Soy Consumption, Markers of Inflammation, and Endothelial Function

A cross-over study in postmenopausal women with the metabolic syndrome

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OBJECTIVE — To determine the effects of soy consumption on markers of inflammation and endothelial function in postmenopausal women with the metabolic syndrome.

RESEARCH DESIGN AND METHODS — This randomized cross-over clinical trial included 42 postmenopausal women with the metabolic syndrome. Participants were randomly assigned to consume a control diet (Dietary Approaches to Stop Hypertension [DASH]), soy protein diet, or soy nut diet, each for 8 weeks. Red meat in the DASH diet (one serving/day) was replaced by soy protein in the soy protein diet and by soy nut in the soy nut diet.

RESULTS — For nitric oxide levels, the difference from the control diet was 9.8% ($P < 0.01$) on the soy nut and −1.7% ($P = 0.10$) on the soy protein diets. The difference from the control diet for serum E-selectin was −11.4% ($P < 0.01$) on the soy nut consumption and −4.7% ($P = 0.19$) on the soy protein diet. Soy nut consumption reduced interleukin-18 compared with the control diet (difference from the control diet: −9.2%, $P < 0.01$), but soy protein did not (difference from the control diet: −4.6%, $P = 0.14$). For C-reactive protein, the difference from the control diet was −8.9% ($P < 0.01$) on the soy nut diet and −1.6% ($P < 0.01$) on the soy protein diet.

CONCLUSIONS — Short-term soy nut consumption reduced some markers of inflammation and increased plasma nitric oxide levels in postmenopausal women with the metabolic syndrome.

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The metabolic syndrome is a heterogeneous condition accompanied by visceral adiposity, dyslipidemia, hypertension, and insulin resistance (1,2). Elevated blood levels of inflammatory markers such as C-reactive protein (CRP), interleukin (IL)-2, IL-6, IL-18, and tumor necrosis factor (TNF)-α and endothelial dysfunction are associated with features of the metabolic syndrome (3–7). Inflammation, insulin resistance, and endothelial function are strongly correlated in individuals with the metabolic syndrome and may influence each other and exacerbate metabolic deterioration (8,9). Estrogen deficiency in postmenopausal women may further aggravate these conditions (10); endothelium-dependent vasodilation declines with aging, particularly in women after menopause (11). Therefore, postmenopausal women with the metabolic syndrome are at greater risk of endothelial dysfunction.

Diet plays an important role in the development of the metabolic syndrome, partly through its effects on proinflammatory markers (12). Several studies have examined the effects of diet on serum levels of inflammatory markers and endothelial function (13–17); some have focused on the effects of soy consumption (18–24). Soy contains fiber, polyunsaturated fat, and phytoestrogens, which are individually associated with lower levels of inflammatory markers and improved endothelial function (12,16,17). Most studies on the effects of soy consumption on these markers have been performed in healthy (18–20,22–24) or hypercholesterolemic (21) postmenopausal women. We are aware of no study regarding such effects among women with the metabolic syndrome. We, therefore, evaluated the effects of soy consumption, in the forms of isolated soy protein and roasted soy nut with naturally occurring isoflavones, on markers of systemic inflammation and endothelial function in postmenopausal women with the metabolic syndrome.

RESEARCH DESIGN AND METHODS

— A total of 120 postmenopausal women with the metabolic syndrome were screened for inclusion in the study. The study was conducted in Tehran, Iran, in 2005. Women were considered postmenopausal if menstrual periods had been absent for >1 year and follicle-stimulating hormone confirmed their status (25). The metabolic syndrome was defined according to Adult Treatment Panel (ATP) III guidelines (26). Exclusion criteria were considered as any secondary cause of hyperglycemia; current or previous (in the preceding 6 months) use of estrogen therapy; treatment with insulin or oral hypoglycemic agents, antihypertensive and antilipemic

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Abbreviations: CRP, C-reactive protein; DASH, Dietary Approaches to Stop Hypertension; IL, interleukin; sICAM-1, soluble intercellular cell adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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agents, or antihypertensive and antilipemic agents; untreated hypothyroidism; smoking; kidney or liver diseases; breast cancer or any cancer; or the presence of inflammatory or infectious disease. Finally, 42 women who had all five components of the metabolic syndrome were included in the present study. All participants provided informed written consent.

