoassays with isoflavones is unknown, although it is <1% with all other mammalian estrogens, except as noted above. The estrone and estradiol kits were sensitive at concentrations of 4.4 and 23.9 pmol/L, respectively.

Plasma isoflavones

Plasma concentrations of the isoflavones and metabolites genistein, daidzein, dihydrodaidzein, equol, ortho-desmethylangolensin, and glycitein were measured by using HPLC with electrochemical detection (25). Briefly, samples were hydrolyzed overnight in the presence of β-glucuronidase in a sodium acetate buffer. Isoflavones were then extracted into methyl *tert*-butyl ether, dehydrated, and reconstituted in methanol and water. Separation was performed with the use of reverse-phase HPLC with a Model 5600 CoulArray 8 Channel Detector (ESA, Inc, Chelmsford, MA) and quantified with the use of standards of known concentration. The minimum concentration for detection for all isoflavones was 5 ng/mL. The intraassay and interassay CVs for all 6 isoflavones ranged from 3% to 15%.

Blood lipids

Fasting serum lipids (total and HDL cholesterol and triacylglycerol) were measured with the use of automated enzymatic methods at the clinical laboratory. Analysis was performed on a Synchron LX-20 System (Beckman Coulter, Inc, Brea, CA). LDL cholesterol was calculated by using the equation of Friedwald et al (26). All samples were processed within 2 h of collection.

Formation of conjugated dienes in whole plasma and LDL oxidation

To determine whether soy protein with isoflavones functioned as a lipid antioxidant in normolipidemic subjects, we determined the resistance of whole plasma and isolated LDL to copper-mediated oxidation. The formation of conjugated dienes (CDs) resulting from the oxidation of polyunsaturated fatty acids in whole plasma exposed to copper ions was measured with the use of a spectrophotometric method adapted from Kontush et al (27). Citrated plasma samples were diluted 1:50 with phosphate-buffered solution (pH 7.4), and 10 μL of a 10-nmol $CuSO_4$/L solution was added to the diluted plasma, for a final copper concentration of 100 μmol/L. Absorption was measured at 245 nm with a 12-cell visible spectrophotometer (UV-1601; Shimadzu Scientific Instruments, Inc, Columbia, MD) with an external temperature controller set at 37 °C. Absorption was read until a maximum CD formation was reached and a plateau was observed (≈120 min). Data were graphed as time (min) versus absorbance. The length of time before CD formation began (lag time) was calculated by the intersection of the lines corresponding to the lag phase and the propagation phase on the graph. Samples from each of the 4 study time

TABLE 2
Subject characteristics at baseline[1]

	Treatment scheme[2]			
	A (n = 11)	B (n = 7)	C (n = 10)	All (n = 28)
Age (y)	54.1 ± 1.0[3]	55.9 ± 2.8	55.0 ± 1.6	54.9 ± 1.0
BMI (kg/m^2)	24.3 ± 0.7	24.8 ± 0.6	24.8 ± 0.6	24.6 ± 0.6
FSH (IU/L)[4]	75.9 ± 3.5	74.8 ± 5.1	81.5 ± 5.5	77.7 ± 5.5
Surgical menopause (n)	0	0	2	2

[1] There were no significant differences between treatments (repeated-measures ANOVA). FSH, follicle-stimulating hormone.

[2] The order in which the subjects received the dietary treatment: A = isolated soy protein with isoflavones (Soy+), total milk protein (TMP), and ethanol-washed isolated soy protein with trace isoflavones (Soy−); B = Soy−, Soy+, and TMP; C = TMP, Soy−, and Soy+.

[3] $\bar{x}$ ± SEM.

[4] Postmenopause range >23 IU/L.

points for each participant were analyzed in triplicate in the same run to minimize variability.

LDL was isolated from plasma by microultracentrifugation according to the method of Brousseau et al (28). EDTA was removed from the LDL by overnight dialysis in phosphate-buffered solution (pH 7.4) treated with Chelex 100 (Bio-Rad, Richmond, CA) and purged with nitrogen. The amount of cholesterol in the samples was quantified with the use of a Boehringer Mannheim cholesterol kit (Boehringer Mannheim, Indianapolis). CD formation was measured as described by Esterbauer (29). A volume of dialyzed LDL sample containing 75 μg of cholesterol was added to a quartz cuvette containing phosphate-buffered solution (pH 7.4) and copper sulfate to yield a final concentration of 5 μmol/L in 1 mL total volume. Absorption was read at 234 nm until maximum CD formation was reached and a plateau was observed (≈200 min). Data were analyzed in the same manner as described above in the whole-plasma oxidation studies. All samples for each participant were analyzed in duplicate in the same run.

