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GENERAL GYNECOLOGY

The impact of combined nutraceutical supplementation on quality of life and metabolic changes during the menopausal transition: a pilot randomized trial

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Abstract
Purpose The aim of this study was to assess the efficacy of a combined nutraceutical supplement on symptoms and early metabolic alterations during the menopausal transition. This pilot randomized study was conducted at the service for menopause disorders of the Department of Obstetrics and Gynecology, Catholic University of the Sacred Heart, Rome, Italy.
Methods Ninety women in menopausal transition who attended our service with menopausal symptoms were enrolled in the study. Sixty patients, randomly assigned to the treatment group, were prescribed one daily tablet of a combined nutraceutical compound with phytoestrogen substances, vitamins, micronutrients and passion flower herbal medicine for 6 months. Thirty patients did not receive any treatment and comprised the control group. The intensity of perimenopausal symptoms was assessed by the modified Kuppermann Index (KI) at enrollment and at 3 and 6 months of treatment. At baseline and at the end of the study, patients underwent a clinical evaluation, a pelvic ultrasound and analysis of blood samples.
Results In the nutraceutical supplemented group, a significant reduction in menopausal symptoms was demonstrated according to the KI after 3 and 6 months of supplementation ($p < 0.01$). The within-group analysis of different KI parameters in the treated group showed a significant improvement in hot flushes ($p < 0.001$), insomnia ($p < 0.01$), fatigue ($p < 0.01$) and irritability ($p < 0.01$). Metabolic parameters did not change significantly in the nutraceutical supplemented group. In the control group, total cholesterol level showed a significant increase ($p < 0.05$).
Conclusions Combined nutraceutical supplementation provides an effective and safe solution for early symptoms occurring during menopausal transition.

Keywords Menopause · Nutraceuticals · Hot flushes · Soy isoflavones · Phytoestrogens

Introduction

Perimenopause is typically defined as the period in which the first biological, endocrine and clinical features of upcoming menopause commence during the period around the final menstrual period (FMP). The Stages of Reproductive Aging Workshop (STRAW) staging system [1], widely accepted as the gold standard for characterizing reproductive aging during menopause, accurately describes the different stages of menopausal transition through simple criteria involving change in menstrual pattern, endocrine and ovarian markers of reproductive aging.

Menopausal transition is characterized as incorporating three stages: early menopausal transition (−2 stage): persistent cycle irregularity, characterized by a ≥7 day difference in length of consecutive cycles at least twice over the previous 10 cycles; late menopausal transition (−1 stage): amenorrhea interval longer than 60 days in the prior 12 months; and early postmenopause (+1 stage): the first year subsequent to the FMP [2]. Therefore the menopausal transition begins with variations in menstrual cycle length and ends one year after the FMP [2]. Adopting these criteria, it has been shown that the menopausal transition may even last several years [3]. During the menopausal transition phase,

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levels of estradiol as well as of follicle-stimulating hormone (FSH) often show a significant fluctuation.

Postmenopausal status is significantly associated with the risk of metabolic syndrome, that may even be induced by the physiological changes in lipid and glucose metabolism that take place in this period [4]. In fact, postmenopausal women show higher total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TG) levels and lower high-density lipoprotein (HDL) cholesterol levels compared with premenopausal women. Moreover, the greatest change in LDL and TG concentration occurs early in the menopausal transition [5]. Increased fasting insulin levels [6] and increased fasting glucose levels [7] have been reported in postmenopausal women, which could indicate a progressive deterioration of insulin sensitivity during the menopause.

Hormone replacement therapy (HRT) successfully decreases vasomotor symptoms linked to the reduction in estrogen levels during early menopause, and thus constitutes the most effective therapy in this period, while during the fluctuating menopausal transitional phase, HRT could be unsuitable. Traditional Chinese medicine has long since approached the perimenopausal period with the aim of balancing and harmonizing these changes, using herbal medicines and nutritional principles [8]. In this connection, other therapeutic choices may be taken into consideration, even though data concerning the best therapeutic approaches in menopausal transition are insufficient.

Moreover, HRT must be avoided in patients at increased risk for breast cancer and cardiovascular pathology [9] as well as in patients who prefer not to start HRT in conjunction with the beginning of the transitional phase when the symptoms are still not well defined. Instead, those women may prefer a nutraceutical/alternative approach.

The term “nutraceutical” is used to describe products derived from food sources, for which extra health benefits are proposed, in addition to the basic nutritional value found in the foods. A range of claims may be made, depending on the specific properties of a nutraceutical product, including prevention of chronic diseases, health improvements, delaying of the aging process, support for the structure or functions of the body, and increased life expectancy [10].

