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The effect of Promensil™, an isoflavone extract, on menopausal symptoms

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ABSTRACT

Objectives The primary aim was to assess whether the use of an isoflavone extract containing 40 mg or 160 mg of total isoflavones affects the frequency of menopausal flushes and other symptoms. The secondary aims were assessments of possible effects on menopause symptom scores and biological measures of estrogen activity.

Methods A randomized, double-blind, placebo-controlled prospective trial of 37 postmenopausal women with symptoms of estrogen deficiency was performed over a 12-week period. The women were randomized to three treatment groups: placebo, 40 mg or 160 mg, delivered in tablet form.

Results There was no significant difference in the incidence of flushes between the three groups at trial conclusion. There was no difference between the groups in Greene Menopause Symptom Scores, vaginal pH, levels of follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG) or total cholesterol, liver function or blood parameters. A statistically significant increase in high-density lipoprotein (HDL) cholesterol of 18.1% ($p = 0.038$) occurred in the 40-mg group.

Conclusion A large placebo response and inadvertent use of dietary isoflavones in the placebo group may have obscured a significant change in flushing frequency. Previous uncontrolled studies claiming a beneficial effect of foods with a high isoflavone content on menopausal symptoms may have been confounded by a large placebo response.

INTRODUCTION

Menopausal hot flushes are the most common symptom of the climacteric, and occur in 60–75% of women undergoing a natural menopause with a higher incidence after surgical menopause[1]. Flushes may occur at any time, and are characterized by a flush usually starting in the face, spreading to the neck, head, chest and rest of the body, and can severely disrupt a woman's life. Flushes are often accompanied by the subjective feeling that others notice the occurrence of these symptoms. Flushes also occur at night, awaking women from sleep, with consequences including insomnia, irritability, tiredness and general lethargy. Estrogen is a very effective treatment of flushes; however, in certain clinical settings, the use of estrogen in some women is undesirable or contraindicated. Other women may have a resolution of symptoms with estrogen replacement but find the side-effects of estrogen more undesirable, even in small amounts. Despite clinical benefits attributed to the use of hormone replacement therapy (HRT) in postmenopausal women, compliance ranges from 10 to 50%[2], and alternatives to estrogen replacement would be therapeutically valuable in these cases.

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The apparent cultural differences in the incidence of menopausal flushing[3,4] have been attributed to differences in dietary intake of isoflavones, naturally occurring plant compounds with a similar spatial structure to estrogen and estrogen-like biological activity[5,6]. Much of the evidence regarding the effects of isoflavones on menopausal symptoms is epidemiological, and has been linked to the consumption of soy products in different populations. This indicates soy products to be a major source of isoflavones. Soybean and its products appear to have high levels of isoflavones, the extent depending on the type and manner of preparation[7,8]. Intervention by dietary modification, with foods containing isoflavones, has been shown to alter biochemical markers associated with reproductive function[9]. The consumption of soy products is estimated to be highest in Japanese populations, with levels in the diet of up to 200 mg per day[10]. There is a gradation in consumption of isoflavones in the diet from Asia, where consumption is estimated to be 25–45 mg of total measurable isoflavones per day, to Western countries, where less than 5 mg per day are consumed[11]. A Western-type diet elevates plasma levels of sex hormones and decreases the sex hormone binding globulin (SHBG) concentration, increasing the bioavailability of the sex steroids[12]. The same diet also results in low formation of isoflavone[12].

The hypothesis that supplementation with isoflavones has an estrogenic effect in postmenopausal women and relieves menopausal symptoms was investigated. The primary outcome measure was the frequency of hot flushes, with secondary outcomes being menopause symptom scores and biological measures of estrogen activity. Supplementation was achieved using Promensil™(Novogen Ltd, Sydney, Australia), a tablet isoflavone supplement prepared from red clover extract.

MATERIALS AND METHODS

A double-blinded, randomized, placebo-controlled trial was performed consisting of three arms: placebo, one tablet (40 mg) of Promensil and four tablets (160 mg) of Promensil. Promensil is a standardized isoflavone supplement prepared from red clover extract, in tablet form. Each tablet contained 40 mg of total isoflavones comprising the four primary isoflavones: genistein (4.0 mg) and daidzein (3.5 mg), and their methylated precursors biochanin (24.5 mg) and formononetin (8.0 mg).