This study was approved by the research council and the ethical committee of the National Nutrition and Food Technology Research Institute of Shaheed Beheshti University of Medical Sciences.

Study procedures

We used a randomized cross-over design. After 3 weeks of run-in period on a usual diet, we randomly assigned women to a control diet (red meat–Dietary Approaches to Stop Hypertension [DASH] diet), DASH diet with soy nut (soy nut period), or DASH diet with soy protein (soy protein period), each one for 8 weeks. Each woman followed the three diets and had two washout periods (each washout for 4 weeks) (27). Participants were asked not to change their habitual physical activity levels for the duration of the study, and they recorded their physical activities for 3 days each month.

Diets

We used three diets: *1*) a control diet, which was a DASH diet with 55% carbohydrates, 17% protein, and 28% total fat and had one serving of red meat per day (red meat–DASH) and was rich in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fat, total fat, cholesterol, refined grains, and sweets; Na intake was 2,400 mg/day (28); *2*) a diet with soy nut, which was the same as the control diet, but we replaced red meat with roasted soy nut; 30 g soy nut was considered as one serving of red meat; and *3*) a diet with soy protein, which was the same as the control diet, but we replaced red meat with soy protein; 30 g soy protein was considered as one serving of red meat. The nutrient composition of soy nut and soy protein per 100 g are as follows: soy nut: protein (37.5 g), fat (20.5 g), fiber (30 g), and total phytoestrogen (340 mg) and soy protein: protein (50 g), fat (0.9 g), fiber (33 g), and total phytoestrogen (281 mg). The amount of tea and other phytochemical-rich food was similar in all three periods of study.

Calorie requirements of each participant were calculated individually based on equations suggested by the Institute of Medicine, Food and Nutrition Board (29). For measuring food intake, 3-day diet records were used at baseline and during intervention for each month. Every participant had to bring her 3-day diet record and physical activity records every month, where they were reviewed by the study staff and used for checking the diet compliance. Patients' compliance was assessed by analyzing 3-day diet records and plasma levels of phytoestrogens.

Measurements

Body weight was measured while the subjects were minimally clothed, without shoes, using digital scales and recorded to the nearest 0.1 kg. Height was measured in a standing position, without shoes, using a tape meter while the shoulders were in a normal state. Waist circumference was measured to the nearest 0.1 cm at the narrowest level over light clothing, using an unstretched tape measure, without any pressure to body surface.

The 12-h fasting blood samples were collected into tubes containing 0.1% EDTA and were centrifuged at 4°C and 500*g* for 10 min to separate plasma. Plasma total nitrite concentration was assayed with an enzymatic kit (Diagnostic Biochem Canada), which uses the reduction of nitrates to nitrites and the addition of reagents for detection at 550 nm. Sensitivity of the assay was 2.0 mmol/l. Serum endothelin-1, soluble intercellular cell adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), serum E-selectin, IL-2, IL-6, IL-18, TNF-α, serum amyloid A, and CRP were measured using enzyme-linked immunosorbent assay (Diaclone Besancon, France). The sensitivity of the assay for endothelin-1 was 0.15 pg/ml. Serum samples were diluted 20-fold for measurement of sICAM-1 and serum E-selectin and 50-fold for measurement of sVCAM-1. The sensitivity of the assay for sICAM-1, sVCAM-1, and serum E-selectin was 0.5, 3, and 0.1 ng/ml, respectively, and there was no cross-reactivity with other adhesion molecules. The sensitivity of the assay for IL-2, IL-6, and IL-18 was 2, 1, and 0.9 pg/ml, respectively. The sensitivity of the assay for TNF-α, serum amyloid A, and CRP was 3 pg/ml, 1 pg/ml, and 0.6 mg/l. Inter- and intra-assay coefficients of variation for all tests were <10%. Serum follicle-stimulating hormone was measured by radioimmunoassay. Plasma phytoestrogen levels were measured by high-performance liquid chromatography according to Franke et al. (30,31) to check the soy trial compliance.

