Isoflavone treatment for acute menopausal symptoms

Guojun Cheng, MD, PhD,1 Brigitte Wilczek, MD, PhD,2 Margaret Warner, PhD,1 Jan-Åke Gustafsson, MD, PhD,1 and Britth-Marie Landgren, MD, PhD2

Abstract

Objective: The onset of climacteric symptoms (hot flashes and night sweats) is the primary reason for perimenopausal women to start hormone therapy. The association of a lower incidence of postmenopausal symptoms with high intake of soybeans in Asian women suggests that phytoestrogens are an alternative to estrogen therapy. The main effective compounds in soybeans are isoflavones, which have a higher binding affinity to estrogen receptor β than to estrogen receptor α. The aim of present study was to evaluate the effects of isoflavone treatment in postmenopausal women.

Design: This was a double-blind prospective study. Sixty healthy postmenopausal women were randomly assigned by computer into two groups to receive 60 mg isoflavones or placebo daily for 3 months. Before and after treatment, climacteric symptoms were recorded; serum was collected to measure the levels of lipoprotein lipids, estradiol, and follicle-stimulating hormone; and biopsy specimens from endometrium and breast were analyzed to investigate the expression level of steroid receptors and proliferation. Endometrial thickness was measured by ultrasound.

Results: Fifty-one women finished the 12-week study. In women receiving 60 mg isoflavones daily, hot flashes and night sweats were reduced by 57% and 43%, respectively. The treatment did not change the levels of circulating estradiol or follicle-stimulating hormone. Immunohistochemical staining of endometrial and breast biopsy specimens revealed that isoflavones did not affect expression levels of steroid receptors; estrogen receptors α, β, and βC; progesterone receptors A and B; or the proliferation marker Ki67. No side effects on body weight or lipoprotein lipids were observed.

Conclusions: This short-term prospective study implies that isoflavones could be used to relieve acute menopausal symptoms.

Key Words: Estrogen receptor – Phytoestrogen – Selective estrogen receptor modulator – Hormonal therapy – Menopause.

Menopausal symptoms include hot flashes, night sweats, and vaginal dryness, which result from the decline in the circulating estradiol (E2) level after menopause. The occurrence of hot flashes is the primary reason that women seek medical treatment for menopausal symptoms.1 Estrogen therapy is highly effective for relieving menopausal symptoms as well as for preventing osteoporosis and colon and gastric cancer, thus improving quality of life and increasing life span.2,3 Reports in the 1970s that the incidence of endometrial cancer increased in women taking estrogen alone led to the development of standard hormone therapy (HT) with estrogen plus progestin.2,4 However, recent studies indicated that the progestin component in HT increases the risk of breast cancer and stroke and counteracts the beneficial effects of estrogen on the heart and lipoprotein lipids.2,5 Although HT is still widely used, there is increasing interest in effective and safe alternatives to HT for treatment of menopausal symptoms.5,6,7 In Asia, only 10% to 20% of women experience hot flashes, compared with 70% to 80% of women in Western countries.2 A popular hypothesis to explain this difference is that isoflavones found in soy, a staple of the traditional Asian diet, influence the body’s response to the changing hormonal levels of menopause.6,7 In addition, high intake of dietary phytoestrogens has been suggested to account for the

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lower rates of osteoporosis, breast cancer, endometrial cancer, and cardiovascular disease in Asian women. \textsuperscript{3,8-10} The lower incidence of cardiovascular disease has been related to the beneficial effects of estrogen on circulating lipids and lipoprotein levels. \textsuperscript{11,12}

Plant-derived estrogens, termed phytoestrogens, are currently used by many women as alternatives to HT. However, the purity, potency, and effectiveness of these botanical preparations have not been well established.\textsuperscript{2,3} The three main classes of phytoestrogens are isoflavones, lignans, and coumestans. Of these, isoflavones, which are found primarily in soybeans and other legumes, are the most widely used.\textsuperscript{1,2} Isoflavones, including genistein, daidzein, and glycitein, have been shown to have estrogenic effects in the laboratory. They bind weakly to estrogen receptor (ER) α but more strongly to ER-β and compete with E\textsubscript{2} for binding to both ERs. Women who have contraindications for HT or who want a “natural” alternative might possibly use phytoestrogens for treatment of climacteric complaints.\textsuperscript{1,2} In the present double-blind, prospective study, we studied the efficiency of isoflavones in relieving climacteric symptoms and their potential effects on lipoprotein lipids, endometrium, and breast.

