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New equol supplement for relieving menopausal symptoms: Randomized, placebo-controlled trial of Japanese women

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Abstract

Objectives: Equol, a metabolite of the isoflavone daidzein, is hypothesized to play a major role in the health benefits of soy. We examined the effect of a new S-equol supplement on menopausal symptoms and mood states.

Design: We conducted a randomized, double-blind, placebo-controlled trial with our new equol supplement for 12 weeks with 134 Japanese women (aged 40-59 years). They were randomly assigned to three groups: placebo (n = 44), 10 mg of equol per day (EQ-1; n = 44), and 10 mg of equol three times per day (EQ-3; n = 46). Habitual isoflavone intake was limited to 20 mg/d. Participants completed menopausal symptom and Profile of Mood States questionnaires at baseline and postintervention. Physical examination and blood and 24-hour urine collection were performed at baseline and postintervention.

Results: At baseline, total menopausal symptom score varied by menopausal and equol producer status (34.3% producers). A total of 127 participants (94.8%) completed the trial. No adverse effects were reported, except for a systemic rash in one EQ-3 woman. The anxiety scores of equol producers were lower than those of nonproducers ($P < 0.05$). Significant differences between premenopausal and perimenopausal/postmenopausal symptom scores were observed for anxiety, somatic, and total scores. After the EQ-3 intervention, perimenopausal/postmenopausal equol nonproducers showed significant decreases from baseline in all menopausal symptom scores except depression ($P < 0.01$). Compared with placebo, the EQ-3 group showed significant decreases in depression scores ($P < 0.05$), as well as significant decreases in Tension-Anxiety ($P < 0.05$), Depression-Dejection ($P < 0.05$) and Fatigue ($P < 0.01$) and increases in Vigor ($P < 0.05$) of the Profile of Mood States.

Conclusion: S-equol supplement improved mood-related symptoms in perimenopausal/postmenopausal equol nonproducers.

Key Words: Equol – Isoflavones – Supplement – RCT – Menopausal symptoms – Mood.

Epidemiological studies suggest that high consumption of soy isoflavones may account for the lower frequency of menopausal symptoms in Asian populations.[1] Recent studies found a 2-week hot flash prevalence of 22.1% among Japanese women compared with 36.6% and 46.5% for white American and African American women, respectively.[2-4] Adlercreutz et al[5] suggested that the estrogen-like properties of isoflavones might account for the low prevalence of hot flashes experienced by Japanese women.

Although estrogen therapy effectively alleviates vasomotor symptoms,[6,7] the results of the Women's Health Initiative showed overall harmful effects of estrogen plus progestin on risks of myocardial infarctions, strokes, and venous thrombosis in the lungs and legs.[8-10] Isoflavone supplements may provide an alternative to estrogen for relief of menopausal symptoms because Japanese women with high soy intake experience fewer hot flashes.[11] However, intervention studies with soy protein or isoflavones showed small or inconclusive results.[12-15]

The clinical effectiveness of soy isoflavones for menopausal symptoms may depend on the ability to metabolize the isoflavone daidzein to the isoflavan equol, mediated by equol's greater estrogenic activity and affinity for both estrogen receptors.[16,17] The ability to produce equol depends on the presence of certain intestinal microflora.[18,19] The prevalence of equol producers varies from 30% to 59% in human populations and seems to be higher in Asian and vegetarian populations.[20-24] Uchiyama et al[25] successfully used isolated *Lactococcus garvieae* spp to yield S-equol from daidzein-rich soy germ. Preliminary research indicated that equol producers have fewer hot flashes.[26] Thus, efficacy of isoflavones for alleviation of menopausal symptoms may depend on an individual's ability to produce equol.

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The objectives of this double-blind, randomized, placebo controlled trial were to examine differences in symptoms, mood states, and biomarkers (1) between Japanese midlife female equol producers and nonproducers with habitual soy diet and (2) after an intervention with a recently developed S-equol supplement administered once or three times daily. We hypothesized that equol producers would have fewer symptoms and better mood states at baseline compared with equol nonproducers and that equol nonproducers would show more significant improvements after intervention, approaching scores similar to those of equol producers at baseline.