Phytoestrogens are nutraceutical products whose estrogen-like properties may represent safer alternatives to relief menopausal symptoms, and oftentimes patients themselves ask for their prescription. Phytoestrogens appear to exert an estrogenic effect if the circulating estrogen level is low or an anti-estrogenic effect when the estrogen level is high. A recent Japanese randomized controlled trial demonstrated that low-dose isoflavone aglycones also have an effect on psychological symptoms of menopause [11]. The soy isoflavone daidzein has estrogen-like effects that depend predominantly on its intestinal absorption. In fact, daidzein is metabolized by gut bacteria to the active metabolite equol [12]. Among women only 30–50% have the bacteria able to generate equol after ingestion of soy foods [12, 13]. Individuals who can metabolize daidzein to equol are designated ‘equol producers’, and those who cannot convert daidzein to equol as ‘equol nonproducers’. The high variability in equol production is presumably attributable to inter-individual differences in the composition of the intestinal microflora. Certainly, the prevalence of individuals with the equol-producing intestinal bacteria is higher among Asians and vegetarians [13, 14].

Although the estrogen-like properties of S-equol have been investigated for almost 50 years, its action has only more recently been defined. In particular, Setchell et al. [12] described in a broad review the biological and pharmacokinetic properties of equol, together with a summary of the history of research on this molecule. Unlike estradiol, which binds with similar affinity to both estrogen receptors (ERalpha and ERbeta), S-equol preferentially binds to ERbeta [15]. Two observational studies provide support for the efficacy of equol. In one of them, a study of 108 Japanese peri- and postmenopausal women of whom 51.6% were equol producers, there was no difference in the urinary excretion of daidzein and genistein between women with high and low menopausal symptom scores, while patients with lower menopausal symptomatology showed higher S-equol urinary levels [16]. A similar population-based observational study conducted in the U.S. involved 355 women in the menopause transition or in postmenopause. The study demonstrated that among equol producers, higher equol availability attributable to higher soy consumption contributes to a decrease in the number of hot flushes per day [17]. The effects of S-equol on vasomotor symptoms were first evaluated in a 12-week placebo-controlled pilot study involving 134 postmenopausal Japanese women, which included both producers and nonproducers of equol. During the intervention phase, the consumption of soy isoflavones via dietary soy-food intake was limited to no more than 20 mg/day. In the subgroup of equol nonproducing women taking S-equol 10 mg three times daily decreases in the somatic sub-score of the menopausal symptom score, as well as decreases in tension/anxiety, depression and fatigue, were statistically significant. However, no benefits were observed among women who received the lower dose of 10 mg of S-equol once daily [18]. Aso et al. found in a 12-week study involving 99 Japanese non-equol producing women that equol supplementation improved menopausal symptoms [19]. A later study, also by Aso et al., showed beneficial effects of a 10-mg natural S-(-) equol supplement, consumed daily for 12 weeks, on major menopausal symptoms, specifically hot flushes and neck or shoulder muscle stiffness, in postmenopausal Japanese women [20]. A recent U.S. trial [21] had a 4-week placebo run-in phase and an 8-week

intervention in 102 postmenopausal women aged between 45 and 65 years. Most of the subjects were White (81%) and Black/African American (16%). This trial consisted of four groups, three receiving different dosages of S-equol, with a fourth group, receiving soy isoflavones, that served as a positive control. S-equol, 10 mg/day, appears to be effective in reducing the frequency of hot flushes and more effective for relieving muscle and joint pain in postmenopausal women. S-equol, ≥20 mg/day, has been shown to alleviate hot flushes to a greater extent than soy isoflavones in those women who experienced more than 8 hot flushes/day.

The effects of S-equol on cardiovascular disease have been investigated in a small number of clinical trials in Japan. A 12-week study of 54 overweight or obese Japanese subjects, male and female, compared S-equol supplementation (10 mg/day) to placebo and found significantly lower hemoglobin A1c levels, serum LDL cholesterol concentrations and cardio-ankle vascular index scores in patients treated with S-equol. Of particular note, the effects were more prominent in female nonproducers of equol than in female equol producers [22].

However, to be effective, nutraceutical supplementation not only requires a full absorption process, but also finding the right association between different active substances. In particular, the properties shown by two flavonoids, resveratrol and quercetin, appear of interest.

Resveratrol (RES), a bioflavonoid found naturally in grapes, has had cardio-protective and antioxidant activities demonstrated both in vitro and in animal models [10, 23]. Several preclinical studies focused on the properties of RES: it is known for its antioxidant and anti-inflammatory actions and for its ability to upregulate endothelial nitric oxide (NO) synthase (eNOS). RES was able to scavenge OH/O_2 and peroxyl radicals, which can limit the lipid peroxidation processes. However, clinical studies with RES have not been as promising as the preclinical findings of potential benefit in cardiovascular diseases. This could be partly related to the low bioavailability of RES, due to its rapid metabolism [24]. Only a few in vivo studies have demonstrated efficacy for RES, many of them mainly conducted in patients at risk for cardiovascular disease. Wong et al. [25] demonstrated that daily RES consumption was well tolerated and had the potential to maintain healthy circulatory function in obese adults. Another study conducted by Tomé-Carnero et al. [26] demonstrated the anti-inflammatory activities of RES in hypertensive patients with type 2 diabetes mellitus and coronary disease. Resveratrol has also been characterized as a weak phytoestrogen based on its capability to bind to and activate in part the estrogen receptors (ER) [27]. A number of points about the activity of RES remain to be investigated, relating not only to its poor bioavailability [24], but also to dose, length of treatment and best time for initiating treatment.

