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Effects of soy germ isoflavones and hormone therapy on nitric oxide derivatives, low-density lipoprotein oxidation, and vascular reactivity in hypercholesterolemic postmenopausal women

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ABSTRACT

Objective: To evaluate the effects of soy germ isoflavones and hormone therapy on vascular reactivity, the formation of nitric oxide derivatives, and lipid peroxidation in hypercholesterolemic postmenopausal women.

Design: Women were treated with soy germ, 17β-estradiol or 17β-estradiol + noretisterone acetate for 3 months after taking placebo for 1 month. The plasma concentrations of nitrite + nitrate and S-nitrosothiols were evaluated by gaseous phase chemiluminescence; nitrotyrosine, electronegative low-density lipoprotein, and estradiol levels were determined by enzyme-linked immunosorbent assay; cholesterol oxides and isoflavones were determined by gas chromatography and high-performance liquid chromatography, respectively. Vascular reactivity was analyzed by high-resolution ultrasonography.

Results: Soy germ isoflavones and hormone therapy induced a decrease in nitrite + nitrate, electronegative low-density lipoprotein, and cholesterol oxides, as well as an increase in S-nitrosothiols. Soy germ isoflavones lowered electronegative low-density lipoprotein, and cholesterol oxides more efficiently than did hormone therapy. Only soy isoflavones inhibited nitrotyrosine formation. A significant improvement of vascular reactivity was only seen in women treated with 17β-estradiol.

Conclusions: The soy germ isoflavones and 17β-estradiol, alone or associated with noretisterone acetate, in the doses and forms used here, have similar effects on the bioavailability of nitric oxide. Soy germ treatment inhibited lipid peroxidation more effectively than hormone therapy.

Key Words: Isoflavones – Soy germ – Nitric oxide – Cholesterol oxides – Electronegative low-density lipoprotein – Vascular reactivity.

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Estrogen therapy and estrogen/progestogen therapy provide antioxidant protection and have favorable effects on blood lipid and lipoprotein concentrations, endothelial function, and vascular reactivity.[1] However, recent reports have shown that exposure to estrogen may induce endometrial proliferation, increase the risk of breast cancer, and increase the risk of acute cardiac events, vascular cerebral accidents, and venous

thromboembolism.[2-4] Thus, the clinical cardiovascular health benefits of estrogen/progestogen therapy for postmenopausal women remain controversial, and alternatives for hormone therapy (HT) have been sought. Soy is a rich source of the isoflavones genistein, daidzein, and glycitein. Isoflavones are structurally similar to estradiol and have a higher binding affinity for estrogen receptor β, the primary estrogen receptor in the vascular wall, than for estrogen receptor α.[5]

The bioactivity of nitric oxide is reduced in postmenopausal women. However, it is not clear whether this reduction is a consequence of decreased production by nitric oxide synthase, enhanced inactivation by reaction with superoxide radical, forming the peroxynitrite anion ($ONOO^-$), or both. Peroxynitrite is decomposed into other reactive oxygen species,[6] reacts with the tyrosine residues of proteins to form nitrotyrosine (N-Tyr),[7] and may initiate lipid peroxidation of human low-density lipoprotein (LDL),[8] causing endothelial lesions and increased vascular permeability.[6] Several studies indicate that the endothelium-dependent deficiency in vascular relaxation is associated with inactivation of nitric oxide by the formation of peroxynitrite.[9-11]

Cholesterol molecules may also be oxidized, resulting in the formation of cholesterol oxides (oxysterols). Cholesterol oxides (COx) may reduce endothelium-dependent relaxation and nitric oxide production by endothelial cells.[12] High concentrations of cholesterol oxides are found in atherosclerotic arteries and in lipoproteins from hypercholesterolemic women.[13] Some cholesterol oxides, especially 7-ketocholesterol, have already been shown to have antiestrogenic activity.[14,15]

Electronegative LDL (LDL^-) is a minimally oxidized lipoprotein found in human plasma and is characterized by an increased content of conjugated dienes and malondialdehyde and reduced concentration of vitamin E.[16] LDL^- has proinflammatory and atherogenic properties.[17] The amount of LDL^- becomes increased in women with elevated cardiovascular risk, such as familial hypercholesterolemia and type 2 diabetes.[17] To our knowledge, there are no current studies to evaluate the influence of HT or phytoestrogens in the formation of cholesterol oxides and LDL^-.

