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THE EFFECT OF ISOFLAVONE SOY PROTEIN SUPPLEMENTATION ON ENDOMETRIAL THICKNESS, HYPERPLASIA AND ENDOMETRIAL CANCER RISK IN POSTMENOPAUSAL WOMEN: A RANDOMIZED CONTROLLED TRIAL

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Abstract

Objective—To determine whether long-term isoflavone soy protein (ISP) supplementation affects endometrial thickness and rates of endometrial hyperplasia and cancer in postmenopausal women.

Methods—In this randomized, double-blind, placebo-controlled trial, 350 postmenopausal women 45–92 years of age were randomized to a total daily dose of 154 mg of ISP or a milk protein matched placebo for a 3-year period. Women with a surgically absent uterus were excluded from the analysis (final study population: n=224). The main outcome measures were the mean change in endometrial thickness on transvaginal ultrasound from baseline until up to 36 months of follow-up; the incidence of endometrial sampling, endometrial hyperplasia and endometrial cancer.

Results—A total of 666 visits among 224 participants were evaluated. Treatment groups did not significantly differ on the mean baseline or on-trial changes in endometrial thickness. Of the 103 placebo-treated participants, 7 (6.8%) underwent an endometrial biopsy; 6 (85.7%) of these biopsies were benign. One woman in the placebo group was diagnosed with complex endometrial hyperplasia with atypia and underwent a hysterectomy. The pathology result from this surgery was Stage IB endometrial cancer. Of the 121 participants in the soy group, 9 (7.4%) underwent an endometrial biopsy. The results were benign in all 9 cases (100%). Although the rate of hyperplasia / malignancy was higher in the placebo group (14.3% versus 0%), the difference was not statistically significant.

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Conclusion—Three-year isoflavone soy protein (ISP) supplementation has no effect on endometrial thickness or rates of endometrial hyperplasia and cancer in postmenopausal women.

LEVEL OF EVIDENCE—I

Keywords

Isoflavones; menopause; endometrium; randomized controlled trial

Introduction

Because of concerns over traditional estrogen preparations for postmenopausal hormone therapy, an increasing number of women in the US turn to alternative therapies such as phytoestrogens, nonsteroidal compounds contained in plants, fruits and vegetables [1,2]. One type of phytoestrogen, the isoflavones, are structurally similar to 17β-estradiol. Isoflavones therefore possess selective estrogen receptor modulator (SERM)-like activity, with varying estrogenic and anti-estrogenic effects depending on the receptor characteristics of the target tissue [3].

Although a high percentage of women use isoflavone soy protein (ISP) supplementation as an alternative to hormone replacement therapy to help manage the effects of menopause [4], research on the efficacy of ISP in this context has yielded conflicting results. The possibility that ISP has biological activity similar to estrogen with the concordant benefits and risks has been studied with respect to vasomotor symptoms [5,6], bone [7–11], heart disease [12,13], atherosclerosis [14–16], and breast cancer [17]. The effect of ISP supplementation on the endometrium remains unclear. A pilot study of phytoestrogen supplementation over a period of six months among 27 participants demonstrated no effect on endometrial histology [18], whereas a randomized controlled trial among 376 women suggested that 5-year phytoestrogen supplementation is associated with increased rates of endometrial hyperplasia [19].

The objective of the current study was to determine whether long-term ISP supplementation affects endometrial thickness and rates of endometrial hyperplasia and cancer in postmenopausal women in a randomized controlled setting.

Materials and Methods

The Women's Isoflavone Soy Health (WISH) trial was a randomized, double-blind, placebo-controlled trial conducted from April 2004 to March 2009. 350 postmenopausal women 45–92 years of age were randomly assigned in a 1:1 ratio to daily 25 g soy protein or daily total milk protein-matched placebo, for a 3-year period.