Statistical analysis

Data are presented as means (±SEMs), unless stated otherwise, of duplicate or triplicate sample values. Statistical analysis was performed with the use of SPSS for WINDOWS software, version 10.0 (SPSS Inc, Chicago) and SAS for WINDOWS software (SAS Institute, Inc, Cary, NC). Comparisons among the 3 treatments were done by repeated-measures analysis of variance and two-factor repeated-measures analysis of variance with interaction terms for comparison of brachial artery reactivity with and without nitroglycerin. The Tukey-Kramer post hoc test for multiple comparisons was used. Differences were considered significant if $P \leq 0.05$.

RESULTS

Clinical characteristics

Forty-two women were enrolled, and 28 completed the study. Twenty-six of the participants were white, 1 was African American, and 1 was Ethiopian. A total of 14 women withdrew from the study: 3 stated they were unable to tolerate the protein, 2 because of gastrointestinal disturbances (constipation or diarrhea) and 1 because of taste intolerance; 8 women withdrew because of time constraints related to attending clinic visits or participating in the intervention; and 3 women did not finish the study for reasons unrelated to the intervention. There were no differences in the baseline characteristics between these 14 women and those completing the study.

The baseline characteristics of the 28 women completing the study (**Table 2**) were comparable among treatment groups with regard to age, body mass index, follicle-stimulating hormone, and blood lipids. Body weight did not change significantly in any of the treatment groups throughout the 26-wk study. Serum estrone and estradiol concentrations were consistent with a lack of endogenous ovarian estrogen production in all of the women and did not vary after any of the treatments (observations not shown). No effect of treatment order was seen for any of the variables examined.

Diet

Dietary intake was evaluated at 3 time points during the study by the collection of 3-d food records. Macronutrients as a percentage of total energy were well matched among the 3 groups. The mean intakes in the Soy+, Soy−, and TMP treatment groups were, respectively, 54%, 52%, and 55% for carbohydrate; 20%, 20%, and 19% for protein; and 26%, 27%, and 25% for fat. No significant differences were found among treatment groups in the intakes of energy, macronutrients, fiber, β-carotene, vitamins C and E, or any other nutrients (**Table 3**).

Eleven subjects consumed vitamin and mineral supplements on a regular basis, and therefore their mean intake of dietary antioxidants exceeded the recommended dietary intake throughout the study. The mean intakes of vitamins C and E in all groups were, respectively, ≈525% and ≈450% above the recommended daily allowances for this population. Vitamin supplement users were included in the study to evaluate the potential for soy protein to modulate the oxidative susceptibility of whole plasma and LDL under these conditions, because of the widespread use of supplements in this age group. Subgroup analysis of nonsupplement users did not reveal any trends in analyses compared with vitamin supplement users, but the number of subjects was not large enough for the detection of significance.

The consumption of meatless meals, fruit, and vegetables was also common in this study population. During the entire study

TABLE 3
Dietary intake of subjects in each dietary period[1]

Nutrient	TMP	Soy−	Soy+
Energy (MJ)	8.0 ± 0.4	7.2 ± 0.4	7.6 ± 0.3
Carbohydrate (g/d)	263.7 ± 15.0	225.7 ± 14.2	247.9 ± 12.7
Protein (g/d)	91.1 ± 4.0	84.3 ± 5.2	89.6 ± 4.5
Total fat (g/d)	55.3 ± 4.8	52.1 ± 4.5	52.4 ± 4.1
Saturated	17.1 ± 1.7	16.3 ± 1.5	15.5 ± 1.4
Polyunsaturated	9.1 ± 1.0	9.2 ± 1.1	8.8 ± 0.8
Monounsaturated	16.4 ± 1.8	16.6 ± 2.2	15.7 ± 1.8
Cholesterol (mg/d)	178.1 ± 20.1	175.9 ± 18.4	164.4 ± 16.8
Fiber (mg/d)	21.2 ± 1.7	19.4 ± 1.7	21.6 ± 1.9
Vitamin C (mg/d)[2]	395.2 ± 77.9	377.3 ± 94.0	410.1 ± 87.2
α-Tocopherol equivalents[2]	55.8 ± 17.9	66.5 ± 29.0	85.8 ± 34.6
β-Carotene (μg/d)	990.9 ± 253.5	970.2 ± 224.7	422.0 ± 88.4

[1] $\bar{x}$ ± SEM; n = 28. TMP, total milk protein; Soy−, ethanol-washed isolated soy protein with trace isoflavones; Soy+, isolated soy protein with isoflavones. There were no significant differences between treatments (repeated-measures ANOVA).