Thus, the objective of this study was to evaluate the effect of HT and soy germ isoflavones on vascular reactivity and the bioavailability of nitric oxide by determining the concentration of its derivatives, such as nitrite and nitrate (NOx) , S-nitrosothiols, and N-Tyr, and the formation of LDL^- and COx in the plasma of hypercholesterolemic, moderately hypertensive postmenopausal women treated with either estrogen, estrogen/progestogen, or isoflavone soy therapy.

METHODS

This study is part of a major study previously described[18] that investigated the effect of statins and HT on lipid profile and vascular reactivity. The primary focus of our study was on the comparison of isoflavones with HT on nitric oxide metabolism and lipoprotein oxidation. Fifty-five women aged 50 to 65 years (average, 57 ± 2.55) were selected to participate in the study. With respect to racial distribution, 70.9% were white, 27.3% were black (21.8% of mixed blood and 5.5% pure black), and 1.8% were Asian. The average duration of menopause was 8.1 years (range, 6.15 to 10.15), and body mass index in week 12 averaged 27.83 (range, 26.82 to 29.55).

All the women suffered from mild to moderate arterial hypertension, which was controlled with a low-sodium diet and/or antihypertensive drugs. They had hypercholesterolemia with or without mild hypertriglyceridemia and natural menopause-related estrogen deficiency. The trial was approved by the Ethical Committees of the University of São Paulo School of Pharmaceutical Sciences and the Dante Pazzanese Institute of Cardiology. Before beginning the trial, the women signed an informed consent form.

The inclusion criteria were natural menopause (no menstrual cycle for at least 1 year), all racial backgrounds, age between 50 and 65 years, LDL cholesterol levels higher than 130 mg/dL and triglycerides less than 400 mg/dL during the randomization phase, mild to moderate arterial hypertension controlled with low-sodium diet and/or pharmacological therapy (systolic pressure ≤139 mm Hg and diastolic pressure ≤89 mm Hg), and nonsmoking status. The exclusion criteria were premenopause status; overt atherosclerotic disease in any vascular territory; surgically induced menopause with or without intact ovaries and tubes; a history of intolerance or hypersensitivity to statins or HT; history of oral, parenteral, vaginal, or transdermal HT during the 3 months before the initial visit; history of confirmed breast cancer or suspected image on the mammogram; history of endometrial cancer, abnormal uterine bleeding, or endometrial hyperplasia; history of pulmonary embolism or venous thrombosis; uncontrolled hypertension; smoking and/or alcohol consumption; activities of alanine aminotransferase, aspartate aminotransferase, and/or creatine kinase above the upper normal limit; poor compliance during the placebo phase (before randomization); any contraindication to the use

of estradiol or noretisterone; and history of intolerance or hypersensitivity to soy or soy products. The criteria for discontinuing the study were patient desire to withdraw; hospitalization for any reason; serum concentrations of alanine aminotransferase and/or aspartate aminotransferase, or creatine kinase, 3- or 10-fold above the upper normal limits respectively; or the presence of any severe adverse event.

After a 4-week period of dietary counseling by a dietitian aimed at reducing cholesterol levels and a 4-week period on placebo, 55 women were randomly assigned, according to a double-blind design, to any of three treatments (HT or isoflavones), which they followed for 12 weeks: group E (17β-estradiol, 2 mg/day; n = 17), group E + P (17β-estradiol, 2 mg/day, + noretisterone acetate, 1 mg/day; n = 18), or group I (soy germ, 2 g/day; n = 20). Throughout the study, all the women followed the same diet established in the initial recommendations. At the end of the placebo period and at the end of the treatment period, the women were subjected to a compliance evaluation, a clinical assessment (medical history and physical examination), and clinical chemistry blood tests. Half the women from each subgroup, randomly selected, were subjected to vascular reactivity measurements.

