

GENISTEINA

Am J Clin Nutr. 2014 Jul;100 Suppl 1:423S-30S. doi: 10.3945/ajcn.113.071464.

Soy foods, isoflavones, and the health of postmenopausal women.

Messina M.

Abstract

Over the past 2 decades, soy foods have been the subject of a vast amount of research, primarily because they are uniquely rich sources of isoflavones. Isoflavones are classified as both phytoestrogens and selective estrogen receptor modulators. The phytoestrogenic effects of isoflavones have led some to view soy foods and isoflavone supplements as alternatives to conventional hormone therapy. However, clinical research shows that isoflavones and estrogen exert differing effects on a variety of health outcomes. Nevertheless, there is substantial evidence that soy foods have the potential to address several conditions and diseases associated with the menopausal transition. For example, data suggest that soy foods can potentially reduce ischemic heart disease through multiple mechanisms. Soy protein directly lowers blood low-density lipoprotein-cholesterol concentrations, and the soybean is low in saturated fat and a source of both essential fatty acids, the omega-6 fatty acid linoleic acid and the omega-3 fatty acid alpha-linolenic acid. In addition, isoflavones improve endothelial function and possibly slow the progression of subclinical atherosclerosis. Isoflavone supplements also consistently alleviate menopausal hot flashes provided they contain sufficient amounts of the predominant soybean isoflavone genistein. In contrast, the evidence that isoflavones reduce bone loss in postmenopausal women is unimpressive. Whether adult soy food intake reduces breast cancer risk is unclear. Considerable evidence suggests that for soy to reduce risk, consumption during childhood and/or adolescence is required. Although concerns have been raised that soy food consumption may be harmful to breast cancer patients, an analysis in 9514 breast cancer survivors who were followed for 7.4 y found that higher postdiagnosis soy intake was associated with a significant 25% reduction in tumor recurrence. In summary, the clinical and epidemiologic data indicate that adding soy foods to the diet can contribute to the health of postmenopausal women.

Climacteric. 2016 Oct 6:1-11.

Effects of phytoestrogens on bone mineral density during the menopause transition: a systematic review of randomized, controlled trials.

Abdi F, Alimoradi Z, Haqi P, Mahdizad F.

Abstract

INTRODUCTION:

Menopause is associated with increased bone resorption and decreased bone mineral density (BMD). Phytoestrogens are believed to prevent bone loss. This study reviewed relevant randomized, controlled trials to determine the effects of phytoestrogens on BMD in postmenopausal women.

METHODS:

In order to perform this systematic review, PubMed, Science Direct, Scopus, Cochrane Library, ISI Web of Knowledge, and ProQuest databases were searched for articles published during 2005-2016. The main keywords used during the searches were "phytoestrogen" and "bone mineral density" and "menopause". The Cochrane Risk of Bias Assessment Tool was used to evaluate the quality of the selected studies and to assess the risk of bias.

RESULTS:

A total of 23 eligible studies were included in this systematic review. Most selected studies used a double-blind, placebo-controlled design. In total, 3494 participants were enrolled in the selected trials. Different types of soy isoflavone extracts, including genistein extracts (either alone or in combination with daidzein), dietary products containing different amounts of phytoestrogens, and red clover extracts were used in the designed interventions. The duration of the interventions ranged from 7 weeks to 3 years. In most studies, the primary outcome was the efficacy of the designed intervention which was assessed through measuring whole body or regional BMD or bone mineral content, T-scores, and biomarkers of bone metabolism.

CONCLUSIONS:

Isoflavones probably have beneficial effects on bone health in menopausal women. Nevertheless, there were controversial reports about changes in BMD. Supplementation with a phytoestrogen can probably prevent the reduction in BMD and maintain a healthy bone structure during menopause.

Osteoporos Int. 2014 Mar;25(3):1123-9. doi: 10.1007/s00198-013-2512-5.

Genistein effects on quality of life and depression symptoms in osteopenic postmenopausal women: a 2-year randomized, double-blind, controlled study.

Atteritano M, Mazzaferro S, Bitto A, Cannata ML, D'Anna R, Squadrito F, Macrì I, Frisina A, Frisina N, Bagnato G.

Abstract

SUMMARY:

Postmenopausal estrogen decline is implicated in several age-related physical and psychological changes in women, including decreases in perceived quality of life. The phytoestrogen genistein at a dose of 54 mg daily in osteopenic postmenopausal women after 2 years implies an improvement on quality of life and depression symptoms.

INTRODUCTION:

Postmenopausal estrogen decline is implicated in several age-related physical and psychological changes in women, including decreases in perceived quality of life (QoL). A number of trials with hormone therapy showed beneficial effects of the intervention on quality of life parameters. However, because of known or suspected serious side effects of conventional hormone therapy, there is a need for alternatives.

METHODS:

We conducted a double-blind randomized placebo-controlled trial using the isoflavone genistein, 54 mg, or placebo for 2 years. In this trial, we recruited 262 postmenopausal women aged 49 to 67 years.

RESULTS:

At baseline, after 1 year, and at final visit, participants filled in the Short Form of 36 questions (SF-36) and the Zung Self-rating Depression Scale (ZSDS). For the placebo group, scores on all dimensions of the SF-36 decreased after 1 and 2 years. The genistein group showed increases on all dimensions of the SF-36 at the end of the study. There were, however, statistically significant differences in changes of scores between the two intervention groups. For the ZSDS, similarly, significant differences were found between groups.

CONCLUSION:

In conclusion, the findings of this randomized trial showed that genistein improves quality of life (health status, life satisfaction, and depression) in osteopenic postmenopausal women.

CIMICIFUGA

Springerplus. 2015 Feb 10;4:65. doi: 10.1186/s40064-015-0808-y. eCollection 2015.

A systematic review of non-hormonal treatments of vasomotor symptoms in climacteric and cancer patients.

Drewe J, Bucher KA, Zahner C.

Abstract

The cardinal climacteric symptoms of hot flushes and night sweats affect 24-93% of all women during the physiological transition from reproductive to post-reproductive life. Though efficacious, hormonal therapy and partial oestrogenic compounds are linked to a significant increase in breast cancer. Non-hormonal treatments are thus greatly appreciated. This systematic review of published hormonal and non-hormonal treatments for climacteric, and breast and prostate cancer-associated hot flushes, examines clinical efficacy and therapy-related cancer risk modulation. A PubMed search included literature up to June 19, 2014 without limits for initial dates or language, with the search terms, (hot flush* OR hot flash*) AND (clinical trial* OR clinical stud*) AND (randomi* OR observational) NOT review). Retrieved references identified further papers. The focus was on hot flushes; other symptoms (night sweats, irritability, etc.) were not specifically screened. Included were some 610 clinical studies where a measured effect of the intervention, intensity and severity were documented, and where patients received treatment of pharmaceutical quality. Only 147 of these references described studies with alternative non-hormonal treatments in post-menopausal women and in breast and prostate cancer survivors; these results are presented in Additional file 1. The most effective hot flush treatment is oestrogenic hormones, or a combination of oestrogen and progestins, though benefits are partially outweighed by a significantly increased risk for breast cancer development. This review illustrates that certain non-hormonal treatments, including selective serotonin reuptake inhibitors, gabapentin/pregabalin, and Cimicifuga racemosa extracts, show a positive risk-benefit ratio. Key points. Several non-hormonal alternatives to hormonal therapy have been established and registered for the treatment of vasomotor climacteric symptoms in peri- and post-menopausal women. There are indications that non-hormonal treatments are useful alternatives in patients with a history of breast and prostate cancer. However, confirmation by larger clinical trials is required.

Chin Med. 2013 Nov 1;8(1):20. doi: 10.1186/1749-8546-8-20.

Efficacy of black cohosh (*Cimicifuga racemosa* L.) in treating early symptoms of menopause: a randomized clinical trial.

Mohammad-Alizadeh-Charandabi S, Shahnazi M, Nahaei J, Bayatipayan S.

Abstract

BACKGROUND:

This study aims to evaluate the efficacy of Black cohosh (*Cimicifuga racemosa* L.) in treating early menopausal symptoms.

METHODS:

This randomized, double-blind, placebo-controlled clinical trial was conducted on 84 early post-menopausal participants with Greene climacteric scale (GCS) scores of 15 to 42, who were referred to two public health care centers in Tehran, Iran, in 2011-2012. The participants were randomly allocated into treatment (6.5 mg of dried extract of Black cohosh roots daily) and control (placebo) groups with a ratio of 1:1. The participants took one tablet per day for 8 weeks. The GCS scores were recorded at baseline, and after 4 and 8 weeks of treatment. Data analysis was carried out using a general linear model with repeated measures with SPSS software. The level of significance was set at $P < 0.05$.

RESULTS:

There was no loss to follow-up during the 8 weeks of treatment. The GCS total score (primary outcome) in the treatment group was significantly lower than that in the control group at both week 4 [adjusted mean difference: -7.8 (95% confidence interval: -11.1 to -4.4)] and week 8 [-12.9 (-16.2 to -9.3)]. The treatment group showed significantly more improvement than the control group in all GCS subscale scores (vasomotor, psychiatric, physical, and sexual symptoms; secondary outcomes). The differences between the treatment and control groups at week 8 were significantly higher ($P < 0.001$) than those at week 4 in terms of the total scores and the vasomotor and psychiatric subscale scores. No side effects were reported.

CONCLUSIONS:

Black cohosh reduced the GCS total score and all GCS subscale scores (vasomotor, psychiatric, physical, and sexual symptoms) during 4 and 8 weeks of treatment.

CLINICAL TRIAL REGISTRATION:

This study was approved (Code 9061) by the Ethics Committee of Tabriz University of Medical Sciences and registered at the Iranian Registry of Clinical Trials with IRCT201107186709N4 on 15 January 2012.

Gynecol Endocrinol. 2011 Oct;27(10):844-8. doi: 10.3109/09513590.2010.538097. Epub 2011 Jan 13.

Black cohosh (*Cimicifuga racemosa*) in tamoxifen-treated breast cancer patients with climacteric complaints - a prospective observational study.

Rostock M, Fischer J, Mumm A, Stammwitz U, Saller R, Bartsch HH.

Abstract

OBJECTIVE:

The antihormonal therapy of breast cancer patients with the antiestrogen tamoxifen often induces or aggravates menopausal complaints. As estrogen substitution is contraindicated, herbal alternatives, e.g. extracts of black cohosh are often used.

DESIGN:

A prospective observational study was carried out in 50 breast cancer patients with tamoxifen treatment. All patients had had surgery, most of them had undergone radiation therapy (87%) and approximately 50% had received chemotherapy. Every patient was treated with an isopropanolic extract of black cohosh (1-4 tablets, 2.5 mg) for 6 months. Patients recorded their complaints before therapy and after 1, 3, and 6 months of therapy using the menopause rating scale (MRS II).

RESULTS:

The reduction of the total MRS II score under black cohosh treatment from 17.6 to 13.6 was statistically significant. Hot flashes, sweating, sleep problems, and anxiety improved, whereas urogenital and musculoskeletal complaints did not change. In all, 22 patients reported adverse events, none of which were linked with the study medication; 90% reported the tolerability of the black cohosh extract as very good or good.

CONCLUSIONS:

Black cohosh extract seems to be a reasonable treatment approach in tamoxifen treated breast cancer patients with predominantly psychovegetative symptoms.

Phytother Res. 2011 Aug;25(8):1153-9. doi: 10.1002/ptr.3400. Epub 2011 Feb 3.

A double-blind, placebo-controlled investigation of the effects of *Passiflora incarnata* (passionflower) herbal tea on subjective sleep quality.

Ngan A, Conduit R.

Abstract

Passiflora incarnata is a traditional herbal sedative, anxiolytic and a popular sleep aid used for the treatment of sleep disturbance. Several controlled experiments have demonstrated enhanced sleep in laboratory animals, but clinical trials in humans are lacking. The aim of the present study was to investigate the efficacy of *Passiflora incarnata* herbal tea on human sleep, as measured using sleep diaries validated by polysomnography (PSG). This study featured a double-blind, placebo-controlled, repeated-measures design with a counterbalanced order of treatments (passionflower vs placebo tea), separated by a 1 week 'washout' period. Forty-one participants (18-35 years) were exposed to each treatment for a week, whereby they consumed a cup of the tea and filled out a sleep diary for 7 days, and completed Spielberger's state-trait anxiety inventory on the seventh morning. Ten participants also underwent overnight PSG on the last night of each treatment period. Of six sleep-diary measures analysed, sleep quality showed a significantly better rating for passionflower compared with placebo ($t(40) = 2.70$, $p < 0.01$). These initial findings suggest that the consumption of a low dose of *Passiflora incarnata*, in the form of tea, yields short-term subjective sleep benefits for healthy adults with mild fluctuations in sleep quality.

ZINCO

Arch Biochem Biophys. 2016 Dec 1;611:113-119. doi: 10.1016/j.abb.2016.06.003. Epub 2016 Jun 7.

Zinc and skin biology.

Ogawa Y, Kawamura T, Shimada S.

Abstract

Of all tissues, the skin has the third highest abundance of zinc in the body. In the skin, the zinc concentration is higher in the epidermis than in the dermis, owing to a zinc requirement for the active proliferation and differentiation of epidermal keratinocytes. Here we review the dynamics and functions of zinc in the skin as well as skin disorders associated with zinc deficiency, zinc finger domain-containing proteins, and zinc transporters. Among skin disorders associated with zinc deficiency, acrodermatitis enteropathica is a disorder caused by mutations in the ZIP4 transporter and subsequent zinc deficiency. The triad acrodermatitis enteropathica is characterized by alopecia, diarrhea, and skin lesions in acral, periorificial, and anogenital areas. We highlight the underlying mechanism of the development of acrodermatitis because of zinc deficiency by describing our new findings. We also discuss the accumulating evidence on zinc deficiency in alopecia and necrolytic migratory erythema, which is typically associated with glucagonomas.

ARTICOLI DISPONIBILI SOLO IN ABSTRACT

Effectiveness of red clover in alleviating menopausal symptoms: a 12-week randomized, controlled trial

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Climateric Vol 18- 2015, Pages 568-573 , Published online: 24 Feb 2015

Abstract

Objectives Menopausal women are interested in alternative therapy to alleviate climacteric symptoms. This study aimed to investigate the effect of red clover on the severity of menopausal symptoms.

Methods This randomized, placebo-controlled, clinical trial included 72 healthy postmenopausal women, who were randomly divided into intervention and placebo groups. Women in the intervention group received two capsules containing 40 mg dried leaves of red clover daily for 12 weeks, while those in the control group received two capsules containing 40 mg starch daily for 12 weeks. Outcome measures of this study were menopausal symptoms determined using the Menopause Rating Scale (MRS) at baseline and at the end of the study. Sociodemographic data of the included women were collected using a questionnaire.

Results No significant differences were observed between the two groups with respect to the demographic characteristics and menopausal symptoms before the intervention. At the end of the study, the total score on the MRS decreased from 20.41 to 10.08 in the intervention group and from 20.77 to 17.20 in the control group. This decrease in the total score was attributed to the scores of vegetative somatic and psychological categories of menopausal symptoms. Thus, a remarkable difference was observed in the severity of the menopausal symptoms (95% confidence interval, $p = 0.0001$).

Conclusion These results indicated that, compared to placebo, dried leaves of red clover were more effective in reducing the severity of menopausal symptoms in postmenopausal women.

The effect of Hop (*Humulus lupulus* L.) on early menopausal symptoms and hot flashes: A randomized placebo-controlled trial

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Complement Ther Clin Pract, 2016 May;23:130-5.

Abstract

Objective: This study aimed to evaluate the efficacy of Hop on early menopausal symptoms and hot flashes.

Methods: In this randomized controlled trial, 120 women were randomly allocated into two groups, receiving the Hop or placebo tablets for 12 weeks. Early menopausal symptoms were assessed using Greene scale and hot flashes were recorded in a diary before, and 4, 8 and 12 weeks after intervention.

Results: The mean Greene score was significantly lower in the Hop group than the placebo group at the end of weeks 4 (adjusted difference: -10.0, 95% confidence interval: -11.1--8.9), 8 (-18.6, -20.1--17.1) and 12 (-23.4, -25.1--21.6). The number of hot flashes was significantly lower in the Hop group than the control group during the weeks 4 (-8.4, -9.8--7.1), 8 (-17.1, -14.9--19.3) and 12 (-23.8, -21.1--26.4).

Conclusions: Hop effectively reduced the early menopausal symptoms.

Clinical trial registration: This study was approved (code 91209) by the Ethic Committee of Tabriz university of Medical Sciences and registered at the Iranian registry of clinical trials, with IRCT 2013010110324N7 on April 2013.

Keywords: Early menopausal symptoms; Hop (*Humulus lupulus* L.); Menopause; Randomized clinical trial.

The Therapeutic Role of Genistein in Perimenopausal and Postmenopausal Women

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J Clin Aesthet Dermatol. 2024;17(10):45–53.

OBJECTIVE: We sought to review the biology and clinical benefits of genistein, a plant-derived isoflavone with emphasis on perimenopausal and postmenopausal women. The focus is on assessing its impact on skin health and aesthetics as well as bone density and cardiovascular and metabolic functions. **METHODS:** This narrative review used PubMed to collect studies relating to the biology and clinical effects of genistein on postmenopausal signs and symptoms, including bone density loss, metabolic issues and symptoms, and skin aging. Articles were selected based on relevance to the scope of genistein's influence on estrogen receptors and their downstream effects. This review included *in vitro*, *in vivo*, animal, and human studies. **RESULTS:** According to the current literature, genistein demonstrates efficacy in mitigating menopausal signs and symptoms such as hot flashes, bone density loss and rate of osteoporosis, and skin aging. It shows a protective effect against cardiovascular diseases by improving lipid profiles, weight changes, and reducing low-density lipoprotein cholesterol. It also displays benefits in increasing bone mineral density but has not displayed the side effects commonly associated with estrogen replacement. Regarding skin health, genistein appears to enhance photoprotection, wound healing, elasticity, and hydration, inhibits skin cancer, and reduces wrinkles. **CONCLUSION:** Genistein acts as a selective estrogen receptor modulator (SERM) with benefits across a spectrum of menopausal signs and symptoms, presenting a viable alternative to estrogen replacement in perimenopausal and postmenopausal women. Its utility extends to improving cardiovascular health, bone density, and skin quality, making it a comprehensive treatment option for peri and postmenopausal women. **KEYWORDS:** Flavonoid, genistein, estrogen, hormone replacement therapy, skin health, treatment

As humans age, virtually all organ systems lose their full functional ability. Bone loss and cardiovascular compromise are common; and skin becomes thinner, drier, and less elastic. This decline in structure and function is tied to the cumulative effects of factors like time, photoaging, hormonal imbalances, environmental stressors, and metabolic slowing.^{1,2} In women, many deleterious organ system changes are emphasized during and after menopause, the natural end of menstruation due to ovarian failure, which occurs at a mean of 51.5 years of age in Western societies.³ The process of menopause causes significant fluctuations followed by sustained declines in ovarian hormones. Decreases in the sex hormone estrogen have been linked to numerous physiologic effects, including vasomotor symptoms (VMS), an increased risk of cardiovascular disease, bone loss and increased risk of significant osteoporosis, and changes to the skin.⁴ Management of declining estrogen levels through exogenous hormone supplementation has been shown to mitigate some of the effects of menopause; however, estrogen supplementation is associated with an increased risk for blood clots (including heart attacks and strokes), weight gain, and breast and endometrial cancers, and some women elect not to treat their menopause symptoms because of these associated risks. Therapeutic alternatives to estrogen supplementation with better risk profiles may make clinical

improvements and symptomatic relief more accessible for women in menopause or the post-menopausal period.⁵

Flavonoids stand out as a potential alternative to exogenous estrogen supplements. Certain flavonoids are considered to be phytoestrogens, plant-produced compounds whose structural characteristics enable them to mimic the hormonal functions of endogenous estrogens. Derived from a wide range of plants, flavonoids are abundant polyphenolic compounds commonly associated with various health-promoting effects, including anti-inflammatory, anti-cancer, and antioxidant activities. Isoflavones, which are a structural subgroup of flavonoids, are among the more biologically active phytoestrogens and exhibit selectivity to which estrogen receptors they bind, with a preference for estrogen receptor (ER) beta (ER β) over ER alpha (ER α). Evidence suggests that the selective estrogenic activity of isoflavones may provide clinical improvements and symptomatic relief for patients with an estrogen deficit, without the risk associated with typical estrogen supplementation.⁶

Genistein (5,7,4'-trihydroxyisoflavone), found in soybeans and other legumes, is an isoflavone that functions as a selective estrogen receptor modulator (SERM) with a preference for ER β .⁶ By activating ER β , genistein triggers several pathways that mitigate the effects of unopposed estrogen, such as by modulation of cell proliferation and apoptosis, which are

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TABLE 1. Effects of estrogen replacement therapy versus genistein

EFFECTS	ESTROGEN	GENISTEIN
Body Mass Index	-/↑	↓
Bone loss	↓	↓↓
Breast cancer	-/↑	↓
Endometrial cancer	-/↑	↓
Heart disease	-/↑	↓
Hot flashes	↓	↓

TABLE 2. Relative binding affinity of genistein

COMPOUND	ER α	ER β
17 β -Estradiol	100	100
Raloxifene	69	16
Genistein	4	87

critical factors in estrogen-associated cancer development and progression.^{7,8} Currently, genistein is used clinically as the main ingredient of two medical foods indicated for osteoporosis.⁹ Genistein aglycone is available as prescription medical food in combination with vitamin D3 and zinc (Fosteum; Primus Pharmaceuticals) or in combination with phosphate, zinc, calcium, vitamins D3 and K2 (Fosteum Plus; Primus Pharmaceuticals). Medical foods are intended to provide a targeted nutrient-oriented therapeutic approach, must be administered under a physician's supervision, and are available by prescription only for specific disease states.^{10,11} Evidence indicates that the effects of genistein-based medical foods are pleiotropic, and they may include protective health benefits in osteoporosis, obesity, diabetes mellitus, hyperlipidemia, and cardiovascular disease.¹² Furthermore, as an antioxidant, genistein may protect against ultraviolet (UV)-induced skin damage, and it could also reduce the risk of diseases associated with oxidative stress, such as heart disease and cancer.⁷ Genistein also appears to improve skin elasticity and enhance collagen synthesis, showcasing its utility in mitigating the effects of skin aging.³ This review will focus on the therapeutic role of genistein in perimenopausal and postmenopausal women as an anti-aging and regenerative treatment through exploration of its potential applications, analysis of its proposed mechanisms of action, and narrative review of existing evidence on its clinical efficacy.

METHODS

A narrative literature review was conducted on PubMed to examine the effects of genistein on multiple organ systems, including the skin. This review was based on articles sourced through targeted searches using terms like "Genistein AND skin," "Genistein AND osteoporosis," "Genistein AND vasomotor symptoms," "Genistein AND metabolic issues," "Genistein AND prostate cancer," and "Genistein AND fertility." The search included articles pertaining to *in vitro*, *in vivo*, animal, and human studies. The review specifically excluded non-English articles and studies involving mixed isoflavones, focusing solely on studies discussing pure genistein. Further investigation into genistein's effects on the various organ systems was enhanced through citation tracking, additional PubMed searches, and consultations with experts.

Biochemistry of estrogen and genistein. Estrogen plays a complex role in regulating multiple organ systems such as the skeletal, cardiovascular, central nervous, and integumentary systems. Therefore, estrogen deficiency secondary to menopause can have several negative consequences. Effects across these organ systems can differ, through estrogen's various interactions with its receptors.¹³ Estrogen's effects are mediated through two receptors: ER α and ER β .¹⁴ These receptors act as transcription factors when bound to estrogen, regulating targeted genes. ER β , prevalent in adult human bone, skin, and in cells like the outer root sheath and epithelial matrix, contrasts with ER α , which is mainly found in the dermal papilla cells of hair follicles.^{14–17} The widespread distribution of estrogen receptors in the skin could benefit greatly from estrogen replacement, if not for the significant potential adverse effects associated with this treatment option, such as weight gain, breast cancer, endometrial cancer, and cardiovascular disease (Table 1).^{5,18–21}

Genistein's molecular structure allows it to mimic estrogen, engaging estrogen receptors with significant biological effects. It shows a strong preference for ER β over ER α , with about 30 times greater affinity. When bound to ER β , genistein alters the conformation of helix 12 in the receptor's active site to resemble an antagonist, despite its structural similarities to estradiol, the primary human estrogen. Functioning as a partial agonist for ER β , genistein achieves 60 to 70 percent of estradiol's

efficacy. This unique interaction with helix 12 highlights genistein's distinct influence on estrogenic activities, emphasizing its nuanced role (Table 2).³

Genistein exists primarily in two forms: glycosylated genistein and aglycone genistein, with the latter being crucial for biological studies due to its higher bioavailability. Once ingested, aglycone genistein is absorbed in the intestines, conjugates with glucuronic acid, and may be transported to the liver and secreted into the bile for further intestinal metabolism and reabsorption.²² Comparative studies show that aglycone genistein is absorbed more effectively than glycosylated genistein due to its lower molecular weight and less hydrophilic nature, making it a focal point for extensive research in both preclinical and clinical settings.²³

Genistein for osteoporosis. Osteoporosis poses a significant health issue for the aging population globally, especially postmenopausal women.²⁴ Treatments such as hormone replacement therapy (HRT), bisphosphonates, calcitonin, and SERMs necessitate prolonged dosing schedules, provide limited symptom relief, and may have associated side effects.²⁵ Soy protein consumption in postmenopausal women correlates with increased bone mineral density (BMD), and isoflavones such as genistein have promising effects in treating postmenopausal osteoporosis.^{26–28} Bone primarily contains ER- β receptors, which genistein binds to more effectively, potentially acting as an agonist to benefit bone cells without harming the breast and uterus.²⁹

Bone remodeling is an intricate process that involves osteoblasts, osteoclasts, and osteocytes, and is governed by several intracellular signaling pathways, notably the canonical Wnt/ β -catenin pathway. This pathway is essential for the intracellular activities that promote osteoblast maturation and the formation of bone.^{30,31} During secondary osteoporosis, disruptions in the Wnt/ β -catenin pathway can lead to decreased osteoblast differentiation and activity, alongside increased apoptosis of osteoblasts and osteocytes.³² An *in vitro* model showed genistein's capabilities in promoting osteoblast proliferation and differentiation, while also inhibiting the formation and activity of osteoclasts.³³ WNT10b is crucial in the Wnt canonical pathway, essential for promoting osteoblast formation. Genistein

significantly increased Wnt10b expression at doses of 10, 50, 100, and 200µM, and enhanced protein and transcription factor levels in primary osteoblasts.³¹ This highlights genistein's potential influence on the Wnt/β-catenin signaling pathway in osteocytes, stimulating Wnt10b, facilitating β-catenin's nuclear translocation, and reducing sclerostin, especially at doses of 200µM.³¹ Sclerostin is a protein found in osteocytes that functions to inhibit the Wnt pathway.^{31,34} Its downstream effects include suppressing the production of osteoblasts. By reducing levels of sclerostin, genistein assists in indirectly promoting osteoblast production.³¹

In vitro studies showcased genistein's ability to inhibit osteoclast activity and induce their apoptosis through intracellular Ca²⁺ signaling pathways.³⁵ It significantly reduced osteoclast numbers in rat femoral tissues and showed strong inhibition of osteoclast-like cell formation in mouse marrow cultures. Genistein's inhibitory effects on osteoclasts, at 10⁻⁵ M, were equal to that of 17 β-estradiol's (at 10⁻⁸ M). Additionally, its effects were even more potent than other isoflavones and phytoestrogens.^{36,37} Genistein may also impact the osteoprotegerin (OPG)-receptor activator of nuclear factor κB ligand (RANKL) system. Changes in this system may underlie various metabolic bone disorders, including osteoporosis, as recent studies have suggested.³⁸⁻⁴¹ OPG and RANKL are key components involved in regulating bone resorption.^{42,43} OPG is a part of the TNF receptor superfamily and is produced by osteoblasts, while RANKL is secreted by cells of the osteoblastic lineage.⁴⁴ OPG acts as a decoy by binding to RANKL, preventing its interaction with RANKL. This binding stops osteoclast formation, activation, and survival, thus promoting bone formation.⁴⁵ Serum levels of RANKL, OPG, and their ratio were assessed in postmenopausal women who were treated with genistein for 24 months. Participants received either genistein (n=198; 54mg/d) or placebo (n=191) and the genistein group showed significantly lower RANKL levels and higher OPG levels compared to placebo, at both 12 and 24 months. By 24 months, genistein also significantly lowered the RANKL/OPG ratio compared to placebo. This reduction in RANKL/OPG ratio may contribute to genistein's beneficial impact on BMD.^{44,45} Genistein is the only known agent to collectively inhibit RANKL, stimulate Wnt-beta-catenin, and inhibit

TABLE 3. Osteoporosis treatments

TREATMENTS	HISTOLOGICAL SCORE: BONE QUALITY	FEMORAL BREAKING STRENGTH	SIDE EFFECTS
Estradiol	1.4	113	VTE, stroke, breast cancer, endometrial cancer [†]
Raloxifene	1.0	115	VTE, hot flashes, arthralgia, insomnia, flu-like symptoms [†]
Alendronate	0.6	130	Atypical fractures, GI side effects, bone, joint, and muscle pain
Genistein	0.2	132	Nausea, upset stomach*

VTE: Venous thromboembolism

[†]2017 guidelines from the American College of Physicians recommend against using raloxifene or HRT to treat osteoporosis in women

*In clinical trials, women who took genistein and those who took a placebo (calcium and vitamin D) had a similar incidence of stomach upset

sclerostin.³¹

Bitto A et al²⁵ compared genistein's effectiveness to common treatments like alendronate, raloxifene, and estradiol in ovariectomized rats (Table 3).⁴⁶⁻⁴⁹ Genistein, at 10mg kg⁻¹, outperformed these treatments in improving BMD and bone mineral content (BMC), demonstrating greater bone-building capabilities than alendronate, the mainstay of postmenopausal osteoporosis treatment. Genistein more effectively increased bone formation markers such as bone-alkaline phosphatase (b-ALP) and decreased bone resorption markers like collagen C-telopeptide (CTX). Histological analyses also indicated increased femoral fracture resistance and enhanced overall bone quality.

Morabito et al⁵⁰ compared the effects of genistein and HRT (composed of estradiol combined with norethisterone acetate) on bone metabolism and BMD in postmenopausal women, finding that both significantly enhanced BMD in the femur and lumbar spine. Genistein's effect on bone resorption was measured through decreased urinary excretion of pyridinoline and deoxypyridinoline, markers of bone resorption. After 6 to 12 months of daily genistein (54mg/day) administration, there was a significant decrease in excretion of these markers.^{47,50,51} Marini et al⁴⁷ performed a similar trial that lasted 24 months, using genistein (54mg/day) in combination with vitamins D3 and calcium. Results were comparable and showed genistein's ability to raise femoral and lumbar spinal BMD, while reducing excretion of bone resorption markers in postmenopausal women.⁴⁷ Additionally, 24 months of genistein

treatment, at 54mg/day, did not pose a clinically significant risk to the uterus.^{47,50} This reduction in bone loss, and lack of significant consequences to the uterine lining, underscores genistein's potential as an effective treatment for bone loss in postmenopausal women.

Genistein for metabolic issues: fat accumulation and cardiovascular health.

Menopause is closely tied to an elevation in body weight, accumulation of visceral fat, and changes in body fat distribution, which can increase the risk of metabolic issues such as insulin resistance, Type 2 diabetes, and hyperlipidemia, which may collectively potentiate cardiovascular issues.⁵² Genistein has been shown to possess cardioprotective effects that help reduce the progression of such complications.