The primary trial outcome was progression of subclinical atherosclerosis, assessed by the rate of change in the right distal common carotid artery intima media thickness (CIMT). Primary trial endpoint data are published elsewhere [20]. Secondary endpoints were safety of ISP supplementation, including endometrial safety. Participants with a surgically absent uterus (n=75) and participants with incomplete ultrasound surveillance of the endometrium during the trial (n=51) were excluded from this analysis, resulting in a final study population of 224 subjects.

The 25 g soy protein contained 91 mg aglycon equivalents of naturally occurring isoflavones and its glycosides (154 mg total isoflavone conjugates plus aglycons): genistein 52 mg aglycon equivalents (88 mg total), daidzein 36 mg aglycon equivalents (61 mg total), and

glycitein 3 mg aglycon equivalents (5 mg total). The milk protein-matched placebo contained 0 isoflavones.

Randomization occurred within 2 strata of carotid artery intima-media thickness (CIMT; <0.75 mm, ≥0.75 mm). Within each stratum, blocked randomization was implemented with a masked block size. Randomization lists from a computerized random number generator (SAS statistical software) were prepared prior to trial initiation by the trial statistician. For each stratum, the randomization list included the product identification number and treatment code (active or placebo). Blinded study product was prepared based on the randomization list. Upon determination of trial eligibility for a given participant, clinic staff pulled the next study product in sequence from the appropriate stratum and recorded the product identification number. The statistician monitored the fidelity of the randomization process. Participants, investigators, staff, imaging specialists, and data monitors were masked to treatment assignment [20]. The placebo and active treatments were taken in 2 evenly divided doses daily delivered in either beverage powder-food packs or food bars to provide variety and to maintain compliance. ISP and placebo products were prepared without charge by the Solae Company (St Louis, MO) and were identical in taste and appearance.

Trial inclusion criteria were the absence of vaginal bleeding for at least one year and a serum estradiol level of <20 pg/ml. Exclusion criteria were clinical signs, symptoms or personal history of cardiovascular disease, diabetes mellitus or fasting serum glucose >126 mg/dl, fasting triglycerides >500 mg/dL, systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg, untreated thyroid disease, serum creatinine >2 mg/dL, life-threatening illness with prognosis <5 years, alcohol intake >5 drinks per day or substance abuse, using postmenopausal hormone therapy (HT) and soy, nut or related food allergies.

The endometrial echocomplex (EEC) was measured by transvaginal ultrasound at baseline and at 18 and 30 months of follow-up, as well as at 36 months in some participants. All EEC measurements were done by the same physician (DS). Participants with an EEC >5 mm and those with vaginal bleeding underwent endometrial biopsies. Endometrial biopsies were performed using suction pipelles. Endometrial biopsies were fixed in 10% neutral buffered formalin, routinely processed, and paraffin embedded. Serial 5 micron sections were stained with hematoxylin and eosin and examined under the light microscope by a gynecological pathologist (JCF). Compliance with ISP supplementation was confirmed by plasma and urine isoflavone measurements. The University of Southern California Institutional Review Board approved the study protocol; all participants provided written informed consent.

Statistical analysis

All analyses were by intent to treat, by which participants were analyzed according to their randomized intervention. Treatment group comparisons on demographic and other baseline characteristics used chi-square tests for categorical and t-tests for continuous variables. For each participant, the change in the EEC from baseline was computed at each EEC follow-up visit. Baseline EEC and baseline and on-trial blood levels of genistein, daidzein and glycitein were compared between treatment groups using a Wilcoxon rank sum tests. Because of multiple EEC measurements within participants over the trial, treatment groups were compared on mean EEC change from baseline using generalized estimating equations (GEE), specifying an identity link and an exchangeable correlation structure among repeated measurements within each participant. A covariate specifying the randomization stratification factor of baseline carotid artery intima-media thickness was included in the GEE model. Treatment group comparisons on incidence of endometrial sampling, endometrial hyperplasia and endometrial cancer used the Fisher's exact test. All statistical

analyses used SAS 9.2 software (SAS, Inc., Cary, North Carolina); statistical testing was conducted at a two-sided 0.05 significance level.