Quercetin is a flavonoid compound, widely distributed in many vegetables, that has antioxidative and anti-inflammatory properties [28, 29]. Arteaga et al. [28] showed that quercetin has potent antioxidant activity, higher than estradiol. Quercetin may also act synergistically with equol to counter the effects of menopause on cardiovascular risk.

Activity on mood disorders and anxiety has traditionally been associated with the *Passiflora incarnata* L. flower herbal medicine, and is presumably mediated by the modulation of the gamma-aminobutyric acid (GABA) system [30]. Numerous *P. incarnata* derivative products have been commercialized as alternative anxiolytic and sedative remedies based on their long tradition of use and their efficacy that, however, does not appear to be adequately corroborated by the literature data. A recent review [31] suggests that more rigorous methodology should be adopted in new clinical trials conducted to assess the traditional efficacy thought to be associated with *P. incarnata*.

In this connection, we assessed a new nutraceutical product (Zemiar®, Biofutura, Pomezia, Rome, Italy) that has gained approval for the treatment of perimenopausal disorders. The product has been formulated to contain a combination of different molecules: equol (derived from fermented soy standardized to 40% isoflavones) 80 mg/tablet, passiflora 178 mg/tablet, quercetin 150 mg/tablet, resveratrol 10 mg/tablet, magnesium (Mg) 60 mg/tablet, calcium (Ca) 120 mg/tablet, and vitamins D and K 5 and 15 µg/tablet, respectively.

The aim of this prospective study was to assess the efficacy of this combined nutraceutical supplementation on perimenopausal symptoms and early metabolic alterations in women in menopausal transition.

Materials and methods

This pilot non-blinded, randomized, parallel group, efficacy study was conducted between March 2015 and March 2016 at the service for menopause disorders of the Department of Obstetrics and Gynecology, Catholic University of the Sacred Heart, Rome, Italy. The study protocol was approved by the Institutional Review Board of the Department of Obstetrics and Gynecology, Catholic University of the Sacred Heart, Rome, Italy (registration number 14394/14, January 10th 2015) and conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all individuals enrolled in the study.

The primary end point of the study was to evaluate the efficacy of the combination nutraceutical supplementation (Zemiar®) on perimenopausal symptoms as evaluated by the modified Kuppermann Index (KI) score after 6 months.

Ninety women who attended our service, in menopausal transition according to the STRAW staging [1] and with menopausal symptoms, were enrolled in the study. Sixty women were randomly assigned to the nutraceutical treatment and thirty did not receive any treatment; all were followed for 6 months. The random allocation sequence was planned based on a minimal size of the control (untreated) group, for ethical considerations, and was computer-generated using a sequential number of blocks. Exclusion criteria comprised women with abnormal uterine bleeding, diagnosis of endometrial hyperplasia, HRT in the last 3 months, or women who presented other contraindications such as an allergy to one or more of the nutraceutical components. Furthermore, women engaged in particular dietetic protocols, women having diabetes, hypertension, or being treated with lipid-lowering drugs were excluded. Women with a body mass index (BMI) >28 were also excluded.

Of the 60 treated patients, 48 completed the 6-month study (Fig. 1). Five patients were lost to follow-up; three discontinued the treatment after only 3 months due to improvement in symptoms; one woman discontinued the protocol due to a scheduled surgical intervention; and three failed to reach the control visit scheduled after 6 months of treatment. Of the 30 non-treated patients, 27 completed the 6-month study; two were lost to follow-up; one discontinued the protocol because of the initiation of hormone replacement therapy due to significant menopausal symptomatology.

The intensity of perimenopausal symptoms was assessed by the modified KI at enrollment and at 3 and 6 months of treatment. Widely used to evaluate menopause symptoms, the modified KI comprises 13 items, including hot flushes/night sweats, paresthesia, dizziness, arthralgia/myalgia, headache, palpitations and formication (somatic symptoms); insomnia and/or sleep disturbance, depression, irritability, and fatigue (psychological symptoms); and urinary infection and sexual complaints, categorized as urogenital symptoms [32]. The individual items scores are 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The total KI score, calculated as the sum of all of the scores for each item, ranges from 0 to 63. Scores in the ranges of 0–6 (none), 7–15 (mild), 16–30 (moderate), and >30 (severe) were used to grade the degree of severity.