Studies involving ovariectomized mice demonstrated genistein's effects on dampening numerous postmenopausal metabolic symptoms such as weight gain, elevated build-up of fat, insulin resistance, and worsening lipid profile.^{53,54} Genistein may also have promising utility in regulating the proliferation and migration of leptin, a peptide hormone involved in the maintenance of hunger and satiety.⁵⁵ Its potential ability to modulate body fat mobilization through induction of adipose tissue apoptosis can provide another mechanism for mitigating elevations in postmenopausal adiposity.^{53,56,57}

Genistein's ability to improve lipid profiles plays a crucial role in reducing cardiovascular risk. In ovariectomized rats, it significantly lowered serum cholesterol and triglyceride levels.⁵⁴ Human studies demonstrated that

genistein, at 36mg/day, significantly lowers LDL cholesterol and the LDL-HDL ratio.^{58,59}

The LDL-HDL ratio represents the metric most strongly associated with a higher risk of cardiovascular disease among women.⁶⁰ Additionally, genistein stabilized insulin levels by modifying membrane binding sites and was the only compound in the study that did not increase uterus weight, unlike coumestrol and zearalenone.^{61–66} In a randomized, double-blind, placebo-controlled study, 120 postmenopausal women with metabolic syndrome were treated with either 54mg of genistein daily or a placebo, for one year. After one year, those receiving genistein experienced significant reductions in fasting glucose, fasting insulin, and systolic/diastolic blood pressures, while no changes were observed in the placebo group. Genistein recipients also saw statistically significant health improvements, including increased HDL and decreased LDL and triglycerides. There were no significant differences in side effects or discontinuation rates between the genistein and placebo groups.⁶⁷ 54mg/day of genistein demonstrated its potential in comprehensively reducing the risk of developing Type 2 diabetes mellitus, while avoiding major side effects.⁶⁸

Genistein for VMS. VMS can include hot flashes which are characterized by sudden intense heat, sweating, and flushing, and often linked to hormonal shifts such as estrogen deficiency. Postmenopausal Asian women have low VMS prevalence, possibly due to their high soy diet. While estrogen supplementation is effective in treating these symptoms, it poses risks.²⁰ A study administering 54mg/day of genistein for 12 months showed significant reductions in daily hot flashes by 22 percent, 29 percent, and 24 percent after three, six, and 12 months, respectively.⁶⁹ D'Anna et al²⁰ strengthened these results in a larger study (n=389), noting a nearly 60-percent reduction in hot flashes at the study's conclusion, without significant endometrial lining changes, highlighting genistein's efficacy without the adverse effects associated with estrogen supplementation.

Genistein: skin effects. Menopause plays a substantial role in aging of the skin. Skin thinning, fragility, laxity, and delayed wound healing are all potential sequelae of ovarian failure and a subsequent decline in estrogen. Genistein has shown promise in treating these symptoms in addition to functioning as a

protective factor against UV radiation and skin cancer. Due to its anti-inflammatory, anti-tumor, and estrogen-like effects, genistein can be useful in improving the quality, appearance, and health of the skin.

Genistein for UV protection. Exposure to ultraviolet (UV) rays, particularly UVB (280–320nm), can cause oxidative damage, leading to premature skin aging, sunburn, and skin cancer.^{70,71} UV exposure results in epidermal DNA damage, triggering an inflammatory response marked by erythema, swelling, and increased cell proliferation, which can indicate the risk of photocarcinogenesis. This response is mediated by histamine and pro-inflammatory prostaglandins, and is reliant on oxygen radicals, making it vulnerable to antioxidant intervention.⁷² Topical antioxidants are effective in protecting skin from UV-induced oxidative damage and have anti-inflammatory properties that help address issues like photoaging and potentially skin cancer.^{3,73}

Research has shown that genistein can reduce free radicals involved in skin aging and may protect against oxidative stress from UVB radiation.⁷⁰ In mice, dietary genistein (250ppm) significantly boosts the production of endogenous antioxidants like superoxide dismutase (SOD) and various forms of glutathione, particularly impacting the small intestine and skin.⁷⁴ These antioxidants help maintain balanced reactive oxygen species (ROS) levels and mitigate cellular stress. An imbalance, with a higher oxidant/antioxidant ratio, can elevate ROS levels, causing oxidative stress that may damage DNA, proteins, and lipids, leading to premature aging.⁷⁵

Nitric oxide (NO) is implicated in photoaging and plays a role in various inflammatory skin conditions, making it a target for treatment.^{76–78} It has been noted that the inducible nitric oxide synthase (iNOS) isoform produces excess NO in response to UV light exposure.⁷⁹ Genistein has been shown to reduce endothelial NOS (eNOS) and iNOS expression dose-dependently in cells pre-treated with hydrogen peroxide.⁸⁰ Additionally, genistein effectively inhibits prostaglandin production in human cell cultures and skin, both under normal and UV-induced conditions, comparable to the antioxidant N-acetylcysteine. This supports the role of ROS in mediating UV radiation's effects, such as sunburn and immunosuppression.⁸¹

When administered before UV exposure,

genistein (15mmol/L) inhibited the UV-induced rise in cyclooxygenase-2 (COX-2) expression in human keratinocyte cultures, suggesting anti-inflammatory properties.^{81,82} Additionally, 60 µM of genistein significantly reduced COX-2 and increased expression of the *Gadd45* gene in UVB-irradiated BJ-5ta cells (human skin fibroblast cells preserved with human telomerase reverse transcriptase (hTERT)), which is known to activate in stressful conditions, such as ionizing radiation.⁸³ *Gadd45* is crucial for cell repair, acting as a stress sensor and influencing cell cycle arrest, DNA repair, and cell fate (survival or apoptosis).^{83–85} This evidence supports genistein's potential as a protective agent against photodamage.

Genistein for skin cancer prevention.

Skin cancer is the most common cancer globally. It is categorized into two main types: cutaneous melanoma and non-melanoma skin cancer (NMSC), which includes basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). The development of skin cancer is influenced by various factors, such as ultraviolet radiation, tanning beds, genetic factors, and organ transplantation.^{86,87} Studies have proposed genistein as a promising candidate for skin cancer therapy, due to its antitumor, chemopreventative, and anti-inflammatory capabilities.^{86,88,89}

Genistein appears to inhibit DNA helicase and topoisomerase I and II, enzymes essential for DNA replication, and can arrest the cell cycle and induce apoptosis at critical points like G1 to S and G2 to M, reducing cell growth.^{90,91} It is shown to also reduce tumor angiogenesis by inhibiting vascular endothelial growth and promoting breakdown of extracellular matrix (ECM) proteins.^{92,93} As a tyrosine kinase inhibitor, genistein may limit cancer cell proliferation and has anti-inflammatory properties that enhance lymphocyte immunostimulatory effects.^{92,94} It boosts both non-specific and humoral immunity, thereby enhancing immune responses, and stimulates interleukin production, supporting its role in cancer chemoprevention.^{90,92,95}

Cyclin D1, a nuclear protein encoded by the *CCND1* gene, is vital for cell growth and tumor cell survival.^{96,97} It facilitates the transition from the G1 to S phase of the cell cycle and is often overexpressed in melanoma, enhancing tumor progression and proliferation due to abnormal degradation that increases its stability.^{97–99}

Reductions in p-16 and/or p-21 proteins can trigger melanoma by failing to effectively block Cyclin D1 activity.¹⁰⁰ Genistein has been shown to reduce Cyclin D1 levels, leading to an arrest of the G2/M phase. In melanoma cells, low doses (25 and 50 μ M) of genistein increased Cyclin D1 expression, while higher doses (100 μ M) decreased it, leading to reduced cell growth and increased apoptosis.⁹⁷

Studies showed that T-lymphokine-activated killer (T-LAK) cell-originated protein kinase (TOPK), a serine-threonine kinase, is crucial in skin cancer development. Both phosphorylated and total levels of TOPK are significantly higher in human actinic keratosis (AK) and cutaneous SCC, compared to normal skin.^{88,101} This may suggest that inhibiting TOPK could be an effective strategy for treating skin cancer. Roh et al⁸⁸ investigated the therapeutic effects of a genistein derivative, in a 1% topical formulation, on SCC caused by chronic solar-simulated light (SSL).⁸⁸ In mouse models, early treatment significantly reduced chronic SSL-induced SCC development and demonstrated therapeutic potential by effectively reducing tumor regrowth post-treatment.^{88,102}

Genistein for wound healing and aberrant scar prevention. Perimenopausal and postmenopausal estrogen deficiency can cause skin atrophy, increased dryness, reduced collagen and water content, diminished sebaceous secretion, and loss of elasticity. These changes are believed to impair wound healing.¹ Delays in wound healing with age have also been linked to several other factors including reduced levels of transforming growth factor-beta1 (TGF- β 1) and vascular endothelial growth factor (VEGF), reduced collagen production, and increased elastase concentration.^{103–105} Studies have indicated that genistein may play a beneficial role in this process by modulating TGF- β 1 secretion from dermal fibroblasts, enhancing healing.^{103,106,107}

In addition to TGF- β 1 secretion, fibroblasts also induce collagen production which is vital for wound repair. Genistein's impact on collagen production was assessed in human dermal fibroblasts undergoing oxidative stress induced by t-butylhydroperoxide (t-BHP). t-BHP's inhibitory effects on collagen production were counteracted at 1 μ M genistein, demonstrating a protective effect. The findings indicate that at dietary levels (1 μ M), genistein protects human dermal fibroblasts against the reduction

in collagen production triggered by oxidative stress.¹⁰⁸

To further assess genistein's impact on wound healing, mice were given a standard diet (control group) or one enriched with genistein at either 0.025% or 0.1%. After inducing skin wounds, daily measurements showed that the control group's wounds reduced in size by 20 percent within three days. In contrast, the 0.1% genistein group saw a 50 percent wound size reduction in the same timeframe, significantly outperforming the control. Although the 0.025% genistein group initially showed a notable reduction on day one, its effects didn't significantly differ from the control thereafter. This indicates that a higher dose of genistein (0.1%) accelerates wound healing more effectively, suggesting a dose-dependent relationship.¹⁰⁹

Ovariectomy hastens the aging process in skin, given that sufficient levels of estrogen are necessary to maintain the skin's structural integrity and functional abilities. In skin samples from untreated ovariectomized rats, there was a reduction in TGF- β 1, VEGF, MMP-2 and MMP-9, and TIMP-1 and TIMP-2, compared to control.^{110,111} In a study where ovariectomized rats were treated with genistein for six months (at doses of 1 and 10mg/kg/day via subcutaneous injections), full-thickness incisional wounds were created to assess healing at 7 and 14 days. Genistein effectively improved wound healing by enhancing the remodeling and turnover of the ECM in these rats. Notably, at both 7- and 14-days post-incision, 1mg/kg genistein demonstrated greater efficacy in enhancing various skin healing parameters compared to estradiol or raloxifene.¹¹¹

The abnormal wound and scar tissue microenvironment, characterized by increased growth factors, drives fibroblast changes and scar progression, leading to hypertrophic scars or keloids. Many of these growth factors utilize the tyrosine protein kinase (TPK) pathway to enhance cell growth, development, and differentiation.^{112–117} Genistein, which may function as a TPK inhibitor, was used to evaluate its effects on inhibiting cultured human hypertrophic scar fibroblast (HSFB) proliferation and TPK signal transduction. Significant differences were observed in both the 50 μ M/l and 100 μ M/l treatment groups compared to the control group at all time points ($p < 0.01$), demonstrating a dose- and time-dependent

relationship on HSFB proliferation. HSFB growth and proliferation, collagen synthesis, type I or III pre-collagen mRNA expression, and intracellular TPK activity were significantly decreased at the 50 μ M/l or 100 μ M/l concentrations of genistein.¹¹⁸

Keloids arise from excessive connective tissue growth during abnormal wound healing, characterized by an imbalance between profibrotic and antifibrotic cytokines that manage ECM synthesis and remodeling.¹¹⁹ Central to this process are TGF β isoforms and connective tissue growth factor (CTGF). TGF β 1 primarily promotes ECM synthesis and contraction, while CTGF extends collagen deposition, further perpetuating fibrosis. Platelet derived growth factor (PDGF) also plays a role in this process as it is mitogenic and chemotactic for connective tissue cells and stimulates collagenase production and synthesis of ECM components, such as fibronectin and hyaluronic acid. There are several other mediators that play a role in keloid development through facilitating increased fibroblast proliferation, apoptosis resistance, and inflammation. Other growth factors and cytokines such as insulin-like growth factor-2 (IGF-2) and prostaglandin E2 (PGE2), as well as key genes such as the tumor suppressor *p53*, cyclin-dependent kinase inhibitor CDKN1A (*p21*), and *BCL2* family genes have all been implicated.^{120–124} *In vitro*, genistein exerted antifibrotic effects through suppression of CTGF mRNA and protein levels, modulation of *p53*, *p21*, and *BCL2* family gene expression, all in a dose-dependent manner. Genistein, at 370 μ M, showed the greatest level of CTGF mRNA suppression.¹⁰⁷

Genistein for skin aging, body fat, and aesthetics. The skin and body undergo noticeable changes with aging, especially after menopause due to their heavy dependence on estrogen. As menopause shifts body fat to the abdomen, thighs, and buttocks, wrinkles are potentiated through diminished supportive facial fat, effectively accelerating skin aging.³

Wrinkles, however, although influenced by environmental and hormonal factors, are ultimately caused by a reduction in skin elasticity, stemming from the degeneration of elastic fibers and the loss of connective tissue through the accumulation of collagen degradation products.¹²⁵ During aging, changes occur in two types of collagen: type I, the main

collagen in adult skin, and type III, which is found throughout the body.¹²⁶ A decrease in both collagen types and in the type III/I ratio within the dermis occurs with aging. Estrogen deficiency can lead to a reduction in collagen by two percent, a reduction in skin elasticity by 1.5 percent, and a reduction in skin thickness by approximately 1.13 percent annually after menopause.^{126,127} The first five years post-menopause however can see up to a 30-percent reduction in type I and III skin collagen, a trend that mirrors the bone mass decline found in postmenopausal women. This reduction in collagen content and skin thickness is more closely linked to estrogen deficiency than to chronological aging.¹²⁸

Promising outcomes were demonstrated by genistein when tested in anti-aging cosmetic products. These involve improving skin elasticity, reducing photoaging, and mitigating the development of skin cancer. Creams infused with genistein have been effective in reducing wrinkles and improving skin dryness.¹²⁹ Genistein's ability to induce subcutaneous VEGF production and enhance TGF- β levels in the skin creates downstream effects that increase skin collagen thickness. Additionally, genistein counteracts matrix metalloproteinases (MMPs) by elevating tissue inhibitors of metalloproteinases (TIMP) protein levels, thereby reducing breakdown of collagen. Consequently, genistein may significantly improve collagen thickness and slow the skin aging process.^{110,126}

A topical anti-aging formulation (GEN), featuring genistein, vitamin E, vitamin B3, and ceramide, was developed to counteract menopausal skin effects. In a randomized, controlled trial with 50 postmenopausal women, 25 received the GEN product and 25 a placebo cream, applied twice daily for six weeks. Results indicated that the GEN product significantly improved skin hydration, reduced the appearance of pores, and diminished several wrinkling metrics compared to the placebo. This suggests that genistein could be effective in improving skin health and appearance in postmenopausal women.¹³⁰ Additionally, a double-blind study with 26 middle-aged women tested the effects of genistein aglycone. Participants received either 40 mg of genistein aglycone daily or a placebo. The treatment group showed significant improvements in fine wrinkles and cheek skin elasticity after

12 weeks, further supporting the benefits of genistein for skin health and appearance.¹³¹

Genistein in males. Prostate cancer accounts for 15 percent of all cancer diagnoses in men. The incidence of prostate cancer is lower in Asian countries, however, where soy intake is also higher. It has been observed that genistein accumulates in prostatic tissue and may be protective against the development of prostate cancer. One mouse model highlighted genistein's protective effects against the development of prostate cancer. Mice were provided three different diets: control, low-dose genistein (300mg/kg), and high-dose genistein (750mg/kg) and results of prostate cancer incidence were 70 percent, 47 percent, and 32 percent, respectively.¹³² This indicated the possibility that higher dosages of genistein can reduce the risk of prostate cancer development. A recent meta-analysis supports previous studies showing that soy intake, and genistein intake specifically, are associated with a decreased risk of prostate cancer growth and development ($p < 0.001$ and $p = 0.008$, respectively).¹³³ A case-control study in Japanese men looked at isoflavone consumption and assessed its association with prostate cancer risk. Individuals consuming the most genistein (≥ 2.5 mg/day) versus the least (< 1.1 mg/day) had an odds ratio (OR) of 0.42, demonstrating a significant linear trend ($P < 0.05$).¹³⁴ Additionally, another study assessed 100 Chinese men for prostate cancer and diagnosed 46 of them with prostate cancer. The median plasma concentration of genistein in patients without prostate cancer (728.6ng/mL) was significantly higher compared to those with prostate cancer (513.0ng/mL) ($P < 0.05$). That study's multivariate analysis showed plasma genistein's potential impact, alongside age and smoking history, in determining risk of prostate cancer.¹³⁵ As the second most common cancer worldwide, it is crucial to identify potentially modifiable factors in the development of prostate cancer, and these results show promise in genistein's utility as a preventative agent.

Nonetheless, as a phytoestrogen, it may be possible that genistein intake also has potentially deleterious effects on the reproductive health of males. A study on adult male rats exposed to genistein *in utero*, demonstrated a dose-dependent relationship between genistein and its negative impact on fertility. Epididymal sperm counts were

significantly lower in the 20mg and 100mg groups, compared to control. Sperm counts in the 2mg group were not significantly different from control. Additionally, motile and viable sperm percentages were significantly reduced in the 100mg group compared to controls. The 2mg and 20mg groups did not seem to significantly impact motility and viability.¹³⁶ Shi et al¹³⁷ tested genistein's impact on fertility in male mice by providing a low-dose (40mg/kg) and high-dose (800mg/kg) genistein diet. The low-dose diet demonstrated improved testes weight, seminiferous tubule diameter, serum testosterone levels, and spermatogenesis. Conversely, the high-dose diet significantly reduced body weight, testes weight, and testes size. This was strengthened by another study showing that high-dose (300mg/kg genistein) can negatively impact antral follicular growth in mice.¹³⁸ Interestingly, one mouse study demonstrated an improvement in fertility when treated with genistein, although a dose-response relationship was not found. Intraperitoneal administration of genistein occurred at varying dosages: 1, 2, and 4mg/kg. Administration of genistein significantly enhanced sperm count, motility, and viability. It also improved testosterone, LH, and FSH levels.¹³⁹ These findings show the potentially detrimental effects of genistein exposure on the male reproductive system, particularly when exposure occurs in utero, but are likely attributed to the very high doses of genistein. Additionally, the studies included were performed on mice and rats with doses significantly higher than human consumption levels.¹⁴⁰

DISCUSSION

The sex hormone estrogen is critically involved in the regulation of key biological processes across the entire body, and a chronic estrogen deficiency or imbalance is associated with an increased risk for the development of various pathologies, such as dyslipidemia, cardiovascular disease, VMS, osteoporosis, xerosis, dermal atrophy, poor wound healing, and cancer. Following menopause, women enter a state of chronic estrogen deficiency and may experience a decline in quality of life because of the associated symptoms and/or disease states. Some of the health risks of estrogen deficiency may be mitigated by restoring a portion of normal estrogen activity,

which is conventionally accomplished with estrogen supplementation. However, the risks associated with estrogen supplementation, such as VTE and cancer, may contraindicate therapy or otherwise deter patients from pursuing treatment. An alternative therapeutic solution with a better risk profile would enable more patients to pursue and achieve symptomatic relief and forestall the development of estrogen osteoporotic women treated orally with genistein. Moreover, genistein outperforms alendronate and raloxifene in its ability to reduce osteoclastic markers.²⁵ Its selective binding to ER- β , strongly expressed in human bone, particularly trabecular bone, underscores its role in bone formation and potential as a selective ER- β agonist for osteoporosis treatment.^{16,27,29,142}

Genistein has shown significant anti-inflammatory effects and enhanced DNA repair.⁸³ This aligns with recent research displaying genistein's anti-inflammatory and anticarcinogenic properties, including its ability to modulate COX-2 in various cellular systems and prevent UV-induced DNA damage in skin models. As UV damage is also a risk factor for skin cancer development, genistein's anti-tumor properties may also play a role in reducing its incidence, a valuable function given the prevalence of skin cancer worldwide. A genistein derivative was found to reduce tumor growth and oncogenic expression, and a topical application of this derivative was protective against tumor regrowth.⁸⁸ Genistein has also been found to promote cell growth at lower doses and induce apoptosis at higher doses, highlighting its potential as a therapeutic agent for malignant melanoma at appropriate dosages.⁹⁷ The findings of studies investigating genistein and its derivatives further underscore its potential as a therapeutic agent, offering avenues for targeted interventions in skin cancer treatment.

Estrogen depletion during menopause contributes to skin atrophy, diminished collagen production, and impaired wound healing. However, genistein shows promise as an agent capable of ameliorating these effects. Genistein has demonstrated antioxidant properties through mitigation of the effects of oxidative stress and downregulation of proinflammatory mediators, by which it contributes to improved wound healing.¹⁰⁹ Additionally, genistein shows promise in inhibiting hypertrophic scar

fibroblast proliferation and modulating key genes implicated in keloid development.¹⁰⁷ The topical application and oral administration of genistein was shown to alleviate skin aging, reduce fine wrinkles, and improve skin elasticity.^{130,131} These findings collectively underscore genistein's multifaceted mechanisms in promoting skin health and wound healing, suggesting its therapeutic utility in mitigating the effects of estrogen deficiency, including aberrant wound healing processes and aging skin.

Limitations. The studies on genistein show very significant promise however, one of the main limitations is that since genistein is not a drug, individual studies are using different sources as well as different amounts of genistein. Unless reproducible medical food grade sourcing is used, different studies cannot necessarily be correlated to each other. There is also little data on the use of genistein in women taking hormonal supplements during menopause. Further studies should be conducted on this population with medical food grade dosing. Overall, future research should prioritize larger, long-term clinical trials with standardized dosages, and assess efficacy and side effects in diverse populations to further establish genistein's role in clinical practice.

CONCLUSION

Genistein functions as a SERM and offers various health benefits, particularly for postmenopausal women. By mimicking estrogen, it can alleviate menopausal symptoms and conditions. This is strengthened by the availability of medical foods that improves upon genistein's bioavailability and therapeutic efficacy in mitigating the downstream effects of estrogen deficiency. Genistein aids in metabolic and cardiovascular health and is a potential alternative for estrogen supplementation, reducing the risks of obesity, diabetes, and cardiovascular disease. Furthermore, it benefits bone health by increasing density and lowering resorption, offering a safer osteoporosis treatment. It also promotes skin health by enhancing elasticity, reducing wrinkles, and improving wound healing, through collagen and elastin production, while also providing protection against UV damage and skin cancer. Genistein's therapeutic potential makes it a valuable supplement for postmenopausal women and

may even support prostate cancer prevention in men.

REFERENCES

1. Calleja-Agius J, Brincat M. The effect of menopause on the skin and other connective tissues. *Gynecol Endocrinol*. 2012;28(4):273–277.
2. Faubion SS, Kuhle CL, Shuster LT, et al. Long-term health consequences of premature or early menopause and considerations for management. *Climacteric*. 2015;18(4):483–491.
3. Irrera N, Pizzino G, D'Anna R, et al. Dietary management of skin health: the role of genistein. *Nutrients*. 2017;9(6).
4. Wines N, Willsted E. Menopause and the skin. *Australas J Dermatol*. 2001;42(3):149–148.
5. Colditz GA. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. *J Natl Cancer Inst*. 1998;90(11):814–823.
6. Munson PL, Mueller RA, Breese GR. Principles of pharmacology: basic concepts and clinical applications; 1995.
7. Kurzer MS, Xu X. Dietary phytoestrogens. *Annu Rev Nutr*. 1997;17:353–381.
8. Messina MJ, Persky V, Setchell KD, et al. Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr Cancer*. 1994;21(2):113–131.
9. Nieves JW. Nutritional therapies (including foseum). *Curr Osteoporos Rep*. 2009;7(1):5–11.
10. Hur JY, Lee S, Shin WR, et al. The emerging role of medical foods and therapeutic potential of medical food-derived exosomes. *Nanoscale Adv*. 2023;6(1):32–50.
11. Bitto A, Burnett BP, Polito F, et al. The steady-state serum concentration of genistein aglycone is affected by formulation: a bioequivalence study of bone products. *Biomed Res Int*. 2013;2013:273498.
12. Yu L, Rios E, Castro L, et al. Genistein: dual role in women's health. *Nutrients*. 2021;13(9).
13. Delgado BJ, Lopez-Ojeda W. Estrogen. [Updated 26 Jun 2023]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK538260/>
14. Thornton MJ. The biological actions of estrogens on skin. *Exp Dermatol*. 2002;11(6):487–502.
15. Younes M, Honma N. Estrogen receptor β . *Arch Pathol Lab Med*. 2011;135(1):63–66.
16. Kuiper GG, Carlsson B, Grandien K, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology*. 1997;138(3):863–870.
17. Taylor AH, Al-Azzawi F. Immunolocalisation of oestrogen receptor beta in human tissues. *J Mol Endocrinol*. 2000;24(1):145–155.
18. Laing A, Hillard T. Oestrogen-based therapies for menopausal symptoms. *Best Pract Res Clin Endocrinol Metab*. 2024;38(1):101789.
19. Boardman HM, Hartley L, Eisinga A, et al. Hormone therapy for preventing cardiovascular disease in post-menopausal women. *Cochrane Database Syst Rev*. 2015;2015(3):Cd002229.
20. D'Anna R, Cannata ML, Atteritano M, et al. Effects of the phytoestrogen genistein on hot flashes, endometrium, and vaginal epithelium in postmenopausal women: a 1-year randomized, double-blind, placebo-controlled study. *Menopause*. 2007;14(4):648–655.
21. Castelo-Branco C SI. Clinical efficacy of estradiol transdermal system in the treatment of hot flashes in postmenopausal women. *Research and Reports in Transdermal Drug Delivery*. 2014;3:1–8.

22. Steensma A, Faassen-Peters MA, Noteborn HP, et al. Bioavailability of genistein and its glycoside genistin as measured in the portal vein of freely moving unanesthetized rats. *J Agric Food Chem*. 2006;54(21):8006–8012.
23. Cederroth CR, Zimmermann C, Nef S. Soy, phytoestrogens and their impact on reproductive health. *Mol Cell Endocrinol*. 2012;355(2):192–200.
24. Kanis JA, Melton LJ 3rd, Christiansen C, et al. The diagnosis of osteoporosis. *J Bone Miner Res*. 1994;9(8):1137–1141.
25. Bitto A, Burnett BP, Polito F, et al. Effects of genistein aglycone in ovariectomized, ovariectomized rats: a comparison with alendronate, raloxifene and oestradiol. *Br J Pharmacol*. 2008;155(6):896–905.
26. Horiuchi T, Onouchi T, Takahashi M, et al. Effect of soy protein on bone metabolism in postmenopausal Japanese women. *Osteoporos Int*. 2000;11(8):721–724.
27. Setchell KD, Lydeking-Olsen E. Dietary phytoestrogens and their effect on bone: evidence from in vitro and in vivo, human observational, and dietary intervention studies. *Am J Clin Nutr*. 2003;78(3 Suppl):593S–609S.
28. Messina M, Ho S, Alekel DL. Skeletal benefits of soy isoflavones: a review of the clinical trial and epidemiologic data. *Curr Opin Clin Nutr Metab Care*. 2004;7(6):649–658.
29. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*. 1998;139(10):4252–4263.
30. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. *J Clin Invest*. 2006;116(5):1202–1209.
31. Mannino F, Imbesi C, Irrera N, et al. Insights into the antiosteoporotic mechanism of the soy-derived isoflavone genistein: modulation of the Wnt/beta-catenin signaling. *Biofactors*. 2024;50(2):347–359.
32. Frenkel B, White W, Tuckermann J. Glucocorticoid-induced osteoporosis. *Adv Exp Med Biol*. 2015;872:179–215.
33. Ming LG, Chen KM, Xian CJ. Functions and action mechanisms of flavonoids genistein and icaritin in regulating bone remodeling. *J Cell Physiol*. 2013;228(3):513–521.
34. Lewiecki EM. Role of sclerostin in bone and cartilage and its potential as a therapeutic target in bone diseases. *Ther Adv Musculoskelet Dis*. 2014;6(2):48–57.
35. Gao YH, Yamaguchi M. Suppressive effect of genistein on rat bone osteoclasts: apoptosis is induced through Ca²⁺ signaling. *Biol Pharm Bull*. 1999;22(8):805–809.
36. Gao YH, Yamaguchi M. Suppressive effect of genistein on rat bone osteoclasts: involvement of protein kinase inhibition and protein tyrosine phosphatase activation. *Int J Mol Med*. 2000;5(3):261–267.
37. Gao YH, Yamaguchi M. Inhibitory effect of genistein on osteoclast-like cell formation in mouse marrow cultures. *Biochem Pharmacol*. 1999;58(5):767–772.
38. Hofbauer LC, Heufelder AE. Clinical review 114: hot topic. The role of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin in the pathogenesis and treatment of metabolic bone diseases. *J Clin Endocrinol Metab*. 2000;85(7):2355–2363.
39. Simonet WS, Lacey DL, Dunstan CR, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*. 1997;89(2):309–319.
40. Dougall WC, Glaccum M, Charrier K, et al. RANK is essential for osteoclast and lymph node development. *Genes Dev*. 1999;13(18):2412–2424.
41. Bucay N, Sarosi I, Dunstan CR, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev*. 1998;12(9):1260–1268.
42. Yamagishi T, Otsuka E, Hagiwara H. Reciprocal control of expression of mRNAs for osteoclast differentiation factor and OPG in osteogenic stromal cells by genistein: evidence for the involvement of topoisomerase II in osteoclastogenesis. *Endocrinology*. 2001;142(8):3632–3637.
43. Viereck V, Gründker C, Blaschke S, et al. Phytoestrogen genistein stimulates the production of osteoprotegerin by human trabecular osteoblasts. *J Cell Biochem*. 2002;84(4):725–735.
44. Crisafulli A, Altavilla D, Squadrito G, et al. Effects of the phytoestrogen genistein on the circulating soluble receptor activator of nuclear factor kappaB ligand-osteoprotegerin system in early postmenopausal women. *J Clin Endocrinol Metab*. 2004;89(1):188–192.
45. Marini H, Minutoli L, Polito F, et al. OPG and sRANKL serum concentrations in osteopenic, postmenopausal women after 2-year genistein administration. *J Bone Miner Res*. 2008;23(5):715–720.
46. Qaseem A, Forciea MA, McLean RM, et al. Treatment of low bone density or osteoporosis to prevent fractures in men and women: a clinical practice guideline update from the American College of Physicians. *Ann Intern Med*. 2017;166(11):818–839.
47. Marini H, Minutoli L, Polito F, et al. Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. *Ann Intern Med*. 2007;146(12):839–847.
48. Quintanilla Rodriguez BS, Correa R. Raloxifene. [Updated 13 Feb 2023]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK544233/>
49. Ozaras N, Rezvani A. Diffuse skeletal pain after administration of alendronate. *Indian J Pharmacol*. 2010;42(4):245–246.
50. Morabito N, Crisafulli A, Vergara C, et al. Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. *J Bone Miner Res*. 2002;17(10):1904–1912.
51. Hesley RP, Shepard KA, Jenkins DK, et al. Monitoring estrogen replacement therapy and identifying rapid bone losers with an immunoassay for deoxyypyridinoline. *Osteoporos Int*. 1998;8(2):159–164.
52. Carr MC. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab*. 2003;88(6):2404–2411.
53. Shen HH, Huang SY, Kung CW, et al. Genistein ameliorated obesity accompanied with adipose tissue browning and attenuation of hepatic lipogenesis in ovariectomized rats with high-fat diet. *J Nutr Biochem*. 2019;67:111–122.
54. Nogowski L, Maćkowiak P, Kandulska K, et al. Genistein-induced changes in lipid metabolism of ovariectomized rats. *Ann Nutr Metab*. 1998;42(6):360–366.
55. Tsai YC, Leu SY, Peng YJ, et al. Genistein suppresses leptin-induced proliferation and migration of vascular smooth muscle cells and neointima formation. *J Cell Mol Med*. 2017;21(3):422–431.
56. Kim HK, Nelson-Dooley C, Della-Fera MA, et al. Genistein decreases food intake, body weight, and fat pad weight and causes adipose tissue apoptosis in ovariectomized female mice. *J Nutr*. 2006;136(2):409–414.
57. Naaz A, Yellayi S, Zakroczyński MA, et al. The soy isoflavone genistein decreases adipose deposition in mice. *Endocrinology*. 2003;144(8):3315–3320.
58. Romualdi D, Costantini B, Campagna G, et al. Is there a role for soy isoflavones in the therapeutic approach to polycystic ovary syndrome? Results from a pilot study. *Fertil Steril*. 2008;90(5):1826–1833.
59. Khani B, Mehrabian F, Khalesi E, et al. Effect of soy phytoestrogen on metabolic and hormonal disturbance of women with polycystic ovary syndrome. *J Res Med Sci*. 2011;16(3):297–302.
60. Scavo M. Cardiovascular risk factors and prevention in women: similarities and differences. *Ital Heart J Suppl*. 2001;2(2):125–141.
61. Abler A, Smith JA, Randazzo PA, et al. Genistein differentially inhibits postreceptor effects of insulin in rat adipocytes without inhibiting the insulin receptor kinase. *J Biol Chem*. 1992;267(6):3946–3951.
62. Smith RM, Tiesinga JJ, Shah N, et al. Genistein inhibits insulin-stimulated glucose transport and decreases immunocytochemical labeling of GLUT4 carboxyl-terminus without affecting translocation of GLUT4 in isolated rat adipocytes: additional evidence of GLUT4 activation by insulin. *Arch Biochem Biophys*. 1993;300(1):238–246.
63. Kandulska K, Nogowski L, Szkudelski T. Effect of some phytoestrogens on metabolism of rat adipocytes. *Reprod Nutr Dev*. 1999;39(4):497–501.
64. Nogowski L, Maćkowiak P, Nowak K. Isoflavone—genistein changes tissue glycogen and blood glucose concentration in ovariectomized rats: possible ways of action. *J Anim Physiol Anim Nutr (Berl)*. 1998;80(1–5):1–9.
65. Nogowski L. Effect of the myco-oestrogen zearalenone on carbohydrate and lipid metabolism indices in ovariectomized female rats. Possible role of insulin and its receptor. *J Anim Physiol Anim Nutr (Berl)*. 1996;75(1–5):156–163.
66. Nogowski L. Effects of phytoestrogen-coumestrol on lipid and carbohydrate metabolism in young ovariectomized rats may be independent of its estrogenicity. *J Nutr Biochem*. 1999;10(11):664–669.
67. Squadrito F, Marini H, Bitto A, et al. Genistein in the metabolic syndrome: results of a randomized clinical trial. *J Clin Endocrinol Metab*. 2013;98(8):3366–3374.
68. Jain R, Bolch C, Al-Nakkash L, et al. Systematic review of the impact of genistein on diabetes-related outcomes. *Am J Physiol Regul Integr Comp Physiol*. 2022;323(3):R279–R288.
69. Crisafulli A, Marini H, Bitto A, et al. Effects of genistein on hot flashes in early postmenopausal women: a randomized, double-blind EPT- and placebo-controlled study. *Menopause*. 2004;11(4):400–404.
70. Afaq F, Mukhtar H. Botanical antioxidants in the prevention of photocarcinogenesis and photoaging. *Exp Dermatol*. 2006;15(9):678–684.
71. Wei H, Saladi R, Lu Y, et al. Isoflavone genistein: photoprotection and clinical implications in dermatology. *J Nutr*. 2003;133(11 Suppl 1):3811S–3819S.
72. Widayari S, Spinks N, Husband AJ, et al. Isoflavonoid compounds from red clover (*Trifolium pratense*) protect from inflammation and immune suppression induced by UV radiation. *Photochem Photobiol*. 2001;74(3):465–470.
73. Tang SC, Hsiao YP, Ko JL. Genistein protects against ultraviolet B-induced wrinkling and photoinflammation in in vitro and in vivo models. *Genes Nutr*. 2022;17(1):4.
74. Cai Q, Wei H. Effect of dietary genistein on antioxidant enzyme activities in SENCAR mice. *Nutr Cancer*. 1996;25(1):1–7.
75. Masaki H. Role of antioxidants in the skin: anti-aging effects. *J Dermatol Sci*. 2010;58(2):85–90.
76. Grossini E, Molinari C, Mary DA, et al. Intracoronary genistein acutely increases coronary blood flow in anesthetized pigs through β -adrenergic mediated nitric oxide release and estrogenic receptors. *Endocrinology*. 2008;149(5):2678–2687.
77. Yang J, Min S, Hong S. Therapeutic effects of fermented flax seed oil on NC/Nga mice with atopic dermatitis-like skin lesions. *Evid Based Complement Alternat Med*. 2017;2017:5469125.
78. Park JH, Choi JY, Son DJ, et al. Anti-inflammatory effect of titrated extract of *Centella asiatica* in phthalic anhydride-induced allergic dermatitis animal model. *Int J Mol Sci*. 2017;18(4):738.
79. Deliconstantinos G, Villiotou V, Stavrides JC. Nitric oxide and