MATERIALS AND METHODS

Participants

Women aged 40 to 59 years were recruited by direct mail from the mothers of students in a Japanese women's university. The response rate was 21% (n = 141), and 134 women participated in the study. A total of 127 women completed the 12-week intervention, and their data were used for all analyses. Women were excluded if they reported previous or current use of hormone therapy, history of ovariectomy or hysterectomy, or no menopausal symptoms. They were enrolled between February and May 2006. Using baseline menopausal symptom scores, the women were randomly assigned to one of four treatment groups: EQ-1, EQ-3, P-1, or P-3 (described in the "Equol Supplements" section). Because no significant differences were observed between the two placebo groups, results for only one combined placebo group are reported. Menopausal status was assigned based on self-reported menstrual patterns in the previous 12 months according to the following definitions: no changes, premenopausal; irregular menstrual periods, perimenopausal; no menstrual periods, postmenopausal. The study protocol was approved by the institutional review board of the National Institute of Health and Nutrition, and all women gave written informed consent to participate before beginning the study and were free to withdraw from the study at any time without obligation.

Equol supplements

The equol supplement was produced from soy germ by fermentation with *L garvieae* spp by Otsuka Pharmaceutical Co Ltd.[25] One capsule contained 10.9 mg (41.7 μmol) equol; 66.7% of the powder was fermented soy material, and 33.3% was erythritol and other additives to improve taste. One equol supplement package contained 10.0 mg S-equol, 0.8 mg daidzein, 2.0 mg genistein, and 4.5 mg glycitein in granulated form. The EQ-1 group consumed one pack containing 10 mg equol per day at breakfast; the EQ-3 group consumed one pack containing 10 mg equol at each meal (three per day). The placebo groups took one (P-1) or three (P-3) placebo packs that were identical in appearance, size, color, taste, and smell to equol supplements. The placebo supplements contained lactose instead of equol and isoflavones. Participants were permitted to ingest up to 20 mg isoflavones daily from meals during the 12-week protocol. Participants were given an illustrated isoflavone content table of soy products, and they recorded their soy product intake amount in a daily diary during the intervention period.

Intervention

The study design was a randomized, double-blind, placebo-controlled, 12-week clinical trial. After completing the baseline tests, women were randomly assigned to the EQ-1, EQ-3, P-1 or P-3 group. Group assignments were generated using a computer algorithm that allocated participants by baseline menopausal symptom score into four groups with approximately equal numbers. Participants, investigators, study staff, and laboratory technicians were blinded to group assignment until the final analysis of the trial. To determine compliance, the participants were asked to report how many supplement packs they had missed or forgotten to take in their daily diaries. Compliance was also assessed by counting returned empty supplement packs at the end of intervention.

Measurements

Participants were asked to collect 24-hour urine samples using Urinmate (Sumitomo Bakelite Co Ltd) at baseline and after 12 weeks. To determine equol producer status, participants consumed soy products containing approximately 50 mg isoflavones at dinner and began 24-hour urine collection the following morning starting with the second-void urine. Participants who excreted more than 10 ng/mL (41.28 nM) equol (HPLC detection limit) in 24-hour urine samples were defined as equol producers.

Urinary concentrations of equol were measured by HPLC using a modified method of Lundh et al.[27] Urine samples were extracted twice in ethyl acetate and evaporated. Mobile phase consisted of 17% methanol and 3% ethyl acetate in 0.05% phosphate (A) and 2% ethyl acetate in methanol (B). Isoflavones and metabolites were separated at 40°C by reversed-phase HPLC on a 4.5 × 250 mm Capcell pak C18 column (Shiseido Co, Tokyo, Japan) using a linear gradient of 0% to 40% B. Equol detection was at 280 nM using a model SPD-10A VU-VIS detector (Shiseido Co, Tokyo, Japan). The equol sensitivity and interassay CV were 0.04 μmol/L and 1.5%, respectively. Given the sensitivity of the assay, women with urinary equol concentrations less than 10 ng/mL (0.041 μmol/L) were considered to be nonproducers.

Fasting blood samples were collected at baseline and after 12 weeks of intervention. Samples were centrifuged at 3,000 rpm for 10 min at 4°C within 2 hours of blood collection. Serum estradiol, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured by fluoroimmunoassay at SRL Inc (Tokyo, Japan). Height, body weight, body fat, and blood pressure were measured at baseline and the end of intervention.

Questionnaires

General questionnaire

Questionnaires (self-report) were filled out at baseline and at the end of intervention. It included information

TABLE 1. *Component symptoms grouped according to the Greene Climacteric Scale factors in this study (symptom score out of 69 total)*

	Psychological (24)			
Vasomotor (9)	Anxiety (18)	Depression (6)	Somatic (21)	Other (15)
Hot flash	Difficulty falling asleep	Depression	Dizziness	Ringing in the ear
Sweating	Sleep lightly	Fatigue	Chest tightness	Eye fatigue
Chilliness	Excitement		Headache	Forgetfulness
	Anxiety		Shoulder stiffness	Vaginal dryness
	Nervousness		Backache	Decreased libido
	Palpitations		Arthritis	
			Numbness	

regarding general health, physical activity, smoking status, menstruation states, years since last menstrual cycle, medication, and soy product consumption. A 1-month food frequency questionnaire was used to assess dietary intake at baseline.