Each woman received a diary in which she reported her daily nutraceutical usage. The diary was also used for registering menstrual bleeding dates as well as declaring any adverse events should they take place during the treatment. The diary consisted of monthly sections that were further divided into days. At baseline, patients had a clinical evaluation, a pelvic ultrasound exam was performed and blood samples withdrawn, and these examinations were repeated at the end of the study. Pelvic ultrasound examinations were performed on the same day of the baseline metabolic determinations. Standardized transvaginal ultrasonography was performed on each patient using a 7.5 MHz transvaginal probe (MyLab25Gold, Esaote, Milan, Italy). Two different operators performed the ultrasound examinations. For all measurements, inter-observer variation was verified not to exceed 3%. Each woman enrolled in the active treatment group was prescribed one tablet daily, to be taken orally for 6 months. All assessments were made at baseline and at the third and sixth months. For each enrolled woman, a blood chemistry panel, along with metabolic and hormonal parameters, was collected (estradiol, FSH, HDL cholesterol, LDL cholesterol, triglycerides, vitamin D, fasting glucose and insulin levels) at baseline and after 6 months of treatment. All hormones were measured by commercial

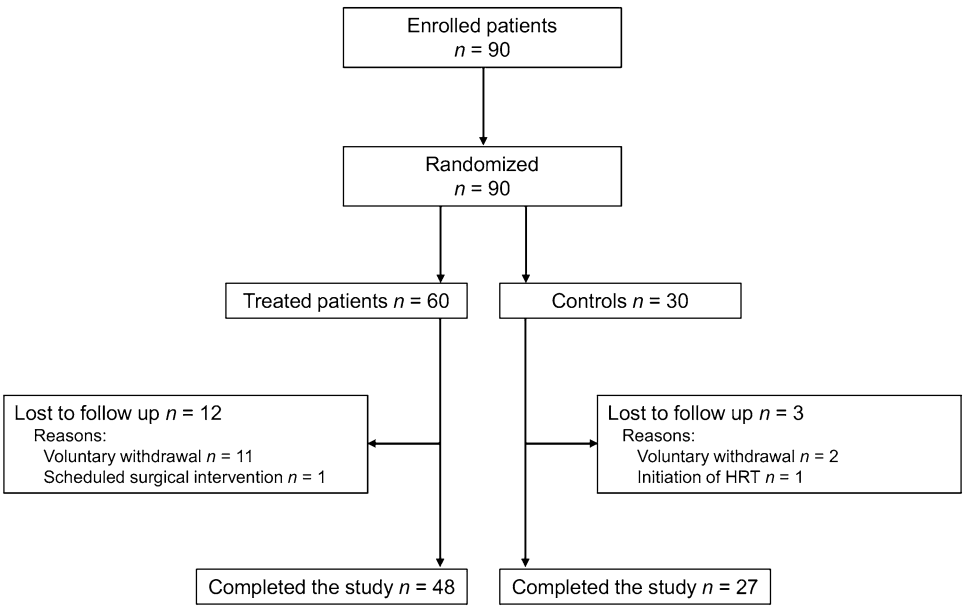

Fig. 1 Flow of patients through the study. *HRT* hormone replacement therapy

radioimmunoassay kits (Radim, Pomezia, Italy). The intra- and interassay coefficients of variation were less than 8 and 15% for all hormones. Plasma glucose concentrations were determined by the glucose oxidase technique, using a glucose analyzer (Beckman Instruments, Palo Alto, CA, USA). Plasma total cholesterol, lipoproteins and triglycerides concentrations were measured using commercially available kits. The safety and tolerability of Zemiar was recorded.

Statistical analysis

The Friedman test was used to compare the values obtained at baseline with those of the follow-ups for the KI score and each of the 13 items. The non-parametric Wilcoxon rank-sum test with z values was used to compare blood chemistry data at baseline and at the 2nd follow-up. The Mann–Whitney test was used to compare results in the two groups at baseline and at 6 months. The result was considered statistically significant when $p < 0.05$.

The sample size was calculated according to literature data reporting the correlation between the modified KI score and the Menopause Rating Scale in Chinese women [33]. The response of modified KI within each subject group was normally distributed with a standard deviation of 9.9. Considering an expected delta between the experimental and control group means of 7.0, at a ratio of 2:1 we needed to analyze 48 experimental subjects and 24 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups were equal with a probability (power) of 0.8. The Type I error probability associated with this test of the null hypothesis is 0.05. Considering a drop-out rate of 20%, we needed to enroll 60 subjects in the experimental group and 30 subjects in the control group.

Statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp, Armonk, NY, USA, Released 2013).

Results

Baseline characteristics of the nutraceutical supplemented group (study group) and of the control group are illustrated in Table 1. At baseline, no differences were found between the nutraceutical supplementation group and the control group. All women were in menopausal transition without significant differences related to the different stages. No serious adverse events were reported. Gastric discomfort was reported in one patient. The number of cycles recorded by women during the 6-month observation was higher in the study group in comparison with the control group ($p < 0.05$) (Fig. 2).

Figure 3 shows the endometrial ultrasound evaluation in the study group and in control group. No significant change in endometrial thickness was noticed at the end of the treatment in the supplemented group, while the control group shows lower endometrial thickness values at 6 months than at baseline evaluation ($p < 0.01$) as well as lower values than the supplemented group ($p < 0.05$).