- peroxynitrite released by ultraviolet B-irradiated human endothelial cells are possibly involved in skin erythema and inflammation. *Exp Physiol*. 1996;81(6):1021–1033.
80. Savoia P, Raina G, Camillo L, et al. Anti-oxidative effects of 17 β -estradiol and genistein in human skin fibroblasts and keratinocytes. *J Dermatol Sci*. 2018;92(1):62–77.
81. Isoharranen K, Punnonen K, Jansen C, et al. Ultraviolet irradiation induces cyclooxygenase-2 expression in keratinocytes. *Br J Dermatol*. 1999;140(6):1017–1022.
82. Buckman SY, Gresham A, Hale P, et al. COX-2 expression is induced by UVB exposure in human skin: implications for the development of skin cancer. *Carcinogenesis*. 1998;19(5):723–729.
83. Iovine B, Iannella ML, Gasparri F, et al. Synergic effect of genistein and daidzein on UVB-induced DNA damage: an effective photoprotective combination. *J Biomed Biotechnol*. 2011;2011:692846.
84. Liebermann DA, Hoffman B. Gadd45 in the response of hematopoietic cells to genotoxic stress. *Blood Cells Mol Dis*. 2007;39(3):329–335.
85. Liebermann DA, Hoffman B. Gadd45 in stress signaling. *J Mol Signal*. 2008;3:15.
86. Peterle L, Sanfilippo S, Borgia F, et al. The role of nutraceuticals and functional foods in skin cancer: mechanisms and therapeutic potential. *Foods*. 2023;12(13).
87. Watson M, Holman DM, Maguire-Eisen M. Ultraviolet radiation exposure and its impact on skin cancer risk. *Semin Oncol Nurs*. 2016;32(3):241–254.
88. Roh E, Kim JE, Zhang T, et al. Orbol, 3'-hydroxy-genistein, suppresses the development and regrowth of cutaneous SCC. *Biochem Pharmacol*. 2023;209:115415.
89. Banerjee S, Li Y, Wang Z, et al. Multi-targeted therapy of cancer by genistein. *Cancer Lett*. 2008;269(2):226–242.
90. Russo M, Russo GL, Daglia M, et al. Understanding genistein in cancer: The "good" and the "bad" effects: a review. *Food Chem*. 2016;196:589–600.
91. Peeters PH, Keinan-Boker L, van der Schouw YT, et al. Phytoestrogens and breast cancer risk. Review of the epidemiological evidence. *Breast Cancer Res Treat*. 2003;77(2):171–183.
92. Pawlicka MA, Filip A. Can genistein be a potential agent against skin side effects associated with the treatment of breast cancer? *Postepy Dermatol Alergol*. 2022;39(1):7–12.
93. Mense SM, Hei TK, Ganju RK, et al. Phytoestrogens and breast cancer prevention: possible mechanisms of action. *Environ Health Perspect*. 2008;116(4):426–433.
94. Bilal I, Chowdhury A, Davidson J, et al. Phytoestrogens and prevention of breast cancer: the contentious debate. *World J Clin Oncol*. 2014;5(4):705–712.
95. Mukund V, Mukund D, Sharma V, et al. Genistein: its role in metabolic diseases and cancer. *Crit Rev Oncol Hematol*. 2017;119:13–22.
96. Sauter ER, Yeo UC, von Stemm A, et al. Cyclin D1 is a candidate oncogene in cutaneous melanoma. *Cancer Res*. 2002;62(11):3200–3206.
97. Apriliantina YS, Novita HD, Sadono EG, et al. Protective Effect of Genistein on Cyclin D1 Expression in Malignant Ocular Melanoma Cells. *Med Arch*. 2021;75(3):180–183.
98. Kim JK, Diehl JA. Nuclear cyclin D1: an oncogenic driver in human cancer. *J Cell Physiol*. 2009;220(2):292–296.
99. Oba J, Nakahara T, Abe T, et al. Expression of c-Kit, p-ERK and cyclin D1 in malignant melanoma: an immunohistochemical study and analysis of prognostic value. *J Dermatol Sci*. 2011;62(2):116–123.
100. Ji Z, Flaherty KT, Tsao H. Molecular therapeutic approaches to melanoma. *Mol Aspects Med*. 2010;31(2):194–204.
101. Roh E, Lee M-H, Zykyova TA, et al. Targeting PRPK and TOPK for skin cancer prevention and therapy. *Oncogene*. 2018;37(42):5633–5647.
102. Roh E, Han Y, Reddy K, et al. Suppression of the solar ultraviolet-induced skin carcinogenesis by TOPK inhibitor HI-TOPK-032. *Oncogene*. 2020;39(21):4170–4182.
103. Ashcroft GS, Dodsworth J, Boxtel EV, et al. Estrogen accelerates cutaneous wound healing associated with an increase in TGF- β 1 levels. *Nature medicine*. 1997;3(11):1209–1215.
104. Ashcroft GS, Mills SJ. Androgen receptor-mediated inhibition of cutaneous wound healing. *J Clin Invest*. 2002;110(5):615–624.
105. Herrick S, Ashcroft G, Ireland G, et al. Up-regulation of elastase in acute wounds of healthy aged humans and chronic venous leg ulcers are associated with matrix degradation. *Lab Invest*. 1997;77(3):281–288.
106. Schmidt JB, Binder M, Demschik G, et al. Treatment of skin aging with topical estrogens. *Int J Dermatol*. 1996;35(9):669–674.
107. Jurzak M, Adamczyk K, Antończak P, et al. Evaluation of genistein ability to modulate CTGF mRNA/protein expression, genes expression of TGF β isoforms and expression of selected genes regulating cell cycle in keloid fibroblasts in vitro. *Acta Pol Pharm*. 2014;71(6):972–986.
108. Sienkiewicz P, Surazynski A, Palka J, et al. Nutritional concentration of genistein protects human dermal fibroblasts from oxidative stress-induced collagen biosynthesis inhibition through IGF-1 receptor-mediated signaling. *Acta Pol Pharm*. 2008;65(2):203–211.
109. Park E, Lee SM, Jung IK, et al. Effects of genistein on early-stage cutaneous wound healing. *Biochem Biophys Res Commun*. 2011;410(3):514–519.
110. Polito F, Marini H, Bitto A, et al. Genistein aglycone, a soy-derived isoflavone, improves skin changes induced by ovariectomy in rats. *Br J Pharmacol*. 2012;165(4):994–1005.
111. Marini H, Polito F, Altavilla D, et al. Genistein aglycone improves skin repair in an incisional model of wound healing: a comparison with raloxifene and oestradiol in ovariectomized rats. *Br J Pharmacol*. 2010;160(5):1185–1194.
112. Su CW, Alizadeh K, Boddie A, et al. The problem scar. *Clin Plast Surg*. 1998;25(3):451–465.
113. O'leary R, Wood E, Guillou P. Pathological scarring: strategic interventions. *Eur J Surg*. 2002;168(10):523–534.
114. Nanney LB. Epidermal and dermal effects of epidermal growth factor during wound repair. *J Invest Dermatol*. 1990;94(5):624–629.
115. Niessen FB, Andriessen MP, Schalkwijk J, et al. Keratinocyte-derived growth factors play a role in the formation of hypertrophic scars. *J Pathol*. 2001;194(2):207–216.
116. Uhl D, PDGF, EGF and TGF- α . Promoters of cancer growth and wound healing. *Med Monatsschr Pharm*. 1991;14(5):130–131.
117. Phan T-T, Lim IJ, Bay BH, et al. Role of IGF system of mitogens in the induction of fibroblast proliferation by keloid-derived keratinocytes in vitro. *Am J Physiol Cell Physiol*. 2003;284(4):C860–C869.
118. Cao C, Li S, Dai X, et al. Genistein inhibits proliferation and functions of hypertrophic scar fibroblasts. *Burns*. 2009;35(1):89–97.
119. Wolfram D, Tzankov A, Püzl P, et al. Hypertrophic scars and keloids—a review of their pathophysiology, risk factors, and therapeutic management. *Dermatol Surg*. 2009;35(2):171–181.
120. Ashcroft KJ, Syed F, Bayat A. Site-specific keloid fibroblasts alter the behaviour of normal skin and normal scar fibroblasts through paracrine signalling. *PLoS One*. 2013;8(12):e75600.
121. Leask A, Abraham DJ. TGF- β signaling and the fibrotic response. *FASEB J*. 2004;18(7):816–827.
122. Colwell AS, Phan TT, Kong W, et al. Hypertrophic scar fibroblasts have increased connective tissue growth factor expression after transforming growth factor-beta stimulation. *Plast Reconstr Surg*. 2005;116(5):1387–1392.
123. Grotendorst GR, Rahmanie H, Duncan MR. Combinatorial signaling pathways determine fibroblast proliferation and myofibroblast differentiation. *FASEB J*. 2004;18(3):469–479.
124. Butler PD, Longaker MT, Yang GP. Current progress in keloid research and treatment. *J Am Coll Surg*. 2008;206(4):731–741.
125. Shah MG, Maibach HI. Estrogen and skin. An overview. *Am J Clin Dermatol*. 2001;2(3):143–150.
126. Liu T, Li N, Yan YQ, et al. Recent advances in the anti-aging effects of phytoestrogens on collagen, water content, and oxidative stress. *Phytother Res*. 2020;34(3):435–447.
127. Brinca M, Versi E, Moniz CF, et al. Skin collagen changes in postmenopausal women receiving different regimens of estrogen therapy. *Obstet Gynecol*. 1987;70(1):123–127.
128. Thornton MJ. Estrogens and aging skin. *Dermatoendocrinol*. 2013;5(2):264–270.
129. Rona C, Vailati F, Berardesca E. The cosmetic treatment of wrinkles. *J Cosmet Dermatol*. 2004;3(1):26–34.
130. Na Takuathung M, Klinjan P, Sakuludomkan W, et al. Efficacy and safety of the genistein nutraceutical product containing vitamin E, vitamin B3, and ceramide on skin health in postmenopausal women: a randomized, double-blind, placebo-controlled clinical trial. *J Clin Med*. 2023;12(4).
131. Izumi T, Saito M, Obata A, et al. Oral intake of soy isoflavone aglycone improves the aged skin of adult women. *J Nutr Sci Vitaminol (Tokyo)*. 2007;53(1):57–62.
132. Slusarz A, Jackson GA, Day JK, et al. Aggressive prostate cancer is prevented in ER α KO mice and stimulated in ER β KO TRAMP mice. *Endocrinology*. 2012;153(9):4160–4170.
133. Applegate CC, Rowles JL, Ranard KM, et al. Soy consumption and the risk of prostate cancer: an updated systematic review and meta-analysis. *Nutrients*. 2018;10(1).
134. Nagata Y, Sonoda T, Mori M, et al. Dietary isoflavones may protect against prostate cancer in Japanese men. *J Nutr*. 2007;137(8):1974–1979.
135. Wu Y, Zhang L, Na R, et al. Plasma genistein and risk of prostate cancer in Chinese population. *Int Urol Nephrol*. 2015;47(6):965–970.
136. Meena R, Supriya C, Pratap Reddy K, et al. Altered spermatogenesis, steroidogenesis and suppressed fertility in adult male rats exposed to genistein, a non-steroidal phytoestrogen during embryonic development. *Food Chem Toxicol*. 2017;99:70–77.
137. Shi Z, Lv Z, Hu C, et al. Oral exposure to genistein during conception and lactation period affects the testicular development of male offspring mice. *Animals (Basel)*. 2020;10(3).
138. Patel S, Peretz J, Pan YX, et al. Genistein exposure inhibits growth and alters steroidogenesis in adult mouse antral follicles. *Toxicol Appl Pharmacol*. 2016;293:53–62.
139. Jalili C, Ahmadi S, Roshankhah S, et al. Effect of genistein on reproductive parameter and serum nitric oxide levels in morphine-treated mice. *Int J Reprod Biomed*. 2016;14(2):95–102.
140. Rashid R, Kumari A, Chattopadhyay N, et al. Genistein lowers fertility with pronounced effect in males: meta-analyses on pre-clinical studies. *Andrologia*. 2022;54(9):e14511.
141. Morgan SL, Baggott JE. Medical foods: products for the management of chronic diseases. *Nutr Rev*. 2006;64(11):495–501.
142. Bord S, Horner A, Beavan S, et al. Estrogen receptors alpha and beta are differentially expressed in developing human bone. *J Clin Endocrinol Metab*. 2001;86(5):2309–2314. **JCAD**

Review Article

Herbal Medicinal Products from *Passiflora* for Anxiety: An Unexploited Potential

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Herbal medicines containing *Passiflora* species have been widely used to treat anxiety since ancient times. The species *Passiflora incarnata* L. is included in many Pharmacopoeias, and it is the most used species in food, cosmetic, and pharmaceutical industries. However, there are around 600 species of the genus *Passiflora* and probably other species that can be used safely. Thus, this article was based on a search into the uses of the main species of the genus *Passiflora* with anxiolytic activity and its main secondary metabolites and some pharmacological studies, patents, and registered products containing *Passiflora*. Furthermore, the Brazilian Regulatory Health Agency Datavisa, Medicines and Healthcare Products Regulatory Agency of the United Kingdom, and the European Medicines Agency websites were consulted. The results showed that *Passiflora* species have health benefits but clinical trials are still scarce. The complexity of *Passiflora* extracts creates challenges for the development of herbal medicines. *P. incarnata* is the most studied species of the genus and the most used in natural anxiolytic herbal medicine formulations. However, there are hundreds of *Passiflora* species potentially useful for medicinal and nutraceutical purposes that are still little explored.

1. Introduction

The contemporaneous tense lifestyle is responsible for the wide use of anxiolytic substances, mainly those of the benzodiazepine group. This class of synthetic drugs is one of the most prescribed antianxiety drugs in the world to treat stress and anxiety. However, many risks are associated to their use, such as dependence, abstinence syndrome, and the reduction of motor reflexes. Therefore, the use and acceptance of herbal medicines and nutraceuticals for these purposes, including

Passiflora species, have been growing among doctors and patients [1]. *In vitro* and *in vivo* studies are needed to establish the bioequivalence of herbal formulations. In the case of *Passiflora* formulations, the standardization can be based on the potency in animal models, such as the open arm plus-maze using rats or mice. After the isolation of the substances responsible for the anxiolytic activity, the pharmaceutical equivalence can be applied [2].

Many countries have laws regulating herbal medicinal products. Germany, that is considered the world's largest

market for these products [3], defines herbal medicinal products as “medicinal products that exclusively contain, as active substances, one or more herbal substances, one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations, according to the German Drug Law [4].” Estimates suggest that Germany spends more than 2 billion dollars on herbal medicinal products each year [3].

The Directive 2001/83/EC [5] and Directive 2004/24/EC [6] of the European Parliament of the Council of the European Union are codes for medicinal products and traditional herbal medicinal products for human use. The code defines herbal medicinal products as any medicinal product exclusively containing, as active ingredients, one or more herbal substances (whole or fragmented plants) or herbal preparations (tinctures, extracts, essential oils, etc). In the same code, traditional herbal medicinal products have simplified registration, because they can be used without prescription or monitoring treatment and the products are not harmful when used according to the instructions of the manufacture. They may be administered orally, topically, or by inhalation. The pharmacological effects or efficacy are based on long experience (at least 30 years, including at least 15 years within the European Community) but the safety of the product must be ensured, and the quality must be verified according to the European Pharmacopoeia monographs, or those in the Pharmacopoeia of a Member State [7].

The regulatory requirements for the registration of herbal medicines in Brazil are similar to those in Europe. In Brazil, these preparations are classified as herbal medicines if they are obtained only from active raw vegetal material, of which the efficacy and safety have been validated through ethnopharmacological surveys, scientific and technical documentation, or clinical evidence [8]. These products may be registered and marketed as traditional herbal medicine, if evidence of their safe and effective use has been published in scientific and technical literature, and there is evidence of continuous safe use for a minimum period of thirty years. Furthermore, they may not be used for any serious diseases; they may not contain materials in toxic concentrations; and they should not be administered by the ophthalmic or intravenous routes. The safety and efficacy of herbal medicines are based on clinical evidence, and they are characterized by consistent quality [9]. Any registrations or marketing authorizations in other countries, and the reasons for refusal of registration or marketing authorization must be stated [3]. Traditional herbal medicinal products are designed for use without the supervision of a physician in terms of diagnosis, prescription, or monitoring.

Arifin and coworkers [10] list and discuss several challenges that impact the development of a herbal medicinal product. Normally, the difficulties present in the production of a herbal medicine are related to the isolation of the active ingredient(s) and its pharmacological evidence, safety, and therapeutic efficacy. The complex nature of plants creates difficulties, and consequently, there are challenges for the development of herbal medicinal products, which is not a quick and simple process. Plant species have various phytochemicals with different characteristics, e.g.,

absorption, solubility that affect their bioavailability. Additionally, there are the possible unknown toxic effects, potential harmful interactions of herbal medicinal products and the lack of pharmacokinetic parameters such as gastrointestinal absorption, protein binding, and plasma clearance by the liver and kidneys, which are all important challenges to overcome to be able to produce new medicines from medicinal plants. The quality of herbal products from a technological point of view is ensured for the intermediate and finished products, using adequate production planning, appropriate production processes, and strict control of the raw materials from the plant species used. All of these cares must ultimately result in products with constant quality, efficacy, and security.

This article was based on a literature search into the widespread use of the main species of the genus *Passiflora* and their main secondary metabolites to treat anxiety. The search covered pharmacological studies, patents, and registered products that were focused on the promising Brazilian market. This report about *Passiflora* herbal medicinal products comprises eight sections: *Passiflora* genus (*Passifloraceae*); chemical constituents; mechanism of anxiolytic action; *Passiflora* extract: standardization and chemical marker; pharmacology, pharmacokinetics and safety; patents and intellectual property in Brazil; registered medicines containing *Passiflora* in Brazil; and patents and registered products containing *Passiflora* outside Brazil.

2. Methods

Empirical searches were conducted via the databases Scopus® (<http://www.scopus.com>) [11], Scirus (<http://www.scirus.com>) [12], SciFinder® (<http://scifinder.cas.org/>) [13], Google Scholar (<https://scholar.google.com.br/>) [14], as well as periodicals using the following three keywords: ‘*Passiflora incarnata*’, ‘*Passiflora edulis*’, and ‘*Passiflora alata*’. Other keywords used with the genus *Passiflora* were extraction, pharmacophores, ADMET, flavonoids, and formulation. A search for clinical trials using the keywords ‘anxiety’ and ‘*Passiflora*’ were performed in the database ClinicalTrials.gov (<https://clinicaltrials.gov>), of the National Institutes of Health (NIH), an agency of the U.S. Department of Health and Human Services [15]. All of the information about the plants, their past and present therapeutic uses, and data about marketing of herbal medicines in Brazil and outside Brazil, including species of *Passiflora* in combination with other active ingredients, were taken directly from selected papers and searched in the following websites: the National Institute of Industrial Property (Instituto Nacional de Propriedade Industrial, INPI), <https://gru.inpi.gov.br/pePI/servlet/PatenteServletController> [16]; National Health Regulatory Agency (Agência Nacional de Vigilância Sanitária, ANVISA), <https://consultas.anvisa.gov.br/#/medicamentos/>, an agency of the Ministry of Health [17]; Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement) [18], the Brazilian Pharmacopoeia [19], Medicines and Healthcare Products Regulatory Agency (MHRA) of the United Kingdom [20], and European Medicines Agency [21].

The graphs and tables were constructed using Excel 2013.

2.1. *Passiflora* Genus (*Passifloraceae*). According Muschner and colleagues [22], the *Passifloraceae* family is divided into two tribes: *Paropsieae*, with six genera, is found throughout Europe, Africa, and Madagascar, and *Passiflorieae*, with 14 genera, is found in the Americas and in Europe. The genus *Passiflora*, belonging to the *Passifloraceae* family, contains approximately 530 species and 400 artificial hybrids [23] or, according to other authors, more than 600 species [22, 24, 25]. At least 140 are native to Brazil, and 70 of them produce edible fruits. Brazil is the largest producer of *Passiflora* fruits in the world, with a reported annual production of 923,035t [26]. It is popularly known as passion fruit in English and “maracujazeiro” in Portuguese [27, 28]. Besides the nutritional use of the fruits, the passion flower is cultivated as an ornamental plant and used in the cosmetic and pharmaceutical industries. The sedative properties of its leaves and hypoglycemic activity of the flour produced from the epicarp of the fruit have already been described [26, 29]. Species of *Passiflora* are rich in flavonoids, which have anxiolytic activity, among other activities. *P. incarnata* L. is the most widely studied species, due to its chemical composition and its pharmacological effects [30]. However, biological activities similar to those described for *P. incarnata* have been reported for other Brazilian species of the same genus, providing an alternative source for the formulation of herbal medicines.

Despite the many different species of *Passiflora*, only two are reported in the Brazilian Pharmacopoeia, 6th Edition [19], *Passiflora edulis* Sims and *Passiflora alata* Curtis. In Brazil, herbal medicines are prepared using the native species *P. alata*, *P. edulis* f. *flavicarpa* [31], and the exotic *P. incarnata*. *P. incarnata* is the most widely studied from a pharmacological point of view and it is present in the official pharmaceutical codes of different countries, including the British Herbal Pharmacopoeia, the United States Homoeopathic Pharmacopoeia, the Homoeopathic Pharmacopoeia of India, the Pharmacopoeia Helvetica, the Egyptian, French, German and Swiss Pharmacopoeias, and the British Herbal Compendium [27, 31, 32].

There have been numerous taxonomical, pharmacological, and toxicological studies on *P. edulis* and *P. alata*, but for other species native to Brazil, although cataloged for a long time, there is still a lack of studies on their potential uses in food, cosmetics, and pharmaceuticals [32]. Different activities have been reported for *P. incarnata*, especially in the pharmaceutical field. These include its action on the central nervous system for the treatment of anxiety disorders and neuropsychic symptoms of menopause, such as pain, anxiety, sexual dysfunction, and sleep disorders.

2.2. Chemical Constituents. In phytochemical studies, some papers have reported that the flavonoids and alkaloids of *Passiflora* may be related to its anxiolytic properties [33–35]. Some variability of flavonoids may occur within this botanical genus throughout the year, and there are different methods to increase the concentration of these metabolites

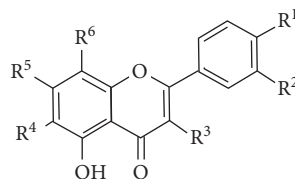
in the leaves [36]. The literature indicates that some species of *Passiflora* have differences in their flavonoid C-glycosides contents, and these differences are relevant to the characterization of their specific origins. For example, isovitexin is found in higher amounts in *P. incarnata* [33, 37], *P. alata* [38], and *P. edulis* var. *flavicarpa* and *P. edulis* var. *edulis* [38], although it was not detected in *P. edulis* var. *edulis* by Zucolotto and colleagues [39]. Moreover, orientin and vitexin are found in small amounts in the leaves of *P. incarnata*, *P. alata*, and *P. edulis* [37–40]. Furthermore, iso-orientin is one of the major flavonoids in *P. incarnata* and *P. edulis* var. *flavicarpa*, but it is only detected in small concentrations in *P. alata* [37–39]. Additionally some C-glycosides were identified in just one species, such as luteolin-6-C-chinovoside and luteolin-6-C-fucoside in *P. edulis* [24, 33] and 2''-xylosylvitexin in *P. alata* [33]. A comparative metabolite profiling and fingerprinting of the genus *Passiflora* leaves were reported using a multiplex approach of UPLC-MS and NMR analyzed by chemometric tools [41]. Thin layer chromatography can also be used in order to differentiate species [42]. Recently, new components were isolated and identified in the fruit peel of *Passiflora edulis*, including five flavonoids [43, 44].

Based on studies that indicate the presence of flavonoids in extracts of *Passiflora* and their pharmacological properties, a total flavonoid analysis has been proposed for the preliminary evaluation of this plant species [42, 45–49]. Furthermore, the C-glycosylated flavonoid may represent a particularly important chemical marker for the quality control of herbal medicines, enabling the identification of different species of *Passiflora* [46, 50, 51].

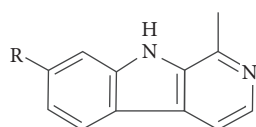
C-glycosylated flavonoids found in *P. incarnata* consist mainly of a glucose group directly linked to the aromatic nucleus only in positions 6 and 8 of the flavonoid core [52]. The aqueous extract is phytochemically characterized by a set of C-glycoside flavonoids such as vitexin (1), isovitexin (2), schaftoside (3), isoschaftoside (4), orientin (5), iso-orientin (6), and swertisin (7) [53, 54]. Moreover, the free flavonoids apigenin (8), luteolin (9), quercetin (10), kaempferol (11), and chrysin (12) are also found [33]. Figure 1 illustrates the chemical structures of the flavonoids of *P. incarnata*.

The alkaloids present in *Passiflora* are of the indole type (β -carboline), which are the second major known group of alkaloids [54]. Some of them have value in medicine as tranquilizers and for the treatment of hypertension [55]. *P. incarnata* is the most widely studied species containing alkaloids [24]. In studies carried out in the 1960s, harmine (13), harmol (14), harmaline (15), harmalol (16), and harman (17) were detected (Figure 2) [56]. Although the presence of traces of these alkaloids was confirmed [24], they were undetectable in most commercial materials [57]. Figure 2 shows the principal alkaloids mentioned above.

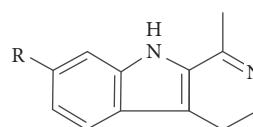
2.3. Mechanism of Anxiolytic Action. Events related to depression and anxiety, among others in the central nervous system, refer to the balance between chemical excitation and inhibition [58]. One of the mechanisms involves the



Flavonoids	R1	R2	R3	R4	R5	R6
(1) Vitexin	OH	H	H	H	OH	Glucose
(2) Isovitexin	OH	H	H	Glucose	OH	H
(3) Schaftoside	OH	H	H	Glucose	OH	Arabinose
(4) Isoschaftoside	OH	H	H	Arabinose	OH	Glucose
(5) Orientin	OH	OH	H	H	OH	Glucose
(6) Isoorientin	OH	OH	H	Glucose	OH	H
(7) Swertisin	OH	H	H	Glucose	OCH ₃	H
(8) Apigenin	OH	H	H	H	OH	H
(9) Luteolin	OH	OH	H	H	OH	H
(10) Quercetin	OH	OH	OH	H	OH	H
(11) Kaempferol	H	OH	OH	H	OH	H
(12) Chrysin	H	H	H	H	OH	H

FIGURE 1: Flavonoids of *Passiflora incarnata*.

