Lúcio Omar Carmignani[1]
Adriana Orcesi Pedro[1]
Lúcia Helena Simões da Costa-Paiva[1]
Aarão Mendes Pinto-Neto[1]

The effect of soy dietary supplement and low dose of hormone therapy on main cardiovascular health biomarkers: a randomized controlled trial

O efeito de um suplemento alimentar à base de soja e terapia hormonal em baixa dose sobre os principais marcadores de risco cardiovascular: ensaio clínico randomizado controlado

Original Article

Keywords
Menopause
Soy food
Lipid profile
Biological markers
Risk factors
Phytoestrogens
Estrogen replacement therapy
Placebos

Palavras-chave
Menopausa
Alimentos de soja
Perfil lipídico
Marcadores biológicos
Fatores de risco
Fitoestrógenos
Terapia de reposição de estrogênios
Placebos

Abstract

PURPOSE: To assess the effects of a soy dietary supplement on the main biomarkers of cardiovascular health in postmenopausal women compared with the effects of low-dose hormone therapy (HT) and placebo. **METHODS:** Double-blind, randomized and controlled intention-to-treat trial. Sixty healthy postmenopausal women, aged 40–60 years, 4.1 years mean time since menopause were recruited and randomly assigned to 3 groups: a soy dietary supplement group (isoflavone 90mg), a low-dose HT group (estradiol 1 mg plus noretisterone 0.5 mg) and a placebo group. Lipid profile, glucose level, body mass index, blood pressure and abdominal/hip ratio were evaluated in all the participants at baseline and after 16 weeks. Statistical analyses were performed using the χ^2 test, Fisher's exact test, Kruskal-Wallis non-parametric test, analysis of variance (ANOVA), paired Student's *t*-test and Wilcoxon test. **RESULTS:** After a 16-week intervention period, total cholesterol decreased 11.3% and LDL-cholesterol decreased 18.6% in the HT group, but both did not change in the soy dietary supplement and placebo groups. Values for triglycerides, HDL-cholesterol, glucose level, body mass index, blood pressure and abdominal/hip ratio did not change over time in any of the three groups. **CONCLUSION:** The use of dietary soy supplement did not show any significant favorable effect on cardiovascular health biomarkers compared with HT. **Clinical Trial Registry:** The trial is registered at the Brazilian Clinical Trials Registry (Registro Brasileiro de Ensaios Clínicos – ReBEC), number RBR-76mm75.

Resumo

OBJETIVO: Avaliar os efeitos do uso de um suplemento alimentar à base de soja sobre os principais marcadores de risco cardiovascular e compará-los com o uso da terapia hormonal (TH) de baixa dose e grupo placebo em mulheres na pós-menopausa. **MÉTODOS:** Foram selecionadas 60 participantes do ambulatório de menopausa com idade entre 40 e 60 anos, com idade média de 4,1 anos na menopausa para participar de um ensaio clínico randomizado, duplo-cego e controlado com duração de 16 semanas. As pacientes foram randomizadas em 3 grupos: um grupo que recebeu suplemento dietético à base de soja (isoflavona 90 mg), um grupo que recebeu TH em baixa dose (estradiol 1 mg e noretisterona 0,5 mg) e um grupo placebo. Os seguintes parâmetros foram avaliados no início e ao término das 16 semanas de intervenção: perfil lipídico, glicemia de jejum, índice de massa corpórea, pressão sanguínea arterial e circunferência abdominal. A análise estatística foi realizada usando-se o teste do χ^2, teste exato de Fisher, teste não paramétrico de Kruskal-Wallis, análise de variância (ANOVA), teste *t* de Student pareado e teste de Wilcoxon. **RESULTADOS:** Ao final do período de intervenção de 16 semanas, houve uma diminuição do colesterol total em 11,3% e do LDL-colesterol em 18,6% no grupo da TH, porém ambos não tiveram mudanças tanto no grupo do suplemento alimentar à base de soja quanto no grupo placebo. Os valores de triglicérides, HDL-colesterol, glicemia de jejum, índice de massa corpórea, pressão sanguínea arterial e circunferência abdominal não mudaram ao longo da intervenção em nenhum dos grupos estudados. **CONCLUSÃO:** Do ponto de vista cardiovascular, o suplemento alimentar à base de soja não mostrou efeito favorável significativo nos marcadores de risco cardiovascular, quando comparado ao uso da TH.

Correspondence
Adriana Orcesi Pedro
Departament of Obstetrics and Gynecology,
Universidade Estadual de Campinas – UNICAMP
Rua Alexander Fleming, 101 – Cidade Universitária Zeferino Vaz
CEP: 13083-881
Campinas (SP), Brazil

Received on
02/27/2014

Accept with modifications
04/16/2014

DOI: 10.1590/S0100-720320140004976

Department of Obstetrics and Gynecology of the Universidade Estadual de Campinas – UNICAMP – Campinas (SP), Brazil.
[1]Department of Obstetrics and Gynecology, Universidade Estadual de Campinas – UNICAMP – Campinas (SP), Brazil.
Conflict of interests: none.