Menopausal symptom scale

At baseline and the end of intervention, participants reported the severity of 23 menopausal symptoms on a four-point scale (0, not at all; 1, a little, but daily life is not affected; 2, quite a lot; 3, very much) in the past 1 month. Twenty-one symptoms were derived from the Menopausal Symptom Scale for Japanese women[28] and two additional symptoms were added to assess sexual and urogenital dysfunction. In addition to the total symptom score, the Greene Climacteric Scale[29] was used to calculate component symptom scores for vasomotor, psychological (anxiety and depression), and somatic symptom factors (Table 1).

Profile of Mood States

To evaluate the effects of the equol supplement on mood, participants answered the validated Japanese version of the Profile of Mood States (POMS) at baseline and the end of intervention.[30] The POMS consists of 65 questions concerning 1-week recall of six mood states: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor, Fatigue, and Confusion.

Sample size calculation

To detect a difference of 4 points in menopausal symptom score, using $\alpha = 0.05$ and $\beta = 0.2$ (power, 80%), and assuming a mean menopausal score of 20 points and SD of 8 points, the necessary sample size was estimated to be 47 per group. With two placebo and two treatment groups, a total sample size of 141 was needed. We were able to recruit 141 women, although the sample size of women who began the intervention was 134 and the number of women who completed the intervention was 127. Because perimenopausal and postmenopausal women were expected to have the most frequent and severe symptoms, we also calculated sample size for this group and determined that 90 individuals would give sufficient power. Thus, we recruited 90 perimenopausal and postmenopausal women for the study.

Statistical analysis

We compared baseline symptom scores and changes after intervention among the three study groups and two menopausal groups (premenopausal vs perimenopausal and postmenopausal groups combined) using non-parametric Mann-Whitney and Wilcoxon tests. Based on previous literature, perimenopausal and postmenopausal women were expected to have the highest prevalence of symptoms, compared with premenopausal women of similar age. Thus, to increase sample size and power of analyses, perimenopausal and postmenopausal groups were combined into a perimenopausal/postmenopausal group. Statistical analyses were performed using SPSS statistical software version 14.0 (SPSS, Tokyo, Japan). A P value of <0.05 was considered significant.

RESULTS

General

Of 134 women who began the study, 39 were premenopausal (age, 46.6 ± 3.8 years), 25 were perimenopausal (age, 48.4 ± 4.2 years), and 70 were postmenopausal (age, 53.3 ± 3.1 years). Seven individuals did not complete the post-intervention questionnaire and sample collection. Therefore, statistical analysis was conducted using the complete data of 127 participants (94.8%).

Baseline characteristics at randomization indicated that there were no significant differences among the three treatment groups (Table 2). As expected, significant differences were observed in hormones between menopausal groups (Table 3). The frequency of equol producers among all participants was 34.3%. Except for one woman from the EQ-3 group who experienced a generalized rash at the second week of intervention, no adverse effects were reported during the study. There were no significant changes in FSH, LH, estradiol, and progesterone between baseline and after the 12-week intervention (Tables 4 and 5) in perimenopausal/postmenopausal women.

TABLE 2. *Baseline characteristics of participants by treatment group*

	Placebo (n = 44)	EQ-1 (n = 44)	EQ-3 (n = 46)
Age, mean ± SD, y	50.6 ± 4.9	50.5 ± 4.7	50.5 ± 4.7
Height, mean ± SD, cm	155.5 ± 3.7	157.6 ± 4.4	154.7 ± 5.0
Body weight, mean ± SD, kg	55 ± 8.7	57.9 ± 9.1	54.9 ± 6.9
Body mass index, mean ± SD, kg/m^2	22.3 ± 3.0	22.3 ± 3.0	22.0 ± 3.0
Body fat, mean ± SD, %	30.3 ± 4.0	31.2 ± 4.1	30.9 ± 3.8
Age of menarche, mean ± SD, y	12.9 ± 1.1	12.9 ± 1.4	13.0 ± 1.5
Menopausal symptom score, mean ± SD	19.7 ± 10.7	17.4 ± 9.9	18.4 ± 8.1
Parity, mean ± SD	2.47 ± 1.5	2.61 ± 1.5	2.3 ± 1.1
Premenopausal women, % (n)	22.7 (10)	29.5 (13)	34.8 (16)
Perimenopausal women, % (n)	25.0 (11)	18.2 (8)	13.0 (6)
Postmenopausal women, % (n)	52.3 (23)	52.3 (23)	52.2 (24)
Smoking, %	18.2	22.7	15.2
Equol producer, %	41.9	29.3	37.2

The EQ-1 group took 10 mg equol once a day, the EQ-3 group took 10 mg equol three times a day. There were no significant differences in groups by analysis of variance.