(a)



(b)

Alkaloids			
A	R	B	R
(13) Harmine	OCH ₃	(16) Harmaline	OCH ₃
(14) Harmol	OH	(17) Harmalol	OH
(15) Harman	H		

FIGURE 2: Alkaloids of *Passiflora incarnata*.

γ -aminobutyric acid (GABA) system via binding to the benzodiazepine-site of the GABA type A receptor (GABA_A), which can be modulated by allosteric agents, for example, benzodiazepines, which promote the regulation of the chloride flow through the ion channel complex [59]. The possibility of elaborating or identifying new drugs with chemical characteristics of benzodiazepines for the treatment of anxiety led to the formulation of pharmacophoric models, and the model designed by Cook is the reference in this field of study. The pharmacophoric model designed by Cook describes a lipophilic region, two hydrogen bond donor sites (HB1 and HB2), and a hydrogen bond acceptor site (A2) [59, 60]. These characteristics are consistent with the chemical structure of flavonoids, mainly the two hydrogen bond-donating sites that are related to ether oxygen and a carbonyl group present in flavones, and the aromatic rings A and B corresponding to the lipophilic regions L1 and L2, respectively [61]. In order to elucidate some incongruencies related to actions not predicted by this model in

the study of 6-methyl-3'-nitroflavone [62], a new pharmacophoric model was designed. In this new model, in addition to the regions described by Cook's model, there are two sites classified as S1 and S2 relative to electrostatic interaction of substituents in 6-position of flavone [61, 63]. Additional studies revealed two new regions of steric repulsive around the 4'- and 5'-regions of the B-ring [61, 64].

The chemical constituents responsible for the anxiolytic activity of *Passiflora* are not yet fully understood but the majority of published works suggest that phenolic substances, especially the flavonoid class, are related to this property [65–68]. The flavonoids identified in *P. incarnata* (Figure 1) probably act according to the pharmacophoric models described. The mechanism of action is probably related to the modulation of the γ -aminobutyric acid (GABA) system, because *Passiflora* flavonoids are partial agonists of GABA_A receptors and inhibit the uptake of [3H]-GABA into rat cortical synaptosomes [30, 67, 69, 70]. The review article written by Wasowski and Marder [58]

describes flavonoids as GABA_A receptor ligands, including apigenin and chrysin that bind to the benzodiazepine-binding site of the GABA_A receptor, exhibiting anxiolytic activity, without evidencing sedation and muscle relaxant effects [58, 71]. *In vitro* assays demonstrated that the flavonoid chrysin (5,7-dihydroxyflavone) has affinity for the benzodiazepine receptors and that it increases pentobarbital-induced hypnosis and reduces locomotor activity in mice after intraperitoneal administration of 30.0 mg/kg body weight (bw) [57]. This is reinforced by studies that indicate that the pre-administration of flumazenil, an antagonist of GABA_A receptor, attenuates the anxiolytic effects of passionflower like diazepam. However, this is possible not the unique mechanism involved [30].

In one study, kaempferol exhibited a very low affinity for the benzodiazepine-binding site and was devoid of anxiolytic actions by the intraperitoneal route [58]. However, in another study to evaluate the anxiolytic activity of the flavonols, kaempferol, quercetin, and myricetin, in the elevated plus-maze after oral and intraperitoneal (i.p.) administration in mice, only kaempferol and quercetin were active after oral administration, although they were inactive via i.p. [72].

The harman alkaloids found in *P. incarnata* which belong to the β -carboline class have different structural characteristics that interact with benzodiazepine receptors. This class of alkaloids attracted the attention of Braestrup and colleagues [73] during studies with endogenous ligands for benzodiazepine receptors after the detection of β -carboline-3-carboxylic acid ethyl ester in normal human urine. This discovery was important because it was foreshadowing the synthesis of several derivatives of β -carboline compounds with important biological activity [74]. In addition, research involving β -carboline compounds has led to conceptual and experimental considerations, covering the role of ligands in the GABA_A receptor modulation process in a spectrum that ranges from full agonists to full inverse agonists [75]. Furthermore, β -carboline alkaloids are consistent with the pharmacophoric model developed by Cook and coworkers [59, 60], enabling the recognition of a hydrogen bond acceptor (A2) site in the NH group, a hydrogen bond donor (H1) in N(2) [75], in addition to the positions in C6 and C3 framed in the lipid domain (LDi) [76].

2.4. *Passiflora* Extract: Standardization and Chemical Marker.

The marketing of extracts obtained from plant drugs is advantageous for the industry in several ways, as it reduces the steps involved in the manufacturing of herbal medicine products, it minimizes waste production, and it simplifies analytical quality control. However, an important point for evaluation is the standardization of this extract obtained commercially. Standardization of raw plant materials can be done by the chemical quantification of substances or class of substances related to the therapeutic effects, or through the presence of chemical marker substances of the species. The most abundant substances in the leaves of this species belong to the class of flavonoids, especially C-glycosides, which are directly related to its pharmacological activities [24].

According to the current Brazilian regulations for herbal medicines with simplified registration, Normative Rule no. 02 determines that the main chemical marker of the *P. incarnata* extract is vitexin, which is considered to be the active ingredient of its aqueous extract. The range of a daily dosage varies from 30 mg to 120 mg of total flavonoids expressed as vitexin in an herbal medicine made from *Passiflora* [77]. According to the Assessment Report on *P. incarnata*, elaborated by the European Medicines Agency (EMA) that is affiliated to the European Union, the herbal extract prepared with hydroalcohol solution or acetone solution may contain at least 2.0% of flavonoids expressed as vitexin, and the daily dose recommended corresponds in most cases to about 20–30 mg of total flavonoids [21]. The Brazilian Pharmacopoeia does not contain any information about the minimum flavonoid contents in extracts, but British and French Pharmacopoeias recommend at least 1.5% of flavonoids expressed as vitexin [78].

The continuous growing demand for plant derived therapeutic molecules obtained in a sustainable and eco-friendly manner favors biotechnological production leading to the production of plants with increased levels of desirable compounds with potential biological activity. Similarly, the development of innovative extraction techniques to obtain phytoconstituents that can be used in modern medicine is another approach that must be taken into account. On this point, accelerated solvent extraction can be used to obtain extracts enriched in polyphenolic compounds from different species of *Passiflora* sp. [38]. This extraction method offers many advantages such as good reproducibility and shorter extraction time, the possibility to adjust extraction temperature, and purification of extracts online. Fierascu and colleagues [79] suggest some techniques that may be combined and applied in the development of products based on *Passiflora*.

2.5. Pharmacology, Pharmacokinetics, and Safety.

Information on the absorption, distribution, metabolism, elimination, and toxicity (ADMET) of a drug in the human body is of great importance for the characterization of a drug formulation regarding the treatment of a certain disease and its safety for the patient [80, 81]. Knowledge of ADMET properties is usual for synthetic drugs; however, when the universe of substances present is expanded, such as in the case of products from plant species, there is a reduced level of information about these properties [80–82].

The reasons for this gap are the complexity of a formulation based on natural products, which generally includes a high number of substances in its composition that can suffer qualitative and quantitative variation of these constituents due to many factors, such as plant harvest and climatic factors [80, 81]. Currently, it is possible to apply standardized methods for the preparation of herbal medicines with the chemical composition controlled. However, the identification of the active substance is usually difficult, and this fact can compromise the knowledge of the ADMET properties in the body [80, 82].

The alternative to deal with these peculiarities related to herbal medicines is the determination of ADMET properties of pure constituents of the formulation, through *in vitro* tests, for example, Caco-2 cells, human liver microsomes, and cultured human hepatocytes [80]. In this context, the ADMET properties of the flavonoids iso-orientin, isovitexin, luteolin, apigenin, and kaempferol present in *P. incarnata* have been described in the literature [80, 83, 84].

Absorption processes are evaluated by blood-brain barrier (BBB) penetration and human intestinal absorption (HIA) methods that demonstrated high absorption through human intestine and side effects on absorption through BBB for iso-orientin, isovitexin, and luteolin. Apigenin and kaempferol showed positive values for both BBB and HIA [80, 83, 85]. The importance of a substance crossing the BBB is partially related to the required biological activity, and this is based on structural and physicochemical characteristics, such as lipophilicity, molecular size, desolvation potential, pKa-to-charge ratio, and hydrogen bond [80, 86, 87].

In metabolic process, CYP450 is an isoenzyme that metabolizes several substances, including, carcinogens, fatty acids, bile acids, and steroids, which evidences its importance in the characterization of the metabolic properties of various substances. Among the previously mentioned flavonoids, only iso-orientin and isovitexin, have low CYP inhibitory promiscuity. However, all of them were non-substrate for all CYP450 substrates (2C9, 2D6), and most of them inhibited most of the CYP450 inhibitors (2C9 and 2D6) [80, 83, 85]. The inhibition of CYP450 isoforms is closely related to the metabolism of a substance and can lead to increased toxicity [85]. Aspects related to toxicity were measured based on biodegradability, Ames toxicity, and carcinogenicity. In general, the toxicological profile of the flavonoids indicates that they are not carcinogenic and, with the exception of iso-orientin and isovitexin, demonstrated to be negative for Ames toxicity [80, 83, 85].

Within a broader context, the flavonoids present in *P. incarnata* have a good ADMET profile and, considering an herbal formulation containing these substances, i.e., good absorption, metabolism, distribution, elimination, and low toxicity.

Given these premises, knowledge about pharmacokinetic parameters is an extremely important aspect for the development of any oral formulation of *Passiflora* extracts. The water solubility of flavone molecules, such as for most of the flavonoids, is limited, and it is 0.1 g/L for chrysin. Flavonoid aglycones generally tend to have good membrane permeability, whereas flavonoid glycosides have poor membrane permeability, related to their ability to form hydrogen bonds, and consequently, aglycones are absorbed better and faster. Pure chrysin seems to be predominantly absorbed by passive diffusion. All flavones, including those with an unsubstituted B ring, are rapidly metabolized in the small intestine and then supplemented with biotransforms in the liver, while those that are not absorbed are affected by the additional metabolism of bacterial enzymes in the colon. The prolonged metabolism of the first pass of flavonoids, mainly by glucuronidation and sulfation, proved to be the main reason for their low bioavailability. Glucuronidation of

flavones by UDP-glucuronosyltransferases (UGT) 1A1 represents together with sulfation by sulfotransferase (SULT) 1A3 and 1A1 the most important metabolic pathway of these natural molecules when ingested by humans. Chrysin is predominantly glucuronidated by UGT1A1, having preeminence over sulfation [88, 89].

General data of flavonoid excretion indicate that most of these glucuronides are subject to biliary excretion, with little urinary contribution. In the case of chrysin, only about 3% of the dose administered is excreted through the kidneys. However, most of the glucuronides tend to be pharmacologically inactive, although notable exceptions are known [88, 89].

The third volume of the World Health Organization monographs on selected medicinal plants contains the monograph of *P. incarnata* [57]. In this monograph, experimental pharmacology studies related to central nervous system depressant activity in mice are described. The aqueous or hydroalcohol extracts (30–40% of ethanol) of the aerial parts reduces spontaneous motor activity, increased pentobarbital-induced sleeping time, and potentiated pentobarbital-induced sleeping time after intraperitoneal or intragastric administration. However, the aqueous extract seems to be more active than the hydroalcohol extract, since some activity is observed with the aqueous extract at 25.0 mg/kg (bw), while hydroalcohol extracts only have some activity at higher concentrations (50.0 mg/kg or over) [57].

The aqueous extract is toxic to mice at 900.0 mg/kg bw i.p. and the oral median lethal dose (LD₅₀) of a 30% ethanol extract in mice was 37.0 ml/kg bw [57]. Other nonclinical information indicates that the acute and repeated dose toxicity is low, and the LD₅₀ of the hydroalcoholic extract in mice and rats is higher than 15 g/kg orally and higher than 3 g/kg i.p. [21]. There is no evidence of genotoxicity, but *P. incarnata* is contraindicated during pregnancy because it seems to stimulate uterine contractions in animal models. It may cause drowsiness and therefore care is recommended for activities that require attention. Some adverse events that are very rare include hypersensitivity, ventricular tachycardia, nausea, vomiting, drowsiness and prolonged QT interval, thrombocytopenia, left ventricular failure, ventricular fibrillation, hepatic function abnormal, arrhythmia, tremor, agitation, and withdrawal syndrome. However, no correlation of serious adverse events and *P. incarnata* intake has been established. There are no reports regarding safety for nursing mothers and children, and the use of *P. incarnata* in these cases should be monitored by a physician [21, 57]. Furthermore, the concomitant use of *P. incarnata* and sedatives such as benzodiazepines, zolpidem or barbiturates, alcohol, and clonidine is not recommended due to possible pharmacokinetic interactions [90].

Clinical research studies involving the use of *Passiflora* to treat anxiety were searched for in the ClinicalTrials.gov, a database maintained by the National Institutes of Health (NIH), which is an agency of the U.S. Department of Health and Human Services [15]. This database registers private and public clinical studies from throughout the world. There are six research studies registered that are listed in Table 1, all of

TABLE 1: List of clinical trials related to the use of *Passiflora* to treat anxiety registered in NIH.

Trial number	Dosage form	Formulation NAME	Stage	Period	Reference anxiolytic AGENT	Status
NCT00794456	Tablet	Pasalix: <i>Passiflora incarnata</i> L., <i>Crataegus oxyacantha</i> L., <i>Salix alba</i> L.	Phase 3	6 weeks	<i>Valeriana officinalis</i> L. (50 mg)	Completed Oct 2013
NCT01178632	Tablet	Passiflorine: <i>Passiflora incarnata</i> L., <i>Crataegus oxyacantha</i> L., <i>Salix alba</i> L.	Phase 3	4 weeks	<i>Valeriana officinalis</i> L.	Unknown
NCT00944268	Liquid	<i>Passiflora incarnata</i> L., <i>Crataegus oxyacantha</i> L., <i>Salix alba</i> L.	Phase 3	30 days	None	Unknown
NCT02065843	Capsules	<i>Passiflora incarnata</i> L. 100 mg	Phase 2 phase 3	1 h before dental surgery	Midazolam (15 mg)	Completed Sep 2014
NCT03909906	tablets	Euphytose®: 50 mg <i>Valeriana officinalis</i> L., 40 mg <i>Passiflora incarnata</i> L., 10 mg <i>Crataegus</i> sp., 10 mg <i>Ballota nigra</i> L.	Not applicable	14 days	None	Completed Dec 2019
NCT00997490	Film-coated sugar-pill	Neurapas balance: hypericum, valerian, passionflower	Phase 3	6 weeks	None	Completed Feb 2003

Source: <https://www.clinicaltrials.gov/>. Accessed on May 19, 2020.

them with the species *P. incarnata*. Four studies have been concluded or terminated, and two are unknown. The results concerning the effectiveness or the reasons for discontinuation were not informed. Only one of the studies was conducted with monopreparations of *Passiflora*. The concomitant use of plant species in clinical trials makes it difficult to attribute any effect specifically to *P. incarnata*.

In the literature, there are few relevant clinical trials available for monopreparations of *P. incarnata* to support its use to treat anxiety, and the published studies should be considered preliminary and only suggestive of its effectiveness. The most complete study is described by Mori and colleagues [91], which was evaluated by The Cochrane Collaboration [92] and EMA [21]. According to these evaluations, the multicentre double-blind study performed in Japan over a 4-week period was adequately randomized, and the Jadad score was 5. In the study, 63 patients received *P. incarnata* extract (Passiflamin) and 71 patients received mexazolam. The initial doses were 90 mg of Passiflamin or 1.5 mg of mexazolam, which were further doubled. Although mexazolam was more effective, the efficacy of Passiflamin was significant to treat anxiety, tenseness, and irritation.

A small study with 36 patients with general anxiety over a 28-day period demonstrated that the use of passionflower extract (45 drops/day) is as effective as oxazepam (30 mg/day) [93]. The same research group evaluated the effect of *P. incarnata* extract (60 drops) associated with clonidine (0.8 mg) in opiate addicts undergoing withdrawal [94]. In the study with 65 patients, the authors concluded that the daily administration of the combination over 14 days was better than only clonidine at 0.8 mg/day.

Movafegh and colleagues [95] conducted a clinical study with 60 patients that were premedicated 90 min before a surgery. According to the numerical rating scale to assess anxiety and sedation, the anxiety scores of the group that received a 500 mg tablet containing 1.01 mg benzoflavone was lower than the anxiety scores of the group that received placebo, without inducing sedation. Another study

involving preoperative anxiety was conducted with 60 patients 30 minutes before spinal anesthesia [96]. Patients received 5 mL of an aqueous solution containing 700 mg of an aqueous extract of *P. incarnata* containing 2.8 mg benzoflavone or 5 mL of mineral water. A small difference was observed between the two groups in the State Anxiety Inventory score, without sedation. Dantas and colleagues [97] studied the effect of *P. incarnata* (pill containing 260 mg) or midazolam (pill containing 15 mg) in forty volunteers for the control of anxiety before undergoing bilateral extraction of their mandibular third molar. Over 70% of the patients reported that they felt quiet or a little anxious with both treatments, and no significant differences were observed between the groups. However, 20% of the participants reported amnesia with midazolam. The authors found that *P. incarnata* showed an anxiolytic effect similar to midazolam.

The effect of *P. incarnata* on sleep quality was studied in a double-blind study with 41 volunteers [98]. Over a 7-day period, the volunteers received placebo (dried parsley infusion) or a *P. incarnata* infusion prepared with teabags containing 2 g of aerial parts and 250 mL of boiling water during 10 min. The overnight polysomnography on the last night of the treatment indicated a significant increase of 5.2% of the sleep quality, but no significant effect on total sleep time and nocturnal awakenings.

It is important to highlight the lack of detail regarding the composition of *P. incarnata* extracts or even the composition of the formulations. Nonclinical studies suggest that aqueous extracts are more active than the 40% ethanol extracts, which indicates that small variations in chemical composition can be determinants in activity. The standardization of vegetable raw materials is of great importance in this area. The complex chemical composition of *Passiflora* extracts and the lack of knowledge of the pharmacological activity of each component increase the challenges, due to the difficulties in determining the most appropriate chemical marker in the standardization of the extract and in stability studies that any new studies should be directed to, for the

development of formulations containing *Passiflora*. Other important considerations regarding the clinical trials presented here were previously mentioned by Miroddi and colleagues [30] who reviewed ethnopharmacological and clinical trials related to *P. incarnata*. The studies were performed with a small number of participants, and some studies had inadequate comparison between the placebo and the medications recommended for anxiety and gave little importance to the use of *Passiflora* found in some results. Thus, the clinical trials reported are pilot studies and are not enough to recognize the medicinal use of *P. incarnata* as being well-established. Moreover, the actual recommendations are still based on its long-standing use to relieve mild symptoms of mental stress. Thus, *P. incarnata* and other species of the genus offer a wide field for research that is still little explored, which should involve interdisciplinary and interinstitutional partnerships, covering the stages of phytochemical studies for standardization and developments in herbal medicines, along with nonclinical and clinical studies.

2.6. Patents and Intellectual Property in Brazil. The Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement), of which Brazil is a signatory, established the current standards of intellectual property protection worldwide. It came into force on January 1, 1995 and is mandatory for all member countries of the World Trade Organization [18]. The TRIPS Agreement establishes minimum standards under international law related to patents, including standards for drugs. The members of the World Trade Organization (WTO) agreed on the development and implementation of patenting laws according to common patterns. These models state, among other things that patents should be granted for a minimum of twenty years, that patents may be granted for products and processes and that information for drug tests must be protected against “unfair commercial use”.

In Brazil, the Law of Intellectual Property [99] excludes from patentability all plants and animals, or parts thereof, except for transgenic microorganisms with the requirements of patentability (novelty, inventive step, and industrial application) and those that do not constitute mere discoveries. Thus, Brazilian law does not allow the patenting of plant extracts. However, processes for obtaining extracts or isolated active ingredients from plants, pharmaceutical compositions and their methods of preparation, and other uses of the same products obtained from plants, are patentable. In this way, the growing herbal market aligned to government policies to promote regularization of medicinal plants and their derivatives [100], and the innovation for the development of herbal medicines justifies the product patent protection [101].

On that basis, the keyword <Passiflora> was selected as a search term, to search in the website of the National Institute of Industrial Property (Instituto Nacional de Propriedade Industrial, INPI). The results returned a miscellany of food products, cosmetics, packaging materials, manufacturing processes for simple syrups for dietary use, herbal syrups, and formulations containing bronchodilators, among

others. The results of twenty-eight patents are shown in Table 2. Furthermore, there are recent patent files and they are concerned with obtaining new extracts, production processes, and analytical techniques.

2.7. Registered Products Containing *Passiflora* in Brazil.

In Brazil, sanitary control of medicinal plants and herbal medicines is done through the “Agência Nacional de Vigilância Sanitária” (ANVISA—National Health Regulatory Agency), an agency of the Ministry of Health, which has the role of ensuring the health safety of products and services [102]. One of the actions taken by ANVISA to secure the health of the population is drug registration, whereby new drugs are evaluated for their safety, efficacy, and quality, before being marketed for use by the population.

The regulatory requirements for herbal medicines cover a range of aspects, from the supplier of the active ingredients through to the final product, produced under strict sanitary standards. The Collegiate Board of Directors Resolution (Resolução da Diretoria Colegiada, RDC) number 26 of May 13, 2014 provides for the registration and notification of traditional herbal products and herbal medicines to register with this agency [5]. The Normative Rule no 2 [77] published on the same date presents the lists of herbal medicines and traditional herbal products with simplified registration. Resolution RDC number 38 of June 18, 2014 [103] and resolution RDC 235 of June 20, 2018 [8] regulate adjustments, changes, extensions, updates, additions, and post-registration notifications of herbal medicines and traditional herbal products. Resolution RDC number 66 of November 26, 2014 establishes the information which has to be shown on the package leaflet of the traditional herbal product [104]. Resolution RDC number 105 of August 31, 2016 requires the presentation of the pesticide residue analysis reports or proof that the medicinal plants cultivated or collected in Brazil come from organic agriculture [105]. According to Resolution RDC 26/2014, both traditional herbal products and herbal medicines must be obtained exclusively from active raw material from plants and cannot include, in their composition, isolated or highly purified active substances [9]. With technical and legal support in Brazil, it is possible to develop an innovative herbal medicine with simplified registration.

In Brazil, registration with the competent health regulatory agency is required in order to market herbal medicines. Table 3 shows the products and formulations registered on the Datavisa ANVISA website, including species of *Passiflora* in combination with other active ingredients. The search has included products registered since 1985 since ANVISA does not remove any product from the list in order to maintain the historical record, even after their expired registration. The list includes both regular and revalidated products and those for which registration was canceled or has expired. Although 86 products containing *Passiflora* are registered with ANVISA (Table 3), only 34 medicines are currently valid in Brazil (Figure 3).

The pharmaceutical forms widely marketed and used are capsules, tablets, fluid extract, and tincture, besides infusion,

TABLE 2: List of patents in Brazil related to *Passiflora*.

Publication number	Date	Title
BR 10 2017 027632 5	Dec 20, 2017	Extract and flour of the peel fruit of <i>Passiflora cincinnata</i> Mast. (Passifloraceae) for use as hipolipment
BR 10 2017 020281 0	Sep 22, 2017	Preparation of functional goat yogurt with passion fruit (<i>Passiflora edulis</i> Sims) and mandacaru (<i>Cereus jamacaru</i> DC.) jam
BR 10 2017 020087 6	Sep 20, 2017	Preparation and processing of extra jam with passion flower (<i>Passiflora edulis</i> Sims) and mandacaru (<i>Cereus jamacaru</i> DC.) flavor
BR 10 2017 010267 0	May 16, 2017	Food supplement based on fruits from Caatinga
BR 10 2017 001368 5	Jan 23, 2017	Antimicrobial potential of extracts from exotic fruit waste (tamarind (<i>Tamarindus indica</i> L.), grenadilla (<i>Passiflora ligularis</i> Juss.), noni (<i>Morinda citrifolia</i> L.), dekopon (<i>Citrus reticulata</i> 'Blanco shiranui'), sapoti (<i>Manilkara zapota</i> (L.) P. Royen), tamarillo (<i>Solanum betaceum</i> Cav.), and mirtilo (<i>Vaccinium myrtillus</i> L.)
BR 10 2016 022442 0	Nov 28, 2016	Formulation and production of symbiotic fermented dairy produced with flaxseed (<i>Linum usitatissimum</i> L.) and/or waste from the processing of yellow passion fruit (<i>Passiflora edulis</i> var. <i>flavicarpa</i> Deg.)
BR 10 2016 014976 2	Jun 24, 2016	Sequential extraction process of bioactive compounds from passion fruit bagasse and the use of its bioactive compounds
BR 10 2015 032464 2	Dec 15, 2015	Formulation for topical use for photoprotection containing <i>Passiflora cincinnata</i> Mast.
BR 11 2017 015446 3	Dec 10, 2015	Homoeopathic method for treating colic, intestinal gases, and malaise in children
BR 10 2015 006517 5	Mar 24, 2015	Mixed flour; sweet cookie; and process for producing mixed flour and sweet cookies containing plant extracts of Brazil nuts (<i>Bertholletia excelsa</i> Bonpl.), manioc (<i>Manihot esculenta</i> Crantz), peach palm (<i>Bactris gasipaes</i> Kunth), and passion fruit (<i>Passiflora edulis</i> Sims)
BR 10 2015 002902 0	Fev 10, 2015	Lotion based on <i>Ganoderma lucidum</i> (Curtis) P. Karst. Spores
BR 10 2015 001385 0	Jan 22, 2015	Composition of products without added sugar based on yellow passion fruit flour
BR 10 2015 002897 0	Jan 16, 2015	Production of antibiotic containing <i>Passiflora edulis</i> Sims extract and its use
BR 11 2016 006227 2	Sep 25, 2014	Lipid extract of passion fruit seeds
BR 11 2015 014492 6	Dec 20, 2013	Composition of plant extract for treatment of opious and alcohol abuse
BR 11 2015 014351 2	Dec 18, 2013	Extract of <i>Passiflora</i> seeds and cosmetic, dermatological, and nutraceutical compositions comprising the same
BR 10 2012 026263 0	Oct 08, 2012	Composition for hair mask without gluten
BR 10 2012 026281 9	Oct 08, 2012	Composition for hair conditioner without gluten
BR 10 2012 021728 7	Aug 29, 2012	Nanoparticle
BR 10 2012 022381 3	Aug 28, 2012	Compositions for hair oil
BR 10 2012 019583 6	Jul 09, 2012	Compositions for hair mask
PI 0816292-1	Sep 03, 2008	Process for the preparation of an extract of <i>Passiflora alata</i> Curtis plant and its use in cosmetic and pharmaceutical compositions
PI 0800544-3	Feb 01, 2008	Analytical technology based on pyrolysis coupled to gas chromatography/mass spectrometry for the characterization and obtaining chemical compounds from extracts of <i>Passiflora alata</i> Curtis dried by nebulization
PI 0800705-5	Jan 15, 2008	Flavor capsules for decreasing absorption of lipids and carbohydrates
PI 0602106-9	May 25, 2006	Process for preparing a product based on <i>Passiflora incarnata</i> L., pharmaceutical product, and pharmaceutical composition, use, and method of treatment of anxiety and insomnia
PI 0501200-7	Feb 16, 2005	Process for obtaining energy drink based on coconut water and natural passion fruit juice and honey
PI 0501199-0	Feb 16, 2005	Process for obtaining energy drink based on coconut water and natural passion fruit juice
PI 0203346-1	Jun 26, 2002	Composition of synergistic effect with plant active ingredients for higher activities of hydration and tissue regeneration, dermal protection against aggressive external agents, as lotion, cream, gel, or cellulose scarves, presented ready use

TABLE 3: List of ANVISA registered products containing *Passiflora* sp.

Product/category	Dosage form	Manufacturer Code	Status	Active
A SAÚDE DA MULHER/ antispasmodic, anticholinergic, drug combination	Oral solution	57.507.378/0003-65	Valid	Sodium salicilate/ <i>Passiflora alata</i> Curtis (fluid extract)/‘Agoniada pluméria’ (tincture)/bitter orange peel (fluid extract)
A SAÚDE DA MULHER/ antispasmodic, anticholinergic, drug combination	Simple dragee/oral solution	45.992.062/0001-65	Canceled	Sodium salicilate/ <i>Passiflora</i> dry extract/ ‘Agoniada’ dry extract
ACALMIL/herbal compound	Coated tablet	29.333.218/0001-40	Canceled	<i>Passiflora incarnata</i> L./ <i>Salix alba</i> L. (leaves extract)/ <i>Crataegus oxyacantha</i> L. extract
ACALMIL/herbal compound	Coated tablet	92.265.552/0001-40	Canceled	<i>Passiflora incarnata</i> L./ <i>Salix alba</i> L. (leaves extract)/ <i>Crataegus oxyacantha</i> L. extract
ALPHALIV/simple Herbal, simple anxiolytic	Oral solution/coated tablet	09.545.589/0001-88	Valid	<i>Passiflora incarnata</i> L.
ANSIODORON/potentized preparation	Tablet	56.992.217/0001-80	Valid	<i>Avena sativa</i> L./ <i>Passiflora alata</i> Curtis/ <i>Valeriana officinalis</i> L. <i>Caesalpinia ferrea</i> Mart. / <i>Roripa</i> <i>nasturtium</i> Beck/ <i>Mikania glomerata</i> Spreng./ <i>Lantana camara</i> L./ <i>Passiflora</i> <i>alata</i> Curtis/ <i>Melissa officinalis</i> L./ <i>Myrospermum erythroxylum</i> Allem
BRONQUIOGEN/herbal compound	Oral solution	51.603.488/0001-82	Canceled	<i>Crataegus rhipidophylla</i> Gand./ <i>Passiflora</i> <i>incarnata</i> L./ <i>Salix alba</i> L.
CALMAN/herbal compound	Coated tablet/oral solution	02.433.631/0001-20	Valid	<i>Crataegus rhipidophylla</i> Gand./ <i>Salix alba</i> L./ <i>Passiflora incarnata</i> L.
CALMAN/herbal compound	Coated tablet/oral solution	64.088.172/0001-41	Canceled	<i>Melissa officinalis</i> L./ <i>Passiflora alata</i> Curtis / <i>Valeriana officinalis</i> L./ <i>Lavandula</i> <i>officinalis</i> Chaix
CALMANTE FARMAERVAS/ herbal compound	Oral solution	60.565.520/0001-73	Canceled	<i>Passiflora incarnata</i> L./ <i>Erythrina</i> <i>mulungu</i> Mart./ <i>Matricaria chamomilla</i> L./ <i>Melissa officinalis</i> L. (only in oral solution)
CALMAPAX/similar, associated natural products	Coated tablet/oral solution /cropped	33.173.097/0002-74	Canceled	<i>Passiflora incarnata</i> L.
CALMASYN/simple herbal, simple analytic	Oral solution/coated tablet	17.562.075/0001-69	Valid	<i>Passiflora incarnata</i> L.
CALMAVITA/simple herbal	Hard gelatin capsule	56.646.953/0001-86	Canceled	<i>Passiflora incarnata</i> L. <i>Matricaria chamomilla</i> L./ <i>Citrus</i> <i>aurantium</i> L./ <i>Cymbopogon citratus</i> Stapf/ <i>Passiflora alata</i> Curtis
CALMI/herbal compound	Oral solution	17.299.140/0001-05	Canceled	<i>Passiflora incarnata</i> L.
ACALMIL/herbal compound	Syrup	06.597.801/0001-62	Valid	<i>Passiflora incarnata</i> L./ <i>Salix alba</i> L./ <i>Crataegus rhipidophylla</i> Gand.
CALMIPLAN/herbal compound	Coated tablet	47.100.862/0001-50	Canceled	<i>Passiflora incarnata</i> L./ <i>Salix alba</i> L./ <i>Crataegus rhipidophylla</i> Gand.
CALMOPLANTAS/simple herbal, simple anxiolytic	Tincture/hard gelatin capsule	79.634.572/0001-82	Valid	<i>Passiflora incarnata</i> L./ <i>Salix alba</i> L./ <i>Crataegus rhipidophylla</i> Gand.
CALMOXIL/simple herbal, simple anxiolytic	Coated tablet/oral solution	28.643.633/0001-37	Canceled	<i>Passiflora incarnata</i> L.
CARDIOPAX GOTAS/ Homeopathic association	Oral solution	76.440.528/0001-43	Canceled	<i>Passiflora incarnata</i> L./ <i>Crataegus</i> <i>rhipidophylla</i> Gand./ <i>Digitalis</i> sp.
CARDIOSETYL M/simple herbal, simple anxiolytic	Oral solution	21.573.449.0001-19	Canceled	<i>Passiflora incarnata</i> L.
CARDIOSETYL M/herbal compound/antispasmodic	Oral solution	21.573.449/0001-19	Canceled	<i>Passiflora alata</i> Curtis/ <i>Crataegus</i> <i>rhipidophylla</i> Gand./ <i>Erythrina mulungu</i> Mart./ <i>Leptolobio elegans</i> Vog
DALAY/simple herbal	Coated tablet/oral solution	01.858.973/0001-29	Valid	<i>Passiflora</i> dry extract
DYRAJAIA/herbal compound	Tincture	02.007.074/0001-85	Canceled	<i>Passiflora alata</i> Curtis/ <i>Dorstenia</i> <i>multiformis</i> Miq.
ELIXIR DE MARACUJÁ COMPOSTO FARMAERVAS/ herbal compound	Elixir	60.565.520/0001-73	Canceled	<i>Passiflora alata</i> Curtis / <i>Paullinia cupana</i> Kunth/ <i>Valeriana officinalis</i> L.