Introduction

Cardiovascular disease (CVD) remains the leading cause of mortality and morbidity in postmenopausal women[1]. Changes in lipids and lipoproteins at the time of menopause may contribute significantly to an increased risk for CVD development over a woman's lifetime, mainly through a more atherogenic lipid profile[2].

It has been known for a long time that hormone therapy (HT) has favorable effect on lipid profile[3,4]. Following the Women's Health Initiative study (WHI), there was a significant reduction in HT prescription for the treatment of menopause[5]. The search for complementary and alternative medicine (CAM) for the treatment of menopausal women has increased significantly worldwide. Among CAM, there has been widespread the use of phytoestrogens in menopausal women[6,7]. In Brazil, a recent evaluation after the publication of the WHI study, which assessed the medical knowledge of gynecologists with respect to the menopause and the treatment of symptomatic women, besides the repercussions of this knowledge on their attitudes and practice, reported that 46.3% of gynecologists had begun to prescribe isoflavone and other natural therapies for menopausal symptoms[8].

That is the reason for the intense research on the potential benefits of soy protein and soy isoflavones on plasma lipids and lipoproteins as a way of reducing CVD risk.

Consumption of products containing soy is widely variable in the world and there is no accurate statistics about the amount of soy ingested and the type and quality of soy protein ingested. The Study of Women's Health Across the Nation (SWAN), a multisite, longitudinal US cohort study, found substantial variation in dietary isoflavone intake by race/ethnicity among 3,133 women who were premenopausal or early perimenopausal at baseline. Approximately 40% of non-Asian US women in SWAN consumed no daidzein or genistein, the most prevalent isoflavones[6].

Soy is a rich source of the genistein, daidzein and glycitein isoflavones. Isoflavones are structurally similar to estradiol and have a higher binding affinity for the beta-estrogen receptor, the primary estrogen receptor in the vascular wall, than for the alpha-estrogen receptor[4,9]. Although there have been mixed results, some clinical trials have demonstrated a beneficial effect of dietary soy protein on plasma lipids and lipoproteins[10,11]. A number of studies have shown that isoflavones might neutralize or slow down the rate of LDL-cholesterol oxidation, as a result of this antioxidant effect[4,9,12]. In contrast, there are several studies showing a non-significant effect on the reduction of oxidative damage or no favorable alteration in blood lipids[13-15].

Although soybean has been considered food with functional properties, that is capable of reducing cholesterol according to the Food and Drug Administration (FDA)[16], which recommends the intake of 25 g/day of soy protein as part of a low-fat and cholesterol diet in order to reduce the risk of cardiovascular diseases, some reports have indicated that a large amount of industrialized soy-derived products contain varying quantities of isoflavones. Therefore, the purpose of this study was to evaluate the effects of isolated soy protein-containing isoflavones 90 mg on several biomarkers of cardiovascular health in menopause, mainly serum lipoproteins, which are normally used as indexes for cardiovascular disease, and to compare the results with the effects of low-dose hormone therapy and placebo.

Methods

Study participants

Sixty participants were recruited from two Menopause Outpatient Clinics of the Women's Integrated Healthcare Center at the University of Campinas (UNICAMP) in Campinas (SP), and Leonor Mendes de Barros Maternity Hospital in São Paulo (SP), Brazil, to participate in a 16-week double-blind, randomized placebo-controlled trial designed to examine the effect of soy dietary isoflavone supplementation on clinical biomarkers of cardiovascular health and serum changes in lipid profile and fasting glucose. The study design and participant enrollment have been reported in detail previously[17].

Sample size was based on standard power simulation, admitting a 5% threshold for alpha (type I error probability) and adopting a power of 80%. Based on previous studies, subjects selected for the isoflavone treatment had a mean difference in HDL-cholesterol baseline value and after treatment of 4.1 and a standard deviation of 6.7[18]. The sample size for establishing this difference was 23 subjects. Therefore, with a pool of 20 patients and an alpha error of 5%, we had approximately 74% power to detect a difference. Estimates from this statistical analysis suggest that our study had sufficient statistical power to detect real effects, so our findings can be considered reliable.

Inclusion criteria were postmenopausal women between 40 and 60 years of age, who had had their last menstrual period longer than 12 months before the study and a follicle-stimulating hormone greater than 30 mUI/mL, estradiol levels lower than 20 pg/mL, those who had not been on any type of hormonal treatment during the previous six months and were not currently using lipid lowering drugs, antidiabetic medication, soybean derived products, or herbal supplements. Exclusion criteria were history of hysterectomy, chronic

gastrointestinal disorder, any contra-indication for HT or for participation in a conflicting clinical trial. Finally, women were excluded if they had a known allergy or hypersensitivity to soy or cow milk or were not willing to avoid soy products for the 16-week study period. The Research Ethics Committee approved the protocol, and all participants provided a signed Informed Consent Form. The trial is registered at the Brazilian Clinical Trials Registry (Registro Brasileiro de Ensaios Clínicos – ReBEC), number RBR-76mm75[19]. ReBEC is an open access virtual platform for registration of ongoing or concluded experimental and non-experimental studies on humans, performed in Brazil and elsewhere. ReBEC is a joint project of Brazilian Ministry of Health, The Pan-American Health Organization (PAHO) and The Oswaldo Cruz Foundation (FIOCRUZ). The studies data registered on ReBEC comply with the dataset requirements of the International Committee of Medical Journal Editors (ICMJE) and the World Health Organization (WHO)[19].