[2] Intake from diet and supplements.

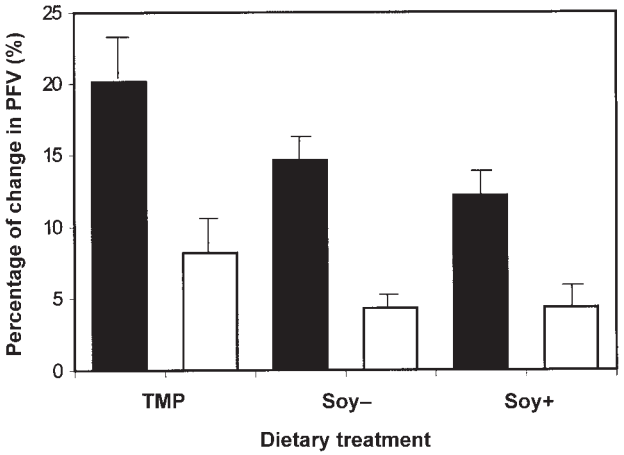

FIGURE 1. Mean (± SEM) percentages of change in brachial artery peak flow velocity (PFV; in cm/s). Postocclusion values (■) represent endothelium-dependent vasodilatory responses. Nitroglycerin-mediated vasodilation (□) represents endothelium-independent responses. The effect of the nitroglycerin-by-treatment interaction was not significant ($P = 0.31$), with a significant main effect difference noted among treatments. PFV after treatment with isolated soy protein containing isoflavones (Soy+) was significantly (37%; $P = 0.03$) lower than that after treatment with total milk protein (TMP). PFV after treatment with ethanol-washed isolated soy protein with trace isoflavones (Soy−) was intermediate in value and did not differ significantly from that after TMP treatment. The nitroglycerin vasodilatory response was more robust than the postocclusion response. Significance ($P < 0.05$) was determined with the use of two-factor repeated-measures ANOVA with interaction term and the Tukey-Kramer post hoc test.

period, <45% of the meals eaten by 13 of the participants were meatless. The number of servings of lean and high-fat meats/d for all participants, calculated with the use of NUTRITIONIST V software, was 4.06 ± 1.67 and 0.45 ± 0.71, respectively. The number of servings of fruit and vegetables/d for all participants was 3.07 ± 1.98 and 1.55 ± 1.67, respectively. Adherence to the daily protein supplements was ≥93% throughout the study.

Plasma isoflavones

The plasma concentrations of isoflavones and metabolites were 5 pg/dL or less in the Soy– and TMP groups. Fasting mean plasma concentrations of daidzein, dihydrodaidzein, equol, genistein, and ortho-desmethylangolensin at completion of the Soy+ treatment were 55.2 ± 1.8, 21.3 ± 7.7, 35.6 ± 10.3, 81.9 ± 14.4, and 30.5 ± 6.5 pg/dL, respectively, and they were significantly ($P \leq 0.01$) higher than values in samples taken at baseline and after the Soy− and TMP treatments (observations not shown). The daidzein concentration in the TMP group was initially not significantly lower, because 2 participants presented with daidzein values at baseline that were comparable to their concentration after the Soy+ treatment, probably as a result of inadvertent dietary indiscretion. An analysis of the data that excluded these 2 participants resulted in a daidzein value after the TMP treatment that was significantly lower than that after the Soy+ treatment. All other data were analyzed with the exclusion of these 2 participants, and no effects on statistical significance were found. Equol was produced by 10 of the subjects. Baseline characteristics of these subjects were similar to those of the rest of the experimental group. Subgroup analysis of the data including only the equol producers did not result in variations from the analysis of all 28 participants, but the number of subjects was not large enough to detect significance (data not shown).