TABLE 3: Continued.

Product/category	Dosage form	Manufacturer Code	Status	Active
EQUILIBRISSE/simple herbal, simple anxiolytic	Coated tablet	05.161.069/0001-10	Valid	<i>Passiflora</i> dry extract
EQUILIBRISSE/simple herbal, simple anxiolytic	Coated tablet	05.161.069/0001-10	Valid	<i>Passiflora incarnata</i> L.
ELIXIR DE CEREUS COMPOSTO/herbal compound	Oral solution	92.695.816/0001-03	Canceled	<i>Crataegus tincture/Cereus grandiflorus</i> (L.) Mill./ <i>Valeriana officinalis</i> L./ <i>Passiflora alata</i> Curtis
ERVA SILVINA COMPOSTA/herbal compound	Elixir	92.943.992/0001-09	Canceled	<i>Casearia sylvestris</i> Sw./ <i>Aristolochia cymbifera</i> Martius/ <i>Passiflora alata</i> Curtis <i>Caesalpinia ferrea</i> Mart./ <i>Roripa nasturtium</i> Beck/ <i>Mikania glomerata</i> Spreng./ <i>Passiflora alata</i> Curtis/ <i>Polypodium vulgare</i> /Myrospermum erythroxylum Allem./ <i>Lantana camara</i> Linné
FIMATOSAN/Associated natural products	Oral solution	33.379.887/0001-96	Canceled	
FIQUEZEN/simple herbal, simple anxiolytic	Coated tablet/oral solution	17.875.154/0001-20	Valid	<i>Passiflora</i> dry extract
FITOCALM/simple herbal, simple anxiolytic	Coated tablet	84.684.620/0001-87	Valid	<i>Passiflora incarnata</i> L.
FITOCALMIN/simple herbal	Hard gelatin capsule	14.186.324/0001-70	Canceled	<i>Passiflora incarnata</i> L.
FLORINY/Anxiolytics—drug combination/herbal compound	Coated tablet/oral solution	64.088.172/0001-41	Canceled	<i>Passiflora incarnata</i> L./ <i>Salix alba</i> L./ <i>Crataegus oxyacantha</i> L.
MARACUGINA COMPOSTA/herbal compound/anxiolytic	Oral solution/coated tablet	61.082.426/0002-07	Canceled	<i>Erythrina mulungu</i> Mart./ <i>Crataegus rhipidophylla</i> Gand./ <i>Passiflora alata</i> Curtis
MARACUGINA COMPOSTA/herbal compound	Oral solution/coated tablet	67.866.665/0002-34	Canceled	<i>Passiflora alata</i> Curtis/ <i>Erythrina mulungu</i> Mart./ <i>Crataegus rhipidophylla</i> Gand.
MARACUGINA COMPOSTA/herbal compound	Oral solution/coated tablet	02.932.074/0001-91	Canceled	<i>Passiflora alata</i> Curtis/ <i>Erythrina mulungu</i> Mart./ <i>Crataegus rhipidophylla</i> Gand.
MARACUGINA PI/ simple herbal, simple anxiolytic	Coated tablet	61.082.426/0002-07	Valid	<i>Passiflora</i> dry extract
MARACUGINA PI/ simple herbal, simple anxiolytic	Coated tablet	61.082.426/0002-07	Valid	<i>Passiflora incarnata</i> L.
MARACUJÁ CONCENTRIX/herbal compound, simple anxiolytic	Simple dragees/oral solution	05.044.984/0001-26	Canceled	<i>Crataegus rhipidophylla</i> Gand./ <i>Passiflora incarnata</i> L./ <i>Salix alba</i> L.
MARACUJÁ GOTAS/simple herbal	Hydroalcoholic solution	78.950.011/0001-20	Canceled	<i>Passiflora incarnata</i> L.
MARACUJÁ HERBARIUM/simple herbal, simple anxiolytic	Hard gelatin capsule/coated tablet	78.950.011/0001-20	Valid	<i>Passiflora incarnata</i> L.
MULUNGU BROMETADO/similar	Syrup	10.419.935/0001-60	Canceled	KBr/NaBr/ NH4Br/ <i>Atropa belladonna</i> tincture/ <i>Hyosciamus niger</i> L. Tincture/ <i>Passiflora alata</i> Curtis <i>Passiflora alata</i> Curtis/ <i>Ptychopetalum olacides</i> Benth/ <i>Cola nitida</i> Schott & Endl./ <i>Paullinia cupana</i> Kunth/thiamine hydrochloride
NERVITON/Associated phytotherapies	Elixir	92.751.965/0001-34	Canceled	<i>Avena sativa</i> L./ <i>Coffea arabica</i> L./ <i>Passiflora incarnata</i> L./ <i>Zincum isovalerianicum</i>
NEUREXAN/Dynamized compound	Sublingual tablet	05.994.539/0001-27	Valid	<i>Passiflora incarnata</i> L./ <i>Valeriana officinalis</i> L./ <i>Crataegus rhipidophylla</i> Gand.
NUIT/herbal compound	Coated tablet	49.475.833/0001-06	Canceled	<i>Crataegus rhipidophylla</i> Gand./ <i>Passiflora incarnata</i> L./ <i>Salix alba</i> L.
PASALIX/herbal compound	Coated tablet	60.726.692/0001-81	Valid	
PASALIX PI/simple herbal, simple anxiolytic	Coated tablet/oral solution	60.726.692/0001-81	Valid	<i>Passiflora incarnata</i> L.
PASIC/herbal compound	Coated tablet/oral aerosol	44.734.671/0001-51	Canceled	<i>Passiflora incarnata</i> L./ <i>Crataegus rhipidophylla</i> Gand./ <i>Salix alba</i> L.

TABLE 3: Continued.

Product/category	Dosage form	Manufacturer Code	Status	Active
PASSANEURO/herbal compound	Coated tablet	47.100.862/0001-50	Canceled	<i>Passiflora</i> dry extract/ <i>Erithrina mulungu</i> Mart./ <i>Matricaria chamomilla</i> L.
PASSICALM/herbal compound	Oral solution	85.776.524/0001-21	Canceled	<i>Erithrina mulungu</i> Mart./ <i>Valeriana officinalis</i> L./ <i>Passiflora alata</i> Curt
PASSIENE/simple herbal	Syrup	78.950.011/0001-20	Canceled	<i>Passiflora</i> dry extract
PASSIENE/simple herbal	Syrup/ coated tablet	78.950.011/0001-20	Valid	<i>Passiflora incarnata</i> L.
PASSIFLORA/herbal compound	Tincture	02.007.074/0001-85	Canceled	<i>Passiflora</i> dry extract/ <i>Erithrina mulungu</i> Mart.
PASSIFLORA ALTHAIA/ simple herbal, simple anxiolytic	Oral solution	48.344.725/0007-19	Valid	<i>Passiflora incarnata</i> L.
PASSIFLORA CATARINENSE/ simple herbal, simple anxiolytic	Coated tablet	84.684.620/0001-87	Valid	<i>Passiflora incarnata</i> L.
PASSIFLORA COMPOSTA/ herbal compound	Oral solution/ simple dragee	42.341.149/0001-84	Canceled	<i>Crataegus rhipidophylla</i> Gand./ <i>Passiflora incarnata</i> L./ <i>Erythrina mulungu</i> Mart.
PASSIFLORA COMPOSTA/ herbal compound	Elixir	61.299.111/0001-35	Canceled	<i>Erythrina mulungu</i> Mart./ <i>Melissa officinalis</i> L./ <i>Passiflora alata</i> Curtis
PASSIFLORA DA ÍNDIA/ associated natural products	Elixir	87.104.170/0001-02	Canceled	<i>Passiflora alata</i> Curtis/ <i>Atropa belladonna</i> L./ <i>Erythrina mulungu</i> Mart./ <i>Citrus limetta</i> Risso/ <i>Cereus grandiflorus</i> (L.) Mill.
PASSIFLORA KLEIN/simple herbal, simple anxiolytic	Tincture	92.762.277/0001-70	Valid	<i>Passiflora incarnata</i> L.
PASSIFLORA ORIENT/simple herbal	Hard gelatin capsule	73.657.876/0001-89	Canceled	<i>Passiflora incarnata</i> L.
PASSIFLORINE/herbal compound	Oral solution/simple dragee	33.388.182/0001-79	Canceled	<i>Passiflora incarnata</i> L./ <i>Salix alba</i> L./ <i>Crataegus rhipidophylla</i> Gand./ <i>Calcium gluconate</i> /Cholecalciferol/low molecular weight peptides/magnesium hyposulfum
PASSIFLORINE PI/simple herbal, simple anxiolytic	Coated tablet	23.668.196/0001-92	Valid	<i>Passiflora</i> dry extract
PASSIPAX/simple herbal	Oral solution	04.656.253/001-79	Valid	<i>Passiflora incarnata</i> L.
PAZINE/simple herbal, simple anxiolytic	Coated tablet	64.088.172/0001-41	Canceled	<i>Passiflora incarnata</i> L.
PAZINE/simple herbal, simple anxiolytic	Coated tablet	07.670.111/0001-54	Valid	<i>Passiflora incarnata</i> L.
PHYTOCALM /herbal compound	Syrup/hard gelatin capsule	84.684.620/0001-87	Canceled	<i>Passiflora incarnata</i> L./ <i>Valeriana officinalis</i> L./ <i>Matricaria chamomilla</i> L./ <i>Crataegus oxyacantha</i> L./ <i>Erythrina mulungu</i> Mart.
PRAKALMAR/Simple herbal, simple anxiolytic	Coated tablet	02.433.631/0001-20	Valid	<i>Passiflora incarnata</i> L.
PRASILENCE/Simple herbal, simple anxiolytic	Oral solution/coated tablet	25.773.037/0001-83	Valid	<i>Passiflora incarnata</i> L.
RITMONEURAN/Herbal compound	Hard gelatin capsule/oral solution	92.695.691/0001-03	Canceled	<i>Passiflora alata</i> Curtis/ <i>Erythrina mulungu</i> Mart./ <i>Leptolobio elegans</i> Vog/ <i>Adonis vernalis</i> L.
RITMONEURAN RTM/simple anxiolytic, simple herbal	Hard gelatin capsule/oral solution/ coated tablet	92.695.691/0001-03	Valid	<i>Passiflora</i> dry extract
SALSAPARILHA COMPOSTO/Associated natural products	Elixir	92.943.992/0001-09	Canceled	<i>Smilax japicanga</i> Griseb./ <i>Baccharis genistelloides</i> Persoon/ <i>Plantago major</i> L./ <i>Aristolochia cymbifera</i> Martius/ <i>Passiflora incarnata</i> L.
SEAKALM/herbal compound	Coated tablet	02.456.955/0001-83	Canceled	<i>Passiflora incarnata</i> L./ <i>Salix alba</i> L./ <i>Crataegus rhipidophylla</i> Gand.
SEAKALM/simple anxiolytic, simple herbal	Coated tablet/oral solution	02.456.955/0001-83	Valid	<i>Passiflora incarnata</i> L.
SEDACAL/ simple herbal	Hard gelatin capsule	01.845.448/0001-79	Canceled	<i>Passiflora incarnata</i> L.
SEDANUS/associated natural products	Oral solution	67.866.665/0002-34	Canceled	<i>Crataegus oxyacantha</i> L./ <i>Passiflora incarnata</i> L./ <i>Erythrina mulungu</i> Mart.
SERENUS/associated phytotherapics	Coated tablet	49.475.833/0001-06	Valid	<i>Crataegus rhipidophylla</i> Gand./ <i>Passiflora incarnata</i> L./ <i>Salix alba</i> L.

TABLE 3: Continued.

Product/category	Dosage form	Manufacturer Code	Status	Active
SINTOCALMY/simple herbal simple anxiolytic	Coated tablet	60.659.463/0001-91	Valid	<i>Passiflora incarnata</i> L.
SOMINEX/herbal compound	Tablet	57.507.378/0001-01	Canceled	<i>Crataegus rhipidophylla</i> Gand./ <i>Valeriana officinalis</i> L./ <i>Passiflora incarnata</i> L.
SOMINEX COMPOSTO/herbal compound anxiolytics—drug combinations	Coated tablet	57.507.378/0001-01	Canceled	<i>Valeriana officinalis</i> L./ <i>Crataegus rhipidophylla</i> Gand./ <i>Passiflora incarnata</i> L.
SONOTABS/herbal compound	Simple dragee	92.695.691/0001-03	Canceled	<i>Passiflora</i> dry extract/ <i>Salix</i> dry extract/ <i>Crataegus rhipidophylla</i> Gand. extract
SONOTABS/herbal compound	Simple dragee	90.455.262/0001-33	Canceled	<i>Passiflora</i> dry extract/ <i>Salix</i> dry extract/ <i>Crataegus rhipidophylla</i> Gand. extract <i>Aconitum napellus</i> L./ Ammonium bromatum/ <i>Agaricus muscarius</i> / <i>Citrullus</i> <i>colocynthis</i> Schrad./ <i>Colocynthis</i> /
SPASCUPREEL/potentized compound	Sublingual tablet/ injectable solution	05.994.539/0001-27	Valid	<i>Gelsemium sempervirens</i> (L.) J. St.-Hil./ Magnesium phosphoricum/ <i>Matricaria</i> <i>recutita</i> L./ <i>Passiflora incarnata</i> L./ <i>Veratrum album</i> L./ Atropinum sulfuricum/Cuprum sulphuricum Atropinum sulfuricum/ <i>Citrullus</i> <i>colocynthis</i> Schrad./ <i>Veratrum album</i> L./
SPASCUPREEL/potentized compound	Sublingual tablet/	74.455.197/0001-90	Canceled	Magnesium phosphoricum/ <i>Gelsemium</i> <i>sempervirens</i> (L.) J. St.-Hil./ <i>Passiflora</i> <i>incarnata</i> L./ <i>Agaricus muscarius</i> / <i>Chamomilla recutita</i> / <i>Aconitum napellus</i>
TENSART/simple herbal	Coated tablet/oral solution	64.088.172/0001-41	Canceled	<i>Passiflora incarnata</i> L.
TENSART/ simple herbal	Coated tablet/oral solution	17.440.261/0001-25	Valid	<i>Passiflora incarnata</i> L.

Source: <https://consultas.anvisa.gov.br/#/medicamentos/>. Accessed on December 03, 2019.

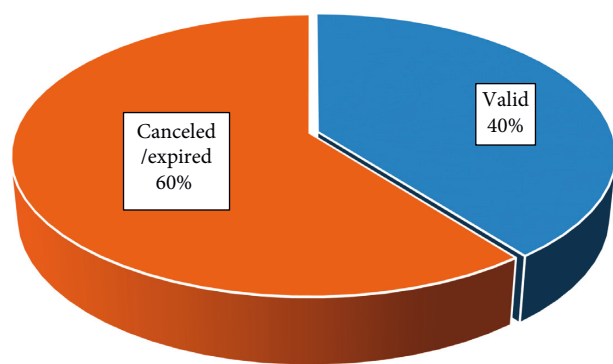


FIGURE 3: Valid and canceled/expired registrations of *Passiflora*-containing medicines registered in Brazil (ANVISA).

which is a folk preparation. Among the 86 products registered with ANVISA, *Passiflora incarnata* is present in 63% of them. Species of *Passiflora* genus are associated with other plant species in 57% of the products, in which *P. incarnata* predominates (Figure 4). Simple herbal medicines correspond to 43% of the total registered products, in which *P. incarnata* has been used in 30 registered products. The registration for the use of herbal products in association

requires proof of the benefits of this composition, and a risk/benefit evaluation [100], as well as the use of *P. alata* as a substituent of *P. incarnata*. Although *P. alata* is registered in the Brazilian Pharmacopoeia [19], this plant species is not listed in the normative rule about simplified registration of herbal medicines [77].

2.8. Patents and Registered Products Containing *Passiflora* outside Brazil. Outside Brazil, empirical searches carried out in the database of the United States Patent and Trademark Office, an Agency of the Department of Commerce North American, indicated the existence of 572, 164 and 4 patents when the keywords “passion flower”, “*Passiflora edulis*” and “*Passiflora alata*” were used, respectively [106].

From 2006 to 2013, 27 traditional herbal products containing *Passiflora* were registered with the Medicines and Healthcare Products Regulatory Agency (MHRA) of the United Kingdom. All of them contain extracts of *P. incarnata* in single formulations or in association with other plant species. These associations represent approximately 74% of all the products, and include, in their formulations, species traditionally used to treat anxiety, such as *Valeriana officinalis* (Valerianaceae), *Lactuca virosa* (Asteraceae),

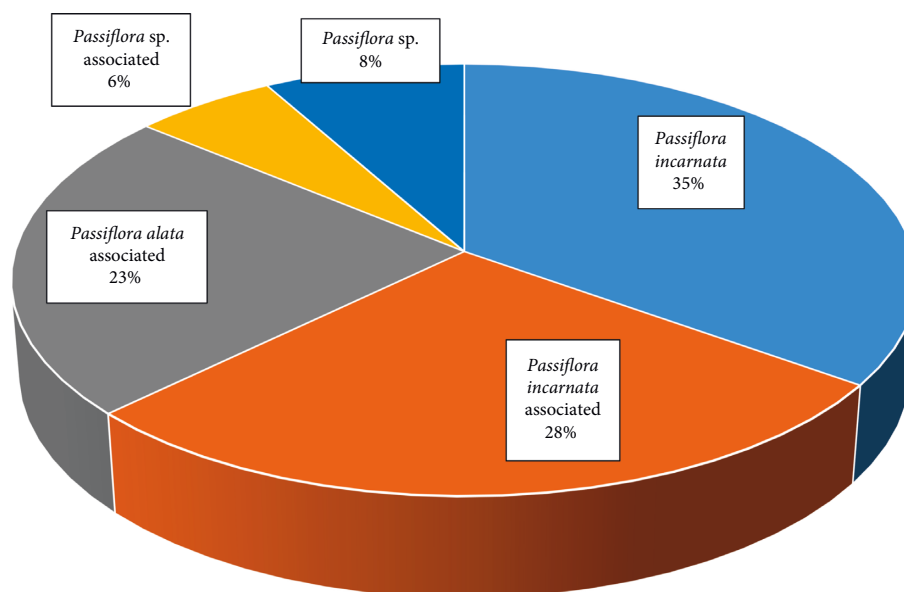


FIGURE 4: Distribution of *Passiflora* species in ANVISA registered products containing *Passiflora* sp.

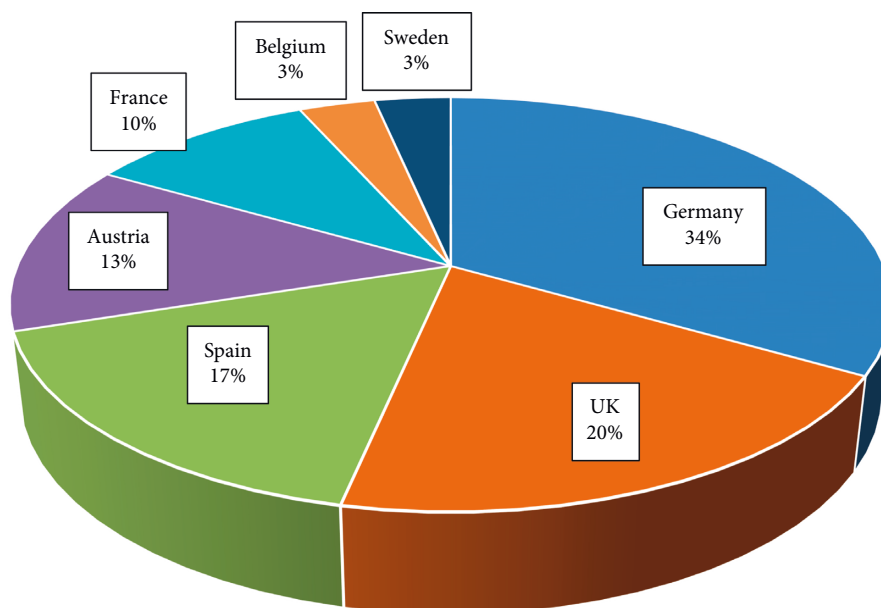


FIGURE 5: Distribution of preparations containing *Passiflora incarnata* registered in Europe Union, according to the European Medicines Agency [21].

Hypericum perforatum (Hypericaceae), and *Melissa officinalis* (Lamiaceae) [20].

According to the European Medicines Agency, affiliated to the European Union, various herbal preparations containing *P. incarnata* are used in the member countries, with marketing authorization and/or registrations for traditional use. Of the total of thirty preparations registered in Europe, Germany has ten, the UK six, Spain five, Austria four, France three, Belgium one, and Sweden one (Figure 5). The main uses are sachets containing fragmented plant material for use in herbal teas, simple or coated tablets containing dry ethanol extract, hard capsules with powdered or fragmented plant material or dry ethanol extract, instant products

containing aqueous extract for the preparation of herbal tea, and liquid products for oral use containing ethanol liquid extract. All these preparations are indicated for the relief of symptoms of mild neurasthenia and for insomnia [21].

3. Conclusions

This work deals with the medicinal plants of the genus *Passiflora* that have effects on generalized anxiety disorders. Although it is the genus most used by humans for therapeutic purposes within the Passifloraceae family, especially for the treatment of phenomena related to the anxiety syndrome, it is still restricted to very few plant species. In

Brazil, the registered and/or patented products containing extracts of *Passiflora* species only include a small number of taxa, highlighting *P. incarnata* and *P. alata*. Among the 86 registered products in Brazil as “*Passiflora*,” those contain *P. incarnata* in single formulations or in combination with other ingredients represent 63% of the total, suggesting that this species of *Passiflora* genus is the most commonly used in medicines. In other countries, *P. incarnata* is also selected as the main component in natural anxiolytic drug formulations, with a long history of use and inclusion as an official plant drug in various Pharmacopoeias, since its effects are documented. Nevertheless, under the criteria of scientific knowledge about the pharmacological properties of *Passiflora* indicated in nonclinical and clinical studies that might be accompanied by the determination of the chemical composition of the extracts, formulations, and products, other studies are still needed to validate the mechanism of action and recognize the medicinal use of *Passiflora* species as well-established. What other species of *Passiflora* can be used safely in therapy as substituents of those most currently used? What are the criteria for this choice? These questions are important, considering that there are around 600 known species little or unexploited for medicinal purposes and that many of them are mainly distributed in tropical regions, such as Brazil. Therefore, research should be implemented immediately, in order to extend the herbal chain production and increase the commercial value of these products.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

- [1] E. A. Carlini, “Plants and the central nervous system,” *Pharmacology Biochemistry and Behavior*, vol. 75, pp. 501–512, 2003.
- [2] K. Dhawan, S. Kumar, and A. Sharma, “Comparative anxiolytic activity profile of various preparations of *Passiflora incarnata* Linnaeus: a comment on medicinal plants’ standardization,” *Journal of Alternative and Complementary Medicine*, vol. 8, no. 3, pp. 283–291, 2002.
- [3] S. Wakdikar, “Global health care challenge: Indian experiences and new prescriptions,” *Electronic Journal of Biotechnology*, vol. 7, pp. 214–220, 2004.
- [4] Germany, Federal Law Gazette [BGBl], “Medicinal products act, part I,” vol. 12, p. 3394, 2005 Updated by Federal Law Gazette I, Law of October 19, 2012, p. 2192. http://www.gesetze-im-internet.de/englisch_amg/medicinal_products_act.pdf.
- [5] E Union and The European parliament and of the Council, *Directive 2001/83/EC*, Official Journal of the European Union, 2001, http://www.wipo.int/wipolex/en/text.jsp?file_id=291864.
- [6] E Union and The European parliament and of the Council, *Directive 2004/24/EC*, Official Journal of the European Union, 2004, http://www.wipo.int/wipolex/en/text.jsp?file_id=179078.
- [7] M. Miroddi, C. Mannucci, F. Mancari, M. Navarra, and G. Calapai, “Research and development for botanical products in medicinals and food supplements market,” *Evidence-Based Complementary Alternative Medicine*, vol. 2013, Article ID 649720, 6 pages, 2013.
- [8] Brazil, Ministry of Health and ANVISA, “RDC no 14/March 31, 2010,” *Diário Oficial da União*, ANVISA, Brasília, Brazil, 2010, http://portal.anvisa.gov.br/documents/10181/2718376/RDC_14_2010_COMP.pdf/a919c09b-0717-451d-849b-067153c02930?version=1.0.
- [9] Brazil, Ministry of Health and ANVISA, “RDCN. 26/May 13, 2014,” *Diário Oficial da União, Brasília, Section 1*, pp. 52–58, ANVISA, Brasília, Brazil, 2014, <http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?jornal=1&pagina=52&data=14/05/2014>.
- [10] S. F. Arifin, A. Al Shami, S. S. S. Omar, M. A. A. Jalil, K. A. Khalid, and H. Hadi, “Impact of modern technology on the development of natural-based products,” *Journal of Ayurvedic and Herbal Medicine*, vol. 5, pp. 133–142, 2019.
- [11] S.V. Scopus®, *Bibliographic Database and Search Site*, Elsevier B.V., Amsterdam, Netherlands, 2019, <http://www.scopus.com>.
- [12] Scirus®, *Bibliographic Database and Search Site*, Elsevier B.V., Amsterdam, Netherlands, 2019, <http://www.scirus.com>.
- [13] SciFinder®, *Bibliographic Database and Search Site*, Chemical Abstracts Service, Columbus, OH, USA, 2019, <http://scifinder.cas.org/>.
- [14] Google Scholar®, *Search Site*, Google LLC, Mountain View, CA, USA, 2019, <https://scholar.google.com.br/>.
- [15] United States, Department of Health and Human Services and National Institutes of Health (NIH), *Database. ClinicalTrials.Gov*, National Institutes of Health (NIH), Bethesda, MA, USA, 2020, <https://clinicaltrials.gov>.
- [16] Brazil, National Institute of Industrial Property (INPI), *Search Website*, National Institute of Industrial Property (INPI), Courbevoie, France, 2019, <https://gru.inpi.gov.br/pePI/servlet/PatenteServletController>.
- [17] Brazil, Ministry of Health and ANVISA, *Datavisa ANVISA Websitae*, Ministry of Health, Brazil, 2019, <https://consultas.anvisa.gov.br/#/medicamentos/>.
- [18] World Trade Organization, *Agreement on Trade-Related Aspects of Intellectual Property Rights*, World Trade Organization, Geneva, Switzerland, 1994, https://www.wto.org/english/tratop_e/trips_e/t_agm1_e.htm.
- [19] Brazil, Ministry of Health and ANVISA, *Brazilian Pharmacopoeia*, ANVISA, Brasília, Brazil, PM061-PM062, 6th edition, 2019.
- [20] MHRA (Medicines and Healthcare Products Regulatory Agency), *Guidance: Herbal Medicines Granted a Traditional Herbal Registration*, Medicines and Healthcare Products Regulatory Agency, London UK, 2014, <https://www.gov.uk/government/publications/herbal-medicines-granted-a-traditional-herbal-registration-thr/herbal-medicines-granted-a-traditional-herbal-registration>.
- [21] HMPC, *Assessment Report on Passiflora Incarnata L. Herba*, https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-passiflora-incarnata-l-herba_en.pdf EMA/HMPC/669738/2013, pp. 1–22, European Medicines Agency, Amsterdam, Netherlands, 2014, .