Randomization and intervention

After initial screening, 60 women were assigned to 3 different treatments in a sequence determined by a computerized random-number generator. All patients received a numerical randomized envelope, with a letter inside labeled #1, #2 or #3, corresponding to HT, isoflavone 90 mg per day and placebo, respectively. During the study, the subjects and study personnel were not informed about the order of treatment. Study drugs were packaged in 30-day flasks. Follow-up visits were conducted by a gynecologist who did not participate in the screening part of this study or in drug distribution. The women were randomly assigned to one of three treatment groups, with daily oral intakes as follows: HT (n=20): 1 tablet of estradiol 1 mg + noretisterone acetate 0.5 mg (Activelle®, Medley Pharmaceuticals, Campinas, SP, Brazil) associated with 2 portions/day of placebo powder; isoflavone group (n=20): 2 portions/day of a food powder with isoflavone 45 mg/portion, totalizing 90 mg of isoflavone/day (Previna®, Sanavita Functional Foods, Piracicaba, SP, Brazil) and 1 placebo tablet; placebo group (n=20): 1 placebo tablet and 2 portions/day of placebo powder.

The isoflavone intervention consisted of 20 g/portion of a food powder containing 12 g of soy protein and 45 mg of total isoflavones (26.5 mg aglycones) to be mixed with 200 mL of any beverage. The soy intervention contained approximately 8 mg of total daidzein, 15 mg of total genistein and 3.5 mg of total glycitein.

The dietary soy supplement (Previna®, Sanavita Functional Foods, Piracicaba, SP, Brazil) consisted of 20 g portions of a food powder containing 12 g of soy protein and a total of 45 mg of isoflavones (26.5 mg of aglycons) to be mixed with 200 mL of water. The soy supplement contained approximately 8 mg of total daidzein, 15 mg of total genistein and 3.5 mg of total glycitein. The placebo powder (Sanavita Functional Foods) contained 20 g of maltodextrin, was identical in appearance to the soy powder and contained the same nutrients and calories except for the isoflavones and soy protein. Both supplements also contained 488 mg of calcium carbonate and 1.2 mg of hydrolyzed collagen per portion. The supplement was taken twice a day for a total of 16 weeks.

The placebo tablet was taken once a day. It was identical in appearance to the HT tablet and was produced by Medley Pharmaceuticals.

Measurements

At the screening visit, women answered a standardized questionnaire, which ascertained information about demographic characteristics including age, ethnicity, educational level and social status. Women were also queried about reproductive history, age at menopause, time since menopause, use of medication, history of cigarette smoking and frequency of alcohol use. Height was measured to the nearest 0.5 cm and weight to the nearest 0.1 kg, in light clothing and without shoes. Body mass index (BMI) was calculated as weight (in kg)/height (in m^2). Blood pressure was measured with a mercury sphygmomanometer after the participant had been seated quietly for at least five minutes. The waist/hip ratio was calculated using the minimum perimeter between the lowest rib and the anterior superior iliac crest as the waist measure, and the maximum perimeter at the gluteus as the hip measure[20].

Data were collected in the three groups at baseline and at 16 weeks of study. Blood was drawn for total lipid levels, lipoprotein levels and glucose analysis after the women had fasted for 12 hours overnight. Plasma glucose was measured by a glucose oxidase assay. Plasma total cholesterol and triglyceride levels were measured using enzymatic techniques, and lipoproteins were determined according to the National Institute of Health lipid research clinics method (commercial kits by Roche®). Inter-run coefficients of variation were 1.5% (TG), 0.8% (TC) and 1.3% (HDL-c). LDL cholesterol was calculated using the Friedwald equation: LDL-cholesterol = total cholesterol – HDL-cholesterol – (triglycerides/5). The Castelli I index was calculated as the ratio between total cholesterol and HDL-cholesterol, and the Castelli II index as the ratio between LDL-cholesterol and HDL-cholesterol[21].

Compliance was assessed by patient self-report of the number of packets of product missed, which was then converted to the percentage of prescribed packets ingested. Compliance was high (99.5%) and no dropouts occurred in any of the three groups during the study period.