Brachial artery reactivity

To assess the effect of the soy protein diets on vascular function, as measured by brachial artery reactivity, we compared the baseline, postocclusion, and postnitroglycerin brachial artery vessel diameter and PFV in the Soy+, Soy−, and TMP (control) treatment groups. The brachial artery postocclusion vessel diameters did not differ significantly among treatment groups and were within 5% of the baseline values. Postnitroglycerin vessel diameters increased 16–18% compared with baseline, and they also did not differ among groups. However, the PFV was significantly ($P = 0.03$) lower at the end of the Soy+ treatment than that at the end of the TMP treatment, which is consistent with a vasodilatory response (**Figure 1**). The Soy− treatment PFV did not differ significantly from the TMP or Soy+ treatment PFV. There was no significant ($P = 0.31$) effect for the nitroglycerin-by-treatment interaction term in the two-factor repeated-measures analysis of variance. Thus, the overall effect of the Soy+ treatment was consistent between conditions (ie, with and without nitroglycerin), although the average values with nitroglycerin tended to be lower.

Endothelial markers

The data for biochemical markers of endothelial function in the 3 treatment groups are summarized in **Table 4**. Plasma nitric oxide–derived products (ie, total nitrate + nitrite concentration) did not differ significantly among treatment groups or when compared with the mean baseline value. Similarly, ET-1 concentrations did not differ significantly among groups or when compared with the mean baseline. There was a trend for nitric oxide to increase and for ET-1 to decrease in the Soy+ group compared with the other diet treatments, but this trend did not reach statistical significance. No significant differences were observed in the serum concentrations of sVCAM-1 and sICAM-1 or in soluble E-selectin among the treatment groups.

Blood lipids and oxidation values

All participants had baseline fasting serum lipid profiles within the recommended National Cholesterol Education Program Step II

TABLE 4
Biochemical markers of endothelial function[1]

	TMP	Soy−	Soy+
Endothelial metabolites			
Nitric oxide products (μmol/L)	31.2 ± 3.3	31.5 ± 3.2	33.7 ± 4.1
ET-1 (pg/mL)	2.2 ± 0.2	2.1 ± 0.2	1.9 ± 0.2
Cell adhesion molecules			
sE-selectin (ng/mL)	45.5 ± 5.1	41.3 ± 4.0	43.5 ± 4.2
sVCAM-1 (ng/mL)	472.1 ± 24.4	458.2 ± 16.8	488.9 ± 24.9
sICAM-1 (ng/mL)	280.7 ± 22.2	250.7 ± 11.5	269.2 ± 12.3

[1] $\bar{x}$ ± SEM. $n = 27$, 24, and 28 for nitric oxide products, ET-1, and cell adhesion molecules, respectively. TMP, total milk protein; Soy−, ethanol-washed isolated soy protein with trace isoflavones; Soy+, isolated soy protein with isoflavones; ET-1, endothelin 1; sE-selectin, soluble E-selectin; sVCAM-1, soluble vascular cell adhesion molecules-1; sICAM-1, soluble intercellular cell adhesion molecules-1. There were no significant differences between treatments for all variables (repeated-measures ANOVA).

TABLE 5
Fasting serum lipids at baseline and by treatment[1]

	Baseline	TMP	Soy−	Soy+
Cholesterol				
Total (mmol/L)	4.91 ± 0.1	5.00 ± 0.1	4.92 ± 0.2	4.82 ± 0.1
LDL (mmol/L)	2.89 ± 0.1	2.94 ± 0.1	2.87 ± 0.1	2.86 ± 0.1
HDL (mmol/L)	1.55 ± 0.1	1.61 ± 0.1	1.55 ± 0.1	1.49 ± 0.1
Total:HDL	3.2 ± 0.2	3.1 ± 0.2	3.2 ± 0.2	3.2 ± 0.2
Triacylglycerol (mmol/L)	1.03 ± 0.1	0.98 ± 0.1	1.08 ± 0.1	1.04 ± 0.1

[1] $\bar{x}$ ± SEM; n = 24. TMP, total milk protein; Soy−, ethanol-washed isolated soy protein with trace isoflavones; Soy+, isolated soy protein with isoflavones. There were no significant differences between treatments (repeated-measures ANOVA). Conversion factors: cholesterol, mg/dL × 0.02586 = mmol/L; triacylglycerol, mg/dL × 0.01129 = mmol/L.

