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Effects of the phytoestrogen genistein on hot flushes, endometrium, and vaginal epithelium in postmenopausal women: a 2-year randomized, double-blind, placebo-controlled study

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Abstract

Objective: To evaluate in a 24-month, prospective, randomized, double-blind, placebo-controlled study whether pure administration of the phytoestrogen genistein (54 mg/d) might reduce the number and severity of hot flushes in postmenopausal women, with no adverse effect on the endometrium and vagina.

Methods: A total of 389 participants met the parent study criteria and were randomly assigned to receive the phytoestrogen genistein (n = 198) or placebo (n = 191). About 40% of participants in both groups did not experience hot flushes, and the evaluation was performed in a subgroup of 236 participants (genistein, n = 119; placebo, n = 117). Reductions from the baseline in the frequency and severity of hot flushes were the principal criteria of efficacy. Endometrial thickness was evaluated by ultrasonography. The maturation value was also used to determine hormonal action on the vaginal cells.

Results: There were no significant differences in vasomotor symptoms between groups at the baseline (4.4 ± 0.33 hot flushes per day in the genistein group and 4.2 ± 0.35 hot flushes per day in the control group). After 12 months of genistein therapy, there was a significant reduction (−56.4%) in the mean number of hot flushes, with a significant difference compared with the control group. After 24 months, there was no further decrease in the number of hot flushes in both groups. No significant difference was found in mean endometrial thickness and the maturation value score between the two groups, either at the baseline or after 24 months.

Conclusions: The phytoestrogen genistein has been shown to be effective on vasomotor symptoms without an adverse effect on the endometrium and vagina, but after the first year, there was no further improvement in the decrease in hot flushes.

Key Words: Postmenopause – Phytoestrogen – Genistein – Hot flushes – Endometrium – Vagina.

The appearance of hot flushes (intense heat with sweating) is a consequence of exhaustion of ovarian function; and it is the major reason for women to attend a menopause center. During the menopausal transition, hot flushes greatly affect the quality of life for about 60% of Western postmenopausal women.[1] Hormone therapy is the most effective treatment for the relief of vasomotor symptoms,[2] but it is associated with a small increased risk of breast cancer and cardiovascular disease.[3] Because only 20% to 25% of postmenopausal Asian women experience hot flushes,[4] it has been suggested that fewer vasomotor symptoms are a consequence of the high soy content in their diet.[5] It is likely that many of the positive effects of soy are derived from its isoflavones.[5] Isoflavones are polyphenolic compounds that are capable of exerting estrogen-like effects. For this reason, they are classified as phytoestrogens: compounds with estrogenic activity derived from plants. The isoflavone genistein is a phytoestrogen found in low concentrations in soybeans and in increased amounts in certain fermented soy foods, whereas genistin, the glucoside form of genistein, is much more abundant in unprocessed soybeans and most soy products. As a natural selective estrogen receptor (ER) modulator,[6] genistein was recently shown to positively regulate

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bone metabolism and favorably affect different cardiovascular risk parameters.[7,8] These effects are probably a direct consequence of genistein's greater affinity for ER-β, which is abundant in bone during the mineralization phase and in the endothelium, rather than for ER-α, which is expressed more in the breast and endometrium. Our research group had shown previously that the administration of the soy isoflavone genistein significantly decreases the number and the severity of hot flushes compared with the placebo group after a 12-month treatment period, without affecting the endometrium.[9]

However, questions about the continued efficacy of genistein on vasomotor symptoms as well as its long-term safety on the endometrium and vagina still remain. On the basis of this background, in this follow-up study, we considered the same endpoints for another 12-month period of treatment to evaluate further not only an eventual decrease in the number and severity of hot flushes but also safety performance regarding effect of the isoflavone genistein on the endometrium and vagina during such a long period.

METHODS

Participants

The protocol is consistent with the principles of the Declaration of Helsinki, and the study participants gave their written informed consent.