Statistical analysis

First, we used general linear models (repeated-measures ANOVA) to compare means of the variables globally at the end of the soy nut, soy protein, and control diets. Then, we used paired *t* tests to compare the end-of-treatment values of each group with each other group. The percent change for each variable was also calculated by the formula $[(E - B)/B \times 100]$, where E is the end of treatment values and B is the baseline values. We compared groups using the percent change using both repeated-measures ANOVA and paired *t* test analyses. We determined the percent difference compared with the control for each group (both soy protein and soy nut) by the formula $[(X - C)/C \times 100]$, where X is the end values of soy protein or soy nut and C is the end values of the control group. Interactions between soy intake and weight were not significant for any of the metabolic features. We compared the percent change of variables in three groups in further models adjusted for lipid profile change. Period effect and carryover effects were tested using the appropriate general linear models.

For skewed variables (all markers of inflammation and endothelial function), we used log-transformed values in all analyses and reported geometric means. Pearson correlation coefficients were used to evaluate the relationship between soy-derived phytoestrogen intake (calculated from self-reported soy intake in 3-day diet records) and plasma phytoestrogen levels. All results were considered significant if the two-tailed P value was <0.05. Statistical analysis was performed using SPSS for Windows version 13.0 (SPSS, Chicago, IL) and SAS version 8.2 (SAS Institute, 1999).

RESULTS — All participants (42 postmenopausal women with the metabolic syndrome) completed the entire cross-over study. Dietary intake of participants in three periods, based on results of 3-day diet records, is shown in Table 1. Both soy nut and soy protein diets were well tolerated. Only one person complained of feeling bloated on soy protein. No significant period effects or carry over effects were identified for any of the observed results.

No significant differences in baseline characteristics of participants were seen across three diet periods. Compared with

Table 1—*Dietary intake of participants separately by intervention period*

	Control*	Soy protein†	Soy nut‡	*P*§	Washout‖
n	42	42	42	—	42
Soy protein (g/day)	0	30	0	—	0
Soy nut (g/day)	0	0	30	—	0
Nutrients					
Energy (kcal)	2,055	2,039	2,049	0.62	2,078
Protein (% of energy)	17	17	17	0.71	15
Total fat (% of energy)	28	25	29	<0.05	31
Saturated fat (% of energy)	7	5	5	0.61	14
Polyunsaturated fat (% of energy)	8	8	11	<0.05	7
Monounsaturated fat (% of energy)	10	10	10	0.73	9
Cholesterol (mg)	189	173	175	0.51	300
Carbohydrate (% of energy)	55	58	57	0.79	54
Fiber (g)	25	32	33	<0.05	11
Potassium (mg)	4,395	4,416	4,426	0.31	1,546
Calcium (mg)	1,209	1,219	1,227	0.49	750
Food groups (servings/day)					
Fruits	5.5	5.5	5.5	0.69	2.4
Vegetable	5.0	5.0	5.0	0.71	3.0
Grains					
Total	8.5	8.5	8.5	0.77	11.0
Whole	3.5	3.4	3.4	0.73	1.0
Low-fat dairy	2.5	2.5	2.5	0.77	0.5
Regular fat dairy	0.5	0.5	0.5	0.73	0.5
Red meat	1.0	0	0	<0.05	1.6
Poultry and fish	1.0	1.0	1.0	0.79	0.5
Fat and oils	3.5	3.5	3.5	0.78	7.9
Sweets	2.5	2.5	2.5	0.77	5.8