Statistical analysis

Data were analyzed according to the intention-to-treat principle, including all original participants in the group to which they were randomly assigned. Data of epidemiologic and clinical characteristics were analyzed including the χ^2 test, Fisher's exact test, the Kruskal-Wallis non-parametric test and analysis of variance (ANOVA). Results shown as the means with observations at baseline and after treatment were compared in the same group, using a paired Student's *t*-test and Wilcoxon test. Intra-group and inter-group differences were evaluated using repeated-measures analysis of covariance, followed by the Tukey test, and non-parametric Kruskal-Wallis test, followed by the Mann-Whitney test[22]. Results were considered statistically significant when alpha error (p-values) was less than 0.05. SAS software, version 9.1.3 (SAS Institute Inc., Carey, NC, USA), was used to perform the analyses[23].

Results

A total of 1,520 patients were screened in both study centers in order to select 60 participants. The study was conducted in a tertiary reference center and the subjects were patients who presented with a high incidence of associated disorders, in addition to the climacteric syndrome. This fact made patient inclusion difficult and prolonged the necessary period to achieve 60 eligible subjects. Women assisted at menopause outpatient clinics were invited to answer a checklist in order to meet study criteria. The majority of screened women (95%) were excluded from the study after failing to meet inclusion criteria and because some of them had no interest in participating in the study (5%). Most (n=1,370) were excluded in the first pre-randomization visit and the main reasons were: 54% – hypertension; 40% – obesity; 28% – hysterectomy; 22% – metabolic syndrome; 8% – diabetes mellitus. In addition, 30% had some type of gynecological cancer and 40% were on HT or non-hormone therapy for climacteric syndrome (more than one condition per patient). At the second pre-randomization visit, 90 women were considered ineligible due to: screening altered for endometrial thickness, some altered findings on mammography, estradiol level higher than 20 pg/mL and lipid profile and/or fasting glucose with high levels, requiring immediate treatment with specific drugs. At the randomization visit, the remaining 60 women were equally randomized into 3 groups, as shown in Figure 1. These groups were observed for 16 weeks and there were no dropouts and no one was lost to follow-up.

Table 1 shows the baseline characteristics of the participants by intervention group. There were no significant differences between the groups. The average age was 52.4 (±3.9) years. Women were on average 4.1 (±3.3) years post-menopause and the mean age at menopause was 48 (±3.7) years. The average educational level was 6.8 (±4.1) years.

Cardiovascular risk parameters such as waist circumference, circumference of the hip, waist/hip ratio, and systolic and diastolic blood pressure did not change over the treatment period among the three groups. There

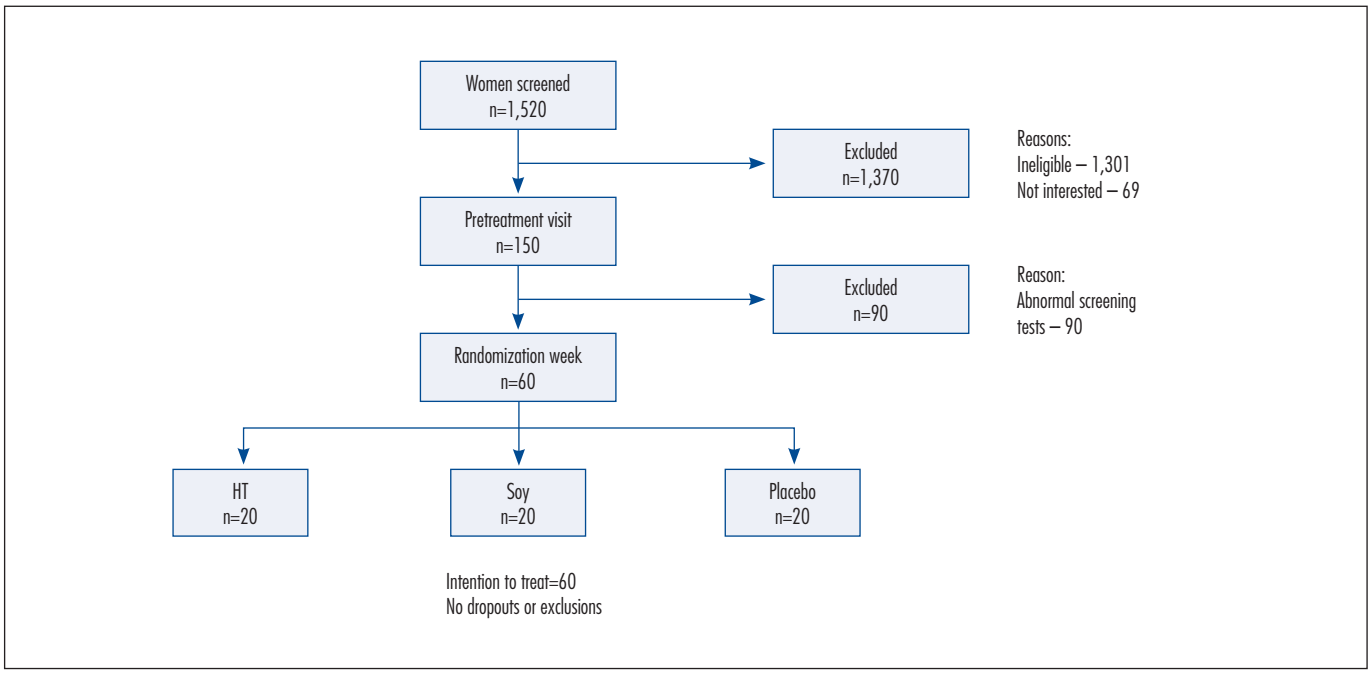

Figure 1. Flow chart of the study participants

was an increase in weight and BMI only in the placebo group, which showed an average increase of 1.3 kg over the 16-week study period. When we compared treatment effect among the groups, no statistically significant differences were observed.