TABLE 3. *Baseline menopausal scores, POMS scores, and hormones by menopausal and equol producer status*

	Equol producer							
	Premenopausal (n = 12)	Perimenopausal (n = 12)	Sig[a]	Postmenopausal (n = 22)	Sig[b]	Sig[c]	Perimenopausal/ postmenopausal (n = 34)	Sig[d]
Menopausal score								
Psychological	2.9 (2.2)	2.8 (3.6)		5.5 (4.8)			4.5 (4.5)	
Anxiety	2.3 (1.9)	2.0 (2.4)		4.1 (4.1)			3.4 (3.7)	
Depression	0.6 (0.7)	0.8 (1.2)		1.4 (1.2)			1.2 (1.2)	
Somatic	3.2 (1.8)	4.8 (2.3)		7.6 (4.4)	[i]		6.6 (4.0)	[i]
Vasomotor	1.6 (1.4)	1.1 (1.2)		3.0 (1.9)	[h]	[i]	2.3 (1.9)	
Total	10.8 (4.9)	14.0 (5.9)		21.3 (11.1)	[i]	[h]	18.7 (10.1)	[i]
POMS								
Tension-Anxiety	46.4 (4.6)	44.3 (4.8)		47.8 (7.5)			46.6 (6.8)	
Depression-Dejection	48.3 (4.1)	44.6 (3.3)	[h]	49.2 (8.4)			47.6 (7.3)	
Anger-Hostility	49.3 (5.6)	46.0 (5.2)		50.0 (9.2)			48.6 (8.2)	
Vigor	49.5 (7.1)	47.2 (7.5)		48.0 (7.9)			47.8 (7.6)	
Fatigue	46.6 (5.5)	44.5 (6.8)		50.1 (9.2)			48.1 (8.7)	
Confusion	47.3 (6.9)	47.1 (5.3)		50.6 (10.1)			49.3 (8.8)	
Hormones								
LH, nmol/L	3.1 (2.3)	12.7 (9.4)	[i]	20.5 (11.6)	[i]		17.8 (11.4)	[i]
FSH, U/L	5.5 (3.3)	27.0 (25.2)	[i]	55.5 (26.3)	[i]	[i]	45.5 (29.0)	[i]
Estradiol, pg/mL	169.0 (139.9)	130.1 (126.7)		30.9 (24.6)	[i]	[h]	78.3 (100.4)	[i]
Progesterone, ng/mL	5.59 (6.76)	0.70 (1.14)		0.60 (1.17)	[i]		0.64 (1.14)	[i]

(Continued on next page)

Values are presented as mean (SD). POMS, Profile of Mood States; Sig, significance; LH, luteinizng hormone; FHS, follicle-stimulating hormone; ANOVA, analysis of variance.
[a]Significant differences between premenopausal and perimenopausal women by Mann-Whitney test.
[b]Significant differences between premenopausal and postmenopausal women by Mann-Whitney test.
[c]Significant differences between perimenopausal and postmenopausal women by Mann-Whitney test.
[d]Significant differences between premenopausal and perimenopausal/postmenopausal women by Mann-Whitney test.
[e]Significant differences between equol non-producer and producer of peri/postmenopausal women by Mann-Whitney test.
[f]Significant differences between premenopausal and perimenopausal/postmenopausal women by Mann-Whitney test.
[g]Significant differences between equol producer status assessed by Mann-Whitney test.
[h]$P < 0.05$.
[i]$P < 0.01$.

Urine S-equol concentrations demonstrated good compliance with the protocol (Fig. 1). After the intervention, mean (median) equol concentrations of equol producers and nonproducers were the following: 6.28 (0.041) μmol/L and 0.33 (0.041) μmol/L for the placebo group, 38.50 (38.25) μmol/L and 25.24 (26.40) μmol/L for the EQ-1 group, and 125.11 (108.15) μmol/L and 88.43 (87.00) μmol/L for the EQ-3 group, respectively. Urine equol concentrations showed good correlation with the equol supplement intake. Compliance with the study protocol was also confirmed by written dietary records. Based on the remnants of supplement packs returned at the end of the intervention, compliance was estimated to be 94.5%.