*Control diet: This diet had one serving of red meat and was rich in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fat, total fat, cholesterol, refined grains, and sweets. The amount of Na intake was 2,400 mg/day (DASH pattern). †Soy protein diet: This diet was the same as the control diet (DASH diet), but we replaced red meat with soy protein. ‡Soy nut diet: This diet was the same as the control diet (DASH diet), but we replaced red meat with soy nut. §*P* values for differences among three trial periods (repeated-measures ANOVA). ‖Washout: In this period, patients used the same diet they were using before the study.

the control diet, plasma phytoestrogen increased significantly after the soy nut regimen (percent change: 64%, $P < 0.01$) or soy protein diet (percent change: 48%, $P < 0.01$).

Effects of the three diets on markers of inflammation and endothelial function are shown in Table 2. Significant global differences were seen between the end values of control diet, soy protein diet, and soy nut diet for nitric oxide (NO), serum E-selectin, IL-18, TNF-α, and CRP. Paired comparisons of diets showed significant differences between control and soy protein diets for CRP and between the soy nut and control diets for NO, serum E-selectin, TNF-α, IL-18, and CRP. For NO levels, the difference from the control diet was 9.8% ($P < 0.01$) on the soy nut and −1.7% ($P = 0.10$) on the soy protein diets. The difference from the control diet for serum E-selectin was −11.4% ($P < 0.01$) on the soy nut consumption and −4.7% ($P = 0.19$) on the soy protein diets. Soy nut consumption reduced IL-18 compared with the control diet (difference from the control diet: −9.2%, $P < 0.01$), but soy protein did not (difference from the control diet: −4.6%, $P = 0.14$). For CRP, the difference from the control diet was −8.9% ($P < 0.01$) on the soy nut diet and −1.6% ($P < 0.01$) on the soy protein diet. End-of-treatment values were lower for the soy nut regimen compared with the soy protein diet for NO, serum E-selectin, TNF-α, IL-18, and CRP.

Figure 1 presents the mean and 95% CI of percent changes of inflammatory markers separately by diets. Percent change in control, soy protein, and soy nut regimens were significantly different for NO (14.3 ± 2.4, 13.9 ± 1.9, and 26.3 ± 2.6%, respectively, global $P < 0.01$), serum E-selectin (−4.6 ± 1.2, −7.9 ± 2.4, and −14 ± 1.6%, respectively, global $P < 0.01$), IL-18 (−5.7 ± 1.4, −6.6 ± 1.8, and −11 ± 1.9%, respectively, global $P < 0.01$), TNF-α (−3.7 ± 2.4, −1.6 ± 2.3, and −11 ± 1.9%, respectively, global $P < 0.05$), and CRP (−1.7 ± 0.6, −2.0 ± 0.3, and −8.5 ± 1.0%, respectively, global $P < 0.01$). The results were not changed when we adjusted the means for the change in lipid profiles in further models (data are not shown).

Neither soy protein nor soy nut consumption changed weight, waist circumference, endothelin-1, sVCAM, sICAM, IL-2, IL-6, or serum amyloid A significantly compared with the control diet.

CONCLUSIONS — We observed that replacement of red meat in the DASH diet by soy nut improved some markers of inflammation and endothelial function in postmenopausal women with the metabolic syndrome, but soy protein did not. We used naturally occurring isoflavones in the form of isolated soy protein and roasted soy nut in our intervention trial instead of purified isoflavones. Although serum concentrations of inflammatory markers are elevated in the metabolic syn-

Table 2—*Means of the markers of inflammation and endothelial function after 8 weeks of intervention in postmenopausal women*