Blood was collected after 12 to 14 hours of fasting in tubes without anticoagulant and in tubes containing ethylenediamine tetraacetic acid or heparin. Serum and plasma were immediately separated for analysis of lipids, isoflavones, estradiol, NOx, S-nitrosothiol, N-Tyr, COx, and LDL^-. The clinical chemistry tests (total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and LDL cholesterol) were done using an automated enzymatic methodology. Estradiol was determined by enzyme-linked immunosorbent assay (Abbott Laboratory). Isoflavones from soy germ and blood plasma were determined by high-performance liquid chromatography.[19] NOx, S-nitrosothiols, N-Tyr, and COx were determined by previously reported techniques.[20] LDL^- was determined in the plasma by sandwich enzyme-linked immunosorbent assay as described by Pereira et al.[21] Brachial artery ultrasound assessments were done in the Echocardiography Department of the Dante Pazzanese Institute of Cardiology, following guidelines for ultrasound assessment of endothelium-dependent flow-mediated vasodilation of the brachial artery.[18] Using video-recorded images, a single observer made all the assessments and analyses. In addition to the measurements taken during the examination, two other measurements were taken after digitization of the video-recorded images with the Microsonic Color Vue analytical system.

Statistical analysis

All results are shown as mean ± SE. Placebo and treatment were compared by variance analysis with repeated measurements and Bonferroni's test. Endothelial function was assessed by comparing the

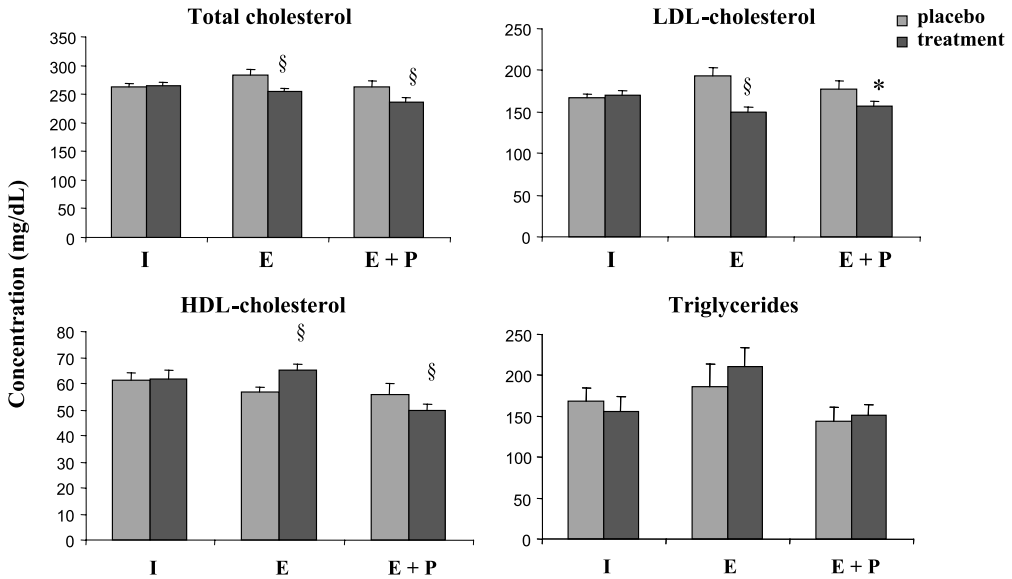

FIG. 1. Concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides after placebo administration and treatment with isoflavones (I), 17β-estradiol (E), or 17β-estradiol + noretisterone acetate (E + P). Data are expressed as mean ± SE. $^*P < 0.05$; $^§P < 0.001$.