**METHODS**

**Clinical material**

Sixty healthy postmenopausal women, with at least 1 year since their last menstruation, follicle-stimulating hormone (FSH) levels greater than 30 IU/mL, and at least 6 months without taking HT volunteered for the study. They were between 49 and 69 years of age. The women received written and verbal information on the purpose and procedures of the study, and informed consent was obtained. All women had hot flashes and night sweats. The women were randomly assigned by computer into two groups, one receiving 60 mg isoflavones daily in a fruit drink with orange or peach flavor and the other receiving placebo (oatmeal drink with orange or peach flavor). The drinks were used as food complements, and the duration of treatment was 12 weeks. The treatment was blinded both to the women and to the doctor.

Before the start of treatment the women went through a general medical examination, including measurement of blood pressure; analyses of circulating lipoproteins; tests of kidney, liver, and thyroid function; and determination of hematological status. A gynecological examination including a Pap smear and vaginal ultrasound with measurements of endometrial thickness and uterine size was also performed. If possible, an endometrial biopsy specimen was obtained using a thin plastic catheter without dilatation of the cervix. Mammography and a middle-needle biopsy of breast tissue were performed, using ultrasound to identify glandular tissue. Women registered the number of hot flashes and night sweats per day as well as the intensity of these vasomotor symptoms. The intensity of climacteric symptoms was scored by the subjects on a five-point self-rating scale adapted from the climacteric symptoms rating scale developed by Collins and Landgren.\textsuperscript{13} Compliance was checked by measurements of urinary isoflavones and by the women returning empty packages of fruit drink once monthly in exchange for new ones. During the study the women were encouraged to lead normal lives with no changes in dietary habits, alcohol consumption, or physical activity.

After 3 months of treatment with isoflavones or placebo, a general examination was performed and included blood pressure, lipoprotein levels, kidney and liver function, hematological status, and thyroid function. Mammography with a needle biopsy of breast tissue, using ultrasound to identify gland tissue, was performed. A gynecological examination was also performed, including ultrasound with measurements of endometrial thickness, and when possible, an endometrial biopsy specimen was obtained. The weekly records of vasomotor symptoms were collected, and questions concerning well-being, possible side effects, and diet were asked.

**Treatment**

The treatment was daily nutritional addition of a fruit drink containing isoflavones (produced by Carlashams Mejeri from soya beans imported from the United States) or placebo. The phytoestrogen contents in the drinks were measured by the chemical laboratory at the University of Lund and at Novum Research Center, Huddinge University Hospital, using the methods described by Adlercreutz et al.\textsuperscript{14}

**Ethical aspects**

The study was approved by the Ethical Committee of Karolinska Institutet (Decision number, 78/03).

**Vaginal ultrasound**

Endometrial thickness were measured using a Siemens Sonoline SI 200 real-time scanner, equipped with a mechanical sector rotating 5.7-7.5 MHz transducer.

**Lipoprotein levels**

Blood samples for lipoprotein levels were collected after a one-night fast. Analysis was performed at the Biochemical Central Laboratory of Karolinska Institutet, Huddinge University Hospital, using standard methods.

**Hormones**

Serum levels of FSH were measured by radioimmunoassay using reagents from the Farmos Group (Oulu, Finland) at the Central Laboratory for Clinical Chemistry, Karolinska Institutet, Huddinge University Hospital. The intraassay and interassay coefficients of variation were 2.7% and 6.3%, respectively. E\textsubscript{2} levels were determined by radioimmunoassay using reagents from Diagnostic Products Corporation (Los Angeles, CA). The intraassay and interassay coefficients of variation were 7.2% and 6.8%, respectively.