Results

A total of 666 visits among 224 participants were evaluated. The baseline characteristics between the women randomized to placebo (n=103) and ISP (n=121) were similar; treatment groups did not differ with respect to age, BMI, smoking status, past HT use, years since menopause, ethnic background, and education level [Table 1].

Baseline levels of plasma genistein, daidzein, and glycitein concentrations did not differ between treatment groups [all p>0.05, Table 2]. During the study, mean plasma concentrations of genistein, daidzein and glycitein were statistically significantly higher in the ISP group than in the placebo group [p<0.001, Table 2], suggesting compliance with the ISP supplements in the intervention group. Compliance was also assessed by package and bar count and found to be >85% among both the placebo-treated and ISP-treated participants [20] (data not shown).

The median baseline EEC in the placebo and ISP groups was 2.5 mm and 2.4 mm, respectively. The EEC decreased by a mean of 0.95 and 0.98 mm, respectively [Table 3]. Neither the baseline EEC measurements nor the changes in EEC over the trial period were statistically significantly different between treatment groups (p>0.8). Of the 103 placebo-treated participants, 7 (6.8%) underwent an endometrial biopsy; 6 (85.7%) of these biopsies were benign. The indication for endometrial sampling was postmenopausal vaginal bleeding in 3 of the 7 cases and asymptomatic endometrial thickening (EEC >5 mm) in the other 4 cases. Of the 121 participants in the soy group, 9 (7.4%) underwent an endometrial biopsy. The indication for endometrial sampling was postmenopausal vaginal bleeding in 3 of the 9 cases and asymptomatic endometrial thickening in the other 6 cases. The results were benign in all 9 cases (100%) [Table 4]. There were no statistically significant differences in the incidence of endometrial sampling between groups (p=0.37) and the rates of the pathology diagnoses “benign, not otherwise specified”, “atrophic”, and “proliferative”. One woman in the placebo group was diagnosed with complex endometrial hyperplasia with atypia and underwent a hysterectomy; the pathology result from this surgery indicated Stage IB endometrial cancer. Although the rate of hyperplasia / malignancy was higher in the placebo group (n=1/7, 14.3%) compared to the ISP group (n=0, 0%), the difference was not statistically significant (p=0.48).

Discussion

More and more postmenopausal women in the US concerned about perceived risks of conventional HT, ingest ISP through food sources and supplements as an alternative therapy for menopausal symptoms[4]. While research regarding the efficacy of these compounds with SERM-like activity on a variety of menopausal symptoms is conflicting [5–17], the clinical reality is that an increasing number of postmenopausal women are using ISP, necessitating careful documentation into its safety.

Previous studies on the effect of ISP on the endometrium have yielded conflicting results. A pilot study by Balk et al. [18] in 2002 demonstrated no effect of phytoestrogen supplementation on endometrial histology, but was limited by a small participant number (27 subjects randomized, 19 completed the study) and a treatment period of only 6 months. A larger study (376 women randomized, 298 completed the study) over 5 years by Unfer et al. [19] from 2004 found increased rates of endometrial hyperplasia. In their study, no cases of hyperplasia were observed in the placebo group, whereas 6/154 women (3.8%) in the ISP

group developed hyperplasia at the end of the 5-year follow-up period. However the vast majority of these cases were cases of simple hyperplasia (5/6 = 83.3%), with only one case of complex hyperplasia without atypia in the entire study population. In addition, the authors did not report on symptoms of postmenopausal bleeding or ultrasound monitoring of the EEC, and instead performed universal biopsies on all subjects, which makes the study less clinically applicable. Our study is the first to assess the effect of long-term ISP supplementation on endometrial thickness and rates of endometrial hyperplasia and cancer in a population of postmenopausal women that were closely monitored by ultrasound and biopsied according to currently used clinical criteria. All EEC measurements were done by the same physician (DS), who has extensive gynecologic ultrasound experience. It has previously been shown that there is an excellent correlation between intraobserver and interobserver measurements of endometrial stripe thickness [21].