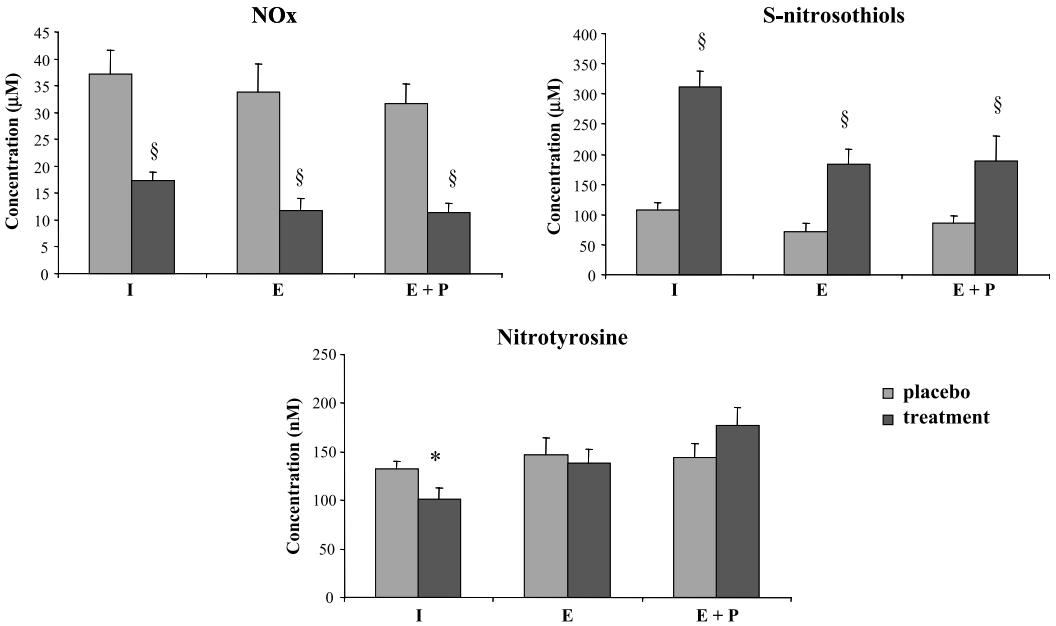

FIG. 2. Concentrations of derivatives of nitric oxide (NOx), S-nitrosothiols, and nitrotyrosine (equivalents of nitroalbumin) after placebo administration and treatment with isoflavones (I), 17β-estradiol (E), or 17β-estradiol + noretisterone acetate (E + P). Data are expressed as mean ± SE. $^{*}P < 0.05$; $^{\S}P < 0.001$.

measurements taken after placebo and treatment using a paired Student's *t* test. Results were considered statistically significant when *P* values were less than 0.05. The software used for the statistical analyses was SPSS for Windows. A Pearson correlation test was done using SigmaStat 1.0.

RESULTS

Effect of treatments on isoflavone and estradiol concentrations

The total dose of isoflavone contained in 2 g of soy germ used by the women in group I was 37 mg (aglycones: daidzein 10.48 mg, glycitein 21.64 mg, and genistein 4.52 mg). Plasma concentrations of daidzein, glycitein, genistein, and equol isoflavones after 12 weeks of treatment (group I) were 87.5 ± 19.9, 152.6 ± 36.3, 113.6 ± 17.9, and 71.47 ± 8.3 nmol/L, respectively. Isoflavones were not found in the plasma from the other groups. The plasma concentrations of estradiol found in the groups at the end of placebo and treatment periods, respectively, were as follows: I = 25.6 ± 9.0 to 24.5 ± 8.6; E = 32.4 ± 5.1 to 211.7 ± 24.4; and E + P = 33.0 ± 5.7 to 179.6 ± 25.4 pg/mL. Only HT elevated the concentration of plasma estradiol. Isoflavones did not affect the estradiol concentration.

TABLE 1. *Plasma concentrations of electronegative low-density lipoprotein (LDL^-) and total cholesterol oxides after placebo administration and treatment with isoflavones (I, n = 20), 17β-estradiol (E, n = 17), or 17β-estradiol + noretisterone acetate (E + P, n = 18)*

		Cholesterol oxides (ng/μL)						
Group	LDL^- (μg/mL)	Total	7α-OH	7β-OH	β-Epoxy	α-Epoxy	Triol	7-Keto/25OH
I: Placebo	8.45 ± 1.59	100.4 ± 14.7	12.7 ± 3.5	37.8 ± 7.5	27.1 ± 5.6	15.1 ± 6.5	3.5 ± 1.1	4.1 ± 1.7
I: Treatment	4.63 ± 0.93[a]	30.9 ± 3.2[b]	10.2 ± 2.7[a]	8.1 ± 1.1[b]	1.7 ± 0.2[a]	4.9 ± 0.7[a]	4.9 ± 0.5	1.0 ± 0.3
E: Placebo	6.66 ± 1.09	76.6 ± 12.6	11.7 ± 3.2	16.3 ± 3.3	17.7 ± 4.4	14.4 ± 3.6	6.8 ± 1.9	9.6 ± 3.1
E: Treatment	4.00 ± 0.63[a]	38.2 ± 7.8[b]	3.0 ± 1.5[a]	11.2 ± 1.8[a]	9.5 ± 3.5	8 ± 1.2[a]	2.6 ± 0.5	4.1 ± 1.0
E + P: Placebo	6.35 ± 1.66	70.2 ± 13.8	13.2 ± 4.2	17.5 ± 4.4	7.1 ± 1.7	18.0 ± 4.2	5.6 ± 1.2	8.7 ± 3.1
E + P: Treatment	5.87 ± 1.06[a]	31.9 ± 6.2[b]	1.5 ± 0.6[a]	10.0 ± 2.2	8.6 ± 3.4	12.4 ± 4.9[a]	2.5 ± 0.4	8.3 ± 1.0