Intervention

Table 3 shows the baseline menopausal scores, POMS scores, and hormones by menopausal and equol producer status. The anxiety scores of equol producers were lower than those of nonproducers ($P < 0.05$). Significant differences between the premenopausal and perimenopausal/postmenopausal groups were observed for anxiety, somatic, and total symptom scores.

Table 4 shows the menopausal scores, POMS scores, and hormone results for the subgroup of perimenopausal/postmenopausal equol producers. Significant differences between baseline and 12 weeks were observed only in the placebo group.

Table 5 shows the results for the subgroup of perimenopausal/postmenopausal equol nonproducers, the group with the highest symptom scores and the group most likely to benefit from the equol supplement. After the EQ-3 intervention, perimenopausal/postmenopausal equol nonproducers showed significant decreases from baseline in all menopausal symptom scores, except depression ($P < 0.05$), and significant decreases in somatic and total menopausal scores ($P < 0.05$) compared with the placebo group (Table 5). The EQ-3 group also showed significant decreases in Depression-Dejection ($P < 0.05$) and increases in Vigor ($P < 0.01$). Compared with the placebo group, the EQ-3 group showed significant decreases in Tension-Anxiety ($P < 0.05$), Depression-Dejection ($P < 0.05$) and Fatigue ($P < 0.01$) and increases in Vigor ($P < 0.05$).

DISCUSSION

Japanese menopausal women with high soy intake have fewer vasomotor symptoms than their North American counterparts do,[11,31] although Japanese menopausal women commonly report chilliness, shoulder stiffness, and psychological symptoms.[2-4,32-34] This observation led to the hypothesis that isoflavones may act as natural selective estrogen receptor modulators at menopause.[35] Although circumstantial evidence for the beneficial effects of isoflavones is increasing, the results of clinical trials are inconsistent.[12-15] Recent evidence suggests that it is important to stratify study

TABLE 3. *Continued*

	Equol nonproducer									Total population	
	Premenopausal (n = 25)	Perimenopausal (n = 10)	Sig[a]	Postmenopausal (n = 46)	Sig[b]	Sig[c]	Perimenopausal/ postmenopausal (n = 56)	Sig[d]	Sig[e]	Perimenopausal/ postmenopausal vs premenopausal significance[e]	Equol nonproducer vs producer significance[f]
Menopausal score											
Psychological	4.6 (4.2)	9.0 (3.9)	i	6.1 (4.1)		h	6.6 (4.2)	h	h	h	i
Anxiety	3.2 (2.2)	6.6 (2.9)	i	4.7 (3.2)	h		5.0 (3.2)	h	i	h	i
Depression	1.4 (1.6)	2.4 (1.6)		1.4 (1.3)		h	1.6 (1.6)				
Somatic	5.5 (3.2)	7.6 (3.2)		6.3 (3.2)			6.5 (3.2)			h	
Vasomotor	2.2 (1.7)	3.1 (1.7)		2.7 (1.9)			2.8 (1.9)				
Total	16.6 (8.6)	25.6 (9.2)	i	19.9 (9.5)			21.0 (9.6)	h		i	h
POMS											
Tension-Anxiety	47.5 (7.2)	51.8 (5.8)	h	48.7 (7.3)			49.3 (7.1)		h		
Depression-Dejection	48.3 (8.6)	53.0 (7.7)		49.1 (6.8)			49.8 (7.0)				h
Anger-Hostility	50.9 (9.3)	53.2 (8.3)		50.0 (8.7)			50.5 (8.6)				
Vigor	48.7 (11.3)	42.2 (5.3)		44.3 (7.4)			43.8 (7.5)		h		h
Fatigue	50.4 (7.8)	51.5 (7.9)		49.5 (7.6)			49.7 (7.6)				
Confusion	49.6 (10.0)	57.1 (7.8)	h	51.9 (9.1)			52.7 (9.0)		h		
Hormones											
LH, nmol/L	4.7 (4.9)	12.4 (9.7)	h	26.0 (14.1)	i	i	23.5 (14.3)	i		i	
FSH, U/L	9.2 (4.4)	21.0 (19.9)	h	65.2 (25.0)	i	i	56.9 (29.3)	i		i	
Estradiol, pg/mL	192.3 (161.6)	206.0 (172.6)		20.3 (23.7)	i	i	87.5 (136.6)	i		i	
Progesterone, ng/mL	4.65 (7.14)	1.73 (2.56)		0.27 (0.12)	i	h	0.53 (1.20)	i		i	