We evaluated the climacteric vasomotor symptoms and the effect on the endometrium and vagina of postmenopausal women enrolled in a 2-year randomized, double-blind, placebo-controlled study of the 2-year parent study, in which the primary endpoints were the effects of the phytoestrogen genistein on bone loss[7] and cardiovascular risk prevention.[8] The patients were not informed about the possible effect of genistein on the reduction of hot flushes to minimize the placebo effect on vasomotor symptoms. Characteristics of the women recruited, inclusion and exclusion criteria, and instructions for the diet have been explained elsewhere.[9]

Treatments

A total of 575 women from the parent study met the inclusion criteria, and 186 of these women refused to participate, leaving 389 women. After a 4-week stabilization with consumption of the standard fat-reduced diet, the 389 women were randomly assigned to receive the phytoestrogen genistein (n = 198) or placebo (n = 191). A computerized database was used for randomization. About 40% of women in both groups did not experience hot flushes at the baseline; therefore, the evaluation of hot flushes was carried out only in 265 participants after randomization: 135 women in the genistein group and 130 women in the placebo group. The flowchart describing the progress of the participants during the trial is represented in Figure 1.

Placebo and genistein tablets had a similar appearance, and two per day were administered (preferably at 8:00 AM and 8:00 PM). Each tablet contained 500 mg of calcium carbonate and 400 IU of vitamin D. In addition, each genistein tablet, obtained from Lab Plants (Messina, Italy), contained 27 mg of total isoflavone. The purity of genistein was approximately 98%.

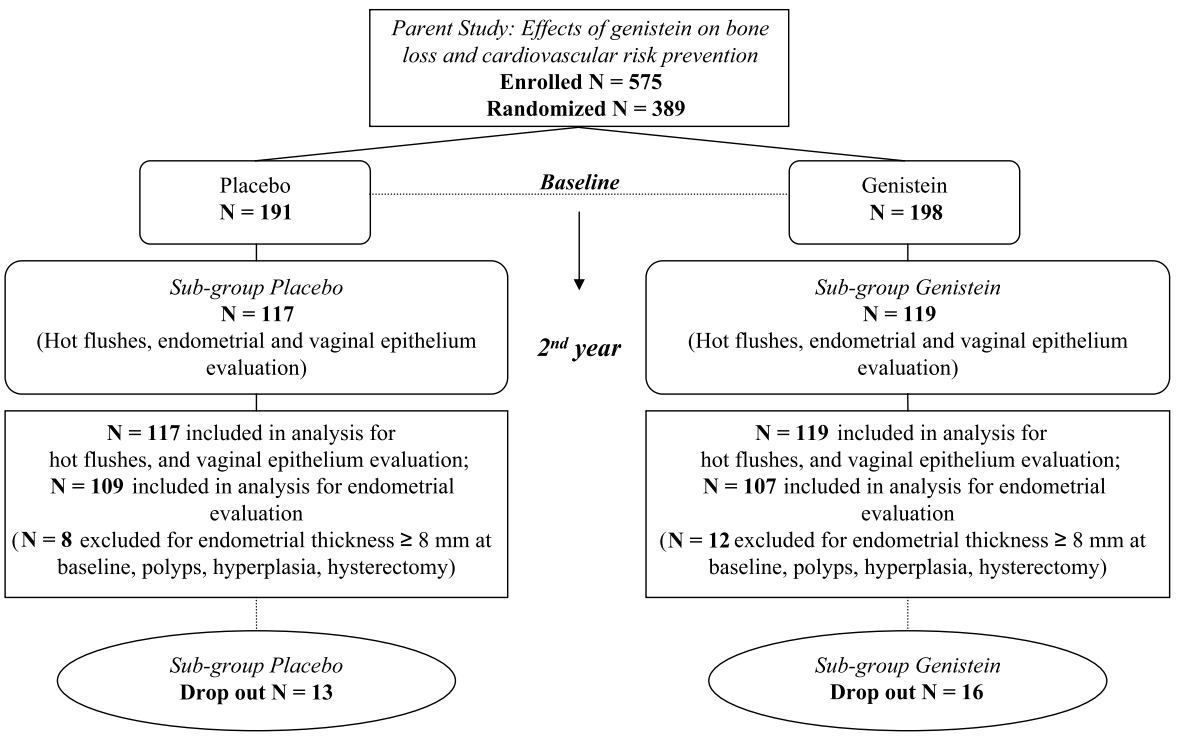

FIG. 1. Flowchart describing the progress of the participants during the trial.