guidelines. After the dietary treatments, there were no statistically significant changes in blood lipids among groups (**Table 5**). However, in comparison to baseline, there were decreases in total and LDL cholesterol in the Soy+ group [0.09 and 0.03 mmol/L (or 3.59 and 1.12 mg/dL), respectively], unchanged lipid values in the Soy− group [0.01 and −0.02 mmol/L (or 0.22 and −0.64 mg/dL), respectively], and increases in lipid values in the TMP group [0.08 and 0.04 mmol/L (or 3.25 and 1.73 mg/dL), respectively]. The latter changes were similar in magnitude but opposite in direction to the changes seen in the Soy+ group. Measurement of lipid oxidation susceptibility by CD formation in whole plasma and isolated LDL showed no significant differences among treatment groups either as absolute values or as the change in lag time from baseline (data not shown).

DISCUSSION

Intact vasomotor tone is an important physiologic characteristic of healthy, nonarteriosclerotic vessels. Endothelium-dependent vasodilation undergoes an age-related decline in both men and women, and, in particular, it decreases significantly in women after menopause (30, 31). Supplemental estrogen and combined HRT improve vascular reactivity in most but not all studies of postmenopausal women through the genomic and nongenomic effects on nitric oxide, ET-1, and intracellular calcium (31–37). Furthermore, genistein infused at or above physiologic plasma concentrations results in acute nitric oxide–dependent vasodilation in forearm vasculature of men and women with a potency that is similar to the dilatation resulting from 17 β-estradiol administration (15). However, clinical trials of the efficacy of soy isoflavones in modulating endothelial function in postmenopausal women have produced conflicting results.

We observed that PFV with the Soy+ treatment was significantly lower than that with the TMP treatment. These findings are consistent with the research of others and suggest that, although both the isoflavone and protein components of soybeans can have a beneficial effect on cardiovascular health, the combination of soy protein and isoflavones probably conveys more benefit than does either component alone (38, 39). The endothelium-independent response to nitroglycerin was robust in all subjects, which indicated normal physiologic responsiveness and supported the possibility of an endothelium-dependent mechanism for the reductions observed in PFV with the consumption of soy protein with isoflavones. It was surprising that an increased vessel diameter was not detected, despite the decrease in flow velocity. However, it is possible that the amount of endothelium-dependent vasodilation was maintained to a greater extent in this healthy population than in other studies of postmenopausal women. Factors possibly contributing to the variable results in this field that have been observed in previous studies include the length of time the soy or isoflavone supplement is consumed, the dose of isoflavones, the presence of CVD risk factors such as hypercholesterolemia that impair endothelial function, and the sensitivity of the values of endothelial responsiveness that are assessed (19–23, 40).

Only one prior study examined endothelial function in postmenopausal women in response to soy protein with isoflavones (containing 54 mg genistein/d), and it did not find a response in brachial artery reactivity but, interestingly, did find significant decreases in the pulse wave velocity of the femorodorsalis arterial segment and in blood pressure, results that are consistent with peripheral vasodilation (23). In a recent study of postmenopausal women receiving isoflavone tablets for 6 mo, investigators found significantly increased flow-mediated brachial artery vasodilation that was correlated with an increased ratio of nitric oxide products to ET-1, which suggested possible pathways through which isoflavones may mediate vascular reactivity (20). Another research group found significant improvements in systemic arterial compliance (19). Both of these studies used tablets that delivered 45–54 mg genistein/d, a dose essentially similar to the 55 mg genistein contained in the daily soy protein in the current study. The remaining studies used soy isoflavone tablets containing 40 mg or unspecified amounts of genistein, taken daily for periods of 2–9 wk, and did not show improvements in brachial artery reactivity (21, 22). Some of these studies have included perimenopausal and postmenopausal women who had mild endothelial dysfunction or hypertension, but these populations were healthy for the most part. Thus, in the context of prior studies, our data support a positive effect on vascular function of the consumption of soy protein with isoflavones containing ≈50 mg genistein/d, even in a very healthy, normolipidemic postmenopausal population.