Values are presented as mean (SD). POMS, Profile of Mood States; Sig, significance; LH, luteinizng hormone; FHS, follicle-stimulating hormone; ANOVA, analysis of variance.
[a]Significant differences between premenopausal and perimenopausal women by Mann-Whitney test.
[b]Significant differences between premenopausal and postmenopausal women by Mann-Whitney test.
[c]Significant differences between perimenopausal and postmenopausal women by Mann-Whitney test.
[d]Significant differences between premenopausal and perimenopausal/postmenopausal women by Mann-Whitney test.
[e]Significant differences between equol non-producer and producer of peri/postmenopausal women by Mann-Whitney test.
[f]Significant differences between premenopausal and perimenopausal/postmenopausal women by Mann-Whitney test.
[g]Significant differences between equol producer status assessed by Mann-Whitney test.
[h]$P < 0.05$.
[i]$P < 0.01$.

TABLE 4. *Menopausal scores, POMS scores, and hormones at baseline and after 12 weeks of equol supplementation in perimenopausal/postmenopausal equol producers*

	Placebo (n = 14)			EQ-1 (n = 10)		EQ-3 (n = 10)	
	Baseline	12 weeks	Significance[a]	Baseline	12 weeks	Baseline	12 weeks
Menopausal scores							
Psychological	5.3 (5.2)	3.4 (3.7)		2.4 (2.1)	1.9 (1.9)	5.6 (4.9)	4.4 (4.5)
Anxiety	4.2 (4.2)	2.3 (2.8)	b	1.7 (1.6)	1.3 (1.5)	3.8 (4.3)	3.2 (3.6)
Depression	1.1 (1.3)	1.1 (1.2)		0.7 (1.1)	0.6 (0.7)	1.8 (1.0)	1.2 (1.4)
Somatic	7.0 (5.1)	5.8 (4.4)		6.1 (3.4)	5.8 (2.6)	6.5 (3.0)	5.7 (3.1)
Vasomotor	3.3 (2.2)	1.8 (2.2)	c	1.2 (1.0)	1.1 (0.9)	2.1 (1.8)	1.6 (1.6)
Total	20.8 (12.5)	14.6 (9.3)	c	13.7 (6.3)	11.7 (4.1)	20.8 (8.3)	15.9 (9.6)
POMS							
Tension-Anxiety	45.6 (7.4)	45.0 (6.1)		45.9 (7.0)	44.7 (5.3)	48.6 (6.0)	47.9 (7.9)
Depression-Dejection	48.0 (9.2)	48.1 (6.3)		46.2 (6.2)	44.5 (3.5)	48.3 (5.8)	50.1 (8.5)
Anger-Hostility	46.8 (9.0)	47.1 (6.4)		47.7 (9.0)	45.8 (4.9)	52 (5.2)	50.6 (7.5)
Vigor	47.4 (9.5)	42.5 (8.2)	b	49.5 (5.9)	46.4 (7.1)	46.6 (6.4)	48.8 (6.8)
Fatigue	46.5 (9.6)	47.6 (10.6)		47.8 (9.9)	47.6 (8.5)	50.6 (6.1)	51.8 (8.1)
Confusion	50.1 (9.3)	48.3 (9.2)		47.1 (8.0)	46.1 (2.2)	50.4 (9.4)	48.5 (7.4)
Hormones							
LH, nmol/L	19.4 (9.0)	19.3 (11.9)		18.0 (16.2)	18.3 (14.9)	15.2 (9.2)	13.7 (6.8)
FSH, U/L	54.7 (32.7)	55.0 (35.8)		37.1 (28.9)	40.1 (27.3)	40.8 (21.7)	40.2 (26.9)
Estradiol, pg/mL	35.1 (25.4)	43.0 (32.8)		103.8 (97.1)	85.2 (55.6)	134.2 (159.5)	143.6 (199.5)
Progesterone, ng/mL	0.27 (0.14)	0.24 (0.12)		1.17 (1.79)	1.72 (2.59)	0.63 (1.01)	0.34 (0.28)

Values are presented as mean (SD). The EQ-1 group took 10 mg equal once a day, and the EQ-3 group took 10 mg equal three times a day. POMS, Profile of Mood States; LH, luteinizing hormone; FSH, follicle-stimulating hormone.
[a]Significant differences between baseline and 12 wk in placebo group by paired Wilcoxon test.
[b]$P < 0.05$.
[c]$P < 0.01$.