- [22] V. C. Muschner, A. P. Lorenz, A. C. Cervi, S. L. Bonatto, and T. T. Souza-Chies, "A first molecular phylogenetic analysis of *Passiflora* (Passifloraceae)," *American Journal of Botany*, vol. 8, pp. 1229–1238, 2003.
- [23] T. Ulmer and J. M. Mac Dougal, *Passiflora: Passionflowers of the World*, Timber Press, Portland, OR, USA, 2004.
- [24] C. A. M. Pereira and J. H. Y. Vilegas, "Constituintes químicos e farmacologia do gênero *Passiflora* com ênfase a *P. alata* Dryander," *Revista Brasileira de Plantas Mediciniais*, vol. 3, pp. 1–12, 2000.
- [25] M. M. Souza, T. N. S. Pereira, and M. C. Vieira, "Cytogenetic studies in some species of *Passiflora* L. (Passifloraceae): a review emphasizing Brazilian species," *Brazilian Archives of Biology Technology*, vol. 51, pp. 247–258, 2008.
- [26] C. B. M. Cerqueira-Silva, L. D. H. C. S. Conceição, A. P. Souza, and R. X. Corrêa, "A history of passion fruit woodiness disease with emphasis on the current situation in Brazil and prospects for Brazilian passion fruit cultivation," *European Journal of Plant Pathology*, vol. 139, pp. 261–270, 2014.
- [27] G. Gosmann, G. Provesnsi, L. N. Comunello, and S. M. K. Rates, "Composição química e aspectos farmacológicos de espécies de *Passiflora* L. (Passifloraceae)," *Revista Brasileira de Biociências*, vol. 9, pp. 88–99, 2011.
- [28] J. C. Oliveira and C. Ruggiero, "Espécies de Maracujá com potencial agrônomo," in *Maracujá germoplasma e melhoramento genético*, F. G. Faleiro, N. T. V. Junqueira, and M. F. Braga, Eds., pp. 141–158, Embrapa Cerrados, Planaltina, Brazil, 2005, http://www.cpac.embrapa.br/ivrtpm/homepage/capitulos/cap_6.pdf.
- [29] A. Braga, T. P. Medeiros, and B. V. Araújo, "Investigação da atividade antihiperlipidêmica da farinha da casca de *Passiflora edulis* Sims, Passifloraceae, em ratos diabéticos induzidos por aloxano," *Revista Brasileira de Farmacognosia*, vol. 20, pp. 186–191, 2010.
- [30] M. Miroddi, G. Calapai, M. Navarra, P. L. Minciullo, and S. Gangemi, "*Passiflora incarnata* L.: ethnopharmacology, clinical application, safety and evaluation of clinical trials," *Journal of Ethnopharmacology*, vol. 150, pp. 791–804, 2013.
- [31] N. Klein, A. C. Gazola, T. C. de Lima, E. Schenkel, K. Nieber, and V. Butterweck, "Assessment of sedative effects of *Passiflora edulis* f. *flavicarpa* and *Passiflora alata* extracts in mice, measured by telemetry," *Phytotherapy Research*, vol. 28, pp. 706–713, 2014.
- [32] A. C. Cervi and W. A. Rodrigues, "Nomenclatural and taxonomic review of Passifloraceae species illustrated and described by Vellozo in Flora Fluminensis," *Acta Botanica Brasílica*, vol. 24, pp. 1109–1111, 2010.
- [33] K. Dhawan, S. Dhawan, and A. Sharma, "*Passiflora*: a review update," *Journal of Ethnopharmacology*, vol. 94, pp. 1–23, 2004.
- [34] F. De-Paris, R. D. Petry, F. H. Reginatto et al., "Pharmacological study of aqueous extract of *Passiflora alata* Dryander and *Passiflora edulis* Sims," *Acta Farmaceutica Bonaerense*, vol. 21, pp. 5–8, 2002.
- [35] R. Soulimani, C. Younos, S. Jarmouni, D. Boust, R. Misslin, and F. Mortier, "Behavioural effects of *Passiflora incarnata* L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse," *Journal of Ethnopharmacology*, vol. 57, pp. 11–20, 1997.
- [36] M. S. Oliveira, I. O. Pinheiro, and F. S. B. V. Silva, "Vermicompost and arbuscular mycorrhizal fungi: an alternative to increase foliar orientin and vitexin-2-O-ramnoside synthesis in *Passiflora alata* Curtis seedlings," *Industrial Crops and Products*, vol. 77, pp. 754–757, 2015.
- [37] A. Rehwald, B. Meier, and O. Sticher, "Qualitative and quantitative reversed-phase high-performance liquid chromatography of flavonoids in *Passiflora incarnata* L.," *Pharmaceutica Acta Helveticae*, vol. 69, pp. 153–158, 1994.
- [38] S. V. F. Gomes, L. A. Portugal, J. P. Anjos et al., "Accelerated solvent extraction of phenolic compounds exploiting a Box-Behnken design and quantification of five flavonoids by HPLC-DAD in *Passiflora* species," *Microchemical Journal*, vol. 132, pp. 28–35, 2017.
- [39] S. M. Zucolotto, C. Fagundes, F. H. Reginatto et al., "Analysis of C-glycosyl flavonoids from south American *Passiflora* species by HPLC-DAD and HPLC-MS," *Phytochemical Analysis*, vol. 23, pp. 232–239, 2012.
- [40] A. Ulubelen, S. Oksuz, T. J. Mabry, G. Dellamonica, and J. Chopin, "C-glycosylflavonoids from *Passiflora pittieri*, *P. alata*, *P. ambigua* and *Adenia mannii*," *Journal of Natural Products*, vol. 45, p. 783, 1982.
- [41] M. A. Farag, A. Otfy, A. Porzel, C. G. Michel, A. Elsayed, and L. A. Wessjohann, "Comparative metabolite profiling and fingerprinting of genus *Passiflora* leaves using a multiplex approach of UPLC-NS and NMR analysed by chemometric tools," *Analytical and Bioanalytical Chemistry*, vol. 408, pp. 3125–3143, 2016.
- [42] L. Wosch, K. C. Santos, and D. C. Imig, "Comparative study of *Passiflora* taxa leaves: II. A chromatographic profile," *Brazilian Journal Pharmacognosy*, vol. 27, pp. 40–49, 2017.
- [43] C. L. Lin, C. L. Kao, S. C. Huang et al., "Chemical constituents of fruit shells of *Passiflora edulis*," *Chemistry Natural Compounds*, vol. 52, pp. 314–315, 2016.
- [44] Y. Hu, L. Jiao, M. H. Jiang et al., "A new C-glycosyl flavone and a new neolignan glycoside from *Passiflora edulis* Sims peel," *Natural Products Research*, vol. 32, pp. 2312–2318, 2018.
- [45] R. D. Petry, K. C. B. De Souza, V. L. Bassani, P. R. Petrovick, and G. G. Ortega, "Doseamento do teor de flavonóides totais em extratos em extratos hidroalcoólicos de *Passiflora alata* Dryander (maracujá)," *Revista Brasileira de Farmácia*, vol. 79, pp. 7–10, 1998.
- [46] R. D. Petry, F. Reginatto, F. De-Paris et al., "Comparative pharmacological study of hydroethanol extracts of *Passiflora alata* and *Passiflora edulis* leaves," *Phytotherapy Research*, vol. 15, pp. 162–164, 2001.
- [47] S. D. Müller, S. B. Vasconcelos, M. Coelho, and M. V. Biavatti, "LC and UV determination of flavonoids from: *Passiflora alata* medicinal extracts and leaves," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 37, pp. 399–403, 2005.
- [48] C. A. M. Pereira, J. H. Yariwake, F. M. Lanças, J. N. Wauters, M. Tits, and L. Angenot, "A HPTLC densitometric determination of flavonoids from *Passiflora alata*, *P. edulis*, *P. incarnata* and *P. caerulea* and comparison with HPLC method," *Phytochemical Analysis*, vol. 15, pp. 241–248, 2004.
- [49] F. Reginatto, H. F. De-Paris, R. D. Petry et al., "Evaluation of anxiolytic activity of spray dried powders of two South Brazilian *Passiflora* species," *Phytotherapy Research*, vol. 20, pp. 348–351, 2006.
- [50] G. Gonzáles Ortega and P. C. Schmidt, "Stability studies on dried extracts of passion flower (*Passiflora incarnata* L.)," *Pharma Sciences*, vol. 5, pp. 385–389, 1995.
- [51] V. Quercia, L. Turchetto, N. Pierini, V. Cuozzo, and G. Percaccio, "Identification and determination of vitexin

- and isovitexin in *Passiflora incarnata* extracts," *Journal of Chromatograph A*, vol. 161, pp. 396–402, 1978.
- [52] M. Jay, "C-glycosylflavonoids," in *The Flavonoids*, J. B. Harbone, Ed., Chapman & Hall, London, UK, 1996.
 - [53] H. Wohlmuth, K. G. Penman, T. Pearson, and R. P. Lehmann, "Pharmacognosy and chemotypes of passionflower (*Passiflora incarnata* L.)," *Biological and Pharmaceutical Bulletin*, vol. 33, pp. 1015–1018, 2010.
 - [54] M. L. Zeraik, C. A. M. Pereira, V. G. Zuin, J. H. Yariwake, and Maracujá, "Um alimento funcional?" *Revista Brasileira de Farmacognosia*, vol. 20, pp. 459–471, 2010.
 - [55] J. B. Harbone and H. Baxter, *Phytochemical Dictionary: A Handbook of Bioactive Compound from: Plants*, Taylor & Francis, London, UK, 1995.
 - [56] J. Lutomski and T. Wrociniski, "Pharmacodynamic properties of *P. incarnata* preparations. The effect of alkaloid and flavonoid components on pharmacodynamic properties of the raw materials," *Biuletyn Instytutu Roslin Leczniczych*, vol. 6, pp. 176–184, 1960.
 - [57] WHO and H. Passiflorae, *WHO Monographs on Selected Medicinal Plants*, pp. 257–267, World Health Organization Press, Geneva, Switzerland, 2007.
 - [58] C. Wasowski and M. Marder, "Flavonoids as GABA_A receptor ligands: the whole story?" *Journal of Experimental Pharmacology*, vol. 4, pp. 9–24, 2012.
 - [59] F. Wang, M. S. Y. Huen, S. Y. Tsang, and H. Xue, "Neuroactive flavonoids interacting with GABA_A receptor complex," *Current Drug Targets*, vol. 4, pp. 575–585, 2005.
 - [60] W. Zhang, K. F. Koehler, P. Zhang, and J. M. Cook, "Development of a comprehensive pharmacophore model for the benzodiazepine receptor," *Drug Design and Discovery*, vol. 12, pp. 193–248, 1995.
 - [61] J. R. Hanrahan, M. Chebib, and G. A. R. Johnston, "Flavonoid modulation of GABA_A receptors," *British Journal of Pharmacology*, vol. 163, pp. 234–245, 2011.
 - [62] K. Dekermendjian, P. Kahnberg, M. R. Witt, O. Sterner, M. Nielsen, and T. Liljefors, "Structure–activity relationships and molecular modeling analysis of flavonoids binding to the benzodiazepine site of the rat brain GABA_A receptor complex," *Journal of Medicinal Chemistry*, vol. 42, pp. 4343–4350, 1999.
 - [63] X. Huang, T. Liu, J. Gu et al., "3D-QSAR Model of flavonoids binding at benzodiazepine site in GABA_A receptors," *Journal of Medicinal Chemistry*, vol. 44, pp. 1883–1891, 2001.
 - [64] P. Kahnberg, E. Lager, C. Rosenberg et al., "Refinement and evaluation of a pharmacophore model for flavone derivatives binding to the benzodiazepine site of the GABA_A receptor," *Journal of Medicinal Chemistry*, vol. 45, pp. 4188–4201, 2002.
 - [65] D. K. R. Holanda, N. J. Wurlitzer, A. P. Dionisio et al., "Garlic passion fruit (*Passiflora tenuifila* Killip): assessment of eventual acute toxicity, anxiolytic, sedative, and anticonvulsant effects using *in vivo* assays," *Food Research International*, vol. 128, Article ID 108813, 2020.
 - [66] J. Zhang, Y. He, X. Jiang, H. Jiang, and J. Shen, "Nature brings new avenues to the therapy of central nervous system diseases—an overview of possible treatments derived from natural products," *Science China Life Sciences*, vol. 62, no. 10, pp. 1332–1367, 2019.
 - [67] L. J. Germán-Ponciano, A. Puga-Olgún, M. J. Rovirosa-Hernández, M. Caba, E. Meza, and J. F. Rodríguez-Landa, "Differential effects of acute and chronic treatment with the flavonoid chrysin on anxiety-like behavior and fos immunoreactivity in the lateral septal nucleus in rat," *Acta Pharmaceutica*, vol. 70, pp. 387–397, 2020.
 - [68] J. S. F. Alves, J. L. Marques, D. P. Demarque et al., "Involvement of isoorientin in the antidepressant bioactivity of a flavonoid-rich extract from *Passiflora edulis* f. *flavicarpa* leaves," *Revista Brasileira de Farmacognosia*, vol. 30, pp. 240–250, 2020.
 - [69] M. Rashno, S. Ghaderi, A. Nesari, L. Khoesandi, Y. Farbood, and A. Sarkaki, "Chrysin attenuates traumatic brain injury-induced recognition memory decline, and anxiety/depression-like behaviors in rats: insights into underlying mechanisms," *Psychopharmacology*, vol. 237, pp. 1607–1619, 2020.
 - [70] K. Appel, T. Rose, B. Fiebich, T. Kammler, C. Hoffmann, and G. Weiss, "Modulation of the γ -aminobutyric acid (GABA) system by *Passiflora incarnata* L.," *Phytotherapy Research*, vol. 25, pp. 838–843, 2011, <https://onlinelibrary.wiley.com/doi/epdf/10.1002/ptr.3352>.
 - [71] H. Viola, C. Wasowski, M. L. Stein et al., "Apigenin, a component of *Matricaria recutita* flowers, is a central benzodiazepine receptors-ligand with anxiolytic effects," *Planta Medica*, vol. 61, pp. 213–216, 1995.
 - [72] C. Vissiennona, K. Nieber, O. Kelber, and V. Butterweck, "Route of administration determines the anxiolytic activity of the flavonols kaempferol, quercetin and myricetin—are they prodrugs?" *The Journal of Nutritional Biochemistry*, vol. 23, pp. 733–774, 2012.
 - [73] C. Braestrup, M. Nielsen, and C. E. Olsen, "Urinary and brain β -carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 77, pp. 2288–2292, 1980.
 - [74] S. S. Bharate, S. Mignani, and R. A. Vishwakarma, "Why are the majority of active compounds in the CNS domain natural products? A critical analysis," *Journal of Medicinal Chemistry*, vol. 61, pp. 10345–10374, 2018.
 - [75] S. Salerno, F. Settimo, S. Taliani et al., "Medicinal chemistry of indolylglyoxylamide GABA_A/BzR high affinity ligands: identification of novel anxiolytic/non sedative agents," *Current Topics in Medicinal Chemistry*, vol. 12, pp. 286–311, 2012.
 - [76] T. Clayton, M. M. Poe, S. Rallapalli et al., "A review of the updated pharmacophore for the alpha 5 GABA(A) benzodiazepine receptor model," *International Journal of Medicinal Chemistry*, vol. 2015, Article ID 430248, 54 pages, 2015.
 - [77] Brazil, Ministry of Health and ANVISA, "Normative instruction n. 2./May 13, 2014," *Diário Oficial da União, Brasília, Section 1*, pp. 58–61, ANVISA, Brasília, Brazil, 2014, <http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=14/05/2014&jornal=1&pagina=58&totalArquivos=100>.
 - [78] Brazil, Ministry of Health and ANVISA, *Monografia da espécie Passiflora incarnata Linnaeus (maracujá-vermelho)*, ANVISA, Brasília, Brazil, 2015, <https://www.saude.gov.br/images/pdf/2016/fevereiro/05/Monografia-Passiflora-incarnata.pdf>.
 - [79] R. C. Fierascu, I. Fierascu, A. Ortan, M. I. Georgiew, and E. Sieniawska, "Innovative approaches for recovery of phytoconstituents from medicinal/aromatic plants and biotechnological production," *Molecules*, vol. 25, no. 2, p. 309, 2020.
 - [80] S. M. He, E. Chan, and S. F. Zhou, "ADME Properties of herbal medicines in humans: evidence, challenges and strategies," *Current Pharmaceutical Design*, vol. 17, pp. 357–407, 2011.
 - [81] C. Huang, C. Zheng, Y. Li, Y. Wang, A. Lu, and L. Yang, "Systems pharmacology in drug discovery and therapeutic

- insight for herbal medicines," *Briefings in Bioinformatics*, vol. 15, pp. 710–733, 2013.
- [82] S. Sut, V. Baldan, M. Faggian, G. Peron, and S. Dall'Acqua, "Nutraceuticals, a new challenge for medicinal chemistry," *Current Medicinal Chemistry*, vol. 23, pp. 1–26, 2016.
- [83] M. Rasool, A. Malik, S. Waquar et al., "In-Silico characterization and in-vivo validation of *Albizia* saponin-A, iso-orientin, and aalvadorin using a rat model of Alzheimer's disease," *Frontiers in Pharmacology*, vol. 9, pp. 99–113, 2018.
- [84] M. Wang, J. Firman, L. Liu, and K. Yam, "A review on flavonoid apigenin: dietary intake, ADME, antimicrobial effects, and interactions with human gut microbiota," *BioMed Research International*, vol. 2019, Article ID 7010467, 18 pages, 2019.
- [85] M. U. Mirza, N.-Ul-H. Ghor, N. Ikram, A. R. Adil, and S. Manzoor, "Pharmacoinformatics approach for investigation of alternative potential hepatitis C virus nonstructural protein 5B inhibitors," *Drug Design, Development and Therapy*, vol. 9, pp. 1825–1841, 2015.
- [86] M. H. Abraham, "The factors that influence permeation across the blood–brain barrier," *European Journal of Medicinal Chemistry*, vol. 39, pp. 235–240, 2004.
- [87] S. M. Stamatovic, R. F. Keep, and A. V. Andjelkovic, "Brain endothelial cell-cell junctions: how to "open" the blood brain barrier," *Current Neuropharmacology*, vol. 6, pp. 179–192, 2008.
- [88] R. Ancuceanu, M. Dinu, C. Dinu-Pirvu, V. Anuta, and V. Negulescu, "Pharmacokinetics of B-ring unsubstituted flavones," *Pharmaceutics*, vol. 11, p. 370, 2019.
- [89] K. Noh, D. G. Oh, M. R. Nepal et al., "Pharmacokinetic interaction of chrysin with caffeine in rats," *Biomolecules & Therapeutics*, vol. 24, no. 4, pp. 446–452, 2016.
- [90] A. F. G. Cicero and A. Colletti, "Handbook of Nutraceuticals for Chemical Use," in *Nutraceuticals Active on Central Nervous System*, pp. 1–23, Springer International Publishing AG, Cham, Switzerland, 2018.
- [91] A. Mori, K. Hasegawa, M. Murasaki et al., "Clinical evaluation of Passiflamin (*Passiflora* extract) on neurosis—multicenter double blind study in comparison with mexazolam," *Clinical Evaluation*, vol. 21, pp. 383–440, 1993.
- [92] L. S. Miyasaka, Á. N. Atallah, and B. Soares, "*Passiflora* for Anxiety Disorder," *Cochrane Database of Systematic Reviews Issue 1. Art. No CD004518*, Cochrane, London, UK. <https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD004518.pub2/full>, 2007.
- [93] S. Akhondzadeh, H. R. Naghavi, M. Vazirian, A. Shayeganpour, and M. Khani, "Passionflower in the treatment of generalized anxiety: a pilot double-blind randomized controlled trial with oxazepam," *Journal of Clinical Pharmacy and Therapeutics*, vol. 26, pp. 363–367, 2001.
- [94] S. Akhondzadeh, L. Kashani, M. Mobaseri, S. H. Hosseini, S. Nikzad, and M. Khani, "Passionflower in the treatment of opiates withdrawal: a double-blind randomized controlled trial," *Journal of Clinical Pharmacy and Therapeutics*, vol. 26, pp. 369–373, 2001.
- [95] A. Movafegh, R. Alizadeh, F. Hajimohamadi, F. Esfehiani, and M. Nejatfar, "Preoperative oral *Passiflora incarnata* reduces anxiety in ambulatory surgery patients: a double-blind, placebo-controlled study," *Anesthesia & Analgesia*, vol. 106, pp. 1728–1732, 2008.
- [96] P. Aslanargun, O. Cuvas, B. Dikmen, E. Aslan, and M. U. Yuksel, "*Passiflora incarnata* Linnaeus as an anxiolytic before spinal anesthesia," *Journal of Anesthesia*, vol. 26, pp. 39–44, 2012.
- [97] L. P. Dantas, A. Oliveira-Ribeiro, L. M. Almeida-Souza, and F. C. Groppo, "Effects of *Passiflora incarnata* and midazolam for control of anxiety in patients undergoing dental extraction," *Medicina Oral, Patologia Oral y Cirurgia Bucal*, vol. 22, pp. e95–101, 2017.
- [98] A. Ngan and R. Conduit, "A double-blind, placebo-controlled investigation of the effects of *Passiflora incarnata* (passionflower) herbal tea on subjective sleep quality," *Phytotherapy Research*, vol. 25, pp. 1153–1159, 2011.
- [99] Brazil, Presidency of the Republic and National Congress, "Law No. 9.279/May 14, 1996," *Diário Oficial da União, Brasília, Section 1*, http://www.planalto.gov.br/ccivil_03/leis/L9279.htm, p. 8353, National Congress, Brasília, Brazil, 1996, .
- [100] Brazil, Presidency of the Republic, "Decree No 5.813/June 22, 2006," *Diário Oficial da União, Brasília, Section 1*, http://www.planalto.gov.br/ccivil_03/_ato2004-2006/2006/decreto/d5813.htm, p. 2, National Congress, Brasília, Brazil, 2006, .
- [101] A. C. Muller and M. F. Macedo, "Patentes de fitomedicamentos: como garantir o compartilhamento dos benefícios de P & D e do uso sustentável de recursos genéticos," *Revista Fitos*, vol. 1, no. 2, pp. 19–24, 2005.
- [102] Brazil, Presidency of the Republic and National Congress, "Law No 9.782/January 26, 1999," *Diário Oficial da União, Brasília, Section 1*, http://www.anvisa.gov.br/legis/consolidada/lei_9782_99.pdf, p. 1, National Congress, Brasília, Brazil, 1999, .
- [103] Brazil, Ministry of Health and ANVISA, "RDC No 38/June 18, 2014," *Diário Oficial da União, Brasília, Section 1*, pp. 58–61, ANVISA, Brasília, Brazil, 2014, http://bvsm.sau.gov.br/bvs/sau/legis/anvisa/2014/rdc0038_18_06_2014.pdfhttp://bvsm.sau.gov.br/bvs/sau/legis/anvisa/2014/rdc0038_18_06_2014.pdfhttp://bvsm.sau.gov.br/bvs/sau/legis/anvisa/2014/rdc0038_18_06_2014.pdf.
- [104] Brazil, Ministry of Health and ANVISA, "RDC 66/November 26, 2014," *Diário Oficial da União, Brasília, Section 1*, pp. 46–47, ANVISA, Brasília, Brazil, 2014, <http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?jornal=1&pagina=46&data=27/11/2014>.
- [105] Brazil, Ministry of Health and ANVISA, "RDC 105/August 31, 2016," *Diário Oficial da União, Brasília, Section 1*, pp. 47–48, ANVISA, Brasília, Brazil, 2016, <http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?jornal=1&pagina=47&data=01/09/2016>.
- [106] United States and Department of Commerce; United States Patent and Trademark Office, "Patent full-text databases," 2019, <http://patft.uspto.gov/netahtml/PTO/search-bool.html> US Patent.

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REVIEW



Review & meta-analysis: isopropanolic black cohosh extract iCR for menopausal symptoms – an update on the evidence

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ABSTRACT

A systematic literature search revealed 35 clinical studies and one meta-analysis comprising 43,759 women, of which 13,096 were treated with isopropanolic *Cimicifuga racemosa* extract (iCR). Compared to placebo, iCR was significantly superior for treating neurovegetative and psychological menopausal symptoms, with a standardized mean difference of -0.694 in favor of iCR ($p < 0.0001$). Effect sizes were larger when higher dosages of iCR as monotherapy or in combination with St. John's wort (*Hypericum perforatum* [HP]) were given (-1.020 and -0.999 , respectively), suggesting a dose-dependency. For psychological symptoms, the iCR+HP combination was superior to iCR monotherapy. Efficacy of iCR was comparable to low-dose transdermal estradiol or tibolone. Yet, due to its better tolerability, iCR had a significantly better benefit–risk profile than tibolone. Treatment with iCR/iCR+HP was well tolerated with few minor adverse events, with a frequency comparable to placebo. The clinical data did not reveal any evidence of hepatotoxicity. Hormone levels remained unchanged and estrogen-sensitive tissues (e.g. breast, endometrium) were unaffected by iCR treatment. As benefits clearly outweigh risks, iCR/iCR+HP should be recommended as an evidence-based treatment option for natural climacteric symptoms. With its good safety profile in general and at estrogen-sensitive organs, iCR as a non-hormonal herbal therapy can also be used in patients with hormone-dependent diseases who suffer from iatrogenic climacteric symptoms.

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Introduction

Up to 80% of climacteric women, especially those suffering from hot flashes, use complementary and alternative medicine, mainly without informing their health-care providers^{1–5}. This highlights not only patients' wishes but also a need for evidence-based information on the efficacy and safety of these treatments.

One of the most popular herbal remedies is *Cimicifuga racemosa* (CR) syn. *Actaea racemosa* (black cohosh). CR has been used by Native Americans and eclectic physicians as a gynecological remedy and for rheumatism and other conditions. The earliest literature references date back to the seventeenth century⁶. The first allopathic herbal medicinal product (HMP) containing CR rootstock extract was introduced in Germany in 1956 and has been extensively studied

since⁷. In Germany, where HMPs are authorized medicines under strict regulatory control requiring state-of-the-art proof of efficacy, safety, and pharmaceutical quality, gynecologists rate CR treatment as well-known and effective for climacteric symptoms⁸. In many other countries, CR products are marketed as food supplements (FS). FS are not as rigorously controlled as HMPs and run the risk of adulteration and contamination. Frequently, FS claiming to consist of authentic North American CR have contained completely different Asian *Cimicifuga* species⁹. While FS often have little or no scientific evidence behind them, they are regarded as complementary and alternative medicine. On the other hand, (CR) HMPs are evidence-based rational phytopharmaceuticals and should be treated as such¹⁰.

In 2011, and again in 2018, the European Medicines Agency issued an official monograph with a positive

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benefit–risk ratio for CR HMPs for the treatment of climacteric symptoms^{11,12}. A Cochrane review doubted CR's superiority versus placebo for menopausal symptoms¹³ but was internationally criticized soon after. Apart from a failure to identify all appropriate randomized controlled trials (RCTs) and an incorrect exclusion of positive results, the review's main shortcoming was a missing differentiation between the extracts used¹⁴. The new meta-analysis showed CR to be significantly superior to placebo for menopausal symptoms¹⁴.

Unlike synthetic drugs, the active ingredient of phytopharmaceuticals is not a single substance but the whole plant extract. Herbal extracts contain a multitude of different constituents and – even if derived from the same plant – their composition varies depending on many factors (e.g. cultivation, harvesting conditions, extraction process, extractant, and standardization) that may lead to different effects and efficacies¹⁵. Therefore, study results gained with a specific extract cannot be transferred one-to-one to different extracts of this herb. For phytopharmaceuticals there are no genuine generics as with synthetic drugs. Consequently, when analyzing scientific data, it is crucial to differentiate between HMPs and non-HMPs/FS and between different extracts. The first differentiated evaluation of extract-specific evidence on CR's efficacy and safety was performed by Beer and Neff in 2013¹⁶. Their analysis of publications from 2000 to 2012 came to the conclusion that only HMPs, but not FS/non-HMPs, of CR demonstrated evidence for their efficacy. Best evidence was provided by the isopropanolic CR extract (iCR); the only extract tested in several thousand women and the only extract that provided confirmatory Level 1 evidence and Grade of Recommendation A¹⁶. These results were confirmed by an additional analysis of newer studies published from 2012 to 2014¹⁷.

This review's purpose is to give a current update and overview of all placebo-controlled clinical data (irrespective of publication date) and additional data from clinical studies with iCR during a broader time span ranging from the establishment of the EU Guideline on Good Clinical Practice E6 in 1997 until January 2020. Furthermore, for the first time, all placebo-controlled data gained with iCR are pooled in a meta-analysis. Efficacy and safety results are analyzed in detail and discussed in order to enable health-care providers to advise patients who refuse or have contraindications to hormone therapy for climacteric symptoms.

Methods

MEDLINE, EMBASE, EMBASE Alert, BIOSIS, and PubMed were searched for clinical studies with iCR (irrespective of design) and meta-analyses thereof, published from 1997 to January 2020, as a basis for our review. Furthermore, all available randomized placebo-controlled data, irrespective of publication date, were collected as a basis for our meta-analysis. The database search was complemented by a manual search in the authors' library and an additional search in ClinicalTrials.gov and the EU Clinical Trials Register.

Inclusion criteria were medical use of iCR, efficacy for neurovegetative and psychological climacteric symptoms,

additional clinical benefits, occurrence and frequency of adverse events, and influence on liver, hormones, and estrogen-sensitive organs. No restrictions regarding patients' ages, menopausal status, and treatment duration were made. Patient-relevant endpoints were reduction of neurovegetative and psychological climacteric symptoms (e.g. scales for climacteric symptoms, symptom frequency and intensity, and objective symptom measurement), additional clinical benefits, and frequency and severity of adverse events, including influence on estrogen-sensitive organs or laboratory parameters.

Studies were sorted according to their quality and the RCTs regarding their control group (placebo, active comparator). The placebo-controlled RCTs were pooled in the meta-analysis and independently assessed for risk of bias with the revised Cochrane RoB 2 tool (2019) by three authors (M.G., H.H.H.-v.Z., and S.P.). Disagreements were discussed until a consensus was reached. The meta-analysis was performed using SAS version 9.4 under the fixed-effect size model. For studies that did not directly report the standardized group difference and the corresponding confidence interval, these parameters were deduced either from the published means, standard deviations, and sample size *N* or from the published means, sample size *N*, and *p*-values.

Results

The search revealed one meta-analysis¹⁸ and 35 clinical studies^{19–53}, including 16 RCTs^{19–31,50,52,53}, that met the inclusion criteria. Altogether, 43,759 patients were included in the studies; 13,096 of the patients were treated with iCR. A detailed overview of populations, interventions, and results for each study is presented in Table S1.

Efficacy for natural menopausal symptoms

Placebo-controlled, double-blind RCTs

The first RCT demonstrated that iCR was superior to placebo in improving the Kupperman Menopause Index (KMI) and the Hamilton Anxiety Rating Scale¹⁹ scores for all assessment points (4, 8, and 12 weeks; $p < 0.001$). In the iCR group, the average symptom intensity decreased from 'moderate' at baseline to 'mild' at study termination, showing a good therapeutic result. In the placebo group, it decreased but still remained 'moderate'.

Significantly greater improvements in the total Menopause Rating Scale (MRS) scores were found in patients taking iCR for 3 months compared to placebo ($p < 0.001$)²². Efficacy was best for vasomotor symptoms (hot flushes, sweating, and sleep disorders). With 0.03–0.05 MRS units, the effect size was similar to that of 0.6 mg conjugated estrogens in a previous study and clinically relevant. The therapeutic benefit was highest in patients who were treated in the early climacteric phase.

In iCR-treated patients, significantly higher reductions in climacteric symptom severity (total KMI and hot flush scores) were seen after 4 weeks ($p < 0.05$ for KMI, $p = 0.041$ for hot flushes) and 12 weeks ($p < 0.001$ for KMI, $p = 0.021$ for hot

flushes) compared to placebo²⁶. iCR noticeably decreased both scores from baseline to study termination (KMI, 26.46 ± 10.46 to 6.37 ± 4.16 ; hot flushes, 7.52 ± 2.37 to 1.52 ± 1.97).

Polysomnography, conducted to objectively measure sleep disturbances in a 6-month trial, showed that patients using iCR had significantly increased sleep efficiency ($p = 0.01$) and decreased wake after sleep onset duration ($p < 0.01$) compared to placebo. Vasomotor and physical domains of the Menopause-specific Quality of Life questionnaire also improved versus placebo³¹.

The fixed combination of high-dose iCR plus low-dose St. John's wort (*Hypericum perforatum*) (iCR+HP) was significantly superior to placebo in improving MRS (−50%) and Hamilton Depression Rating Scale scores (−41.8%) ($p < 0.001$)²⁴.

Active-controlled RCTs

A 3-month study compared the efficacy of iCR to low-dose transdermal estradiol for climacteric symptoms²³. Both treatments significantly reduced the number of hot flushes/day and vasomotor symptoms as well as anxiety and depression ($p < 0.001$ for all). Both treatments showed comparable efficacy without significant differences.

Chinese women with climacteric symptoms received either iCR or tibolone for 3 months in a double-blind, double-dummy trial²⁵. A noticeable and clinically relevant reduction of similar size for the KMI scores was seen in both groups, significantly demonstrating non-inferiority of iCR ($p = 0.002$). Yet, due to its better tolerability, iCR was superior to tibolone in the benefit–risk ratio ($p = 0.01$).

In two further RCTs, significant KMI score reductions of perimenopausal symptoms were shown under iCR after 3 months; they were comparable to those under tibolone^{52,53}. Additionally, vaginal atrophy symptoms were significantly ameliorated in both groups, without significant group differences⁵³.

Treatment with a standard dose (SD) or high dose (HD) of iCR for 6 months significantly decreased menopausal symptoms (KMI score reduced by 75%). The responder rate was already 70% after 3 months. These effects were maintained after 6 months²¹. For the complete study population, no differences between SD and HD could be observed. However, patients who were in the early climacteric phase benefited significantly more from HD compared to SD and also exhibited a response rate above 90% when using HD^{21,54}.

Women with perimenopausal depression were treated with either paroxetine or paroxetine plus iCR for 2 months²⁸. The iCR group showed significantly better improvement in the Hamilton Depression Rating Scale ($p < 0.05$) and KMI ($p < 0.01$) scores compared to patients taking only paroxetine.