There were no previous reports in the clinical literature of the effect of soy isoflavones on the soluble plasma CAMs VCAM-1, ICAM-1, and E-selectin. The CAMs are elevated in persons with coronary artery disease or diabetes and in postmenopausal women, and they also are markers for endothelial dysfunction, inflammation, and CVD risk (41–43). Estrogen replacement therapy results in fewer soluble adhesion markers (44, 45). Our results reveal no significant changes in soluble CAMs with the consumption of soy with or without isoflavones, which suggests no benefit to these risk markers in a healthy postmenopausal population without evidence of inflammation. Thus, the improved vasoreactivity in response to the Soy+ treatment does not appear occur via an inflammation-sensitive mechanism. It is possible, however, that biochemical markers of endothelial function and of endothelial reactivity may be more responsive to soy isoflavones when other CVD risk factors and inflammation are present; this would be analogous to the greater lipid-lowering effects of soy protein with isoflavones that are seen in hypercholesterolemic persons.

Studies examining the effects of soy proteins with isoflavones in postmenopausal women showed modest blood lipid–lowering effects relative to HRT, with responses being more robust in persons with significant hyperlipidemia (39, 46–51) and inconsistent in normolipidemic persons (23, 52). The small changes in plasma lipids in response to soy proteins did not reach statistical significance in our

healthy, normocholesterolemic population, which is consistent with previous results. The subjects in the current study had self-selected diets that met the National Cholesterol Education Program Step II guidelines and often included a large proportion of meatless meals. Two previous studies noted a lack of plasma lipid response to soy proteins when the intake of cholesterol is low (≤200 mg/d) (53, 54). In contrast, purified isoflavones, which are essentially devoid of protein, do not lower lipids or decrease oxidation susceptibility, which suggests a role for the soy protein matrix (21, 22, 55).

Most of the studies that measured oxidation resistance in response to soy treatment showed improvements in either the lag time for CD formation (11, 12) or the in vivo CD formation with control for antioxidant intake (56). We did not find significant improvements in the antioxidant capacity of whole plasma or isolated LDL cholesterol. As predicted, the regular high intake of fruit and vegetables in addition to the use of vitamin and mineral supplements in this free-living population provided sufficient antioxidants, which masked any antioxidant effects of the soy protein. This finding does not preclude the possibility that a beneficial effect could be observed in women with suboptimal diets that are low in antioxidant-containing fruit and vegetables, as would be consistent with previous reports.

In summary, in this study we showed that a diet rich in soy protein with isoflavones resulted in significantly increased plasma isoflavone concentrations and significant favorable effects in brachial artery PFV that are consistent with a vasodilatory response. Our results are significant because heretofore it was unclear whether soy has vascular benefits in a healthy, normolipidemic, menopausal population. The data suggest that the consumption of 25 g soy protein with 55 mg genistein in a total consumption of 107 mg isoflavones/d has a benefit for vascular reactivity that is independent of plasma antioxidant actions and lipid-lowering effects. Our findings have implications for the growing population of healthy postmenopausal women who may wish to consume soy products with isoflavones or naturally occurring selective estrogen-receptor modulators. Further research is needed to clarify both the mechanisms by which soy proteins and isoflavones affect vascular tissues, and the clinical significance of such effects.

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FMS, MJM, ACV, and NLG contributed to the design of the study, collection and analysis of the data, and manuscript preparation; FMS, MJM, NLG, and KK collected the data. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