Data are expressed as mean ± SE. LDL^-, electronegative low-density lipoprotein; 7α–OH, 7α-hydroxycholesterol; 7β-OH, 7β-hydroxycholesterol; α-epoxy, cholestan-5α,6α-epoxy-3β-ol; β-epoxy, cholestan-5α-6β-epoxy-3β-ol; Triol, cholestan-3β,5α,6β-triol; 7-keto, 5-cholesten-3β-ol-7-one; 25OH, 5-cholesten-3β, 25-diol.
[a] $P < 0.05$.
[b] $P < 0.001$.

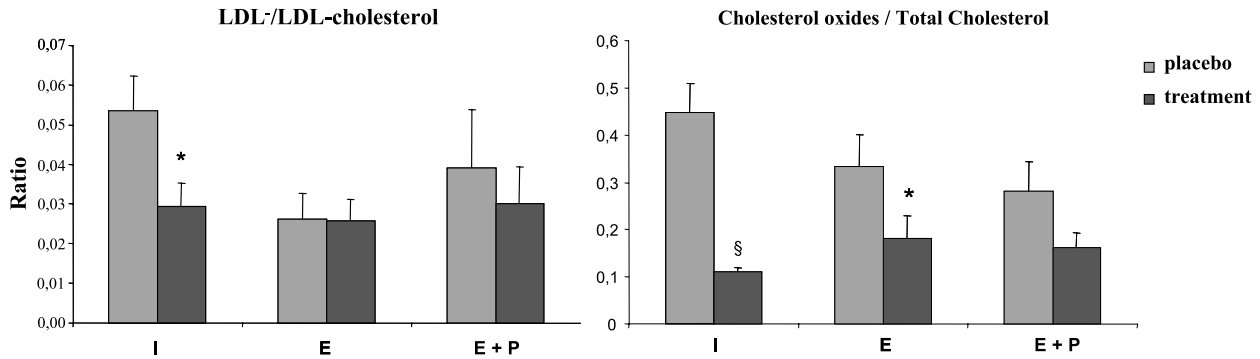

FIG. 3. Ratio electronegative low-density lipoprotein (LDL^-) concentration/LDL cholesterol and total cholesterol oxides/total cholesterol after placebo administration and treatment with isoflavones (I), 17β-estradiol (E), or 17β-estradiol + noretisterone acetate (E + P). Data are expressed as mean ± SE. $^*P < 0.05$; $^{\S}P < 0.001$.

Effect of treatments on blood lipid profile

Figure 1 shows the serum concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. Treatment with soy germ did not affect the blood lipid profile to any great extent. Both hormone treatments significantly reduced serum concentrations of total cholesterol (E = 8.8%, E + P = 10.1%) and LDL cholesterol (E = 20.3% and E + P = 12.1%). Group E, which received only estrogen replacement, presented an increase of 15.5% in the levels of HDL cholesterol. However, the addition of a progesterone derivative caused a reduction in HDL cholesterol concentration (E + P = 9.1%).

Effect of treatments on nitric oxide derivatives

Figure 2 shows a significant reduction in the plasma levels of NOx associated with an increase of S-nitrosothiols in all the groups. N-Tyr levels decreased in group I (Fig. 2). The plasma concentration of glycitein correlated positively with a decrease in N-Tyr (difference between treatment and placebo, $r = 0.568$, $P = 0.04$), suggesting that glycitein was important for this reduction.