Study protocol

All 265 participants included in the study were instructed to record in a diary the number and the severity of hot flushes each day for 1 week before each visit. The baseline, 1-year, and 2-year hot flush counts were calculated as the mean of the last 7 days before treatment and before each visit. Reductions from the baseline in the frequency and severity of hot flushes were the principal criteria of efficacy. The severity of hot flushes was evaluated using a score from 1 to 3: 1, mild; 2, moderate; 3, severe. Mild hot flushes were defined as a warm sensation without sweating that left the woman able to continue her daily activity. Moderate hot flushes were defined as a warm sensation with sweating that left the woman able to continue her daily activity. Severe hot flushes were defined as a hot sensation with sweating so intense that the woman had to stop her activity. Thus, we reported the score for each hot flush, and then the scores were summed and divided by the number of hot flushes.

Ultrasonographic endometrial thickness was evaluated at the baseline, after 12 months and after 24 months. The endometrial thickness at the sagittal plane was measured from one basal layer to the other. If the endometrial thickness measured 8 mm or more or if uterine bleeding was present during the treatment, an hysteroscopic examination and an endometrial biopsy were performed.

Vaginal cytological examination of a specimen from the upper third of the vagina was performed in all participants before the treatment and after 12 and 24 months to evaluate the hormonal action on the vaginal cells by means of a maturation value (MV) in a quantitative analysis.[10] The MV score considers (para)basal, intermediate, and superficial cells, to which the following values were assigned: 0, (para)basal; 0.5, intermediate; and 1.0, superficial. More specifically, the MV can range from 0, when only (para)basal cells are present (atrophic specimens), to 100 for specimens containing only superficial cells (mature specimens). All examinations were interpreted by the same cytopathologist, who was blinded to the treatment.

Statistical analysis

A power analysis was performed with 80% power in two-sided tests and with an expected difference between the two groups of at least 20% with an α of 5%; therefore, the study sample had to include at least 97 women in each group. The Student's t test was initially used to verify the differences between the independent groups. A two-way analysis of variance with repeated measures followed by a post hoc multiple-comparison procedure was performed to verify differences in the numbers of hot flushes among different treatment groups and within groups over time. The demographic characteristics of the study groups are expressed as mean ± SD, whereas the number of hot flushes and severity score are expressed as mean ± SEM. Statistical tests were two tailed; the significance level was set at $P < 0.05$, and data were analyzed with SPSS software, version 13.0 (SPSS Inc, Chicago, IL).

RESULTS

Characteristics of the study population

There were no significant differences in age, time since menopause, and body mass index between the two groups at the baseline (Fig. 2). Eighty-five participants dropped out in the parent study, 48 in the genistein group, and 37 in the placebo arm. Specifically, in our substudy, 29 participants dropped out, 16 in the genistein group (10 occurring within the first 12 months) and 13 in the placebo group (8 occurring

	GENISTEIN	PLACEBO	P
Genistein and placebo groups in parent study			
n	**198**	**191**	
Age (years)	**54.7 ± 3.5**	**54.2 ± 2.7**	**0.2**
Body Mass Index, kg/m^2	**25.0 ± 3.3**	**25.1 ± 4.2**	**0.8**
Time since Menopause, months	**66.8 ± 45.8**	**59.1 ± 38.4**	**0.1**
Genistein, μmol/L	**0.14 ± 0.01**	**0.15 ± 0.02**	**0.8**
Genistein and placebo participants with hot flushes (substudy)			
n	**119**	**117**	
Age (years)	**53.1 ± 2.3**	**53.0 ± 1.8**	**0.6**
Body Mass Index, kg/m^2	**23.8 ± 3.2**	**23.9 ± 4.1**	**1.0**
Time since Menopause, months	**38.8 ± 20.6**	**39.4 ± 18.9**	**0.8**

FIG. 2. Demographic characteristics (mean ± SD) of the genistein and the placebo groups in the parent study and the genistein and placebo participants with hot flushes (substudy).