**Immunohistochemistry**

Breast and endometrium biopsy samples were fixed immediately in 4% paraformaldehyde at 4°C for 16 hours.
Fixed tissues were then gradually dehydrated in ethanol and embedded in paraffin. Paraffin sections (5 μm) were dewaxed in xylene and rehydrated through graduated ethanol to water. Antigen retrieval was performed by microwaving sections in 0.01 M citrate buffer, pH 6.0, for 20 minutes at 800 W. Endogenous peroxidase was blocked by incubation for 30 minutes in a solution of 1% hydrogen peroxide. The sections were incubated for 1 hour at 4°C with normal goat serum diluted 1:10 in phosphate-buffered saline. Antibodies were diluted individually in phosphate-buffered saline (PBS) containing 3% bovine serum albumin. Monoclonal antibodies against ER-α (1D5) and Ki67 (Mib-1 and M7240) were from Dako (Glostrup, Denmark). Monoclonal antibodies against progesterone receptor (PR) (PGR-312) and PR-B (clone san27) were obtained from Novocastra Laboratories LTD, Newcastle, UK. Chicken anti-human ER-β polyclonal antibody (IgY) was made in our laboratory. Dilution of ER-β, PR-312, PR-B, and Ki67 antibodies was 1:100; dilution of ER-α antibody was 1:40. Sections were incubated with antibodies overnight at 4°C. For negative controls, the primary antibody was replaced with PBS alone or with primary antibody after absorption with the corresponding antigen. Before addition of the secondary antibody, sections were rinsed in PBS. Sections were incubated in biotinylated goat anti-rabbit or goat anti-mouse immunoglobulin (1:200 dilution, Vector Laboratories Inc, Burlingame, CA) for 2 hours at room temperature, followed by washing with PBS and incubation in avidin-biotin-horseradish peroxidase for 1 hour. The ABC method was used to visualize signals according to the manual provided by the manufacturer (Vector Laboratories). After thorough washing in PBS, sections were developed with 3,3-diaminobenzidine tetrahydrochloride (Dako), slightly counterstained with Mayer’s hematoxylin, and dehydrated through an ethanol series, followed by exposure to xylene and mounting.

The staining was evaluated independently by two authors (G.C. and M.W.). The percentage of positively stained cells

### TABLE 1. Basic information

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age, y</th>
<th>YSM, y</th>
<th>Weight, kg</th>
<th>BMI</th>
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<tbody>
<tr>
<td>Placebo</td>
<td>25</td>
<td>56.9 ± 4.2</td>
<td>7.0 ± 3.8</td>
<td>66.9 ± 10.4</td>
<td>25.0 ± 3.5</td>
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<tr>
<td>Isoflavones</td>
<td>26</td>
<td>58.4 ± 5.0</td>
<td>8.4 ± 5.3</td>
<td>68.5 ± 9.0</td>
<td>24.8 ± 2.7</td>
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</tbody>
</table>

Data are mean ± SD. YSM, years since menopause; BMI, body mass index = weight (kg)/height (m)².

### TABLE 3. Levels and changes of lipoprotein lipids

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Cholesterol, mmol/L</th>
<th>Triglycerides, mmol/L</th>
<th>HDL-C, mmol/L</th>
<th>LDL-C, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>21</td>
<td>5.8 ± 0.8</td>
<td>1.5 ± 0.9</td>
<td>1.8 ± 0.4</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>After</td>
<td>21</td>
<td>6.2 ± 0.8</td>
<td>1.5 ± 0.7</td>
<td>1.8 ± 0.5</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>26</td>
<td>5.7 ± 0.8</td>
<td>1.6 ± 0.8</td>
<td>1.9 ± 0.4</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>Before</td>
<td>26</td>
<td>5.8 ± 0.9</td>
<td>1.4 ± 0.6</td>
<td>1.9 ± 0.4</td>
<td>3.3 ± 0.8</td>
</tr>
</tbody>
</table>

Data are mean ± SD. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

is an average calculated after counting the stained and the total number of cells from four high-magnification fields.

### Statistical analysis

Statistical differences between groups were analyzed with Student's t test and a paired-sample t test using SPSS software (SPSS Inc, Chicago, IL). A P value less than 0.05 was considered significant.

### RESULTS

Of the 60 women, 2 dropped out during the first month, 7 women continued the treatment but missed some examinations, and 51 women completed the full 12 weeks of study. The women in both groups were of similar age and years since menopause (Table 1). Body weight was recorded for each woman before and after treatment. No significant changes in weight or body mass index were found in either group after treatment (P > 0.05). There were no changes in blood pressure, lipoproteins, kidney and liver function, hematology status, or thyroid function in either group.

### Climacteric symptoms

Climacteric symptoms were analyzed in 51 women. The scores for hot flashes and night sweats before treatment were similar in the two groups (P > 0.05). After 12 weeks of treatment, women taking isoflavones showed a significantly lower score for hot flashes (57%) than those in the placebo group (P < 0.01), but the difference in scores for sweating (43%) was not significant (P > 0.05). No difference in the scores for hot flashes or sweating was found in women taking placebo (P > 0.05) (Table 2).

### Lipoprotein lipids

Blood samples from 47 women were analyzed for cholesterol, triglycerides, high-density lipoprotein cholesterol and low density lipoprotein cholesterol. There were no significant differences between the baseline levels in the two

### TABLE 4. Changes in endometrial thickness

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Endometrial thickness (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>24</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>26</td>
<td>2.3 ± 1.1</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

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FIG. 1. Endometrial biopsy. The expression levels of steroid receptors (estrogen receptor [ER] α, ERβ, ERβcx, progesterone receptor [PR]-A, and PR-B) after isoflavone treatment for 12 weeks showed no significant differences compared with the biopsy sample before treatment. The proliferation marker, Ki67, was seen in 0% to 3% of samples, and no significant change was induced by isoflavone treatment.