Effect of treatments on lipid oxidation

All treatments reduced the plasma levels of LDL^- when expressed as absolute concentration (Table 1). However, the ratio of LDL^- to LDL cholesterol (Fig. 3) was significantly decreased only in group I. Similarly, all the treatments reduced the concentrations of COx (Table 1). However, when these concentrations were considered as the COx:total cholesterol ratio, the E + P group did not show a significant decrease in the concentration of COx (Fig. 3), whereas group I showed a higher decrease than group E ($P = 0.005$). All the groups showed a decrease in the plasma levels of 7α-hydroxycholesterol and α-epoxycholesterol. 7β-Hydroxycholesterol was decreased by soy germ and ET, but not by the association with noretisterone. β-Epoxycholesterol was reduced only by treatment with soy germ. 7-ketocholesterol, 25-hydroxycholesterol, and cholestanotriol were not affected by any treatment (Table 1). The plasma equol concentration was correlated with 7α-hydroxycholesterol ($r = -0.69$, $P = 0.01$). Plasma estradiol was correlated with 7β-hydroxycholesterol ($r = -0.64$, $P = 0.02$), α-epoxycholesterol ($r = -0.72$, $P = 0.006$), β-epoxycholesterol ($r = -0.72$, $P = 0.005$), cholestanotriol ($r = -0.60$, $P = 0.03$),

TABLE 2. *Effects of each therapy on endothelial function after placebo administration and treatment with isoflavones (I), 17β-estradiol (E), or 17β-estradiol + noretisterone acetate (E + P)*

	I (n = 20)		E (n = 17)		E + P (n = 18)	
Brachial artery diameter (mm)	Placebo	Treatment	Placebo	Treatment	Placebo	Treatment
Baseline	3.84 ± 0.28	3.93 ± 0.19	3.80 ± 0.15	3.83 ± 0.15	3.69 ± 0.07	3.93 ± 0.06
Hyperemia	4.31 ± 0.28	4.27 ± 0.20	4.20 ± 0.15	4.44 ± 0.17	4.05 ± 0.13	4.40 ± 0.12
FMV (%)	11.88 ± 2.45	8.78 ± 2.44	10.67 ± 1.62	16.36 ± 2.16[a]	9.78 ± 3.37	11.96 ± 1.73
AD (%)	−3.11		5.69		2.19	
RD (%)	−26.13		53.34		22.38	

Data are expressed as mean ± SE.
FMV, flow-mediated vasodilation; AD, absolute difference; RD, relative difference.
[a] $P < 0.05$.

7-ketocholesterol and 25-hydroxycholesterol ($r = -0.75$, $P = 0.003$), and total oxysterol ($r = -0.79$, $P = 0.001$).

Effect of treatments on vascular reactivity

Of the 55 women who participated in this study, 27 (49%) underwent vascular reactivity assessment before and after each treatment period. They were distributed as follows: group I = 8, group E = 10, and group E + P = 9. Vascular reactivity after treatment was not improved in group I (Table 2). Only women treated with 17β-estradiol (group E) exhibited a significant increase (+64.1%, $P < 0.05$) in flow-mediated dilation compared with baseline values (Table 2). Regarding the vasodilation obtained after administration of sublingual nitrate, the difference between baseline and posttreatment values was comparable in all treatment groups. Although soy germ did not improve vascular reactivity, there was a positive correlation between plasma isoflavone concentrations and vascular reactivity (genistein, $r = 0.899$, $P = 0.006$, and daidzein, $r = 0.948$, $P = 0.04$), suggesting that greater plasma concentrations of genistein and daidzein may improve vascular reactivity.

Power calculation for sample size determination

Based on standard power simulations and by admitting a threshold for α (type I error probability) of 5% (two tailed), our study was designed to have approximately 80% power to detect a 35% relative difference. In fact, based on previous data,[20-23] we assumed a mean 35% difference in treatment response as a (physiologically) significant difference. Therefore, overall, the sample size of our study had a power of 80% or more to detect a mean difference equal to or greater than 35% in the following variables: NOx, S-nitrosothiol, N-Tyr, COx, and LDL^-. Power ranged from 56% to more than 99%, suggesting that our work had an adequate probability of type II error (β). In conclusion, our study had sufficient statistical power to detect real effects, so our findings can be considered reliable.