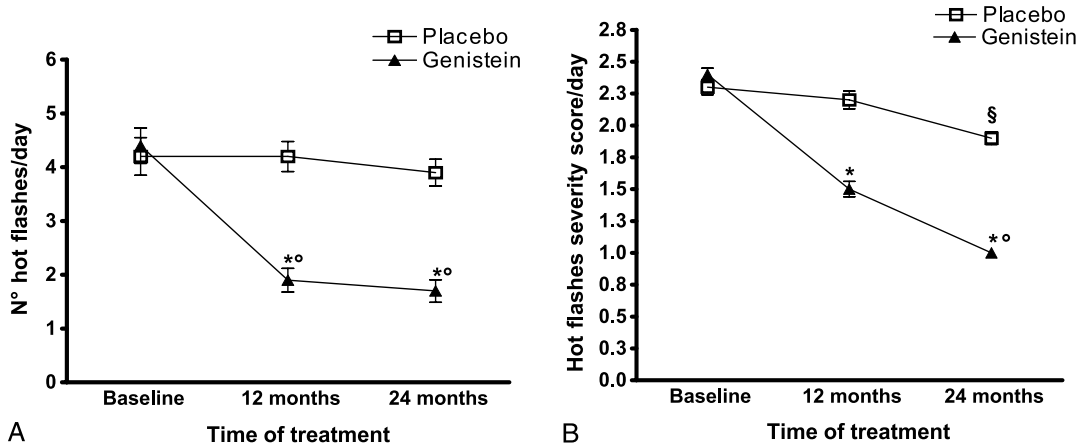

FIG. 3. A: Mean (SEM) number of hot flushes per day at different time points (*$P < 0.001$ vs placebo, °$P < 0.001$ vs genistein baseline) in the two randomized groups. **B:** Mean (SEM) hot flushes severity score per day at different time points (*$P < 0.001$ vs placebo, °$P < 0.001$ vs genistein 12 months; §$P < 0.001$ vs placebo baseline) in the two randomized groups.

within the first 12 months); therefore, 236 women completed the study: 119 in the genistein group and 117 in the placebo group, who were considered for the evaluation of hot flushes (Fig. 1). Gastrointestinal adverse effects were the most common reasons for drop out; other reasons were the presence of other diseases, loss of some participants to follow-up, and possibly inadequate interaction with the woman's family physician. Because women were not treated for the severity of their hot flushes, but rather for the prevention of osteoporosis and cardiovascular risk, we had no withdrawals resulting from treatment inefficacy.

Hot flushes

All postmenopausal women had a moderate number of vasomotor symptoms at baseline: 4.4 ± 0.33 hot flushes per day in the genistein group and 4.2 ± 0.35 hot flushes per day in the control group (Fig. 3A); the mean severity score at the baseline for the genistein group was 2.4 ± 0.05 per day, and for the control group, it was 2.3 ± 0.06 per day (Fig. 3B). The difference in the number of hot flushes and severity score between the genistein group and placebo was highly significant either after 12 ($P < 0.001$) or after 24 ($P < 0.001$) months. After the first year of treatment, in the genistein group, there was a significant decrease in the mean number (−56.4%) and severity score (−37.5%) of the hot flushes. After the 2-year period of treatment, a significant decrease from the first year was evaluated only in the mean severity score (−33.3%) (Fig. 3B), whereas there was no difference at the end of the study in the mean number of hot flushes (Fig. 3A). In the placebo group, there were no significant differences through the 24 months in the mean number of the hot flushes (Fig. 3A); instead, a significant decrease in the mean severity score from the baseline (−17.3%) and from the 12-month period (−13.6%) was observed.