Thirty-seven subjects were recruited through the University Department of Obstetrics and Gynaecology at St. George Hospital, Sydney, Australia. The inclusion criteria for the trial were postmenopausal women who were symptomatic, having at least three flushes per day. Menopause was defined by bilateral oophorectomy or amenorrhea for at least 6 months with typical symptoms of the menopause, and a serum follicle stimulating hormone (FSH) level greater than 40 IU/l. Age was restricted to 40–65 years, upon trial entry. Participants were instructed not to alter their usual diet for the duration of the study. The exclusion criteria included HRT use within the previous 6 weeks; allergy to foodstuffs known to contain isoflavones; current history of active bowel, liver or gallbladder disease; diabetes requiring drug therapy; and malignancy (excluding skin cancers). Women with contraindications to HRT use, vegetarians and/or regular soy product users and those receiving medications that result in liver enzyme induction were also excluded.

Pre-trial flushing was assessed using a daily flush diary for the week prior to trial entry. The severity of menopausal symptoms was assessed during this period, using the Greene Menopause Scale. A 24-h urine collection for isoflavone measurement was performed during this week. Upon fulfillment of inclusion criteria, subjects were entered into the study. The randomization procedure was performed by an external statistician. The allocation schedule was produced in random permuted blocks of six, generated using a computer random number generator. Subsequently, tablets were packed in daily sachets and supplied in individual subject containers to the investigators. Packaged subject containers were received prior to trial recruitment. There was no contact between the generator of randomization and the executor of the trial, prior to, during or after the trial.

After screening, the subjects were randomly assigned to placebo or one of the active treatment groups. Physical and vaginal examinations were performed. A vaginal wall smear for determination of maturation value[13] and pH was performed at trial entry. The vaginal wall smear was performed using a speculum and wooden spatula, with a single pass along each lateral vaginal wall. Blood was collected in a non-fasting state for assessment of hematological profile, liver function and serum levels of FSH and SHBG. Hematology and biochemistry were performed by Southpath Laboratories at St. George Hospital (Sydney,

Australia), with the lipid biochemistry assayed using standard enzymatic colorimetric kits on a BM/Hitachi 747 analyzer. Levels of FSH and SHBG were assayed by the Endocrine Laboratory at the Royal Hospital for Women (Sydney, Australia). The FSH level was determined with an automated chemiluminescence system, Chiron Diagnostic ACS : 180, and the SHBG by chemiluminescent enzyme immunometric assay on an Immulite Automated Analyzer. A 24-h urine collection for isoflavone measurement preceded trial entry. Urinary isoflavone assays were performed by Novogen Ltd. Each sample was analyzed using high-performance liquid chromatography (HPLC) for identification of the isoflavonoids: genistein, daidzein, biochanin A, formononetin and equol. All analyses were run in duplicate. The reproducibility of the method has been previously reported[14]. Synthesized isoflavonoids for use as standards were obtained from the Department of Organic Chemistry, University of Helsinki.

The trial was 12 weeks in length, and the subjects were seen every 4 weeks for clinical assessment, compliance checks and assessment of flush count and Greene Score. Contact between appointments was provided if required by individual subjects. In the final week of the study, physical and vaginal examinations, the vaginal smear, and urine and blood collections were repeated. Compliance was assessed by return of tablet containers and urinary isoflavone levels. All subjects were included in the analysis on an intention-to-treat basis. On a *post hoc* basis, the stored serum was analyzed for serum total cholesterol and high density lipoprotein (HDL) cholesterol levels.

The Greene Menopause Score is a validated menopause symptom self-assessment form, and was completed weekly. For analysis, the Greene Score was calculated as a total and in the accompanying subgroups, the Psychological Scale, Somatic Scale and Vasomotor Scale. Data were entered into Statistica™ (StatSoft™ Inc, Tulsa, USA). Non- parametric data were analyzed using the Kruskal– Wallis non-parametric analysis of variance. Normally distributed data were analyzed using one-way analysis of variance. Any difference between individual groups was re-analyzed using the Newman–Keuls test, provided that the F value from the analysis of variance was significant. This test was preferred to the Bonferoni modification, a corrected Student's t test, as the Newman–Keuls is based on the least significant difference between the means of each group and is a 'protected' test, taking into account the risk of specious results arising from multiple comparisons. Data are presented in the format mean ± standard deviation with p values included when ≤ 0.05.

All subjects consumed four tablets daily. Placebo and active tablets were of the same appearance (in color and size) and taste. Each day's tablets were in an individually marked sachet, containing four placebo tablets, one active and three placebo tablets (40-mg group) or four active tablets (160-mg group). The code was broken only after trial completion, analysis of serum and urine samples, database entry with subsequent checking and locking of the database.