TABLE 5. *Menopausal scores, POMS scores, and hormones at baseline and after 12 weeks of equol supplementation in perimenopausal/postmenopausal equol nonproducers*

	Placebo (n = 19)			EQ-1 (n = 18)		EQ-3 (n = 19)			
	Baseline	12 weeks	Significance[a]	Baseline	12 weeks	Baseline	12 weeks	Significance[b]	Significance[c]
Menopausal scores									
Psychological	6.4 (3.7)	6.4 (4.0)		6.9 (5.4)	5.1 (3.4)	6.5 (3.4)	4.4 (3.1)	[e]	
Anxiety	4.7 (2.8)	4.5 (3.2)		5.3 (4.0)	3.9 (2.7)	5.1 (2.7)	3.4 (2.3)	[e]	
Depression	1.7 (1.5)	1.9 (1.1)		1.7 (1.7)	1.2 (1.0)	1.4 (1.1)	1.0 (1.2)		
Somatic	6.8 (3.2)	6.3 (3.2)		6.7 (3.8)	6.1 (2.0)	6.1 (2.7)	4.7 (2.5)	[d]	[d]
Vasomotor	3.2 (1.9)	2.2 (1.5)	[d]	2.7 (1.8)	2.2 (1.7)	2.4 (1.9)	1.4 (1.3)	[d]	
Total	21.4 (9.0)	18.4 (7.6)		21.3 (12.3)	17.3 (6.9)	20.2 (7.5)	13.6 (6.2)	[e]	
POMS									
Tension-Anxiety	48.9 (6.4)	50.7 (9.1)		49.0 (8.8)	47.9 (7.5)	49.8 (6.2)	46.6 (4.6)		[d]
Depression-Dejection	51.3 (7.6)	53.3 (9.6)		49.4 (7.9)	48.6 (6.7)	48.5 (5.6)	46.5 (4.4)	[d]	[d]
Anger-Hostility	50.4 (8.4)	52.4 (7.1)		50.7 (10.1)	50.1 (7.7)	50.4 (7.7)	48.7 (4.7)		
Vigor	44.0 (7.9)	42.6 (6.5)		44.2 (8.1)	45.2 (7.6)	43.4 (6.7)	45.6 (7.1)	[d]	[d]
Fatigue	50.3 (9.3)	54.3 (9.7)		49.8 (8.4)	49.7 (7.5)	49.2 (4.7)	46.5 (5.6)		[e]
Confusion	54.5 (6.2)	54.3 (8.8)		50.4 (10.1)	50.4 (7.0)	53.1 (10.2)	51.6 (6.6)		
Hormones									
LH, nmol/L	21.3 (9.2)	21.0 (9.1)		25.3 (13.3)	23.9 (12.2)	23.9 (19.0)	23.0 (14.3)		
FSH, U/L	50.8 (24.5)	53.4 (27.9)		63.9 (29.2)	65.4 (31.0)	56.5 (33.5)	59.6 (31.4)		
Estradiol, pg/mL	116.0 (154.6)	178.1 (127.8)		43.0 (56.0)	17.5 (7.9)	129.5 (182.9)	96.8 (166.3)		
Progesterone, ng/mL	0.76 (1.61)	1.38 (3.26)		0.28 (0.12)	0.29 (0.14)	0.55 (1.31)	0.46 (0.92)		

Values are presented as mean (SD). The EQ-1 group took 10 mg equal once a day, and the EQ-3 took 10 mg equal three times a day. POMS, Profile of Mood States; LH, luteinizing hormone; FSH, follicle-stimulating hormone.
[a]Significant differences between baseline and 12 wk in placebo group by Wilcoxon test.
[b]Significant differences between baseline and 12 wk in EQ-3 by Wilcoxon test.
[c]Significant differences in 12-wk's changes between placebo and EQ-3 by Mann-Whitney test.
[d]$P < 0.05$.
[e]$P < 0.01$.

populations by the ability to produce equol,[36] because of equol's greater estrogenic activity and affinity for both estrogen receptors.[16,17] In the present study, mood-related symptom scores showed significant improvements after equol supplementation (10 mg, three times per day) compared with placebo in perimenopausal/postmenopausal equol nonproducers.