Figure 4 indicates the modified KI in the nutraceutical supplemented group and in the control group at baseline and after 3 and 6 months of supplementation. At baseline, the mean score of the global KI corresponded to 22 ± 8.5 in the study group and to 21.4 ± 7.8 in the control group, reflecting a moderate degree of symptoms. The most frequent types of symptoms reported by patients were: hot flushes (mild, moderate, severe) in 94% of patients, insomnia in 91%, fatigue in 93%, irritability 93% and sexual complaints in 79% of patients.

Specifically, in the nutraceutical supplemented group, the global score of the modified KI significantly decreased in comparison with the control group after 3 and 6 months of treatment ($p < 0.01$). Furthermore, although less clinically relevant, the within-treated group changes show a significant improvement of some specific symptoms of the modified KI for hot flushes ($p < 0.001$), insomnia

Table 1 Baseline characteristics of treated patients and controls

	Study group ($n = 48$)	Controls ($n = 27$)
Age, years	49.3 ± 3.9	49.2 ± 2.3
BMI, kg/m^2	23 ± 3.9	23.5 ± 2.6
SBP, mmHg	130 ± 3.8	129.5 ± 3.5
Waist, cm	86 ± 4.6	85.9 ± 4.6
Smoking current status, % of patients	13.3	14.2
Any alcohol consumption, % of patients	78.9	77.8
Menopausal transition stage		
MT −2, % of patients	44	52
MT −1, % of patients	51	46
MT +1, % of patients	5	2

Data are mean ± SD unless otherwise indicated

BMI body mass index, *MT* menopausal transition, *SBP* systolic blood pressure

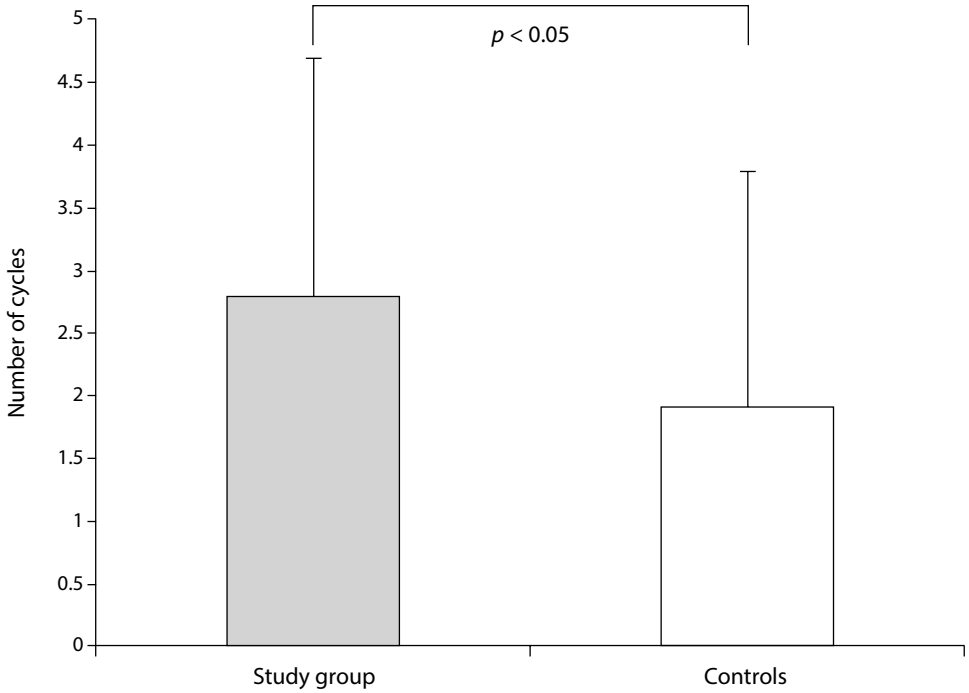

Fig. 2 Number of cycles during the 6-month follow-up of the study in the study group (supplemented patients) and in controls