A 12-week iCR treatment was compared to placebo treatment in patients with postmenopausal atrophic vaginitis²⁷. While atrophic vaginitis symptoms were significantly reduced in the iCR group ($p < 0.05$), there were no significant influences on vaginal pH or the maturation degree of vaginal epithelial cells.

Open, controlled studies

In a prospective, controlled observational study, 6,141 patients were treated for 6–12 months ($n = 736$) with iCR or iCR+HP according to the physicians' choices³². Both treatments led to significant reductions of the MRS total score and the MRS subscore 'psyche'. The iCR+HP combination was superior to the monotherapy in ameliorating psychological climacteric symptoms ($p < 0.001$).

Compared to untreated control, iCR-treated women showed significant decreases in the urinary concentration of N-telopeptides (bone resorption marker) and increases in serum alkaline phosphatase (bone formation marker) after 3 months³³.

Open, uncontrolled studies

Two studies using a 3-month iCR therapy found significant reductions in the KMI scores. The most pronounced improvements were seen for hot flushes and sweating^{42,43}. Sleeping difficulties and anxiety were also greatly reduced⁴³. Postmenopausal women with increased body weight taking iCR for 3 months experienced a significant increase in their quality of life (Cervantes Health-Related Quality-of-Life Scale), especially in the 'menopause/health' and 'psyche' domains⁴⁷. Another 3-month study found that more than 70% of women using iCR had fewer hot flushes, less sweating, and fewer psychological complaints (irritability, depressive moods, and insomnia)⁴⁵. Patients taking HD iCR for 98 days exhibited reductions in 10 typical neurovegetative climacteric symptoms. Efficacy was rated as good or very good in 80% of the cases⁴⁰. The combination of iCR+HP ameliorated not only psychological but also neurovegetative symptoms of menopause significantly after 12 weeks of therapy. Efficacy was judged as good or very good in 81% of the cases³⁹.

Efficacy for iatrogenic menopausal symptoms

Placebo-controlled, double-blind RCTs

Breast cancer patients, most treated with tamoxifen, received either iCR or placebo for 2 months. While both groups had a comparable decline in the number and intensity of hot flushes, the iCR-treated women reported significantly less sweating than the placebo group ($p = 0.04$)²⁰.

Active-controlled RCTs

Endometriosis patients were treated with postoperative goserelin therapy, causing estrogen-deficiency symptoms³⁰. Four weeks after the first goserelin injection, they received add-back therapy with tibolone or iCR for 12 weeks. Both treatments significantly reduced KMI and hot flushes/sweating scores to a comparable extent without significant group differences. The iCR group had significantly fewer adverse events and less vaginal bleeding/spotting or breast pain.

Open, controlled studies

Women with early breast cancer were treated with luteinizing hormone-releasing hormone analogue after surgery,

mostly combined with endocrine therapy. They randomly received iCR or no treatment for 3 months. The iCR group had significantly lower KMI scores compared to control⁵⁰.

Patients with stage 1 endometrial cancer suffering from menopausal symptoms after surgery were treated with iCR for 24 weeks or remained untreated as control³⁵. All patients were followed for 12 months. Therapy with iCR resulted in a significant decrease of the KMI score and a significant increase in bone mineral density (both $p < 0.05$).

Open, uncontrolled studies

A pilot trial in women (breast cancer, high risk, or refused hormone therapy) with significant hot flushes, partly caused by tamoxifen or raloxifene, found a reduction of hot flush frequency (–50%), severity (–22%), and sweating (–80%) after 4 weeks of iCR intake⁴¹. Additionally, patients reported less sleeping problems and less fatigue.

Patients after surgery for gynecological cancers (endometrial, ovarian, cervix, borderline ovarian, and breast cancers) experienced a significant reduction of KMI scores, hot flushes, sweating, and depressive moods after 3 months of treatment with iCR ($p < 0.05$)⁴⁸.

Women after breast cancer surgery suffering from tamoxifen-induced climacteric symptoms were treated with iCR for 6 months. The dosage was adapted to symptom intensity⁴⁹. The iCR treatment led to significant reductions in the total MRS II score ($p < 0.001$). Hot flushes, sweating, sleep problems, and anxiety improved most.

Meta-analysis of the placebo-controlled RCTs

All placebo-controlled RCTs examining efficacy for neurovegetative and psychological climacteric symptoms were included in the meta-analysis^{19,20,22,24,26,31}. Most of the RCTs had a low risk of bias^{19,22,24}. Two RCTs raised some concerns^{20,31} and one RCT was rated as high risk according to RoB 2 criteria²⁶ (Figure 1; extensive assessment presented in Table S2). However, the latter suffered explicitly from low-quality reporting, which may not necessarily imply an actual risk of bias. Detailed characteristics of these RCTs are presented in Table 1. Figure 2 shows a forest plot of these RCTs, revealing an overall standardized mean difference of –0.694 in favor of iCR ($p < 0.0001$). By subgroups, the standardized

mean difference in favor of iCR was –0.568 for natural menopausal symptoms, –0.187 for iatrogenic symptoms, and –0.999 in favor of iCR+HP for natural climacteric symptoms. For studies with higher iCR dosages as monotherapy or in combination with HP^{19,24}, the standardized mean difference in favor of iCR was –1.020 and –0.999, respectively.

Safety in general and at the liver

Treatment with iCR was generally well tolerated with few minor adverse events^{19,21–23,25,30,32,33,39,41,43–45,52} or even no adverse events^{27,31,35}. Compared to placebo, the frequency of adverse events under iCR or iCR+HP was not significantly different^{22,24}. HD iCR demonstrated the same good tolerability as SD iCR²¹. In breast cancer patients treated with tamoxifen, typical tamoxifen-associated adverse effects occurred^{20,49}. Compared to tibolone, iCR treatment had significantly fewer adverse effects, especially for vaginal bleeding or spotting^{25,30,52,53}. No significant influences of iCR on body weight/body mass index^{22,25,35}, heart rate²², blood pressure^{22,28,35}, or electrocardiogram²⁸ were seen. Routine hematology^{21,24,25,28,48}, routine biochemistry^{21,24,25,33,48}, including kidney function tests^{21,24,25,28,30,31,35,48,52,53}, and urinalysis^{25,28,48} remained unaffected by iCR. Regarding lipid metabolism, total cholesterol and triglycerides were not affected^{23,26,30,33,35,44,48,52,53}. However, several investigators reported significant increases of high-density lipoprotein cholesterol and decreases of low-density lipoprotein cholesterol^{23,26,48,52}.

A meta-analysis of five double-blind RCTs including 1117 patients showed that SD or HD iCR treatment for 3–6 months did not significantly influence liver function tests and did not reveal any evidence for hepatotoxicity¹⁸. Ten further studies^{23,26,28,30,31,35,48,52,53,55}, seven of which were RCTs, including 741 patients also did not find any significant changes in liver function parameters.

Safety at estrogen-sensitive organs

Taking SD iCR for 3–6 months did not lead to significant changes in gonadotropin or estradiol levels in otherwise healthy women ($n = 481$)^{21,23,26,27,31,33,40,46,52,53} or in women with estrogen-dependent diseases (breast cancer,

RCT (Author, Year)	Randomization process	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall bias
Jiang, 2015	+	+	+	+	?	?
Li Yilin, 2011	?	–	–	+	?	–
Osmer, 2005	+	+	+	+	+	+
Stoll, 1987	+	+	+	+	+	+
Jacobson, 2001	?	+	+	+	?	?
Uebelhack, 2006	+	+	+	+	+	+

 Low risk
  Some concerns
  High risk

Figure 1. Risk of bias assessment of the placebo-controlled randomized controlled trials (RCTs).

Table 1. Characteristics of the double-blind RCTs comparing iCR with placebo for neurovegetative and psychological menopausal symptoms, used as the basis for the meta-analysis.

First author, year of publication	Number of patients; indication; ethnicity	Daily dose; duration of application	Inclusion criteria	Exclusion criteria	Main efficacy results: standardized difference in means [95% CI]	Impact on evidence; remarks; RoB
Stoll, 1987	30 iCR, 20 placebo; ms; Caucasian	2 × 2 tab. = 8 mg extractum siccum; 3 months	46–58 years old, perimenopausal and postmenopausal patients suffering from neurovegetative, psychological, and somatic menopausal symptoms	Patients after bilateral OVX or pelvic radiation. Contraindication for HT. Other causes for symptoms than menopause. Osteoporosis. Intake of HT ≤ 4 weeks before study entry. Intake of antihypertensives weeks before study entry.	Significant improvement of the KMI and HAMA scores under iCR compared to placebo. Symptom intensity decreasing from moderate to mild; –1.020 [–1.586, –0.454]	⊕⊕ Well-conducted RCT; low RoB
Jacobson, 2001	42 iCR, 43 placebo; iatrogenic ms due to Tam (breast cancer); mainly Caucasian (African-American, Asian)	2 × 1 tab. Δ 40 mg drug; 2 months	Breast cancer patients > 18 years old with daily hot flushes. Completion of primary therapy including chemotherapy and radiation ≥ 2 months before entering study	Use of HT. Pregnancy. Major psychiatric illness. Known recurrent or metastatic breast cancer	Comparable reduction of hot flush frequency and intensity in both groups and significantly stronger reduction of sweating under iCR compared to placebo; –0.187 [–0.659, 0.285]	⊕ Study duration rather short to show efficacy; some concerns on RoB
Osmer, 2005	153 iCR, 151 placebo; ms; Caucasian	2 × 1 tab. Δ 40 mg drug; 3 months	Postmenopausal patients ≥ 45 years old (≥ 12 months of amenorrhea or ≥ 6 months of amenorrhea with FSH ≥ 50 IU/l). Climacteric symptoms of ≥ 0.4 in ≥ 3 MRS items	BMI ≥ 28 kg/m ² . Cancer or other diseases with interference potential. Drug abuse. Participation in other trial ≤ 180 days before study entry. Use of HT during or ≤ 4 weeks before study entry. Use of non-hormonal climacteric drugs, including supplements and homeopathics. Use of anti-epileptics, psycholeptics, or psychoanalitics, including phytotherapeutics	Significant improvement of the MRS total score vs. placebo. Strongest effect in vasomotor complaints (hot flushes, sweating, sleep disorders) and higher superiority during menopausal transition; –0.394 [–0.626, –0.162]	⊕⊕ Well-conducted RCT; low RoB
Uebelhack, 2006	151 iCR+HP 150 placebo; ms; Caucasian	Week 1–8, 2 × 2 tab. Δ 128 mg drug; Week 9–16, 2 × 1 tab. Δ 64 mg drug; 4 months	45–60 years old, perimenopausal and postmenopausal patients with climacteric complaints with pronounced psychological component for ≥ 3 months, untreated for ≥ 2 months. MRS of ≥ 0.4 in ≥ 3 items. HAM-D scale items of ≥ 1 of 2 points	Use of HT, non-hormonal climacteric drugs or other treatment for climacteric symptoms ≤ 12 weeks before study entry. Use of synthetic or herbal anti-epileptics, psycholeptics, or psychoanalitics ≤ 12 weeks before study entry. Psychological or psychiatric therapy. Bilateral OVX. Severe diseases. Abnormal TSH. Treatment with interference potential. Suicidality or attempted suicide in the past year	Significant superiority vs. placebo in the improvement of the MRS and HAM-D scores; –0.999 [–1.225, –0.773]	⊕⊕ Well-conducted RCT; low RoB
Li Y, 2011	45 iCR, 32 placebo; ms; Asian	2 × 1 tab. Δ 40 mg drug; 3 months	45–55 years old, perimenopausal patients with spontaneous menopausal status for ≥ 3 months with E2 ≤ 20 pg/ml and FSH ≥ 40 IU/l. KMI ≥ 17	History of diabetes, serious or unstable physical illness. Use of estrogen, antidepressants, or antipsychotics. Use of supplements for perimenopausal symptoms ≤ 3 months before study entry. Unexplained uterine bleeding, abnormal cervical smear, endometrial thickness ≥ 0.5 cm	Significant improvement of the KMI score and hot flushes compared to placebo; –0.792 [–1.246, –0.338]	⊕ Low quality of reporting, which may imply a high RoB
Jiang, 2015	24 iCR, 24 placebo; ms; Asian	2 × 1 tab. Δ 40 mg drug; 6 months	45–60 years old, postmenopausal patients, STRAW +1b, +1c. BMI ≤ 30 kg/m ² . FSH ≥ 30 IU/l	Bilateral OVX, hysterectomy. Termination of ovarian function by chemotherapy or radiation therapy. Poorly controlled clinical diseases. Historic or present psychiatric disorders. Sleep-related respiratory or movement disorders. Use of opioids, anti-epileptics, anxiolytics, hypnotics, sedatives, antidepressants, or antihistamines ≤ 3 months before study entry. Use of HT or other therapy against menopausal symptoms ≤ 3 months before study entry. Participation in other study ≤ 3 months before study entry. Intake of ≥ 7 alcoholic drinks/week. History of drug/substance abuse	Significantly increased sleep efficiency and decreased WASO duration under iCR compared to placebo. Significantly better improvement of vasomotor and physical domains of Men-QoL by iCR; –0.836 [–1.442, –0.230]	⊕⊕ Well-conducted RCT; some concerns on RoB

BMI, body mass index; CI, confidence interval; E2, estradiol; FSH, follicle stimulating hormone; HAMA, Hamilton Rating Scale for Anxiety; HAM-D, Hamilton Rating Scale for Depression; HT, hormone therapy; iCR, isopropanolol *Cimicifuga racemosa* extract; iCR+HP, fixed combination of isopropanolol *Cimicifuga racemosa* extract plus St. John's wort (*Hypericum perforatum*) extract; IU, international units; KMI, Kupperman Menopause Index; ms, menopausal symptoms; Men-QoL, Menopause-specific Quality of Life Questionnaire; MRS, Menopause Rating Scale; OVX, ovariectomy; RCT, randomized, controlled trial; RoB, risk of bias; STRAW, Stages of Reproductive Aging Workshop; Tam, tamoxifen; TSH, thyroid-stimulating hormone; WASO, wake after sleep onset; Δ: corresponding to; ⊕: exploratory evidence; ⊕⊕: confirmatory evidence.

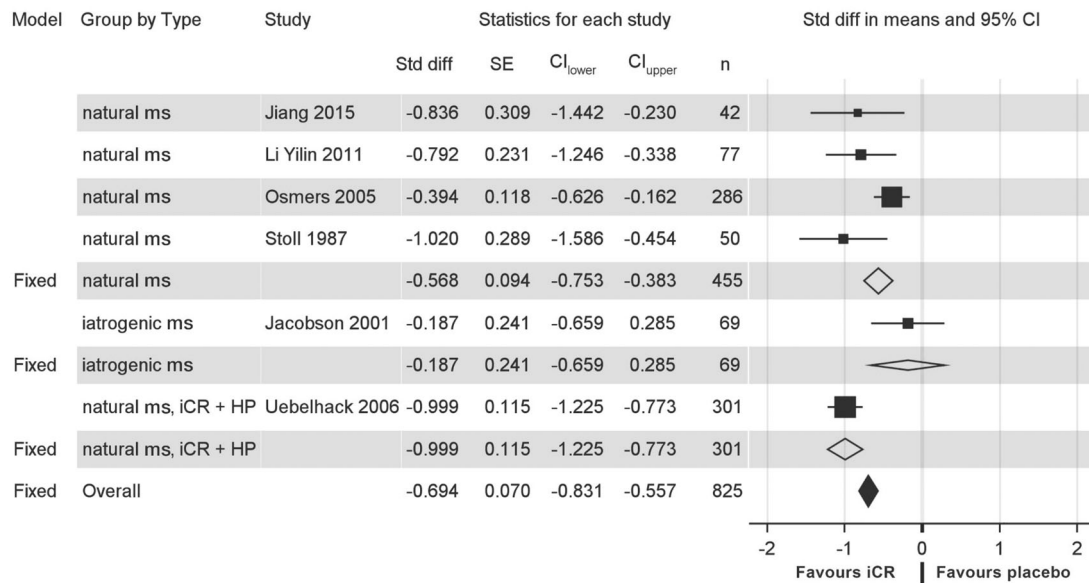


Figure 2. Forest plot of isopropanolic *Cimicifuga racemosa* extract (iCR) versus placebo in neurovegetative and psychological menopausal symptoms. CI, confidence interval; Fixed, summary of the respective group of studies under the fixed-effect size model; HP, *Hypericum perforatum* (St. John's wort); ms, menopausal symptoms; n, number of patients; Std diff, standardized mean difference; SE, standard error of the standardized difference.

gynecological tumors, and endometriosis) ($n = 193$)^{20,30,35,48,50}. HD iCR also did not affect follicle stimulating hormone, luteinizing hormone, or estradiol^{21,40}. Furthermore, prolactin, sex hormone binding globulin, and testosterone remained unchanged^{21,23,33,40,44}.

Ultrasound scans showed that endometrial thickness was not influenced by 3–6-month iCR treatment ($n = 516$)^{23,25–27,31,40,44,50,52,53}. Also, in patients with uterine pathologies like endometriosis or endometrial carcinoma, pelvic ultrasound was unremarkable ($n = 86$)^{30,35}. iCR therapy in goserelin-treated endometriosis patients resulted in significantly less vaginal bleeding/spotting than add-back therapy with tibolone³⁰. Using iCR for 3 months led to a significant reduction of myoma volume compared to baseline (–30%) and compared to tibolone, which had no significant effects (+4.7%)²⁹. Patients with endometrial cancer had no increase of tumor marker CA 125 or recurrences during 6-month iCR treatment and 12-month follow-up³⁵. No significant changes of vaginal cytology/maturation index or pH could be detected after 3–6-month treatment with SD or HD iCR^{21,26,27,40}.

Breast ultrasonography did not reveal any changes after 6-month iCR treatment in healthy women and in women after therapy for estrogen-dependent tumors ($n = 54$)^{31,35}. Furthermore, no signs of increased mammographic breast density (visual assessment) or breast cell proliferation (fine-needle aspiration biopsy) were found after 6-month iCR treatment⁴⁴. These results were confirmed by digitized assessment of the mammograms³⁴. Patients with ductal carcinoma *in situ*, using iCR for 2–5 weeks pre surgery, experienced no significant changes in proliferation marker Ki67, yet a downward trend was seen⁵¹. Two case-control studies demonstrated that iCR use was associated with a reduced risk for breast cancer^{37,38}. The larger study found that risk reduction was independent of lifestyle factors, tumor histology, and receptor status, but improved with longer

treatment duration. A pharmacoepidemiological cohort study in breast cancer survivors demonstrated that women taking iCR for climacteric symptoms compared to women not using iCR had a significantly lower risk for recurrence with a 4.5-year longer recurrence-free survival on average³⁶.

Discussion

All studies with iCR consistently demonstrated its efficacy for natural climacteric symptoms, exhibiting significant superiority versus placebo and an efficacy comparable to low-dose transdermal estradiol or tibolone^{19,21–26,28,31,32,39,40,42,43,45,47,52,53}. Yet, due to its better tolerability, iCR had a better benefit–risk profile than tibolone. These results confirm the NICE guideline meta-analyses. There, CR was significantly superior to placebo in reducing vasomotor symptom frequency, revealing a good effect size, whereas tibolone was associated with high treatment discontinuation⁵⁶. It is noteworthy that among all non-hormonal treatments, CR exhibited the largest effect size and had the highest probability of being the best treatment⁵⁶. However, the guideline calls for caution concerning the great variety of CR products. This makes sense because inconclusive data on CR's efficacy result from products that do not meet the strict criteria for HMPs^{16,17}. With a standardized mean difference of –0.568 in favor of iCR versus placebo for natural menopausal symptoms in our meta-analysis, iCR, as an authorized HMP, offers an evidence-based, recommendable, non-hormonal treatment. The inclusion of one publication with a potential for high risk of bias²⁶ in our meta-analysis could be worth discussing. However, as its effect size does not deviate from the overall treatment effect, its inclusion or exclusion has no impact on the result of this meta-analysis. Most of the included studies had a low risk of bias^{19,22,24}.

Our meta-analysis shows higher effect sizes when higher doses of iCR (without or with HP)^{19,24} were used (standard

mean difference of -1.020 and -0.999 , respectively), suggesting a dose-dependency. Women in their early climacteric phase may benefit from an increase from 40 mg up to 120 mg drug/day⁵⁴. Furthermore, iCR+HP was superior to iCR in ameliorating psychological climacteric symptoms³². With its higher iCR dose and synergistic effects with HP, the combination offers a valuable treatment option not only in terms of its greater effect size shown in our meta-analysis but also because psychological symptoms like depressive moods often occur during perimenopause^{57,58}.

Efficacy data concerning iatrogenic climacteric symptoms are varied, with the findings being predominantly positive. Climacteric symptoms after endometrial cancer surgery were significantly reduced by iCR over 24 weeks compared to untreated control³⁵. These positive effects are in line with results of another 24-week, hormone therapy-controlled study with iCR in hysterectomized patients⁵⁹. For goserelin-induced symptoms in endometriosis patients, 12 weeks of iCR treatment was as effective as add-back therapy with tibolone, yet more tolerable³⁰. These results were recently confirmed by a study in gonadotropin-releasing hormone analogue-treated breast cancer patients. There, iCR treatment significantly lowered KMI scores after 12 weeks compared to control⁵⁰. In tamoxifen-treated breast cancer patients, hot flush reductions over 8 weeks under iCR were not significantly different from placebo, but the sample size was too small to prove superiority; however, iCR-treated patients still benefited from significantly less sweating²⁰. This study was too short to expect significant efficacy results. The authors assumed that 'it is possible that when used for a longer period of time, black cohosh may show greater efficacy'. Excluding this study with a shorter treatment duration than the US Food and Drugs Administration and the European Medicines Agency require would have increased the homogeneity of our meta-analysis results. Significant reductions of iatrogenic hot flushes, sweating, and psychological symptoms were shown in longer open studies (12–24 weeks)^{48,49}. There, tamoxifen-treated breast cancer patients were also allowed to double the standard iCR dose⁴⁹. This could enhance treatment effects, as practical experience showed that patients with tamoxifen-induced symptoms often need higher CR doses than natural menopausal women⁶⁰. Thus, HD iCR or iCR+HP can be used to treat climacteric symptoms induced by endocrine therapy. These patients cannot take hormones and without additional therapy may discontinue the anti-hormonal treatment due to its severe side effects^{60–62}.

Treatment with SD/HD iCR or iCR+HP was well tolerated with only rare minor adverse effects, no significant differences from placebo, and significantly better tolerability compared to tibolone^{19,21–25,27,28,30,31,33,35,39–45,48,49,52,53}. This favorable safety profile of iCR is congruent with data analyzed for CR extracts in general^{13,16,17,63}. None of the iCR studies showed liver-specific side effects. Additionally, a meta-analysis of five RCTs and 10 further studies, 1858 patients in total, showed no influence of iCR on liver function^{18,23,26,28,30,31,35,48,52,53,55}. Single cases of hepatotoxicity had been reported during the use of CR products.

These included FS that do not meet the strict quality criteria of HMPs and are often adulterated with different Asian *Cimicifuga* species⁹. Case report assessment with a liver-specific diagnostic algorithm did not reveal a causal relationship^{64–67}.

In the past it was believed that CR's efficacy may be based on estrogen-like effects due to its reputed constituent formononetin. However, this isoflavone was never detected in CR extracts used for the manufacture of iCR^{68,69}. Activity on estrogen receptors was not shown for iCR⁷⁰. Instead, binding and modulation of central nervous system key receptors for thermoregulation, mood, and sleep (e.g. serotonin, dopamine, γ -aminobutyric acid, μ -opioid) and favorable changes in brain metabolism and activity were observed^{46,71–75}. These mechanisms of action explain not only the efficacy for climacteric symptoms but also the fact that iCR did not influence hormones, breast tissue density or proliferation, endometrial thickness, or vaginal cytology^{20,21,23,25–27,30,31,33–35,40,44,48,50,52,53}. The amelioration of vaginal atrophy symptoms observed by some researchers can possibly be explained by anti-inflammatory and anti-oxidative effects of CR. The reduction of psychological menopausal symptoms by iCR may also play an important role in this regard, as psychological stress impairs vaginal health and sexual function. Against this background and in line with the official monographs, the benefit-risk ratio of iCR/iCR+HP is clearly positive. The resulting recommendation of iCR for patients with climacteric symptoms is fortified by results of the NICE guideline health economic model. Out of all treatments in hysterectomized women and in women with a uterus, CR had the second highest and the highest probability, respectively, of being the most cost-effective treatment⁷⁶.

Furthermore, initial data suggest additional benefits. The longer recurrence-free survival in breast cancer patients taking iCR³⁶ and an association of iCR therapy with a decreased risk of developing breast cancer^{37,38} are substantiated by experimental data. iCR dose-dependently inhibited the proliferation of estrogen-dependent and estrogen-independent breast cancer cells due to caspase activation and induction of apoptosis; it reduced invasiveness and enhanced the effects of tamoxifen^{77–81}. CR downregulated the expression of estrogen receptor- α , progesterone receptor, and BRCA1 in hormone-dependent breast cancer cells and decreased their proliferation⁸². In breast cancer animal models, application of iCR indicated a trend toward or actually led to reduced tumor growth – the latter accompanied with prolonged life span^{83,84}. Combination of tamoxifen with iCR increased the incidence of tumor-free animals and reduced the individual tumor burden⁸⁵.

Further mechanisms of action of CR and its constituents may contribute to iCR's beneficial effects (not only) in breast cancer patients. Actein, a CR triterpene glycoside, had growth inhibitory effects on human breast cancer cells associated with activation of stress response pathways⁸⁶. Actein had anti-angiogenic effects in vitro and decreased breast tumor size and metastasis in the mouse 4T1 breast tumor model⁸⁷. Recently, actein was found to suppress human

bladder cancer cell proliferation and induce autophagy and apoptosis via activation of reactive oxygen species/c-Jun N-terminal kinase and inhibition of the AKT/mammalian target of rapamycin (mTOR) pathway. The latter is activated in breast and other cancers, leading to tumor cell growth, proliferation, metastasis, and angiogenesis, and is implicated in endocrine resistance^{88–90}. Additionally, CR and some of its components activated 5' adenosine monophosphate-activated protein kinase (AMPK) to the same extent as metformin⁹¹. AMPK activation and mTOR inhibition suppressed the proliferation and angiogenesis of leiomyoma cells, and mTOR inhibition decreased the tumor incidence, multiplicity, and size in the Eker rat model for myoma^{92–94}. AMPK activation and mTOR inhibition may explain the observed shrinking of myoma under iCR treatment.

Clinical findings suggesting osteoprotective effects of iCR are supported by experimental data: iCR dose-dependently increased osteoprotegerin production (and thereby the osteoprotegerin–RANK ligand ratio) in human osteoblasts, which inhibits osteoclast differentiation and activity and may impede bone resorption^{95,96}. The osteoblast differentiation markers osteocalcin and alkaline phosphatase activity were increased^{95,96}. In the ovariectomized rat model for osteoporosis, iCR augmented bone density and quality⁹⁷, protected against bone loss, and increased trabecular bone structure and resistance⁹⁸. 25-acetylcimigenol xylopyranoside (ACCX), a CR triterpene glycoside, inhibited osteoclastogenesis by abrogation of the nuclear factor- κ B and ERK pathways induced by RANK ligand or tumor necrosis factor- α ⁹⁹.

Oxidative stress and inflammatory processes play a significant role not only in menopausal vasomotor symptoms but also in the development and progression of osteoporosis, (breast) cancer, myoma, and further age-related conditions (e.g. cardiovascular disease and cognitive decline)^{100–103}. As iCR exhibited anti-oxidative and mitochondria-protective effects¹⁰⁴ and recent evidence demonstrated strong anti-inflammatory effects for CR¹⁰⁵, these effects may contribute to iCR's efficacy for vasomotor symptom and to possible additional benefits for patients with breast cancer, myoma, or risk for osteoporosis. With numerous mechanisms of action that may explain such benefits, iCR offers an interesting field for future research, especially for diseases with underlying inflammatory, AMPK, or mTOR dysregulation.

Conclusion and practical consequences

In summary, the clinical data and our meta-analysis consistently demonstrate that iCR/iCR+HP is an effective and safe, evidence-based treatment option for natural neurovegetative and psychological climacteric symptoms, meeting increasing patients' demands for non-hormonal, herbal therapies. As benefits clearly outweigh risks, iCR/iCR+HP should be recommended to these women. With its good safety profile in general and at estrogen-sensitive organs, iCR can also be used in patients with hormone-dependent tumors suffering from iatrogenic menopausal symptoms. However, this should not take place in self-medication but under medical supervision. Breast-cancer patients are not appropriate candidates for

non-supervised self-medication, and it seems reasonable that HD iCR or alternatively iCR+HP are more effective in patients with medication-induced symptoms. Breast cancer patients taking iCR may possibly benefit from prolonged recurrence-free survival, which should be confirmed by further clinical trials.