DISCUSSION

The effects of HT and soy isoflavone therapy on nitric oxide derivatives, lipid peroxidation, and vascular reactivity were compared in postmenopausal hypercholesterolemic women. We found that treatment with 17β-estradiol, 17β-estradiol + noretisterone, or soy isoflavones had similar effects on NOx and S-nitrosothiol levels. However, the lipid profile, N-Tyr, LDL^-, COx, and vascular reactivity were affected differently by each treatment. HT decreased the plasma concentrations of total cholesterol and LDL cholesterol (Fig. 1). However, the association of estrogen with progestogen eliminated the ability of estrogen to increase HDL cholesterol (Fig. 1), as previously reported.[1] Soy germ isoflavones were not able to modify the lipid profile after 3 months of treatment (Fig. 1). Some previous studies[24,25] have shown that soy isoflavones are able to decrease LDL cholesterol and increase HDL cholesterol under similar treatment conditions, whereas others[26,27] have failed to reproduce these results. The hypocholesterolemic effect of soy isoflavones may depend on the doses ingested and the duration of treatment, as well as differences in isoflavone composition. Isoflavones associated with soy protein seem to have a dose-dependent effect on plasma lipids.[26] However, when isolated isoflavones are administered, they are apparently less effective.[28] One possible explanation for this lack of effect on the lipid profile may be the low bioavailability of isolated isoflavones. However, in our study, we know soy germ isoflavones were bioavailable because they were detected in blood plasma from the women only after treatment. Moreover, equol, the daidzein intestinal metabolite with potent estrogenic and antioxidant activity,[29] was detected in the blood plasma of women treated with soy germ isoflavones. Thus, it can be suggested that, under the conditions of our study, the estrogenic action of isoflavones was not strong enough to modify lipid metabolism. Another important finding of our study is that treatment with soy germ isoflavones did not increase estrogen concentrations in postmenopausal women (Fig. 1), reinforcing previously reported data.[30,31]

In this study, a decrease in plasma nitric oxide derivatives (nitrite + nitrate) was observed in all the treated groups (Fig. 2). This is relevant because both nitrate and N-Tyr concentrations are reportedly increased in postmenopausal women as compared with premenopausal women.[20] This could be due to the estrogen hypocholesterolemic effect, considering that hypercholesterolemia is associated with increased production of nitric oxide ($^{\bullet}NO$), superoxide radical ($O_2^{\bullet -}$), and peroxynitrite.[20,21] However, isoflavones reduced NOx and N-Tyr without affecting cholesterol levels. Estrogens can increase nitric oxide bioactivity without changing endothelial nitric oxide synthase gene expression or activity, probably by inhibiting superoxide radical production.[32] The decrease of endothelium-derived superoxide generation in response to estrogens could enhance the bioactivity of nitric oxide by preventing the formation of peroxynitrite. 17β-Estradiol also inhibits NOx release by

macrophage-inducible nitric oxide synthase in cell culture.[33] The effect of isoflavones on NOx concentration observed here is congruent with that observed by Yen and Lai,[34] indicating that the oral administration of isoflavones and soy-based products significantly decreased serum nitrite, nitrate, and N-Tyr levels in lipopolysaccharide-treated rats. It has been reported that genistein exhibits mild anti-inflammatory properties that may, in part, involve the attenuation of nitric oxide release via inducible nitric oxide synthase and the formation of peroxynitrite, which involves a tyrosine kinase–dependent mechanism.[35] In fact, we observed that N-Tyr levels decreased only in women treated with soy germ isoflavones (Fig. 2). Moreover, the plasma concentrations of glycitein were positively correlated with a reduction in the concentration of N-Tyr (r = 0.568, P = 0.04), suggesting that this isoflavone may affect inducible nitric oxide synthase regulation. Recent studies have demonstrated that the antioxidant activity of isoflavones can provide protection against LDL oxidation and DNA lesion formation mediated by peroxinitrite.[34,36] The effect of isoflavones on N-Tyr reduction seems to be independent of their estrogenic activity, considering that in our study estrogen and estrogen/progestogen therapy did not reduce the N-Tyr concentration. This is in contrast to in vitro data showing that 17β-estradiol blocks peroxynitrite formation in cell culture.[37] Although the plasma concentration of NOx was reduced by all the treatments, a parallel increase in the concentration of S-nitrosothiols was observed (Fig. 2), demonstrating that nitric oxide may be more available for the formation of S-nitrosothiols and is not rapidly degraded. Nitrosothiols may be formed by the reaction of nitric oxide with thiol groups[38] (present in cysteine, homocysteine, and glutathione residues). Nitrosothiols act by promoting vasodilation and inhibiting platelet aggregation.[38] They are considered reservoirs of nitric oxide, and many of their effects can be explained by nitric oxide release. Among other explanations, the altered vasodilation seen in postmenopausal women may result from the accelerated decomposition of nitric oxide, its lower conversion to nitrosothiols, or both. It is important to emphasize that all the treatments evaluated in this study restored the S-nitrosothiol concentration to the reported premenopausal levels.[20]