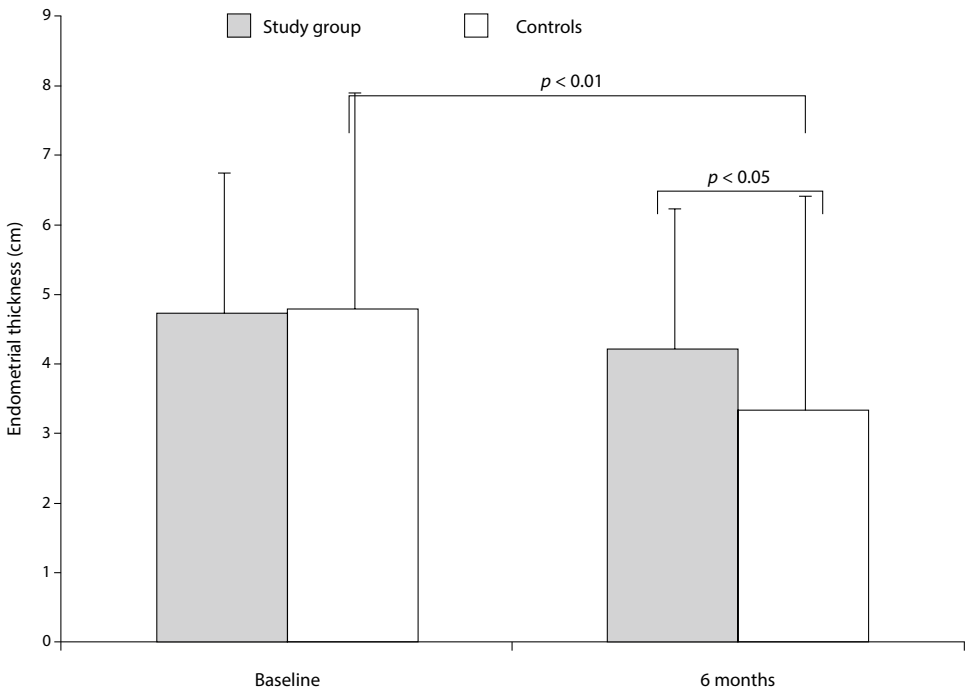

Fig. 3 Ultrasound evaluation of the endometrial thickness in the study group and in controls

($p < 0.01$), fatigue ($p < 0.01$) and irritability ($p < 0.01$) (Fig. 5).

Metabolic characteristics of the study group compared with the control group are shown in Table 2. The two groups do not differ at baseline for any studied features. At the 6-month follow-up, in the treated group, estrogen levels showed a mild, yet non-significant increase. Comparing the metabolic baseline parameters with the values at the 6-month follow-up, no values showed a significant change in the nutraceutical supplemented group, with the exception of vitamin D levels, that significantly increased ($p < 0.001$). In both groups, BMI and glycemic parameters did not change significantly. In the control group, total cholesterol level showed a significant increase ($p < 0.05$). At 6 months,

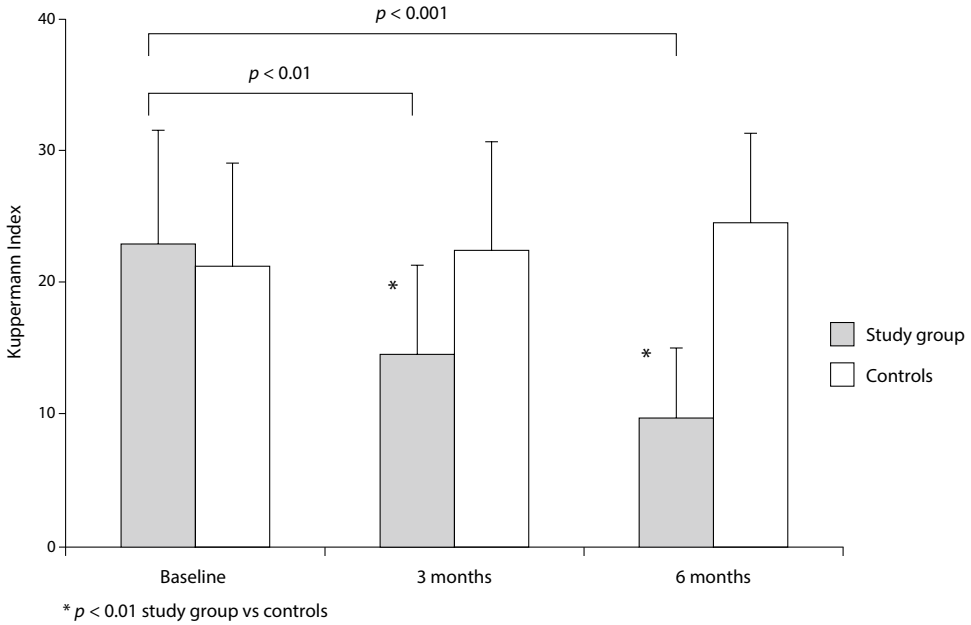

Fig. 4 Modified Kuppermann Index values at 3 and 6 months in the study group and in controls