	End-of-trial values			*P* values			
	Control*	Soy protein†	Soy nut‡	*P*§	*P*‖	*P*#	*P***
Weight (kg)	70.1 ± 0.9	70.7 ± 0.9	70.4 ± 0.8	0.57	0.32	0.43	0.67
Waist circumference (cm)	91.9 ± 0.8	91.5 ± 0.9	91.0 ± 1.0	0.19	0.38	0.09	0.31
NO (μmol/l)	28.8 (27.7–29.6)	28.3 (27.1–29.1)	31.4 (30.3–32.2)	<0.01	0.10	<0.01	<0.01
Endothelin-1 (pg/ml)	3.8 (3.7–3.9)	2.9 (2.7–3.0)	3.5 (3.3–3.6)	0.09	0.06	0.06	0.10
Serum E-selectin (ng/ml)	41.0 (39.6–42.4)	38.4 (36.8–40)	35.7 (33.7–37.7)	<0.01	0.19	<0.01	<0.01
sVCAM-1 (ng/ml)	488 (480–495)	492 (484–502)	485 (477–496)	0.12	0.22	0.46	0.07
sICAM-1 (ng/ml)	286 (278–293)	282 (274–291)	280 (270–289)	0.15	0.08	0.08	0.27
IL-2 (pg/ml)	976 (968–985)	972 (964–981)	968 (957–979)	0.07	0.11	0.07	0.25
IL-6 (pg/ml)	1.7 (1.5–1.8)	1.8 (1.6–2.0)	1.8 (1.6–2.0)	0.17	0.17	0.07	0.97
IL-18 (pg/ml)	146 (136–157)	14 (133–151)	131 (120–143)	<0.01	0.14	<0.01	<0.05
TNF-α (pg/ml)	1.4 (1.3–1.5)	1.4 (1.3–1.5)	1.3 (1.2–1.4)	<0.01	0.65	<0.01	<0.01
Serum amyloid A (μg/l)	18.0 (17.8–18.3)	17.0 (17.6–18.5)	18.0 (17.2–18.8)	0.58	0.20	0.06	0.06
CRP (mg/l)	3.4 (3.3–3.5)	3.3 (3.2–3.4)	3.1 (3.0–3.2)	<0.01	<0.01	<0.01	<0.01

Data are means ± SE or geometric means (95% CI) unless otherwise specified. *Control diet: This diet had one serving of red meat and was rich in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fat, total fat, cholesterol, refined grains, and sweets. The amount of Na intake was 2,400 mg/day (DASH pattern). †Soy protein diet: This diet was the same as the control diet (DASH diet), but we replaced red meat with soy protein. ‡Soy nut diet: This diet was the same as the control diet (DASH diet), but we replaced red meat with soy nut. §*P* values were for global comparison of three diet periods (repeated-measures ANOVA). ‖*P* values were for paired comparison of control and soy protein periods (paired *t* test). #*P* values were for paired comparison of control and soy nut periods (paired *t* test). **P* values were for paired comparison of soy nut and soy protein periods (paired *t* test).

drome, particularly in postmenopausal women, to our knowledge, this is the first study that examines the effect of soy protein or soy nut consumption on markers of inflammation and endothelial function in individuals with the metabolic syndrome. Several trials have evaluated the effect of soy consumption on endothelial function; most of them assessed endothelial function by flow-mediated vasodilation (32–35), and few focused on the biochemical markers of endothelial function such as soluble adhesion molecules and endothelial metabolites (18,21,23).