Endometrium

From the 236 women who were evaluated for hot flushes, only 216 (107 women in the genistein group and 109 in the placebo group) were considered for endometrium evaluation, and the reasons (hysterectomy, hyperplasia, and polyps) are reported in Figure 1. No episode of uterine bleeding was reported in either group during the study. At the beginning of the study, no significant difference was detected between the two groups: 3.1 ± 0.1 mm in the genistein group and 3.2 ± 0.1 mm in the placebo group (Table 1). No significant difference in endometrial thickness was observed between the two groups after 12 and 24 months of treatment. In the placebo group, a significant reduction in endometrial thickness from the baseline was observed only after 24 months when compared with baseline values (Table 1).

Vagina

No statistical difference in the MV score between the two groups was observed at the baseline and after 12 or 24 months (Table 2). In the placebo group the MV score significantly

TABLE 1. *Variation in endometrium through 24 months in the genistein and placebo groups of the substudy*

	Genistein (n = 107)	Placebo (n = 109)	*P*
Baseline	3.1 ± 0.1	3.2 ± 0.1	0.3
12th month	3.0 ± 0.1	3.0 ± 0.1	0.9
24th month	2.9 ± 0.1	2.9 ± 0.1	0.8

Data are presented as mean ± SEM.
Genistein: baseline versus 12th month ($P = 0.2$); baseline versus 24th month ($P = 0.08$); 12th month versus 24th month ($P = 0.2$).
Placebo: baseline versus 12th month ($P = 0.01$); baseline versus 24th month ($P < 0.01$); 12th month versus 24th month ($P = 0.2$).

TABLE 2. *Variation in maturation value score through 24 months in the genistein and placebo groups of the substudy*

	Genistein (n = 107)	Placebo (n = 109)	*P*
Baseline	22.9 ± 1.71	23.7 ± 1.60	0.9
12th month	22.2 ± 1.52	20.6 ± 1.67	0.5
24th month	21.2 ± 1.47	19.2 ± 1.49	0.3

Data are presented as mean ± SEM.
Genistein: baseline versus 12th month ($P = 0.6$); baseline versus 24th month ($P = 0.2$); 12th month versus 24th month ($P = 0.4$).
Placebo: baseline versus 12th month ($P < 0.001$); baseline versus 24th month ($P < 0.001$); 12th month versus 24th month ($P = 0.1$).

decreased after the 12-month evaluation; there was no further decrease until the end of the study. In contrast, the MV score in the genistein group was unchanged through the whole study.

DISCUSSION

Our clinical trial demonstrated that the isoflavone genistein plus calcium and vitamin D_3, along with a healthy diet, is effective for vasomotor symptoms compared with placebo, without any adverse effects on the endometrium and vagina, in a cohort of osteopenic, postmenopausal women after 24 months of treatment. Our postmenopausal women had less than five hot flushes per day, but the Food and Drug Administration has recommended that 7 to 8 moderate or severe hot flushes per day or 50 to 60 hot flushes per week be required for inclusion in this type of study. However, the Food and Drug Administration statement referred to hot flush evaluation as the primary or secondary outcome in a clinical trial. Specifically, in our study, the postmenopausal women were enrolled for their osteopenia, as the primary outcome, and they were completely blind about treatment efficacy for hot flushes.

In the literature, the data on the efficacy of phytoestrogens in the alleviation of menopausal symptoms are conflicting,[11-14] but a recent statement from the Cochrane Library[15] established that there is no evidence of effectiveness in the reduction of hot flushes with the use of phytoestrogen treatments. In effect, the potential positive effect of phytoestrogens on hot flushes is almost always cancelled by the high placebo effect, which may reduce hot flushes by approximately 50%.[16]

The strength and unique characteristic of our study is that women were enrolled and treated for their osteopenia and not for vasomotor symptoms; in fact, no participant withdrew as a consequence of treatment inefficacy. This was the main reason that the placebo effect was probably strongly reduced. However, positive data obtained in our substudy should be evaluated in the context of a fat-restricted diet and in combination with calcium and vitamin D_3.