At the end of the 16-week treatment, total cholesterol plasma levels showed 11.3% decrease in the HT group, but the isoflavone and placebo groups showed no changes. LDL serum levels decreased by 18.6% in the group that received HT and there was no change in the other groups. Plasma triglyceride, HDL and glucose levels did not change in any of the groups. The Castelli I and II indices showed a positive relationship only in the group that used HT, with a reduction of 15.9 and 21.7%, respectively.

Table 1. Characteristics of the women according to study group (n=60)

Characteristics	Groups			p-value
	HT	Soy	Placebo	
Mean age (years±SD)	53.3±4.5	52.9±3.5	50.9±3.4	0.1*
Age at menopause (years) Median (Q_1–Q_3)	48.0 (47.0–50.5)	49.0 (47.5–50.5)	48.0 (46.0–50.0)	0.4**
Time since menopause (years) Median (Q_1–Q_3)	5.6 (1.5–10)	2.5 (1.5–4.0)	2.5 (1.0–5.5)	0.1**
Education (years of schooling±SD)	6.6±4.2	7.6±4.4	6.3±3.9	0.7*
Skin color (%)				
White	65.0	40.0	70.0	0.1***
Non-white	35.0	60.0	30.0	
Parity (%)				
≤2	45.0	65.0	40.0	0.3****
>2	55.0	35.0	60.0	
Social status (%)				
A/B classes	40.0	55.0	45.0	0.6***
C/D/E classes	60.0	45.0	55.0	
Smoking habits (%)				
Current smoker/ex-smoker	60.0	35.0	45.0	0.6***
Never smoked	40.0	65.0	55.0	
Body mass index (kg/m²) Median (Q_1–Q_3)	25.9 (24.0–28.5)	26.4 (24.2–28.8)	26.6 (24.1–30.0)	0.3**

*ANOVA test; **Kruskal-Wallis non-parametric test; ***χ^2 test; ****Fisher's exact test.
HT: hormone therapy; SD: standard deviation; Q_1: first quartile; Q_3: third quartile.

When we compared treatment effect among the groups, a significant improvement in total cholesterol and LDL-cholesterol in the HT group was observed, compared with the soy and placebo groups (Table 2).

There were no statistically significant differences in the adverse effects evaluated (mastalgia, vaginal bleeding, allergy, headache, nausea, weight gain, water retention and intestinal complaints) between the three treatment groups. The adverse effects have been reported in detail previously[17].

Discussion

The results showed that dietary soy supplement had no significantly favorable effect on cardiovascular health biomarkers, when compared to HT use. Of all the clinical markers evaluated, only weight and BMI were slighted increased in the placebo group (1.3 kg). The results have indicated that hormonal changes, in particular, estrogen deficiency that take place in the menopausal transition cause an increase in weight and BMI. Studies have shown that naturally menopausal women have significantly higher BMIs than women treated with HT in the menopausal period[24]. HT protects women against an increase in BMI. This could explain why women receiving a placebo had increased BMI in this study. In contrast, HT use did not change BMI nor did isoflavone use. Maesta et al.[25] found no increase in BMI, waist circumference and body fat in women receiving 25 g of soy protein.

The results of this trial do not support the hypothesis that isoflavones from soy protein have beneficial effects on plasma lipids in post-menopausal women. In this study, there was an increase in HDL-cholesterol (10.2%) and a slight decrease in LDL (-1.9%) and total cholesterol (-0.7%) in soy groups, but these differences compared to baseline values were not statistically significant. These results are similar to previous studies aimed at menopausal women who received soy dietary supplement, which did not demonstrate any favorable effect on lipid

Table 2. Mean percentage variation (confidence interval of 95%) of lipid profile and glucose at baseline and follow-up per treatment group