There are conflicting data in the literature about the effect of soy components on inflammatory markers and endothelial function in humans (18–24). Some studies have assessed the effect of soy on inflammatory markers in healthy (18–20,22–24) or hypercholesterolemic postmenopausal women (21). Nikander et al. (22) showed a neutral effect of phytoestrogen tablet consumption on the concentration of CRP, NO, and serum E-selectin in postmenopausal women. Isolated soy protein consumption caused no significant effect on the biochemical markers of endothelial function in healthy postmenopausal women (23) or vascular inflammation in hypercholesterolemic ones (21). Decreased circulating levels of TNF-α were reported with the consumption of soy milk containing isoflavone in postmenopausal women (19). It seems that the purified phytoestrogens or isolated soy protein alone are not as effective as the combination of soy protein, fatty acids, and phytoestrogens together (23,36). Most studies in this field have used purified phytoestrogens in the form of tablets, or isolated soy protein, which appears to have not as favorable effects as whole soy. This has been reflected to some extent in the current study. Although both soy protein and soy nut regimens had high amount of phytoestrogens compared with the control diet, only the soy nut diet improved metabolic profiles. Thus, the favorable effect might be attributed to higher amount of unsaturated fat or the interaction of fat, phytoestrogens, and other components in soy nut. The levels of several inflammatory and endothelial markers in the soy protein period were between that in the control period and that in the soy nut period. So phytoestrogen itself may have a weak effect on reducing the levels of inflammatory markers. A large sample size may be needed to test its effect. However, the deletion of red meat from the diet in the study periods may play a role in decreased levels of inflammatory markers and increased plasma NO levels. The main novelties of this article are the treatment (soy nut) and the subclass of postmenopausal women (with metabolic syndrome).

The mechanisms through which soy affects inflammatory state and endothelial function are largely unknown but may be related to the effects of soy phytoestrogens (37,38), specific fatty acids (39–42), or fibers (16). Soy phytoestrogens can enhance NO release and bioavailability and so reduce endothelin-1 concentrations (37,38). These phytoestrogens may resemble hormone replacement therapy regimens, hence reducing cell adhesion molecules and inflammatory markers (23). Furthermore, polyunsaturated fat intake, especially the combination of both ω-6 and ω-3 fatty acids, is associated with the lowest levels of inflammation (17), since even ω-6 fatty acids have anti-inflammatory properties (43). Therefore, some beneficial effects of soy nut on inflammatory markers, which contains both ω-3 and ω-6 fatty acids, are likely to be mediated by its fatty acids content. The primary outcomes of the present study were inflammations and the markers of endothelial function. Furthermore, our previous research in this regard showed an improvement in lipid profiles, especially after the soy nut period (27).

We had some limitations in our study. First, women participating in this study were those with all five components of the syndrome. In epidemiological studies, the majority of patients do not fall in the top five criteria category. Second, another limitation of this study is sex. Third, nitrite is a weak surrogate measure of NO. Moreover, nitrite concentration may be affected by foodstuff.

In the present study, the dose of soy isoflavones consumed during the soy protein period was 84 mg/day and during the soy nut period was 102 mg/day. In some