We observed that three-year ISP supplementation had no effect on endometrial thickness or on rates of endometrial hyperplasia and cancer in postmenopausal women. In fact, the only case of endometrial hyperplasia / cancer occurred in the placebo group. On a molecular level, with its structural similarity to 17β-estradiol and SERM-like activity, ISP has been found to predominantly act on the beta-type estrogen receptor (ER- β) [22]. The main estrogen receptor in the endometrium is the alpha-type estrogen receptor (ER-α), as well as the main mediator of endometrial hyperplasia and cancer risk [23]. This may explain our finding of the endometrial safety of 3-year ISP supplementation.

The exclusion criteria for the study included certain comorbid conditions such as hypertension, diabetes, and cardiovascular disease. The mean BMI was in the overweight, but not obese range, and the study population included few smokers. It is unclear what the effects of ISP supplementation are on women with multiple risk factors for carcinoma of the endometrium. In average risk populations of menopausal women taking no estrogens or very low dose unopposed estrogen therapy, studies have shown minimal endometrial proliferation with low incidences of endometrial hyperplasia [24–27]. It is therefore conceivable that our study was underpowered to detect a small difference in the rates of endometrial hyperplasia. This should be taken into account when interpreting our results, and counseling of postmenopausal women should be individualized. Our findings need to be confirmed by larger scale clinical trials and more basic research on the effect of ISP on the endometrium.

Conclusion

Three-year ISP supplementation has no effect on endometrial thickness or rates of endometrial hyperplasia and cancer in postmenopausal women.

Menopausal women who wish to take ISP supplementation for up to three years do not need to be discouraged to do so for endometrial indications.

Acknowledgments

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References

1. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL. Unconventional medicine in the United States. Prevalence, costs, and patterns of use. N Engl J Med. 1993; 328:246–252. [PubMed: 8418405]