Variables	HT (n=20)	Soy (n=20)	Placebo (n=20)	p-value
Total cholesterol*	-11.3 (-15.3 – -7.4)	-0.7 (-5.3 – 4.0)	4.6 (-2 – 11.2)	<0.01
HDL-cholesterol**	8.3 (-0.1 – 16.7)	10.2 (-1.9 – 22.2)	6.1 (-1.5 – 13.6)	0.9
LDL-cholesterol*	-18.6 (-26.0 – -11.2)	-1.9 (-8.6 – 4.8)	5.5 (-4.9 – 15.9)	<0.01
Triglycerides*	-0.1 (-17.6 – 17.4)	-1.1 (-13.7 – 11.5)	21.3 (-15.6 – 58.1)	0.3
Glucose*	-0.8 (-5.1 – 3.5)	3.1 (-0.3 – 6.5)	0,5 (-4.5 – 5.5)	0.4
Castelli index I**	-15.9 (-23.6 – -8.2)	-5.9 (-15.5 – 3.7)	0.8 (-8.5 – 10.2)	0.02
Castelli index II**	-21.7 (-33.5 – -9.8)	-6.1 (-18.4 – 6.1)	1.8 (-10.5 – 14.1)	0.01

*Co-variance ANOVA test (followed by Tukey test); **non-parametric Kruskal-Wallis test (followed by Mann-Whitney test).
p-value for difference between groups: Total cholesterol – HT *versus* soy<0.01; HT *versus* placebo<0.01; soy *versus* placebo=0.3; LDL-cholesterol – HT *versus* soy=0.01; HT *versus* placebo<0.01; soy *versus* placebo=0.4; Castelli Index I – HT *versus* soy=0.2; HT *versus* placebo=0.01; soy *versus* placebo=0.5; Castelli Index II – HT *versus* soy=0.1; HT *versus* placebo=0.01; soy *versus* placebo=0.6.

profile[13,15,26,27]. In a recent review, the North American Menopause Society Isoflavone Report (2011) concluded that any cardiovascular benefit derived from soy protein or isoflavone supplements would be minimal and there is a need for greater standardization and documentation of clinical trial data on soy[28].

On the other hand, some significant trials have reported a generally consistent finding in support of the beneficial role of soy foods or protein in lipid profiles. Anderson et al.[10] published a meta-analysis including 38 trials, observing a decrease in serum lipids: total cholesterol (9.3%), LDL-cholesterol (12.9%) and triglycerides (10.5%), and an increase in HDL-cholesterol (2.4%). A meta-analysis by Zhan and Ho[11] of 23 randomized clinical trials conducted from 1995 to 2002 demonstrated that the intake of soy protein containing isoflavones was associated with a better lipid profile: a 3.77% decrease in serum total cholesterol, 5.25% in LDL-cholesterol and 7.27% in triglycerides, and a significant 3.03% increase in serum HDL-cholesterol. A double-blind placebo controlled trial published by Nahas et al.[29], with 50 menopausal women using 60 mg of isoflavones in tablets in a period of 6 months, showed a decrease in LDL-cholesterol (11.8%) and an increase in HDL-cholesterol (27.3%) in soy group compared to placebo group. A review conducted by Dewell et al.[30] concluded that both soy protein and soy isoflavone extracts resulted in reductions in total plasma cholesterol concentrations, but the authors conclude that these decreases were likely too small to be clinically beneficial in the reduction of CVD.

Research studies have suggested that the beneficial effects of soy might be due to isoflavone activity by three major mechanisms: 1) directly through estrogen receptor (ER) mediated effects; 2) directly through ER-independent effects on cardiovascular risk factors and putative atherogenic risk factors and 3) indirectly by displacement of animal protein intake[28]. However, not all studies have reported a cholesterol-lowering effect of isoflavones[7,15]. Some investigators have used a variety of protocols, including a range of isolated soy proteins, varying doses of isoflavones, different trial lengths, and diverse population subgroups in an attempt to delineate the components of soy protein matrix, doses, and circumstances whereby soy could be effective in improving human lipid profiles[11,28].

Possible explanations for our findings may be gender-related, since the most significant reductions in total cholesterol and LDL-cholesterol levels were observed in men rather than in women, in premenopausal rather than in post-menopausal women and in hyperlipidemic women rather than in normal lipidemic women[11]. However, when comparing isoflavone with low-dose HT, this last intervention was also able to reduce the lipid profile in normal lipidemic post-menopausal women in a period of 16 weeks. Furthermore, the conflicting results obtained from studies on isoflavones may also have been due to differences in supplement form, intake amount, genetic factors and individual absorption ability (equol producers *versus* equol non-producers)[28].

It has been suggested that equol production could be induced with probiotics, the most commonly used being *Lactobacilli* and *Bifidobacteria*. Whether an equal-producing capacity is a determinant of the cardiovascular benefit of soy is uncertain until now[31].

Some critics may question that the length of soy isoflavone use is too short to elicit a satisfactory clinical response. However, other authors observed that the most significant lowering effect of isoflavone soy protein contents on lipid profile occurred within the initial short period of isoflavone exposure, and the extent of the lipid lowering effect decreased as the duration of intervention increased. This fact can be attributed to a physiologic adaptation mechanism to a more prolonged supplementation, by not paying attention to diet or lower adherence in prolonged periods of intervention[11,32]. Therefore, the length of use was satisfactory to induce effects on lipid profiles.