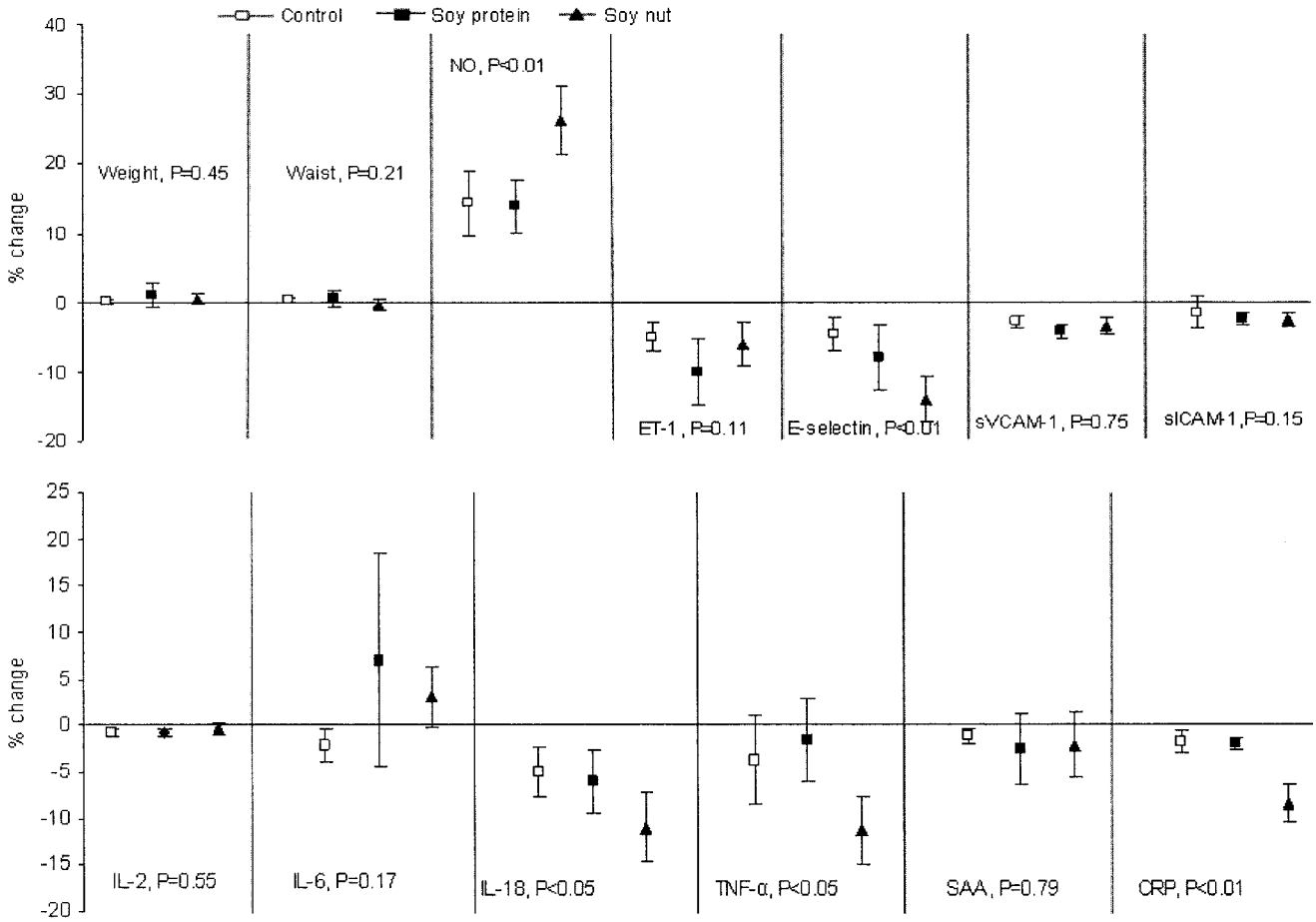

Figure 1—*Mean and 95% CI of percent change in the markers of inflammation and endothelial function in three diets: control, soy protein, and soy nut. Control diet: This diet was a DASH diet and had one serving of red meat and was rich in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fat, total fat, cholesterol, refined grains, and sweets. Diet with soy nut: This diet was the same as the control diet, but we replaced red meat with soy nut. Diet with soy protein: This diet was the same as the control diet, but we replaced red meat with soy protein.* P *values were determined by repeated-measures ANOVA. E-selectin, serum E-selectin; ET-1, endothelin-1; SAA, serum amyloid A.*

situations, excessive soy protein intake could do more harm than good. As animal studies evidence suggest, genistein can stimulate estrogen-receptor positive breast cancers to grow (44,45). However, we excluded patients with breast malignancy or breast cancer from the present study, and we used textured soy protein or soy nut, which were food sources of isoflavones, rather than pure isoflavones or soy protein pills. The washout period of 4 weeks between two treatment phases in our study seems adequate because values of the metabolic risks returned to baseline levels before the start of the next trial.

The effect of the soy nut consumption is difficult to extrapolate to other groups of postmenopausal women (apart from those who meet the five criteria of the metabolic syndrome). In addition, the use of the DASH diet as a control instead of a "typical diet" as a control diet could have weakened these effects. Therefore, more studies will be needed to test this hypothesis.

In summary, the results showed that soy nut consumption, at least for the short term, might reduce plasma concentrations of serum E-selectin, IL-18, TNF-α, and CRP and increase plasma NO levels in postmenopausal women with the metabolic syndrome.

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