FIG. 2. Breast biopsy. The expression levels of steroid receptors (estrogen receptor [ER] α, ERβ, ERβcx, progesterone receptor [PR]-A, and PR-B) after isoflavone treatment for 12 weeks showed no significant differences compared with the biopsy before treatment. The proliferation marker, Ki67, was seen in 0% to 0.5% of samples, and no significant change was induced by isoflavone treatment.

Endometrial thickness was measured in 51 women by ultrasound before and after the 12-week treatment. There was no significant difference at baseline and after treatment between the two groups ($P > 0.05$) (Table 4).

Endometrial samples were obtained from 38 women and were stained with antibodies against ER-α, ER-β, ER-βcx, PR-A, and PR-B. High levels of ER-α expression were found in 67% of samples. Surprisingly, 29% expressed ER-β and 21% expressed ER-βcx. PR-A and PR-B were expressed in 49% and 39%, respectively, of the endometrial samples (Fig. 1). There were no significant differences in the levels of the five steroid receptors in the samples from the two groups of women before or after treatment ($P > 0.05$).
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<table>
<thead>
<tr>
<th>TABLE 5. Changes in E2 and FSH levels</th>
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<tbody>
<tr>
<td>E2, pmol/L</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Placebo</td>
</tr>
<tr>
<td>Isoflavones</td>
</tr>
</tbody>
</table>

Data are mean ± SD. E2, estradiol; FSH, follicle-stimulating hormone.

proliferation marker, Ki67, was expressed in 0% to 3% of cells. No changes in Ki67 expression were found in either group after treatment (P > 0.05).

Breast

Mammography was performed before and after treatment in all participants. No sign of hyperproliferation was recorded. Breast biopsies were performed under the guidance of ultrasound, and 102 samples were obtained. Epithelial cells in lobules or ducts were found in 53 samples, but in 49 samples there were no epithelial cells. Expression of steroid receptors ER-α, ER-β, ER-βx, PR-A, and PR-B was evaluated by immunohistochemistry (Fig. 2). ER-α was expressed in 45% of the samples, and the percentage of the positive cells ranged from 0% to 15%. ER-β was expressed in 79% of the samples, but only 13% of the samples were positively stained with the ER-βx antibody. The levels of PR were quite low, with 20% of PR-A and 14% of PR-B. No significant changes in these steroid receptors were found in either group after treatment (P > 0.05).

Proliferation was evaluated by Ki67 expression. In most Ki67-positive samples, the percentage of Ki67-positive cells was very low, whereas in a few samples up to 0.5% of the cells expressed Ki67. No changes in Ki67-positive expression were found in either group after treatment (P > 0.05).

E2 and FSH level

Levels of E2 and FSH were measured in 51 women. The levels of E2 ranged from 5 to 75 pmol/L. No significant changes of E2 levels were observed in either group after treatment (P > 0.05). The FSH levels were from 41 to 138 IU/L, and no changes occurred in either group (P > 0.05) (Table 5).

DISCUSSION

Phytoestrogens are available in many forms, including manufactured supplements and food with natural phytoestrogen content. Different products and foods contain various amounts of isoflavones. There is no consensus about the best way to deliver phytoestrogens for effects on menopausal symptoms. Accordingly, types and doses of phytoestrogens vary widely in clinical trials.1,3,6,7,9 In our short-term, double-blind, prospective study, we demonstrated that the administration of 60 mg isoflavones daily can relieve hot flashes by 57% and night sweats by 43%. The score for hot flashes in the isoflavone group after treatment was significantly lower than scores before treatment for the same participants and for those in the placebo group (P < 0.01).

The score for night sweats in the isoflavone group after treatment was significantly lower than before treatment in the same participants by paired t test (P < 0.05), but there was no statistical difference compared with the score for the placebo group by independent t test (P > 0.05), which implies that a larger sample size might be needed to assess the effects on sweating.