RESULTS

Thirty-seven subjects were randomized. One subject withdrew for personal reasons before commencing any treatment, and a further subject was recruited to the same randomization position. Twelve subjects were randomized to each of the placebo and 40-mg groups and 13 to the 160-mg group. Two patients were subsequently withdrawn from the 160-mg group because of intervention by their general practitioners. All packages containing tablets were returned. Tablets were not taken by only two patients, with days of missed tablets being less than 7.

The ages of the women participating in the study were 53.1 ± 2.5, 54.5 ± 4.4 and 56.1 ± 3.9 years in the placebo, 40-mg and 160-mg groups, respectively. The age at menopause in these groups was 47.7 ± 8.0, 48.5 ± 3.6 and 51.1 ± 8.8 years. There were no statistically significant differences in age, weight and age at menopause between the groups. Weight was monitored throughout the study and there was no difference in these values within each group, between trial entry and exit.

Flushing frequency decreased in all groups over the 12-week period of the trial. There was no difference in flushing frequency between the active and placebo groups. These data and those of other measures of estrogen activity are presented in Table 1. Analysis of urinary isoflavone levels showed a dose-dependent increase between week 0 and week 12 in groups receiving active tablets. A smaller but notable increase was also observed in the placebo group, indicating that subjects in this group may have adopted altered dietary patterns to include foods containing isoflavones. This occurred despite inclusion criteria requests that dietary patterns should not be altered throughout the course of the trial.

Table 1 Biological and biochemical markers of estrogen activity

	Trial week	*Placebo*	*40 mg Promensil™*	*160 mg Promensil™*
Number of flushes	0	8.6 ± 4.6	6.9 ± 2.1	9.0 ± 5.2
	12	5.8 ± 4.5 (–35%)	4.9 ± 4.8 (–29%)	5.9 ± 4.6 (–34%)
Greene Score	0	18.5 ± 11.4	19.9 ± 10.6	19.9 ± 4.4
	12	9.9 ± 5.9 (–46%)	11.2 ± 8.8 (–44%)	14.7 ± 16.8 (–26%)
FSH (IU/l)	0	75.9 ± 26.6	87.7 ± 31.6	69.9 ± 24.9
	12	76.2 ± 29.4	82.2 ± 30.2	58.7 ± 22.3
SHBG (IU/l)	0	66.2 ± 46.0	72.6 ± 28.2	66.7 ± 28.7
	12	64.7 ± 62.2	64.1 ± 26.0	55.8 ± 19.1
Maturation value	0	51.3 ± 1.7	49.4 ± 2.2	51.1 ± 1.7
	12	49.9 ± 7.8	45.8 ± 24.1	51.1 ± 1.9
Vaginal pH	0	5.3 ± 0.8	5.4 ± 0.7	5.1 ± 0.6
	12	5.4 ± 0.7	5.1 ± 0.9	5.0 ± 0.8
Urinary isoflavone (ng/ml)	0	2.68 ± 1.92	3.43 ± 2.07	3.70 ± 2.96
	12	3.67 ± 2.79	9.40 ± 5.67	28.18 ± 17.52
HDL cholesterol (mmol/l)	0	1.08 ± 0.31	1.05 ± 0.40	1.06 ± 0.80
	12	1.13 ± 0.28	1.24 ± 0.49*	1.19 ± 0.40

*p = 0.038, compared to week 0; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin; HDL, high-density lipoprotein

None of the biological parameters of estrogen activity measured, including FSH, SHBG and climacteric symptom scores, showed any change with time, compared to placebo. Analysis of the vaginal smears showed no difference between the groups in vaginal maturation value, although 20% of the smears were unable to be evaluated owing to severe atrophy. There was no increase in vaginal acidity associated with isoflavone use (Table 1).

Serum HDL cholesterol levels increased significantly by 18% (p = 0.038) in subjects taking the 40-mg dose of isoflavones. The results for 160 mg did not differ from those for placebo. Total cholesterol and triglyceride levels could not be accurately evaluated as the samples were collected in a non-fasting state.

DISCUSSION

The paucity of published data concerning the effects of isoflavones as an intervention in the treatment of menopausal symptoms, in the presence of epidemiological data suggesting that these compounds may modify many physiological processes including the menopause, formed the basis of the present study. A recent larger study has suggested that isoflavones from soy protein may modify flushing frequency in women with severe flushing to a moderate degree (45% reduction in flushes compared to 30% reduction in the placebo group)[15].