REFERENCES

1. Desai MM, Zhang P, Hennessy CH, Surveillance for morbidity and mortality among older adults—United States, 1995–1996. CDC Surveillance Summaries. MMWR Mortal Morb Wkly Rep 1999:48(No. SS–8):7–25.
2. Barrett-Conner E, Stuenkel C. Hormones and heart disease in women: heart and estrogen/progestin replacement study in perspective. J Clin Endocrinol Metab 1999;84:1848–53.
3. Joswig M, Hach-Wunderle V, Ziegler R, Nawroth PP. Postmenopausal hormone replacement therapy and the vascular wall: mechanisms of 17 β-estradiol's effects on vascular biology. Exp Clin Endocrinol Diabetes 1999;107:477–87.
4. Stampfer MJ, Colditz GA, Willett WC, et al. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the Nurses' Health Study. N Engl J Med 1991;325:756–62.
5. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 1998;280:605–13.
6. Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women. Principal results from the women's Health Initiative randomized controlled trial. JAMA 2002;288:321–33.
7. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998;139:4252–63.
8. Hodges YK, Tung L, Yan XD, Graham JD, Horwitz LD. Estrogen receptors alpha and beta: prevalence of estrogen receptor beta mRNA in human vascular smooth muscle and transcriptional effects. Circulation 2000;101:1792–8.
9. Aavik E, du Toit D, Myburgh E, Frosen J, Hayry P. Estrogen receptor beta dominates in baboon carotid after endothelial denudation injury. Mol Cell Endocrinol 2001;182:91–8.
10. Kapiotis S, Hermann M, Held I, Seelos C, Erhinger H, Gmeiner BMK. Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage by atherogenic LDL. Arterioscler Thromb Vasc Biol 1997;17:2868–74.
11. Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci U S A 1998;95:3106–10.
12. Wiseman H, O'Reilly JD, Adlercreutz H, et al. Isoflavone phytoestrogens consumed in soy decrease F_2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am J Clin Nutr 2000;72:395–400.
13. Akiyama T, Ishida J, Nakagava S, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 1987;262:5592–5.
14. Shimokado K, Yokota T, Umezawa K, Sasaguri T. Ogata J. Protein tryosine kinase inhibitors inhibit chemotaxis of vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 1994;14:973–81.
15. Walker HA, Dean TS, Sanders TAB, Jackson G, Ritter JM, Chowienczyk PJ. The phytoestrogen genistein produces acute nitric oxide-dependent dilation of human forearm vasculature with similar potency to 17 β-estradiol. Circulation 2001;103:258–62.
16. Honore ED, Williams JK, Anthony MS, Clarkson TB. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. Fertil Steril 1997;67:148–54.
17. Squadrito F, Altavilla D, Squadritio G, et al. Genistein supplementation and estrogen replacement therapy improve endothelial dysfunction induced by ovariectomy in rats. Cardiovasc Res 2000;45:454–62.
18. Williams JK, Anthony MS, Herrington DM. Interactive effects of soy protein and estradiol on coronary artery reactivity in atherosclerotic, ovariectomized monkeys. Menopause 2001;8:307–13.
19. Nestel PJ, Yamashita T, Sasahara T, et al. Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. Arterioscler Thromb Vasc Biol 1997;17:3392–8.
20. Squadrito F, Altavilla D, Morabito N, et al. The effect of the phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women. Atherosclerosis 2002;163:339–47.
21. Simons LA, von Konigsmark M, Simons J, Celermajer DS. Phytoestrogens do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women. Am J Cardiol 2000;85:1297–301.
22. Hale G, Paul-Labrador M, Dwyer JH, Merz CN. Isoflavone supplementation and endothelial function in menopausal women. Clin Endocrinol 2002;56:693–701.
23. Teede HJ, Dalais FS, Kotsopoulos D, Liang YL, Davis S, McGrath BP.

Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. J Clin Endocrinol Metab 2001;86:3053–60.
24. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. J Am Coll Cardiol 2002;39: 257–65.
25. Coward L, Kirk M, Albin N, Barnes S. Analysis of plasma isoflavones by reversed-phase HPLC-multiple reaction ion monitoring-mass spectrometry. Clin Chim Acta 1996;247:121–42.
26. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
27. Kontush A, Spranger T, Reich A, et al. Whole plasma oxidation assay as a measure of lipoprotein oxidizability. Biofactors 1997;6:99–109.
28. Brousseau T, Clavey V, Bard JM, Fruchart JC. Sequential ultracentrifugation micromethod for separation of serum lipoproteins and assays of lipids, apolipoproteins, and lipoprotein particles. Clin Chem 1993;39:960–4.
29. Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic Res Commun 1989;6:67–75.
30. Gerhard M, Roddy MA, Creager SJ, Creager MA. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. Hypertension 1996;27:849–53.
31. Perregaux D, Chaudhuri A, Mohanty P, et al. Effect of gender differences and estrogen replacement therapy on vascular reactivity. Metabolism 1999;48:227–32.
32. Lieberman EH, Gerhard MD, Uehata A, et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. Ann Intern Med 1994;121:936–41.
33. Al-Khalili F, Eriksson M, Landgren BM, Schenck-Gustafsson K. Effect of conjugated estrogen on peripheral flow-mediated vasodilation in postmenopausal women. Am J Cardiol 1998;82:215–8.
34. Gerhard M, Walsh BW, Tawakol A, et al. Estradiol therapy combined with progesterone and endothelium-dependent vasodilation in postmenopausal women. Circulation 1998;98:1158–63.
35. Koh KK, Blum A, Hathaway L, et al. Vascular effects of estrogen and vitamin E therapies in postmenopausal women. Circulation 1999;100: 1851–7.
36. Sorensen KE, Dorup I, Hermann AP, Mosekilde L. Combined hormone replacement therapy does not protect women against the age-related decline in endothelium-dependent vasomotor function. Circulation 1998;97:1234–8.
37. Cagnacci A, Modena MG, Malmusi S, Muia N, Volpe A. Effect of prolonged administration of transdermal estradiol on flow-mediated endothelium-dependent and endothelium-independent vasodilation in healthy postmenopausal women. Am J Cardiol 1999;84:367–70.
38. Sirtori CR, Lovati MR. Soy proteins and cardiovascular disease. Curr Atheroscler Rep 2001;3:47–53.
39. Crouse JR III, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch Intern Med 1999;159: 2070–6.
40. Yildirir A, Tokgozoglu SL, Oduncu T,et al. Soy protein diet significantly improves endothelial function and lipid parameters. Clin Cardiol 2001;24:711–6.
41. Caulin-Glaser T, Farrell WJ, Pfau SE, et al. Modulation of circulating cellular adhesion molecules in postmenopausal women with coronary artery disease. J Am Coll Cardiol 1998;31:1555–60.
42. Jager A, van Hinsbergh VWM, Kostense PJ, et al. Increased levels of soluble vascular cell adhesion molecule 1 are associated with risk of cardiovascular mortality in type 2 diabetes. The Hoorn study. Diabetes 2000;49:485–91.
43. Demerath E, Towne B, Blangero J, Siervogel RM. The relationship of soluble ICAM-1, VCAM-1, P-selectin and E-selectin to cardiovascular disease risk factors in healthy men and women. Ann Human Biol 2001;28:664–78.
44. Koh KK, Bui MN, Mincemoyer R, Cannon RO. Effects of hormone therapy on inflammatory cell adhesion molecules in postmenopausal healthy women. Am J Cardiol 1997;80:1505–7.
45. Cushman M, Legault C, Barrett-Connor E, et al. Effect of postmenopausal hormones on inflammation-sensitive proteins. The Postmenopausal Estrogen/Progestin Intervention (PEPI) study. Circulation 1999;100:717–22.
46. Baum JA, Teng H, Erdman JW Jr, et al. Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. Am J Clin Nutr 1998;68: 545–51.
47. Wangen KE, Duncan AM, Xu X, Kurzer MS. Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. Am J Clin Nutr 2001;73: 225–31.
48. Gardner CD, Newell KA, Cherin R, Haskell WL. The effect of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolemic postmenopausal women. Am J Clin Nutr 2001;73:728–35.
49. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW Jr. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr 1998;68(suppl): 1375S–9S.
50. Vigna GB, Pansini F, Bonaccorsi G, et al. Plasma lipoproteins in soy-treated postmenopausal women: a double- blind, placebo-controlled trial. Nutr Metab Cardiovasc Dis 2000;10:315–22.
51. Jenkins DAJ, Kendall CWC, Jackson CJC, et al. Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am J Clin Nutr 2002;76:365–72.
52. Dent SB, Peterson CT, Brace LD, et al. Soy protein intake by perimenopausal women does not affect circulating lipids and lipoproteins or coagulation and fibrinolytic factors. J Nutr 2001;131: 2280–7.
53. Meinertz H, Faergeman O, Nilausen K, Chapman MJ, Goldstein S, Laplaud PM. Effects of soy protein and casein in low cholesterol diets on plasma lipoproteins in normolipidemic subjects. Atherosclerosis 1988;72:63–70.
54. Sacks FM, Breslow JL, Wood PG, Kass EH. Lack of an effect of dairy protein (casein) and soy protein on plasma cholesterol of strict vegetarians. An experiment and a critical review. J Lipid Res 1983;24: 1012–20.
55. Hsu CS, Shen WW, Hsueh YM, Yeh SL. Soy isoflavone supplementation in postmenopausal women. Effects on plasma lipids, antioxidant enzyme activities and bone density. J Reprod Med 2001; 46:221–6.
56. Jenkins DJ, Kendall CW, Garsetti M, et al. Effect of soy protein foods on low-density lipoprotein oxidation and ex vivo sex hormone receptor activity—a controlled crossover trial. Metabolism 2000;49: 537–43.