Cardiovascular disease is one of the leading causes of death worldwide. Postmenopausal women have a higher risk of cardiovascular disease because natural or surgical menopause is associated with elevated levels of circulating total cholesterol, low-density lipoprotein cholesterol, and triglycerides but lower levels of high-density lipoprotein cholesterol, which could be reversed by estrogen therapy. Similar effects of phytoestrogens on lipids were observed in some studies, but no effects were observed in others.9 In the present study, we did not observe any effects of isoflavones on total cholesterol, triglycerides, low-density lipoprotein cholesterol, or high-density lipoprotein cholesterol. Isoflavones, like estrogen, are also believed to reduce the risk of heart disease by reducing the susceptibility of low-density lipoprotein to oxidation by an antioxidant action or by improving arterial compliance.9

The risk of breast cancer is an important issue in HT. Many of the major risk factors for breast cancer relate to estrogen exposure. Numerous prospective studies indicate that postmenopausal women with high serum estrogen levels or those taking estrogen-progestin therapy have an increased risk of breast cancer.5,13 To evaluate the effects of isoflavones on breast, we performed middle-needle biopsy of breast tissue by using ultrasound to identify glandular tissue. Of the 102 samples, 53% had lobular or ductal structures. This high recovery of epithelium shows that middle-needle biopsy is superior to fine-needle biopsy, which is widely used in the diagnosis of benign and malignant breast diseases and which, in many cases, does not provide enough epithelium for a reliable diagnosis.14 The proliferation rates in these samples were very low. In many samples no Ki67-positive cells were found. In those that were positive, the percentage of Ki67-positive cells was less than 0.5%. No effect of isoflavones on Ki67 expression was detected in this study. However, in a study using primates, high doses of isoflavones inhibited mammary gland proliferation.15 Surprisingly, the expression of ER-α in the breasts of postmenopausal women was not elevated. ER-α was detected in 45% of the samples, and the percentage of the positive cells ranged from 0% to 15%. Most of what is known about ER expression in the normal postmenopausal breast comes from observations in the normal parts of breast cancer samples, and these have consistently shown elevated levels of ER-α. The explanation given for this phenomenon has been that in the presence of low levels of E2, ER-α levels are increased. Our data show that this is not the case. ER-β expression was also high in the postmenopausal breast, with 79% of the epithelium expressing ER-β. It

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may be of significance that no ER-β was detected in 21% of the samples. If ER-β plays an antiproliferative role in the breast, the loss of ER-β may represent a risk factor for development of proliferative disease. ER-βcx was not frequently observed in the normal postmenopausal breast and was detectable in only 13% of the samples. The significance of ER-βcx in the breast is not clear, but follow-up of these women might provide information about whether it is a normal occurrence or whether it is an early indication of disease.

Endometrial cancer is the main risk of unopposed estrogen therapy, which, since the 1970s, is not recommended for postmenopausal women with intact uteri.1,2 Epidemiological investigations indicated that the consumption of high levels of soy foods is associated with a reduced risk of endometrial cancer.6,7 In the present study, no stimulation of proliferation by isoflavones could be identified by using the proliferation marker Ki67. Our result is consistent with results from a recent study, in which daily administration of 114 mg isoflavones for 3 months had no effect on the endometrium.18

Administration of estrogen reduces the FSH level. In this study, no changes of FSH or E2 levels were induced by isoflavones. ER-α, ER-β, and its variant, ER-βcx, were expressed in breast and endometrium. As a marker for estrogen action, we used PR as an ER target gene. In this study, we did not detect any changes in expression of PR-A or PR-B in breast or endometrium after isoflavone treatment.

In a recent study, the effects of isoflavones were studied in breasts of primates.15 It was found that in the presence of E2, isoflavones can augment the expression of some ER-regulated genes (PR) and inhibit expression of other (PS2). In our study in postmenopausal women, E2 levels were low so that any effects of isoflavones would presumably be mediated by direct interactions of isoflavones with ER. Traditional Chinese medicines are widely used in Asia for treating menopausal symptoms. It has been shown that many herbal medicines contain phytoestrogens and have estrogenic activities.19-21 Isoflavones bind better to ER-β than to ER-α,22 and ER-β can oppose the effects of ER-α on proliferation.23 ER-β was expressed in the postmenopausal endometrium, and interaction of isoflavones with ER-β might have antiproliferative actions in the breast and endometrium. This could perhaps explain why the endometrium of the isoflavone-treated women remained thin, whereas that of the placebo-treated women increased during the study period.

CONCLUSION

This small, short-term, double-blind, prospective study showed that the administration of 60 mg isoflavones daily to postmenopausal women effectively relieved hot flashes with no adverse effects on endometrium, breast, or lipoprotein lipids. Whether isoflavones can substitute for estrogen with regard to the very serious consequences of estrogen deficiency, i.e., osteoporosis, declines in cognition, and disorders of the immune system, requires further evaluation.

REFERENCES