Lipid peroxidation was inhibited in postmenopausal women by all the three treatments, as evidenced by the decreased concentrations of COx (Table 1). A significant reduction of 7β-hydroxycholesterol, 7α-hydroxycholesterol, β-epoxycholesterol, and α-epoxycholesterol was found after treatment with soy germ. The treatment with 17β-estradiol also diminished the concentrations of 7β-hydroxycholesterol, 7α-hydroxycholesterol, and α-epoxycholesterol. This antioxidant effect of 17β-estradiol is in accordance with in vitro studies[39] showing that estradiol inhibits cholesterol oxidation more effectively than α-tocopherol or β-carotene. Although a negative correlation between the plasma concentrations of estradiol and COx was observed in women treated with 17β-estradiol, these correlations were not found in women treated with 17β-estradiol + noretisterone. Furthermore, when data were analyzed as the COx:total cholesterol ratio (Fig. 3), we found that the association with noretisterone eliminated the reducing effect of 17β-estradiol in the formation of COx. In contrast, our data show that isoflavones are more powerful than estradiol in preventing in vivo formation of COx (Fig. 3). The inverse correlation found between the concentrations of equol and 7α-hydroxycholesterol reinforces the antioxidant effect of this metabolite. Salonen et al[40] showed that the high concentration of 7β-hydroxycholesterol is a strong predictor of the increase of carotid thickness in women with atherosclerosis. Thus, the decrease observed in COx, including 7β-hydroxycholesterol, after 17β-estradiol and soy germ therapy would be protective considering that these oxysterols are cytotoxic and atherogenic.[41]

Further strong evidence of the antioxidant effect of HT and soy germ therapy is the significant decrease of LDL^- observed in response to the three treatments. Again, a less intense LDL^- decrease was found to result from the association of 17β-estradiol with noretisterone (Table 1). Moreover, when the LDL^-: LDL cholesterol ratio was considered (Fig. 3), only soy isoflavones significantly reduced LDL^-. This finding is in accordance with previous reports showing the inhibition of LDL oxidation by isoflavones.[42,43] Although soy germ treatment did not reduce total cholesterol and LDL^- cholesterol, it was more efficient in reducing LDL^- and COx than HT. Thus, our data are in agreement with those reported by Yamakoshi et al,[44] who showed that the antioxidant effect of isoflavones on LDL oxidation is independent of modifications in plasma lipid levels.

A significant improvement in vascular reactivity was observed only in women treated with 17β-estradiol (Table 2). Once again, the association of 17β-estradiol with noretisterone eliminated this beneficial estrogen effect. Soy isoflavones did not significantly improve vascular reactivity. This lack of effect may be related to the time of soy germ treatment; Hale et al[45] also found

no improvement of vascular reactivity in postmenopausal women taking 80 mg of isoflavones for 2 weeks. However, positive effects on vascular reactivity were observed in normocholesterolemic and normotensive postmenopausal women treated with isoflavones for longer periods, ie, 6 months or 1 year.[22,23,46] Another important point to be considered is that all the women in our study had hypercholesterolemia and hypertension, which lead to a deficiency in vascular reactivity. In previous studies,[22,23,46] an improvement in vascular reactivity induced by isoflavones was observed in women without these cardiovascular risk factors. Although we did not find a significant improvement in vascular reactivity with isoflavones, positive correlations between genistein and daidzein concentrations and vascular reactivity were observed. This suggests that with higher isoflavones doses, a beneficial effect on vascular endothelial function may occur. In fact, Lissin et al[47] showed that postmenopausal hypercholesterolemic women treated with 90 mg of isoflavones for 6 months had a significant improvement in vascular reactivity. Therefore, in our study, the dose of soy germ isoflavones, as well as the time of treatment, may be related to the absence of positive effects on the vascular endothelial function in hypercholesterolemic and hypertensive postmenopausal women.

CONCLUSIONS

Soy germ isoflavones and 17β-estradiol, alone or associated with noretisterone acetate, in the doses and forms used here, had similar effects on the bioavailability of nitric oxide. Although soy germ treatment did not reduce total or LDL cholesterol, it was more efficient in reducing LDL^{-} and COx than HT. The dose of soy isoflavones administered did not improve vascular reactivity, unlike 17β-estradiol. These results open up new prospects for future research on the effects of soy isoflavones on endothelial function and in the prevention of atherosclerosis in postmenopausal women.

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