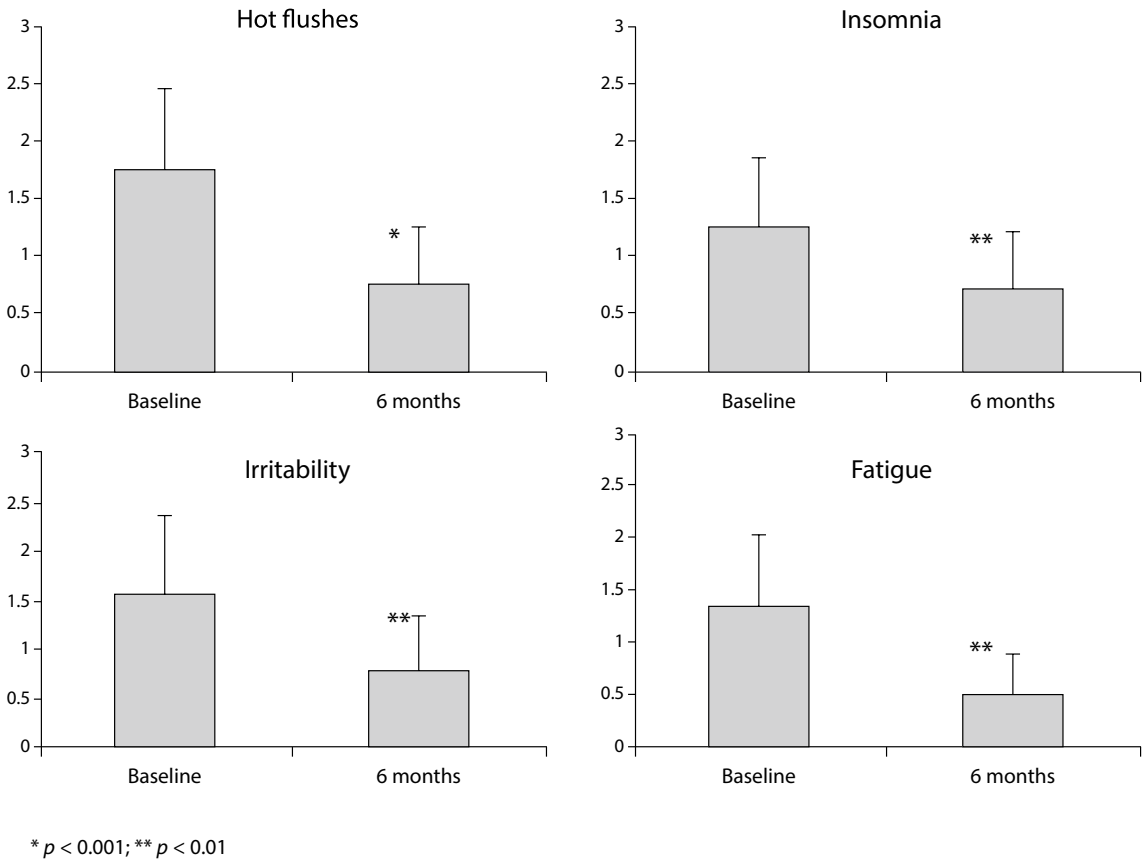

Fig. 5 Single score results of the most frequent symptoms (hot flushes, insomnia, irritability and fatigue) in the study group at baseline and after 6 months

Table 2 Endocrine and metabolic changes before and after 6 months of nutraceutical supplementation in the study group and in controls

	Study group			Controls		
	Baseline	6 months	*p*	Baseline	6 months	*p*
BMI, kg/m^2	23.1 ± 3.9	22.5 ± 2.8		23.5 ± 2.6	24 ± 1.9	
E2, pg/mL	69.1 ± 77.3	75.8 ± 87.7		72.4 ± 46.1	68.8 ± 87.7	
FSH, mUI/mL	48.5 ± 27.9	50.0 ± 28.9		45.4 ± 29.0	50.0 ± 28.9	
LH, mUI/mL	25.7 ± 14.6	30.3 ± 20.4		22.1 ± 13.3	29.5 ± 23.8	
Total cholesterol, mg/dL	208.2 ± 33.7	198.2 ± 27.2		194.7 ± 18.7	212.1 ± 27.1	0.05
LDL cholesterol, mg/dL	122.0 ± 32.0	120.7 ± 25.1		121.8 ± 17.6	114.5 ± 30.1	
HDL cholesterol, mg/dL	64.9 ± 15.0	67.1 ± 12.5*		60.5 ± 11.1	58.1 ± 13.3	
Triglycerides, mg/dL	91.4 ± 30.0	88.0 ± 39.6		92.7 ± 33.1	92.2 ± 43.7	
Glucose mg/mL	88.7 ± 6.2	87.3 ± 5.7		88.9 ± 10.2	89.5 ± 8.5	
Insulin, µUI/mL	8.6 ± 2.5	8.4 ± 2.5		8.9 ± 2.7	9.1 ± 3.3	
Vitamin D, ng/mL	22.6 ± 8.0	30.4 ± 8.8	0.01	19.3 ± 5.0	22.7 ± 4.7	

BMI body mass index, *E2* estradiol, *FSH* follicle-stimulating hormone, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *LH* luteinizing hormone

* $p < 0.05$ for patients vs controls at 6 months

nutraceutical supplemented patients showed significantly higher HDL cholesterol levels in comparison with the control population ($p < 0.01$).

Discussion

Perimenopause is a crucial phase in a woman's life that may vary in duration and intensity of symptoms among women worldwide. In this paper, we analyzed paramount parameters that characterize this period and examined their effect on the quality of life of the women before and after 6 months of supplementation with a nutraceutical chosen for the synergistic activity of its constituents and composed of equol, resveratrol, quercetin, passiflora, vitamin D, vitamin K, Ca and Mg. Our study demonstrated a significant reduction in the global symptoms score and an improvement in the perceptions of hot flushes that could be explained by the estrogen-like action of equol, the active metabolite of the soy isoflavone, daidzein.