In our previous study,[9] in genistein recipients the percentage of decrease in the mean number of hot flushes was highly significant (−56.4%) compared with that in placebo recipients after 12 months of treatment, but in the subsequent 12 months, the reduction was no more significant than that in placebo recipients: there is only a −11.5% reduction from the 1-year evaluation. In the first year of treatment,[9] a significant reduction in the mean number of hot flushes occurred in each step: from the baseline to the 1st month (−25%), from the 1st to the 3rd month (−22%), from the 3rd to the 6th month (−6%), and from the 6th to the 12th month (−12%). Those data agree with the data reported previously by Petri Nahas et al[17]: the major reduction in the number of hot flushes occurs after 3 to 4 months of treatment. Overall, after 2 years, the mean hot flush reduction was −7.2% in the placebo group, whereas in the genistein group, the reduction was −61.4% from baseline levels. Indeed, with the extension of the study, we did not find a further reduction in the number of hot flushes, in agreement with another study in which treatment with isoflavones lasted for 2 years.[18]

At the end of our trial, we also reported a significant reduction in the mean severity score of hot flushes per day from the first year, but a similar reduction occurred also in the placebo group, therefore we can argue that this phenomenon could be time dependent and not related to treatment efficacy. The exact mechanism by which phytoestrogens may reduce the number and severity of hot flushes has not been fully elucidated. A recent study suggests that, similarly to estrogens, phytoestrogens might alter the physiological neuroendocrine mechanism of core body temperature regulation and consequently exert a beneficial effect on hot flushes.[19] This hypothesis might explain, at least in part, the positive effect of genistein on climacteric symptoms in this clinical trial, even if the efficacy of this isoflavone is not the same as that of estrogen. It also appears to have a lesser binding affinity with ERs.[6]

Another major outcome in this follow-up study was the evaluation of endometrial thickness after 24 months of genistein treatment, the safety of this isoflavone being crucial in addition to being effective. Several epidemiological studies highlighted a decreased risk of endometrial cancer in women who consumed soy products.[20,21] This effect could be related to the competitive binding of phytoestrogens to ERs, and because of their weak estrogenic potential (low affinity for the specific receptor ER-α, which is largely predominant in the endometrium, as described above), they show antiestrogenic effects such that they inhibit the growth and proliferation of estrogen-dependent cells.[22] Furthermore, antineoplastic effects have been shown in several in vitro studies for isoflavones such as genistein via enzymatic inhibition, antiangiogenesis effects, stimulation of the immune system, and potent antioxidant capacity, properties that could be protective against cancer development.[23-25] Accordingly, serum concentrations of unconjugated free genistein achieved in our previous study[7,8] with daily intake of 54 mg are near the binding affinity of genistein seen in vitro for ER-β[6,26-29] and are an order of magnitude lower than in vitro affinity of genistein for ER-α.

To date, another Italian research group has used genistein in a smaller population and for a shorter period, with similar results.[30] Moreover, in a long-term phytoestrogen soy treatment (5 years), no case of malignancy was detected during endometrial biopsies, and only a significant increase in simple endometrial hyperplasia was observed.[31] Recently, a 12-month long study of the effects of a 70-mg soy isoflavone extract on the endometrium, evaluated by biopsy and ultrasonography, found atrophic endometrium in 99.67% of the specimens, with no case of hyperplasia or carcinoma.[32]

The weak influence of phytoestrogen on the endometrium was also associated with a minimal effect on vaginal cells, as highlighted by the unchanged MV score in our osteopenic postmenopausal women during genistein treatment; furthermore, no significant difference was observed either at the

baseline or after 24 months compared with placebo. Our results are in agreement with those of a previous report[33] in which the MV score remained unchanged during the isoflavone regimen, although they disagree with those of another report in which the isoflavone supplement was able to improve the MV score.[34]

CONCLUSIONS

In conclusion, with the limitation that our results were based on a number of hot flushes that were lower than the usual number requested for studies on the treatment of hot flushes, our study confirmed that genistein is effective for vasomotor symptoms compared with placebo after 2 years of treatment, even if a further significant decrease in the number of hot flushes from the 12-month treatment period was not demonstrated. In addition, it was well tolerated and had no adverse effects on endometrial thickness or vaginal mucosa compared with placebo. Therefore, for those women who complain of moderate vasomotor symptoms but who prefer not to use hormone therapy for medical or personal reasons, we suggest that genistein might be a reliable therapeutic alternative.

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