A similar pattern was observed in the trial reported here. An overall decrease in flushing frequency in the treatment groups was matched by a large fall also in the control group. The data from this study suggested that a trend towards improvement on active medication may have been obscured by the large placebo effect and the highly variable responses to treatment apparent for both the placebo and active treatments. The decrease in flushing frequency in the placebo group should be interpreted with regard to the increase in urinary isoflavone excretion in this group at trial exit. These effects may also be consistent with the data from small intervention trials already published and represent placebo response and time effects[16].

Closer scrutiny of individual data revealed other difficulties within the control group that may have altered the final analysis. Two subjects were suspected by the investigators of changing the criteria on which flushing frequency was based when completing the diary cards, after enrollment into the trial. Both subjects initiated lengthy discussions at the enrollment visit, and after completion of the weekly pre-trial flush count. Information and education of these patients appear to have altered their perception of determining or characterizing hot flushes. In these two patients, there was a fall in flushing frequency within a week of enrollment from more than ten flushes daily to one or two each day. Another in the placebo group grew alfalfa in her garden and consumed this while taking part in the trial, inadvertently increasing her isoflavone

exposure. This was reflected by a rise in the urinary isoflavone levels for this patient. The increased overall exposure to isoflavones noted in the urinary levels of the control group may have contributed to the placebo response reported. However, the study was performed on an intention-to-treat basis and, therefore, all subjects were included in the analysis.

No differences between the control and the active groups were observed in the subjective scoring of menopausal symptoms. The Greene Climacteric Scale has been previously validated as an assessment of menopausal symptoms. The Vasomotor Symptom Score assesses severity of vasomotor function as a numerical measure of occurrence; however, it does not address subjective assessment of the intensity of flushes or night sweats. Lack of effect is certainly an explanation, and inability to detect an effect is another. In addressing this issue, it is appropriate that questionnaires used for subjective assessment of menopausal symptoms in future studies are capable of detecting changes in the intensity of symptoms as well as the frequency.

While urinary levels of isoflavones increased in the active groups, there were subjects in both the 40- and the 160-mg groups who had a fall in urinary isoflavone levels, compared to trial entry. Return of packaging suggested good compliance, and, while falls in the total urinary isoflavones with time may indicate poor compliance, they may also be indicative of individual variations in absorption profile. There are minimal pharmacokinetic data available for isolated isoflavone use in humans; however, preliminary data suggest individual differences in the metabolism of daidzein to its end-metabolites, equol and *O*-DMA[17]. Recent data suggest that isoflavones are readily absorbed via the gut, and both daidzein and genistein serum levels reflect, in a positive relationship, the oral dose consumed[18]. Of note in this recent trial is that equol was rarely detected in the urine of the study patients while *O*-desmethyl-angolensin levels appeared to rise in these groups.

The present study confirms the results of others, in that isoflavones have no effect on serum levels of FSH and SHBG. The lack of change from baseline and between groups in hematological parameters, liver enzymes and proteins suggests that the doses used in the study cause no gross abnormalities in hematological and liver function in the short or medium term. Debate has also ensued over isoflavone effects on vaginal epithelium and pH, used as biological indicators of estrogen activity. The present data suggest that isoflavones have no effect on vaginal epithelium or vaginal pH.

The hypocholesterolemic effects of dietary soy protein have been previously demonstrated. In a meta-analysis of dietary soy protein and the effects on serum lipids, Anderson and colleagues showed a statistically significant decrease in total cholesterol and low-density lipoprotein (LDL) cholesterol levels in hypercholesterolemic subjects[19]. In this analysis, there was a non-significant trend towards a rise in HDL cholesterol. The authors suggested that 60–70% of the effects on serum lipid levels by soy protein are as a result of the presence of isoflavones in the soy protein. As the lipid levels were assessed on a *post hoc* basis in the present study and were collected in the non-fasting state, LDL cholesterol levels were not determined. The rise in HDL cholesterol of 18.1% seen in the 40-mg Promensil group may represent a window effect. There may be a response to low levels of isoflavones, with diminution or loss of the effect as serum levels increase.

The present study emphasizes many of the problems with pharmaceutical-style intervention studies using naturally occurring dietary compounds. There is a need for further larger studies investigating the areas of clinical effectiveness of isoflavone supplementation in the treatment of menopausal symptoms. Initial indications of biological activity require appropriate assessment of pharmacokinetic properties and dose–response relationships. Although these compounds may well play a complementary role in the treatment of menopausal symptoms and disease associated with estrogen deficiency, issues concerning dose–response relationships, therapeutic variability and side-effect profiles at these doses, and effects on long-term diseases associated with estrogen deficiency, remain to be addressed.

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