This study has primarily demonstrated the effect of equol supplementation in an Italian perimenopausal population. This is in agreement with recent studies demonstrating the efficacy of equol supplementation on vasomotor symptoms both in postmenopausal equol producer Japanese women [19] and in postmenopausal Western equol nonproducers [21]. However, most randomized, blinded, comparative clinical trials of soy isoflavonoids, as reviewed in several recent meta-analyses, have found them to be no more effective than a placebo. On the other hand, most studies have been criticized for numerous study design defects or for the difficulty of comparing different doses and compounds. Moreover, taking into consideration data on the absorption of isoflavonoids, there may be a difference between women who can convert daidzein to equol and nonconverters, who would be unlikely to respond. For this reason, the 2015 Position Statement of the North American Menopause Society on the nonhormonal management of menopause-associated vasomotor symptoms indicated only equol in the summary levels of evidence and recommendation. In particular, studies on equol reached the level II of evidence and equol supplementation was indicated as "recommend", although with caution [34].

Our study demonstrated that nutraceutical supplementation significantly reduced vasomotor symptoms, in patients with moderate intensity of hot flushes at enrollment. Furthermore, our study demonstrated the capability of this combined nutraceutical supplement to also benefit neurological and psychological symptoms such as insomnia, fatigue and irritability, probably by the concomitant interaction of passiflora and resveratrol.

During the transitional menopausal phase there is a fluctuation in estrogenic and in gonadotropic levels [35, 36]. Therefore, during the supplementation period there might have been some phases during which endogenously secreted estrogens may have attenuated the severity of symptoms. In fact, the average estradiol level at the end of the study tended to be elevated compared with its level at enrollment. On the other hand, it is possible that the supplementation "per se" provided a form of preservation of residual ovarian functional activity that contributed to the reduction of the vasomotor symptoms.

Furthermore, the combination of equol and resveratrol might have produced a cumulative efficacy on the vasomotor symptoms greater than the efficacy that might have been produced by each molecule administered separately.

The global effect of the nutraceutical supplementation resulted in a reduction of the total score indicating overall improvement in the patients' quality of life.

The Study of Women's Health Across the Nation (SWAN study) has characterized the biological, psychosocial and endocrine changes that occur during the menopausal transition [37]. Subsequent studies documented that low estradiol and sex hormone binding globulin (SHBG) levels as well as high free androgen index (FAI) levels are strongly associated with cardiovascular risk factors, and that menopausal transition, including the changing hormonal milieu, is associated with elevated vasomotor symptoms together with changes in lipid profile, particularly LDL cholesterol [5, 38–41].

The SWAN Heart Study, an ancillary study to the SWAN study, showed that women with hot flushes had reduced flow mediated dilation and greater aortic calcification, highlighting that hot flushes may mark adverse vascular changes among midlife women even in the transitional period [36].

Our findings showed that, although the lipid pattern did not exhibit a significant improvement during the 6 months of nutraceutical supplementation, patients' blood lipid levels remained similar to those in the basal condition assessment. On the contrary, in the control group a significant increase of total cholesterol levels was noticed.

The association of equol, resveratrol and quercetin may have had a synergistic effect, acting mainly on the reduction of LDL peroxidation and on reducing oxidative stress, as has been shown in some studies in animals and humans [25, 26, 28, 29, 42, 43].

In our study, no improvement in glycemic parameters was observed. Other studies [29, 44] have shown positive effects, mainly in hypercholesterolemic patients and a moderate effect in patients with a normal lipid profile, similar to the patients analyzed in this study.

Therefore, even if the nutraceutical supplementation did not cause any improvement in total cholesterol, LDL cholesterol and triglyceride levels during the short length of the study, after 6 months treated patients showed significantly higher HDL cholesterol levels in comparison with controls. On the other hand, in the control group the total cholesterol level increased significantly. This may mean that nutraceutical supplementation might have counteracted the gradual worsening of the lipid metabolism known to occur in the menopausal period.

In particular, no serious adverse effects associated with the nutraceutical supplementation were reported and the nutraceutical supplementation was generally well tolerated. Moreover, the absence of endometrial growth during the treatment indicates the neutral uterine effect of the nutraceutical supplementation.

Our study presents some limitations. First, this is an efficacy study in a small sample of an Italian homogeneous population. Second, the short-term duration of the study may have prevented the identification of the gradual changes in the menopausal transition. Third, the ideal dose of equol or of the other compounds has not been definitively identified for menopausal women.

In conclusion, our study shows that combined nutraceutical compounds can provide an effective and safe approach for improving early symptoms occurring during menopausal transition. Furthermore, nutraceutical supplementation is well accepted by women and may constitute an alternative to hormonal therapies in this specific period in the woman's life.

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Author contributions PV: project development, manuscript writing, data analysis. IDA: data collection, manuscript editing. CB: data collection, data analysis. CC: data collection. GD: data collection. MCM: data analysis. GS: project development. AL: protocol development.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study protocol was approved by the Institutional Review Board of the Department of Obstetrics and Gynecology, Catholic University of the Sacred Heart, Rome, Italy (registration number 14394/14, January 10th, 2015) and conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Informed consent A written informed consent was obtained from all individuals enrolled in the study.

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