2. Kang HJ, Ansbacher R, Hammoud MM. Use of alternative and complementary medicine in menopause. Int J Gynaecol Obstet. 2002; 79:195–207. [PubMed: 12445983]
3. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology. 1998; 139:4252–4263. [PubMed: 9751507]
4. Newton KM, Buist DS, Keenan NL, Anderson LA, LaCroix AZ. Use of alternative therapies for menopause symptoms: results of a population-based survey. Obstet Gynecol. 2002; 100:18–25. [PubMed: 12100799]
5. Quella SK, Loprinzi CL, Barton DL, et al. Evaluation of soy phytoestrogens for the treatment of hot flashes in breast cancer survivors: A North Central Cancer Treatment Group Trial. J Clin Oncol. 2000; 18:1068–1074. [PubMed: 10694559]
6. Upmalis DH, Lobo R, Bradley L, Warren M, Cone FL, Lamia CA. Vasomotor symptom relief by soy isoflavone extract tablets in postmenopausal women: a multicenter, double-blind, randomized, placebo-controlled study. Menopause. 2000; 7:236–242. [PubMed: 10914616]
7. Alexandersen P, Toussaint A, Christiansen C, et al. Ipriflavone in the treatment of postmenopausal osteoporosis: a randomized controlled trial. JAMA. 2001; 285:1482–1488. [PubMed: 11255425]
8. Cecchini MG, Fleisch H, Muhibauer RC. Ipriflavone inhibits bone resorption in intact and ovariectomized rats. Calcif Tissue Int. 1997; 61 (Suppl 1):S9–11. [PubMed: 9263609]
9. Kreijkamp-Kaspers S, Kok L, Grobbee DE, 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; 292:65–74. [PubMed: 15238592]
10. Mei J, Yeung SS, Kung AW. High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. J Clin Endocrinol Metab. 2001; 86:5217–5221. [PubMed: 11701680]
11. Nikander E, Metsa-Heikkila M, Ylikorkala O, Tiitinen A. Effects of phytoestrogens on bone turnover in postmenopausal women with a history of breast cancer. J Clin Endocrinol Metab. 2004; 89:1207–1212. [PubMed: 15001611]
12. Lissin LW, Cooke JP. Phytoestrogens and cardiovascular health. J Am Coll Cardiol. 2000; 35:1403–1410. [PubMed: 10807439]
13. Zhang X, Shu XO, Gao YT, et al. Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. J Nutr. 2003; 133:2874–2878. [PubMed: 12949380]
14. Clarkson TB, Anthony MS, Morgan TM. Inhibition of postmenopausal atherosclerosis progression: a comparison of the effects of conjugated equine estrogens and soy phytoestrogens. J Clin Endocrinol Metab. 2001; 86:41–47. [PubMed: 11231976]
15. Hwang J, Sevanian A, Hodis HN, Ursini F. Synergistic inhibition of LDL oxidation by phytoestrogens and ascorbic acid. Free Radic Biol Med. 2000; 29:79–89. [PubMed: 10962208]
16. Hwang J, Hodis HN, Sevanian A. Soy and alfalfa phytoestrogen extracts become potent low-density lipoprotein antioxidants in the presence of acerola cherry extract. J Agric Food Chem. 2001; 49:308–314. [PubMed: 11170593]
17. Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian-American women. J Natl Cancer Inst. 1993; 85:1819–1827. [PubMed: 8230262]
18. Balk JL, Whiteside DA, Naus G, DeFerrari E, Roberts JM. A pilot study of the effects of phytoestrogen supplementation on postmenopausal endometrium. J Soc Gynecol Investig. 2002; 9:238–242.
19. Unfer V, Casini ML, Costabile L, Mignosa M, Gerli S, Di Renzo GC. Endometrial effects of long-term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study. Fertil Steril. 2004; 82:145–148. quiz 265. [PubMed: 15237003]
20. Hodis HN, Mack WJ, Kono N, et al. Isoflavone soy protein supplementation and atherosclerosis progression in healthy postmenopausal women: a randomized controlled trial. Stroke. 2011; 42:3168–3175. [PubMed: 21903957]
21. Spandorfer SD, Arrendondo-Soberon F, Loret de Mola JR, Feinberg RF. Reliability of intraobserver and interobserver sonographic endometrial stripe thickness measurements. Fertil Steril. 1998; 70:152–154. [PubMed: 9660438]

22. Mueller SO, Kling M, Arifin Firzani P, et al. Activation of estrogen receptor alpha and ERbeta by 4-methylbenzylidene-camphor in human and rat cells: comparison with phyto-and xenoestrogens. Toxicol Lett. 2003; 142:89–101. [PubMed: 12765243]

23. Wedren S, Lovmar L, Humphreys K, et al. Estrogenreceptor alpha gene polymorphism and endometrial cancer risk--a case-control study. BMC Cancer. 2008; 8:322. [PubMed: 18990228]

24. Notelovitz M. Estrogen therapy and osteoporosis: principles & practice. Am J Med Sci. 1997; 313:2–12. [PubMed: 9001160]

25. Genant HK, Lucas J, Weiss S, et al. Low-dose esterified estrogen therapy: effects on bone, plasma estradiol concentrations, endometrium, and lipid levels. Estratab/Osteoporosis Study Group. Arch Intern Med. 1997; 157:2609–2615. [PubMed: 9531230]

26. Lethaby A, Suckling J, Barlow D, Farquhar CM, Jepson RG, Roberts H. Hormone replacement therapy in postmenopausal women: endometrial hyperplasia and irregular bleeding. Cochrane Database Syst Rev. 2004:CD000402. [PubMed: 15266429]