The first trial assessing the efficacy of soy for the alleviation of menopausal symptoms was published in 1995,[37] and since then, more than 40 others have examined the impact of soy foods or soy-derived isoflavone supplements on hot flash frequency and/or severity. However, numerous published reviews have found inconsistent results.[13,14,35,38] Nagata et al[11] found that the prevalence of hot flashes was inversely related to both the amount of soy foods consumed and the calculated intake of isoflavones. A recent study of 108 Japanese women that examined equol producer status found that hot flashes occurred in only 5% of menopausal women.[26] On the basis of urinary concentrations, equol producers (53.5%) reported less severe symptoms as assessed by a simplified menopausal index score ($P < 0.05$).[26] These data suggest that equol producers comprise a distinct subpopulation that may gain the most benefit from soy isoflavones for relief of menopausal symptoms and that equol nonproducers may benefit from an equol supplement.

Only 34% of participants in this study were equol producers, compared with previous studies that reported close to half for Japanese menopausal women.[26] We recruited women with symptoms, as opposed to conducting a general population study. To the extent that equol-producing ability may lead to decreased symptoms, selecting a population with higher symptom prevalence is likely to result in a lower prevalence of equol-producer ability, such as that observed in this study.

It seems that equol does not have any serious adverse effects. Only one adverse event (generalized rash) occurred in this study, in the second week of the EQ-3 intervention. The participant had previously experienced a similar allergic

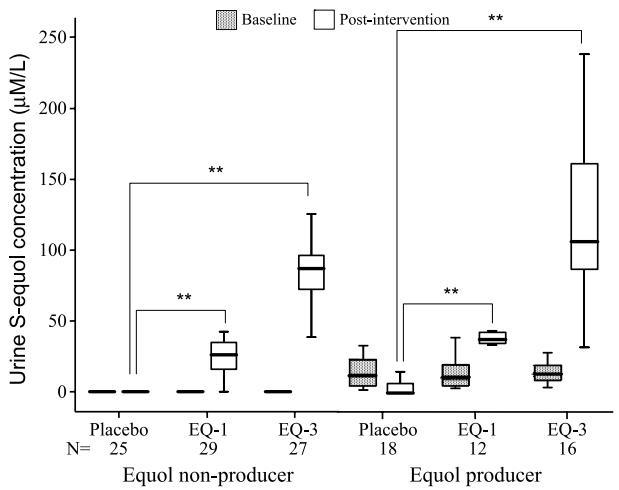

FIG. 1. Equol concentrations (μmol/L) in 24-hour urine samples at baseline and after 12-week intervention. The EQ-1 group took 10 mg equal once a day, and the EQ-3 group took 10 mg equal three times a day. Participants who excreted more than 10 ng/mL (0.041 μmol/L) equol in their 24-hour urine samples were defined as equol producers. Significant differences between baseline and after the intervention by Mann-Whitney rank sum test are shown as $*P < 0.05$ and $**P < 0.01$.

reaction after using propolis. Equol supplementation may affect steroid hormones, but in this study, plasma levels of FSH, LH, and progesterone in premenopausal, perimenopausal, and postmenopausal women did not change after 12 weeks of intervention with 10 mg equol administered once or three times daily. Epidemiological data on populations with habitual soy consumption suggest that long-term therapeutic soy use poses minimal risks.

Most study participants had few symptoms, and thus, sample size limitations may have led to decreased statistical power to identify some true effects. Significant effects of equol supplementation were observed only in perimenopausal/postmenopausal nonproducers, the subpopulation most likely to have symptoms and benefit from equol supplementation. In addition, assessment of long-term effects may be limited by the relatively short 12-week treatment period. Although the short half-life of the S-equol supplement (85 minutes) minimizes cumulative adverse effects,[39] repeat administration is necessary to achieve effective plasma concentrations. In this study, three-times-a-day administration resulted in significant improvement in mood-related symptoms. No significant differences between the EQ-1 and placebo groups (in perimenopausal/postmenopausal equol producers and nonproducers) suggest that a one-time dose of 10 mg equol supplement is insufficient. Given the rapid pharmacokinetic elimination of the equol supplement, it is likely that administration several times daily is required for effectiveness. Lower doses, such as 5 mg, might be sufficient to observe significant improvement in menopausal symptoms and requires further research.

CONCLUSIONS

In conclusion, this is the first study to show the effectiveness of an S-equol supplement made by *L garvieae* spp. For relief of mood-related symptoms in equol nonproducers, three times a day of 10 mg equol supplementation for 12 weeks produced significant improvement in symptoms. This supplement, in combination with 20 mg isoflavones from the habitual diet, did not cause any serious adverse effects, except for one allergic rash. Thus, this equol supplement is a promising alternative to estrogen therapy for menopausal equol nonproducers. Further research is needed on mood-related symptoms and potential underlying hormonal mechanisms.

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