Investigation of fasting glucose in this trial had no significant change in any group. Menopausal transition is accompanied by a decrease in insulin secretion and hepatic insulin clearance[33]. Review studies have reported the beneficial effects of estrogen therapy on glucose homeostasis[34] and some data suggest that postmenopausal women with type 2 diabetes mellitus who use oral estrogen therapy may require lower doses of medications for glycemic control[3]. Soy isoflavones, with structural similarity to estrogens, may also exert their biological effects through estrogen-mediated mechanisms and might be beneficial for glucose homeostasis and alleviation of diabetes[35]. In a recent trial published by Ho et al.[15], moderate but significant differences were observed in changes and percentage of changes in fasting glucose among the studied groups, but the effects were much more apparent in women with high baseline fasting glucose concentration (>100 mg/dL) than in those with lower baseline values (<90 mg/dL). In our trial, the mean fasting glucose was lower than 100 mg/dL and this may be a possible cause for no significant change in fasting glucose level in our intervention. The real influence of soy isoflavones on serum glucose levels remains uncertain.

International societies are cautious about recommending foods or supplements containing isoflavones. Some professionals or even the media have recommended isoflavones, especially for the cardiovascular benefits of these foods, but the observed health effects cannot be clearly attributed to isoflavones alone. Soy and soy isoflavones had such a small effect on plasma lipid

profiles that they probably did not reduce the risk of cardiovascular disease[28,36,37].

This trial is one of the few studies that have compared three intention-to-treat interventions, measuring the effect of low-dose HT, a dietary soy supplement and placebo in the same study, with the purpose of determining the effects of soy isoflavones on several biomarkers of cardiovascular health, mainly serum lipoproteins, which are normally used as indexes for cardiovascular disease and comparing the results with the effects of HT and placebo. Thus, this study may contribute to guide the intake of dietary soy supplement, providing a scientific basis and avoiding the indiscriminate use of any food containing isoflavone.

This 16-week randomized controlled double-blind intention-to-treat trial indicates that consumption of a soy dietary supplement containing 90 mg of isoflavones was not associated with a significant effect on cardiovascular health biomarkers. The studied population was composed of postmenopausal women with previous normal lipid profile and probably that is one of the reasons for our findings. On the other hand, low-dose HT showed a significantly beneficial effect on lipid profile. Future studies with long-term interventions involving different baseline subject characteristics and the inclusion of clinical endpoints would be useful and desirable.

Acknowledgement

Source of Funding: this research was funded by The São Paulo Foundation for the Support of Research (FAPESP), grant # 03/04464-0.

Authors' contributions to manuscript:

Lúcio Omar Carmignani, MD: Conducted research; analyzed data and performed statistical analysis; wrote the paper; had primary responsibility for the final content.

Adriana Orcesi Pedro, MD, PhD: Designed research; conducted research; provided essential reagents and provided essential materials; analyzed data and performed statistical analysis; wrote the paper; had primary responsibility for the final content;

Lúcia Helena Simões da Costa-Paiva, MD, PhD, Associate Professor: Wrote and review the paper.

Aarão Mendes Pinto-Neto, MD, PhD, Associate Professor: Wrote and review the paper.

References

1. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, et al. Heart disease and stroke statistics: 2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2008;117(4):e25-146.
2. Rosano GM, Vitale C, Marazzi G, Volterrani M. Menopause and cardiovascular disease: the evidence. Climacteric. 2007;10 Suppl 1:19-24.
3. The North American Menopause Society. The 2012 hormone therapy position statement of The North American Menopause Society. Menopause. 2012;19(3):257-71.
4. Cano A, García-Pérez MA, Tarín JJ. Isoflavones and cardiovascular disease. Maturitas. 2010;67(3):219-26.
5. Tsai SA, Stefanick ML, Stafford RS. Trends in menopausal hormone therapy use of US office-based physicians, 2000–2009. Menopause. 2011;18(4):385-92.
6. Huang MH, Schocken M, Block G, Sowers M, Gold E, Sternfeld B, et al. Variation in nutrient intakes by ethnicity: results from the Study of Women's Health Across the Nation (SWAN). Menopause. 2002;9(5):309-19.
7. Borrelli F, Ernst E. Alternative and complementary therapies for the menopause. Maturitas. 2010;66(4):333-43.
8. Lazar F Jr, Costa-Paiva L, Morais SS, Pedro AO, Pinto-Neto AM. The attitude of gynecologists in Sao Paulo, Brazil 3 years after the Women's Health Initiative study. Maturitas. 2007;56(2):129-41.
9. Wei H, Bowen R, Cai Q, Barnes S, Wang Y. Antioxidant and antipromotional effects of the soybean isoflavone genistein. Proc Soc Exp Biol Med. 1995;208(1):124-30.
10. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med. 1995;333(5):276-82.
11. Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr. 2005;81(2):397-408.
12. Dong JY, Wang P, He K, Qin LQ. Effect of soy isoflavones on circulating C-reactive protein in postmenopausal women: meta-analysis of randomized controlled trials. Menopause. 2011;18(11):1256-62.
13. Campbell SC, Khalil DA, Payton ME, Arjmandi BH. One-year soy protein supplementation does not improve lipid profile in postmenopausal women. Menopause. 2010;17(3):587-93.
14. Engelman HM, Alekel DL, Hanson LN, Kanthasamy AG, Reddy MB. Blood lipid and oxidative stress responses to soy protein with isoflavones and phytic acid in postmenopausal women. Am J Clin Nutr. 2005;81(3):590-6.
15. Ho SC, Chen YM, Ho SS, Woo JL. Soy isoflavone supplementation and fasting serum glucose and lipid profile among postmenopausal Chinese women: a double-blind, randomized, placebo-controlled trial. Menopause. 2007;14(5):905-12.
16. Food labeling: health claims; soy protein and coronary heart disease. Food and Drug Administration, HHS. Final rule. Fed Regist. 1999;64(206):57700-33.
17. Carmignani LO, Pedro AO, Costa-Paiva LH, Pinto-Neto AM. The effect of dietary soy supplementation compared to estrogen and placebo on menopausal symptoms: a randomized controlled trial. Maturitas. 2010;67(3):262-9.