27. Johnson SR, Ettinger B, Macer JL, Ensrud KE, Quan J, Grady D. Uterine and vaginal effects of unopposed ultralow-dose transdermal estradiol. Obstet Gynecol. 2005; 105:779–787. [PubMed: 15802405]

TABLE 1

Baseline demographics by treatment group (n=224)

Variable	Placebo n = 103	ISP n = 121	P-value
Age, yrs [a]	60.1 ± 6.6	60.9 ± 7.0	0.38
BMI [a]	26.4 ± 5.4	26.3 ± 5.1	0.83
Smoking history			
Current	3 (3%)	1 (<1%)	0.50
Former	41 (40%)	50 (41%)	
Never	59 (57%)	70 (58%)	
Past HT use			
Yes	64 (62%)	83 (69%)	0.31
No	39 (38%)	38 (31%)	
Years since menopause	9.8 ± 6.8	11.0 ± 8.0	0.26
Race			
White (non-hispanic)	69 (67%)	72 (59%)	0.71
Black (non-hispanic)	4 (4%)	8 (7%)	
Hispanic	14 (13%)	20 (17%)	
Asian or Pacific Islander	13 (13%)	15 (12%)	
Other	3 (3%)	6 (5%)	
Weight, lbs [a]	151 ± 34	150 ± 30	0.77
Education			
≤ High school	4 (4%)	9 (7%)	0.26
> High school	99 (96%)	112 (93%)	

Values expressed as number of subjects (%), with chi-square p-value

[a] Age, BMI and weight expressed as mean (±SD), with t-test p-value

TABLE 2

Treatment group comparison of baseline and on-trial plasma isoflavone levels (nmol/l)

	Placebo N = 103	ISP N = 121	P-value*
Daidzein (nmol/l)			
Baseline	11.9 (54.7)	17.6 (52.0)	0.59
On-trial average	41.5 (89.4)	290.5 (330.0)	<.0001
Genistein (nmol/l)			
Baseline	8.4 (35.6)	14.9 (48.5)	0.25
On-trial average	27.1 (87.8)	354.0 (372.0)	<.0001
Glycitein (nmol/l)			
Baseline	2.6 (6.9)	2.0 (6.9)	0.82
On-trial average	3.5 (7.6)	10.4 (18.9)	<.0001

Values are Median (IQR), in nmol/l

*Wilcoxon two-sample test

TABLE 3

Baseline Endometrial Echocomplex (mm) and Mean Change from Baseline Across Trial Visits by Treatment Group

	Placebo n = 103	ISP n = 121	P-value
All participants, baseline [a]	2.50 (1.10)	2.40 (1.00)	0.82
All participants, change [b]	−0.95 (0.26)	−0.98 (0.20)	0.88
Excluding participant with endometrial cancer, change [b]	−1.09 (0.22)	−1.05 (0.19)	0.84

[a] Baseline numbers are median (IQR). Treatment groups compared using Wilcoxon two-sample test.

[b] Mean (SE) change from baseline, adjusted for randomization stratum. Treatment groups compared using generalized estimating equations with identity link function and exchangeable correlation structure

TABLE 4

Endometrial pathology by group

	Placebo	ISP
Endometrial biopsy performed	7/103 (6.8%)	9/121 (7.4%)
Benign endometrial pathology, any type	6/7 (85.7%)	9/9 (100%)
Benign, not otherwise specified	4/7 (57.1%)	5/9 (55.6%)
Atrophic	2/7 (28.6%)	3/9 (33.3%)
Proliferative	0/7 (0%)	1/9 (11.1%)
Endometrial hyperplasia / endometrial cancer	1/7 (14.3%) [a]	0/9 (0%)

p> 0.05 (NS) by Fisher's exact test for all comparisons

[a] Participant with postmenopausal bleeding and EEC=12 mm, endometrial biopsy: atypical endometrial hyperplasia; final pathology from hysterectomy: Stage IB endometrial cancer