18. Han KK, Soares JM Jr, Haidar MA, de Lima GR, Baracat EC. Benefits of soy isoflavone therapeutic regimen on menopausal symptoms. Obstet Gynecol. 2002;99(3):389-94.

19. Brasil. Ministério da Saúde. Registro Brasileiro de Ensaios Clínicos. [RBR-76mm75: the use of phytoestrogens, low dose hormone therapy and placebo to treat psychological somatic and urogenital symptoms in climacteric women: randomized, controlled, double-blind, clinical trial] [Internet]. 2012 [cited 2012 Apr 27]. Available from: http://www.ensaiosclinicos.gov.br/rg/RBR-76mm75. Portuguese.

20. World Health Organization. Obesity: preventing and managing the global epidemic — report of a WHO Consultation. Geneva: WHO; 1997.

21. Castelli WP. Cholesterol and lipids in the risk of coronary artery disease–the Framingham Heart Study. Can J Cardiol. 1988;4 Suppl A:5A-10A.

22. Snedecor GW, Cochran WG. Statistical methods. 8th ed. Ames: Iowa State University Press; 1989.

23. Altman DG. Practical statistics for medical research. London: Chapman & Hall; 1991.

24. Skrzypczak M, Szwed A. Assessment of the body mass index and selected physiological parameters in pre- and post-menopausal women. Homo. 2005;56(2):141-52.

25. Maesta N, Nahas EA, Nahas-Neto J, Orsatti FL, Fernandes CE, Traiman P, et al. Effects of soy protein and resistance exercise on body composition and blood lipids in postmenopausal women. Maturitas. 2007;56(4):350-8.

26. Nahas EA, Nahas-Neto J, Orsatti FL, Carvalho EP, Oliveira ML, Dias R. Efficacy and safety of a soy isoflavone extract in postmenopausal women: a randomized, double-blind, and placebo-controlled study. Maturitas. 2007;58(3):249-58.

27. Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A, Lampe JW, et al. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. JAMA. 2004;292(1):65-74.

28. North American Menopause Society. The role of soy isoflavones in menopausal health: report of The North American Menopause Society/Wulf H. Utian Translational Science Symposium in Chicago, IL (October 2010). Menopause. 2011;18(7):732-53.

29. Nahas EA, Nahas Neto J, De Luca LA, Traiman P, Pontes A, Dalben I. [Effects of isoflavone on menopausal symptoms and blood lipids in postmenopausal women]. Rev Bras Ginecol Obstet. 2003;25(5):337-43. Portuguese.

30. Dewell A, Hollenbeck PL, Hollenbeck CB. A critical evaluation of the role of soy protein and isoflavone supplementation in the control of plasma cholesterol concentrations. J Clin Endocrinol Metab. 2006;91(3):772-80.

31. Fooks LJ, Gibson GR. Probiotics as modulators of the gut flora. Br J Nutr. 2002;88 Suppl 1:S39-S49.

32. Setchell KDR. Absorption and metabolism of isoflavones. Soy Connect Newsl. 1998;6(2):1-3. Available from: http://www40.pair.com/rstevens/nutrition/AbsorptionMetabolism.html

33. Kaaja RJ. Metabolic syndrome and the menopause. Menopause Int. 2008;14(1):21-5.

34. Lobo RA. Metabolic syndrome after menopause and the role of hormones. Maturitas. 2008;60(1):10-8.

35. Jayagopal V, Albertazzi P, Kilpatrick ES, Howarth EM, Jennings PE, Hepburn DA, et al. Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. Diabetes Care. 2002;25(10):1709-14.

36. Sacks FM, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P, Winston M. Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee. Circulation. 2006;113(7):1034-44.

37. Tempfer CB, Bentz EK, Leodolter S, Tscherne G, Reuss F, Cross HS, et al. Phytoestrogens in clinical practice: a review of the literature. Fertil Steril. 2007;87(6):1243-9.