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References

1. Bair YA, Gold EB, Zhang G, *et al.* Use of complementary and alternative medicine during the menopause transition: longitudinal results from the Study of Women's Health Across the Nation. *Menopause* 2008;15:32–43
2. Peng W, Adams J, Hickman L, Sibbritt DW. Longitudinal analysis of associations between women's consultations with complementary and alternative medicine practitioners/use of self-prescribed complementary and alternative medicine and menopause-related symptoms, 2007–2010. *Menopause* 2016;23:74–80
3. Peng W, Adams J, Hickman L, Sibbritt DW. Complementary/alternative and conventional medicine use amongst menopausal women: results from the Australian Longitudinal Study on Women's Health. *Maturitas* 2014;79:340–2
4. Buhling K, Daniels B, Studnitz F, Eulenburg C, Mueck A. The use of complementary and alternative medicine by women transitioning through menopause in Germany: results of a survey of women aged 45–60 years. *Complement Ther Med* 2014;22:94–8
5. Posadzki P, Lee MS, Moon TW, Choi TY, Park TY, Ernst E. Prevalence of complementary and alternative medicine (CAM) use by menopausal women: a systematic review of surveys. *Maturitas* 2013;75:34–43
6. Upton R. Black Cohosh Rhizome *Actaea racemosa* L. syn. *Cimicifuga racemosa* (L) Nutt Standards of analysis, quality control, and therapeutics American Herbal Pharmacopoeia and Therapeutic Compendium Santa Cruz. CA *American Herbal Pharmacopoeia* 2002;1–38
7. Henneicke-von Zepelin HH. 60 years of *Cimicifuga racemosa* medicinal products: clinical research milestones, current study findings and current development. *Wien Med Wochenschr* 2017;167:147–13
8. von Studnitz FS, Eulenburg C, Mueck AO, Buhling KJ. The value of complementary and alternative medicine in the treatment of climacteric symptoms: results of a survey among German gynecologists. *Complement Ther Med* 2013;21:492–5
9. Jiang B, Kronenberg F, Nuntanakorn P, Qiu MH, Kennelly EJ. Evaluation of the botanical authenticity and phytochemical profile of black cohosh products by high-performance liquid chromatography with selected ion monitoring liquid

- chromatography-mass spectrometry. *J Agric Food Chem* 2006;54:3242–53
10. Edwards SE, Da Costa Rocha I, Williamson EM, Heinrich M. *Phytopharmacy-An Evidence-Based Guide to Herbal Medicinal Products*. Chichester: John Wiley & Sons; 2015
11. Community herbal monograph on *Cimicifuga racemosa* (L.) Nutt., rhizoma [Internet]. 2010. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_Community_herbal_monograph/2011/01/WC500100981.pdf
12. European Union herbal monograph on *Cimicifuga racemosa* (L.) Nutt., rhizoma. EMA/HMPC/48745/2017 [Internet]. 2018. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_Herbal_monograph/2017/08/WC500233056.pdf
13. Leach MJ, Moore V. Black cohosh (*Cimicifuga* spp.) for menopausal symptoms. *The Cochrane Library* 2012;1–92
14. Beer A-M, Osmer R, Schnitker J, Bai W, Mueck AO, Meden H. Efficacy of black cohosh (*Cimicifuga racemosa*) medicines for treatment of menopausal symptoms – comments on major statements of the Cochrane Collaboration report 2012 "black cohosh (*Cimicifuga* spp.) for menopausal symptoms (review). *Gynecol Endocrinol* 2013;29:1022–5
15. Schulz V, Hänsel R. *Rationale Phytotherapie: Ratgeber für die ärztliche Praxis*: Springer-Verlag; 2013
16. Beer AM, Neff A. Differentiated evaluation of extract-specific evidence on *Cimicifuga racemosa*'s efficacy and safety for climacteric complaints. *Evid Based Complement Alternat Med* 2013;2013:860602
17. Beer AM. *Cimicifuga racemosa* bei klimakterischen Beschwerden. *Z Phytother* 2015;36:10–17
18. Naser B, Schnitker J, Minkin MJ, de Arriba SG, Nolte KU, Osmer R. Suspected black cohosh hepatotoxicity: no evidence by meta-analysis of randomized controlled clinical trials for isopropanolic black cohosh extract. *Menopause* 2011;18:366–75
19. Stoll W. Phytotherapeutikum beeinflusst atrophisches Vaginalepithel: Doppelblindversuch *Cimicifuga* vs. Oestrogenpräparat. *Therapeutikon* 1987;1:23–31
20. Jacobson JS, Troxel AB, Evans J, et al. Randomized trial of black cohosh for the treatment of hot flashes among women with a history of breast cancer. *J Clin Oncol* 2001;19:2739–45
21. Liske E, Hänggi W, Henneicke-von Zepelin H-H, Boblitz N, Wüstenberg P, Rahlfs VW. Physiological investigation of a unique extract of black cohosh (*Cimicifuga racemosa* rhizoma): a 6-month clinical study demonstrates no systemic estrogenic effect. *J Womens Health Gend Based Med* 2002;11:163–74
22. Osmer R, Friede M, Liske E, Schnitker J, Freudenstein J, Henneicke-von Zepelin HH. Efficacy and safety of isopropanolic black cohosh extract for climacteric symptoms. *Obstet Gynecol* 2005;105:1074–83
23. Nappi RE, Malavasi B, Brundu B, Facchinetti F. Efficacy of *Cimicifuga racemosa* on climacteric complaints: a randomized study versus low-dose transdermal estradiol. *Gynecol Endocrinol* 2005;20:30–5
24. Uebelhack R, Blohmer JU, Graubaum HJ, Busch R, Gruenwald J, Wernecke KD. Black cohosh and St. John's wort for climacteric complaints: a randomized trial. *Obstet Gynecol* 2006;107:247–55
25. Bai W, Henneicke-von Zepelin H-H, Wang S, et al. Efficacy and tolerability of a medicinal product containing an isopropanolic black cohosh extract in Chinese women with menopausal symptoms: a randomized, double blind, parallel-controlled study versus tibolone. *Maturitas* 2007;58:31–41
26. Li Y, Cui M, Gao S. Efficacy of remifemin for control of climacteric symptoms. *Prog Obstet Gynecol* 2011;20:462–5
27. Sun N-x, Jin Z-j, Jia X-f, Li W. Black cohosh improves vaginal atrophy in postmenopausal women. *Academic Journal of Second Military Medical University* 2012;32:339–41
28. Huang YX, Song L, Zhang X, Lun WW, Pan C, Huang YS. [Clinical study of combined treatment of remifemin and paroxetine for perimenopausal depression]. *Zhonghua Yi Xue Za Zhi* 2013;93:600–2
29. Xi S, Liske E, Wang S, et al. Effect of isopropanolic *Cimicifuga racemosa* extract on uterine fibroids in comparison with tibolone among patients of a recent randomized, double blind, parallel-controlled study in Chinese women with menopausal symptoms. *Evid Based Complement Alternat Med* 2014;2014:717686
30. Chen J, Gao H, Li Q, et al. Efficacy and safety of remifemin on peri-menopausal symptoms induced by post-operative GnRH-a therapy for endometriosis: a randomized study versus tibolone. *Med Sci Monit* 2014;20:1950–7
31. Jiang K, Jin Y, Huang L, et al. Black cohosh improves objective sleep in postmenopausal women with sleep disturbance. *Climacteric* 2015;18:559–67
32. Briese V, Stammwitz U, Friede M, Henneicke-von Zepelin HH. Black cohosh with or without St. John's wort for symptom-specific climacteric treatment – results of a large-scale, controlled, observational study. *Maturitas* 2007;57:405–14
33. Garcia-Perez MA, Pineda B, Hermenegildo C, Tarin JJ, Cano A. Isopropanolic *Cimicifuga racemosa* is favorable on bone markers but neutral on an osteoblastic cell line. *Fertil Steril* 2009;91:1347–50
34. Lundström E, Hirschberg AL, Soderqvist G. Digitized assessment of mammographic breast density – effects of continuous combined hormone therapy, tibolone and black cohosh compared to placebo. *Maturitas* 2011;70:361–4
35. Li W, Sun N-X, Chen X, Lang D-F, Jin Z-J. 早期子宫内膜癌患者术后应用黑升麻制剂治疗绝经相关症状 [*Cimicifuga racemosa* for treatment of menopausal symptoms in patients with early endometrial cancer after operation]. *Acad J Second Mil Med Univ* 2013;32:562–4
36. Henneicke-von Zepelin HH, Meden H, Kostev K, Schröder-Bernhardi D, Stammwitz U, Becher H. Isopropanolic black cohosh extract and recurrence-free survival after breast cancer. *Int J Clin Pharmacol Ther* 2007;45:143–54
37. Rebbek TR, Troxel AB, Norman S, et al. A retrospective case-control study of the use of hormone-related supplements and association with breast cancer. *Int J Cancer* 2007;120:1523–8
38. Obi N, Chang-Claude J, Berger J, et al. The use of herbal preparations to alleviate climacteric disorders and risk of postmenopausal breast cancer in a German case-control study. *Cancer Epidemiol Biomarkers Prev* 2009;18:2207–13
39. Liske E. Phytokombination lindert psychovegetative Leiden. [Phytokombination alleviates psychovegetative disorders]. *TW Gynäkologie* 1997;10:172–5
40. Nesselhut T, Liske E, ed. Pharmacological measures in postmenopausal women with an isopropanolic aqueous extract of *Cimicifuga racemosa* rhizoma. *10th Annual Meeting of the North American Menopause Society (NAMS)*; 1999: Menopause.
41. Pockaj BA, Loprinzi CL, Sloan JA, et al. Pilot evaluation of black cohosh for the treatment of hot flashes in women. *Cancer Invest* 2004;22:515–21
42. Schmidt M, Polasek W, Käufeler R. Efficacy and safety of black cohosh (*Cimicifuga racemosa*, Cimifemin®) in menopause discomfort: surveillance study in practical terms [Wirksamkeit und Sicherheit von Traubensilberkerze (*Cimicifuga racemosa*, Cimifemin®) bei Menopausebeschwerden: Therapiebeobachtung unter Praxisbedingungen]. *J Für Menopause* 2005;1:27–32
43. Vermes G, Banhid F, Acs N. The effects of Remifemin® on subjective symptoms of menopause. *Adv Therapy* 2005;22:148–54
44. Hirschberg AL, Edlund M, Svane G, Azavedo E, Skoog L, Schoultz BV. An isopropanolic extract of black cohosh does not increase mammographic breast density or breast cell proliferation in postmenopausal women. *Menopause* 2007;14:89–96
45. Arriaza Peso E, del Carmen Arévalo Páez M, Ángeles Grandas Alonso M, Olleros Izard T. Eficacia de *Cimicifuga racemosa* para el tratamiento de la clínica vasomotora y psíquica en pacientes menopáusicas. [Efficacy of *Cimicifuga racemosa* in the treatment of vasomotor and psychic symptoms in menopausal patients]. *Progresos de Obstetricia y Ginecología* 2008;51:20–7
46. Reame NE, Lukacs JL, Padmanabhan V, Eyvazzadeh AD, Smith YR, Zubietta JK. Black cohosh has central opioid activity in

- postmenopausal women: evidence from naloxone blockade and positron emission tomography neuroimaging. *Menopause* 2008; 15:832–40
47. Molla J, Garcia-Sanchez Y, Romeu Sarri A, Perez-Lopez FR. *Cimicifuga racemosa* treatment and health related quality of life in post-menopausal Spanish women. *Gynecol Endocrinol* 2009;25: 21–6
 48. Wu X. 莉芙敏改善妇科恶性肿瘤患者术后绝经综合征的临床研究 [Remifemin improve gynecological malignant tumor postoperative patients of menopause syndrome for the clinical research]. Dalian Medical University: Master Degree Thesis; 2011
 49. Rostock M, Fischer J, Mumm A, Stammwitz U, Saller R, Bartsch HH. Black cohosh (*Cimicifuga racemosa*) in tamoxifen-treated breast cancer patients with climacteric complaints – a prospective observational study. *Gynecol Endocrinol* 2011;27:844–8
 50. Wang C, Huang Q, Liang CL, et al. Effect of *cimicifuga racemosa* on menopausal syndrome caused by LHRH-a in breast cancer. *J Ethnopharmacol* 2019;238:111840
 51. Hofstatter EW, Trant AA, Stavris K, et al. The effect of black cohosh on Ki67 levels in DCIS patients. *JCO* 2018;36: e13541–e13541
 52. Chen J-y. 莉芙敏与利维爱治疗围绝经期综合征的临床疗效观察 [Comparison on the efficacy and safety of Remifemin and Livial in the treatment of perimenopausal symptoms]. *Hainan Med J* 2013;24:1454–7
 53. Zhang Q, Zhuang Z, Chen T. 莉芙敏治疗围绝经期综合征的安全性和疗效研究 [The efficacy and safety of Remifemin in the treatment of perimenopausal syndrome]. *Journal of Chengdu Medical College* 2015;10:229–32
 54. Henneicke-von Zepelin H-H, ed. High-dose isopropanolic *Cimicifuga racemosa*-extract for climacteric complaints in perimenopausal women. *Human Reproduction, 12th World Congress*; 2005; Venice
 55. Huang X. 黑升麻根茎提取物缓解乳腺癌患者绝经症状的临床研究 [The clinical research of Black cohosh extract in breast cancer patients with climacteric complaints]. Nanjing University of Chinese Medicine: Master Degree Thesis; 2011
 56. Sarri G, Pedder H, Dias S, Guo Y, Lumsden MA. Vasomotor symptoms resulting from natural menopause: a systematic review and network meta-analysis of treatment effects from the National Institute for Health and Care Excellence guideline on menopause. *BJOG* 2017;124:1514–23
 57. Freeman EW, Sammel MD, Lin H, Nelson DB. Associations of hormones and menopausal status with depressed mood in women with no history of depression. *Arch Gen Psychiatry* 2006;63: 375–82
 58. Minuzzi L, Frey BN, Soares CN. Depression during the menopausal transition: an update on epidemiology and biological treatments. *FOC* 2012;10:22–7
 59. Lehmann-Willenbrock E, Riedel HH. Klinische und endokrinologische Untersuchungen zur Therapie ovarieller Ausfallerscheinungen nach Hysterektomie unter Belassung der Adnexe. *Zent bl Gynäkol* 1988;110:611–18
 60. Ruan X, Mueck AO, Beer AM, Naser B, Pickartz S. Benefit-risk profile of black cohosh (isopropanolic *Cimicifuga racemosa* extract) with and without St John's wort in breast cancer patients. *Climacteric* 2019;22:339–47
 61. Hadji P. Improving compliance and persistence to adjuvant tamoxifen and aromatase inhibitor therapy. *Crit Rev Oncol Hematol* 2010;73:156–66
 62. Fallowfield L. Acceptance of adjuvant therapy and quality of life issues. *Breast* 2005;14:612–16
 63. Assessment report on *Cimicifuga racemosa* (L.) Nutt., rhizoma [Internet]. 2011. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_- _HMPC_assessment_report/2011/05/WC500106358.pdf
 64. Teschke R, Schwarzenboeck A. Suspected hepatotoxicity by *Cimicifuga racemosa* rhizoma (black cohosh, root): critical analysis and structured causality assessment. *Phytomedicine* 2009;16: 72–84
 65. Teschke R. Black cohosh and suspected hepatotoxicity: inconsistencies, confounding variables, and prospective use of a diagnostic causality algorithm. A critical review. *Menopause* 2010;17: 426–40
 66. Teschke R, Bahre R, Fuchs J, Wolff A. Black cohosh hepatotoxicity: quantitative causality evaluation in nine suspected cases. *Menopause* 2009;16:956–65
 67. Teschke R, Schmidt-Taenzer W, Wolff A. Spontaneous reports of assumed herbal hepatotoxicity by black cohosh: is the liver-unspecific Naranjo scale precise enough to ascertain causality? *Pharmacoepidemiol Drug Saf* 2011;20:567–82
 68. Struck D, Tegtmeyer M, Harnischfeger G. Flavones in extracts of *Cimicifuga racemosa*. *Planta Med* 1997;63:289
 69. Kennelly EJ, Baggett S, Nuntanakorn P, et al. Analysis of thirteen populations of black cohosh for formononetin. *Phytomedicine* 2002;9:461–7
 70. Beck V, Unterrieder E, Krenn L, Kubelka W, Jungbauer A. Comparison of hormonal activity (estrogen, androgen and progestin) of standardized plant extracts for large scale use in hormone replacement therapy. *J Steroid Biochem Mol Biol* 2003;84: 259–68
 71. Nisslein T, Koetter U, Freudenstein J. In vitro binding of an isopropanolic extract of black cohosh to selected central nervous receptors. *Maturitas* 2006;54:S65
 72. Burdette JE, Liu J, Chen SN, Fabricant DS, et al. Black cohosh acts as a mixed competitive ligand and partial agonist of the serotonin receptor. *J Agric Food Chem* 2003;51:5661–70
 73. Rhyu MR, Lu J, Webster DE, Fabricant DS, Farnsworth NR, Wang ZJ. Black cohosh (*Actaea racemosa*, *Cimicifuga racemosa*) behaves as a mixed competitive ligand and partial agonist at the human mu opiate receptor. *J Agric Food Chem* 2006;54:9852–7
 74. Hui Z, Xiaoyan M, Mukun Y, et al. Effects of black cohosh and estrogen on the hypothalamic nuclei of ovariectomized rats at different temperatures. *J Ethnopharmacol* 2012;142:769–75
 75. Garcia de Arriba S, Henneicke v, Zepelin H-H, Dimpfel W, Nolte K. *Cimicifuga* extract modulates brain activity: electropharmacogram from various brain areas in freely moving rats (Tele-Stereo-EEG). *Maturitas* 2015;82:318
 76. Menopause: diagnosis and management. NICE guideline (NG23). Version 1.5 [Internet]. 2015. <http://www.nice.org.uk/guidance/ng23> <https://www.nice.org.uk/guidance/ng23/evidence/full-guideline-pdf-559549261>
 77. Bodinet C, Freudenstein J. Influence of *Cimicifuga racemosa* on the proliferation of estrogen receptor-positive human breast cancer cells. *Breast Cancer Res Treat* 2002;76:1–10
 78. Bodinet C, Freudenstein J. Influence of marketed herbal menopause preparations on MCF-7 cell proliferation. *Menopause* 2004; 11:281–9
 79. Hostanska K, Nisslein T, Freudenstein J, Reichling J, Saller R. *Cimicifuga racemosa* extract inhibits proliferation of estrogen receptor-positive and negative human breast carcinoma cell lines by induction of apoptosis. *Breast Cancer Res Treat* 2004;84: 151–60
 80. Hostanska K, Nisslein T, Freudenstein J, Reichling J, Saller R. Inhibitory effect of an isopropanolic extract of black cohosh on the invasiveness of MDA-MB 231 human breast cancer cells. *In Vivo* 2007;21:349–55
 81. Nesselhut T, Bodinet C, Schneider P, Freudenstein J. Use of extract of *Cimicifuga racemosa*. US Patent No. 6,267,994 B1 Jul. 31, 2001
 82. Crone M, Hallman K, Lloyd V, et al. The antiestrogenic effects of black cohosh on BRCA1 and steroid receptors in breast cancer cells. *Breast Cancer (Dove Med Press)* 2019;11:99–110
 83. Freudenstein J, Dasenbrock C, Nisslein T. Lack of promotion of estrogen-dependent mammary gland tumors in vivo by an isopropanolic *Cimicifuga racemosa* extract. *Cancer Res* 2002;62: 3448–52
 84. Nisslein T, Freudenstein J, ed. Prepubertal treatment with black cohosh attenuates pathogenicity in the DMBA rat model of

- mammary carcinoma. *10th world congress on the menopause*; 2002 P06-08; Berlin: Climacteric
85. Nisslein T, Freudenstein J, ed. Synergistic effects of Black Cohosh and Tamoxifen in an animal model of mammary carcinoma. *6th European Congress on Menopause*; 2003: Maturitas
 86. Einbond LS, Su T, Wu HA, et al. The growth inhibitory effect of actein on human breast cancer cells is associated with activation of stress response pathways. *Int J Cancer* 2007;121:2073–83
 87. Yue GG, Xie S, Lee JK, et al. New potential beneficial effects of actein, a triterpene glycoside isolated from *Cimicifuga* species, in breast cancer treatment. *Sci Rep* 2016;6:35263
 88. Ji L, Zhong B, Jiang X, et al. Actein induces autophagy and apoptosis in human bladder cancer by potentiating ROS/JNK and inhibiting AKT pathways. *Oncotarget* 2017;8:112498–515
 89. Paplomata E, O'Regan R. The PI3K/AKT/mTOR pathway in breast cancer: targets, trials and biomarkers. *Ther Adv Med Oncol* 2014;6:154–66
 90. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. *Front Oncol* 2014;4:64
 91. Moser C, Vickers SP, Brammer R, Cheetham SC, Drewe J. Antidiabetic effects of the *Cimicifuga racemosa* extract Ze 450 in vitro and in vivo in ob/ob mice. *Phytomedicine* 2014;21:1382–9
 92. Li B, Takeda H, Tsuiji K, et al. The antidiabetic drug metformin inhibits uterine leiomyoma cell proliferation via an AMP-activated protein kinase signaling pathway. *Gynecol Endocrinol* 2013;29:97–0
 93. Tadakawa M, Takeda T, Li B, Tsuiji K, Yaegashi N. The anti-diabetic drug metformin inhibits vascular endothelial growth factor expression via the mammalian target of rapamycin complex 1/hypoxia-inducible factor-1 α signaling pathway in ELT-3 cells. *Mol Cell Endocrinol* 2015;399:1–8
 94. Ciarmela P, Islam MS, Reis FM, et al. Growth factors and myometrium: biological effects in uterine fibroid and possible clinical implications. *Hum Reprod Update* 2011;17:772–90
 95. Viereck V, Grundker C, Friess SC, et al. Isopropanolic extract of black cohosh stimulates osteoprotegerin production by human osteoblasts. *J Bone Miner Res* 2005;20:2036–43
 96. Stute P, Pickartz S. Zusatznutzen eines isopropanolischen Cimicifuga-racemosa-Extrakts (iCR): positive Beeinflussung von Knochenstoffwechsel und -qualität. *Z Phytother* 2015;36:18–22
 97. Nisslein T, Freudenstein J. Effects of an isopropanolic extract of *Cimicifuga racemosa* on urinary crosslinks and other parameters of bone quality in an ovariectomized rat model of osteoporosis. *J Bone Miner Metab* 2003;21:370–6
 98. Cui G, Leng H, Wang K, et al. Effects of remifemin treatment on bone integrity and remodeling in rats with ovariectomy-induced osteoporosis. *PLoS One* 2013;8:e82815
 99. Qiu SX, Dan C, Ding L-S, et al. A triterpene glycoside from black cohosh that inhibits osteoclastogenesis by modulating RANKL and TNF α signaling pathways. *Chem Biol* 2007;14:860–9
 100. Biglia N, Cagnacci A, Gambacciani M, Lello S, Maffei S, Nappi RE. Vasomotor symptoms in menopause: a biomarker of cardiovascular disease risk and other chronic diseases? *Climacteric* 2017;20:306–12
 101. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med* 2010;49:1603–16
 102. Islam MS, Akhtar MM, Ciavattini A, et al. Use of dietary phytochemicals to target inflammation, fibrosis, proliferation, and angiogenesis in uterine tissues: promising options for prevention and treatment of uterine fibroids? *Mol Nutr Food Res* 2014;58:1667–84
 103. Gordon JL, Rubinow DR, Thurston RC, Paulson J, Schmidt PJ, Girdler SS. Cardiovascular, hemodynamic, neuroendocrine, and inflammatory markers in women with and without vasomotor symptoms. *Menopause* 2016;23:1189–98
 104. Da Y, Niu K, Wang K, et al. A comparison of the effects of estrogen and *Cimicifuga racemosa* on the lacrimal gland and submandibular gland in ovariectomized rats. *PLoS One* 2015;10:e0121470
 105. Yang CL, Or TC, Ho MH, Lau AS. Scientific basis of botanical medicine as alternative remedies for rheumatoid arthritis. *Clin Rev Allergy Immunol* 2013;44:284–300

Review

Menopause-Associated Lipid Metabolic Disorders and Foods Beneficial for Postmenopausal Women

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Abstract: Menopause is clinically diagnosed as a condition when a woman has not menstruated for one year. During the menopausal transition period, there is an emergence of various lipid metabolic disorders due to hormonal changes, such as decreased levels of estrogens and increased levels of circulating androgens; these may lead to the development of metabolic syndromes including cardiovascular diseases and type 2 diabetes. Dysregulation of lipid metabolism affects the body fat mass, fat-free mass, fatty acid metabolism, and various aspects of energy metabolism, such as basal metabolic ratio, adiposity, and obesity. Moreover, menopause is also associated with alterations in the levels of various lipids circulating in the blood, such as lipoproteins, apolipoproteins, low-density lipoproteins (LDLs), high-density lipoproteins (HDL) and triacylglycerol (TG). Alterations in lipid metabolism and excessive adipose tissue play a key role in the synthesis of excess fatty acids, adipocytokines, proinflammatory cytokines, and reactive oxygen species, which cause lipid peroxidation and result in the development of insulin resistance, abdominal adiposity, and dyslipidemia. This review discusses dietary recommendations and beneficial compounds, such as vitamin D, omega-3 fatty acids, antioxidants, phytochemicals—and their food sources—to aid the management of abnormal lipid metabolism in postmenopausal women.

Keywords: menopause; estrogen deficiency; lipid metabolic disorder; beneficial foods

1. Introduction

1.1. What Is Menopause?

Clinically, menopause is diagnosed when a woman has not menstruated for one year due to the loss of ovarian follicular activity, which typically occurs at around 45–55 years of age [1]. In the USA, spontaneous menopause occurs at 51 years of age, on an average. Generally, women live longer than men, and the global average life span of women has been increasing. The median age of women has been increasing gradually and is expected to reach 82 years by 2025 in developed countries. Therefore, approximately one-third of women's lives would be lived after menopause [2]. Menopause occurs over several years and not at a single point in time. It is preceded by a stage where women experience irregular menstrual cycle, referred to as menopausal transition (perimenopause), which involves coping with the cessation of oocyte production in the ovaries [3].

Hormonal change is among the major physiological changes associated with menopause. Estrogen, a primary female sex hormone, dictates the secondary sex characteristics and affects the development and functioning of the female reproductive system. All estrogens are C18 steroids [4]. In addition to its indispensable role in the development of female reproductive tissues and organs—such as breasts, vagina, and uterus—estrogen is also involved in maintaining the function of these tissues and organs during puberty, adulthood, and pregnancy. Estradiol (E2) is almost always present in the body of

women of reproductive age, whereas estriol (E3)—which is produced by the placenta—is primarily abundant during pregnancy and plays an important role in maintaining early-stage pregnancy [4]. During pregnancy, the E3 levels increase up to 1000-fold, while the estrone and E2 levels increase up to 100-fold. Thus, E3 can be measured in urine. Throughout the follicular phase of the menstrual cycle, the thecal cells produce androstenedione, which functions as a metabolic precursor to estrone and testosterone in the ovaries and peripheral tissues [5].

1.2. Dysregulation of Lipid Metabolism Due to Alterations in E2

During a woman's fertile life, the average level of total estrogen is 100–250 pg/mL. However, the concentration of E2 in circulation declines up to 10 pg/mL postmenopause [6]. This hormonal menopause is associated with pathological menopausal syndromes, such as disturbances in sleep/mood, vasomotor symptoms (including hot flashes and night sweats), urogenital atrophy, osteopenia and osteoporosis, psychiatric disorders, sexual dysfunction, skin lesions, cardiovascular diseases (CVDs), cancer, metabolic disorders, and obesity [7]. Women are at a higher risk of developing CVDs after menopause due to estrogen deficiency and dysregulated lipid metabolism [8]. Estrogens, especially E2, exert a protective role in the cardiovascular system and are produced primarily in the ovaries via a process that uses low-density lipoprotein (LDL) cholesterol (LDL-C) as a substrate. However, circulatory LDL-C cannot be utilized to synthesize estrogen during menopause, thereby resulting in decreased estrogen production. Therefore, menopause is associated with increased blood LDL-C levels and enhanced CVD risk [9].

Experimental data have demonstrated that E2 plays an essential role in the β -oxidation of fatty acids in the mitochondria. In an aromatase (estrogen synthase) knockout mouse model, E2 was not detectable in the liver tissue; additionally, decreased expression of mRNA was observed, along with a lowered activity of enzymes involved in fatty acid metabolism. Importantly, E2 treatment resulted in recovered mRNA expression and increased the activity of enzymes involved in fatty acid metabolism in an estrogen synthase knockout mouse model [10]. In a postmenopausal model, female mice (aged 2–4 weeks)—after ovariectomy—exhibited decreased expression of nuclear receptors and proteins needed for efficient energy expenditure, such as peroxisome proliferator-activated receptor (PPAR) gamma γ , PPAR δ , PCG1 α , PCG1 β , and ERR1—in the adipose tissue and muscle—relative to that in the sham-operated control mice. Additionally, the ovariectomized (OVX) mice exhibited decreased expression of enzymes involved in the β -oxidation of fatty acids and transcription factors required for lipolysis [11]. Therefore, the depletion of E2 by ovariectomy or after menopause may downregulate the expression of genes required for efficient energy expenditure as fuel sources in the human body and genes involved in fatty acid metabolism or lipid catabolism, which may induce obesity or metabolic disorders in postmenopausal women [12].

Menopause-induced estrogen deficiency may also lead to various metabolic disorders, including dysregulated lipid metabolism. This review has two distinctive parts. The first part of the review comprehensively summarizes the definition of menopause and postmenopausal physiology, including hormonal change and dysregulation of lipid metabolism caused primarily due to alterations in E2 levels. The second half of the review provides practical and applicable daily dietary recommendations and proposes food sources that would prove to be beneficial for postmenopausal women.

2. Lipid Metabolic Disorders Associated with Menopause

2.1. Menopause-Associated Changes in Fat Mass and Fatty Acid Metabolism

Aging is associated with changes in body composition, which affect physical activity and health in humans [13]. Most studies suggest that the changes in the composition of the female body coincide with menopause. Some of these changes can be partly explained by the impairment of the protective role of estrogens and a relative increase in circulating androgen levels [14]. Previous studies have demonstrated that endogenous sex hormones can influence the lipid profile in premenopausal and

postmenopausal women because the receptors for estrogen and androgen are expressed in both visceral and subcutaneous adipocytes. Therefore, changes in the levels of endogenous sex hormones may affect lipid metabolism in the fat tissues of middle-aged women [15]. Postmenopausal women are also reported to exhibit higher total body fat mass, fat percentage, and accumulation of central fat than premenopausal women [16]. Kim et al. reported that although both premenopausal and postmenopausal women exhibited similar mean body mass index (BMI) values, postmenopausal women exhibited larger waist circumference [17]. A controlled longitudinal study reported that postmenopausal women were at a significantly higher (2.88-fold) risk of developing abdominal obesity than premenopausal women [18,19].

During menopausal transition—that usually spans 2–7 years—clinical changes occur in the body composition due to aging and hormonal changes [20]. Ovarian estrogens increase the storage of peripheral fat mainly in the gluteal and femoral subcutaneous regions, while androgens—primarily bioavailable testosterone—augment the accumulation of visceral abdominal fat. The marked decrease in estrogen concentrations accompanying relative hyperandrogenism is regarded as the main factor that causes weight gain and redistribution of body fat in postmenopausal women [21].

A longitudinal community-based Study of Women's Health Across the Nation (SWAN)—a five-year follow-up study—reported that relative androgen excess (a higher baseline testosterone/E2 ratio) can predict incident of metabolic syndrome, including dysregulated lipid metabolism and obesity, during menopausal transition [22]. This study reported that postmenopausal women had 2-fold higher visceral abdominal fat and subcutaneous adipose tissue (SAT) than premenopausal women. However, the testosterone levels were similar among premenopausal and postmenopausal women. This suggested that fat redistribution may be affected by a marked decrease in estrogen levels but not by increased testosterone level [23]. Estrogen and androgen receptors are expressed in visceral and subcutaneous fat cells. Therefore, changes in the levels of sex hormones can influence lipid metabolism [24]. However, the levels of sex hormone-binding globulin also decrease at the onset of menopause, which increases the levels of bioavailable testosterone. Thus, several studies have suggested that augmented bioavailable testosterone can directly regulate the accumulation of visceral fat through androgen receptors in the adipocytes of the abdominal fat [25]. Additionally, Ziaei et al. reported that free testosterone correlated with the BMI and waist circumference in postmenopausal women [26]. Oral administration of androgens to postmenopausal women preferentially augmented the levels of visceral abdominal fat [27].

Excessive visceral abdominal fat can cause metabolic alterations, especially in fatty acid metabolism, in postmenopausal women. Visceral fat is reported to be associated with a high lipolysis rate (breakdown of triacylglycerol (TG) into glycerol and free fatty acid (FFA)), which results in an increased flux of FFA to the liver and enhanced hepatic insulin resistance. One of the strategies for treating insulin resistance involves elimination of the excess FFAs [21,28]. The adipose tissues of postmenopausal and premenopausal women exhibit differential lipid metabolism. The basal lipolysis rate in the gluteal adipose tissue of postmenopausal women was 77% lower than that in the gluteal adipose tissue of premenopausal women. Further, the activity of adipose tissue lipoprotein lipase (AT-LPL)—which catalyzes the conversion of TG into FFAs for uptake and storage of TG by the adipocytes and plays a major role in the accumulation and distribution of fat stores—was significantly higher in the gluteal and abdominal adipose tissues of postmenopausal women than that of premenopausal women. Thus, lower lipolysis rate and higher AT-LPL activity may predispose postmenopausal women to increased accumulation of body fat after menopause [29,30]. Takahashi et al. comparatively evaluated fatty acid metabolism between premenopausal and postmenopausal women. Interestingly, postmenopausal women exhibited significantly higher concentrations of fatty acid metabolites, such as heptanoate (C7:0), octanoate (C8:0), and pelargonate (C9:0), in the visceral fat (but not in subcutaneous fat) than premenopausal women. This may result in enhanced fatty acid metabolite accumulation in the visceral fat due to elevated lipolysis. The expression of transcripts encoding adiponectin, PPAR- γ , and fatty acid transporter in the gluteal fat of estrogen-depleted postmenopausal women was significantly

higher than in the gluteal fat of premenopausal women. These findings indicate that gluteal fat may be insulin-resistant and might contribute to the accumulation of body fat after menopause, which may be related to the development of metabolic syndrome [30,31]. Metabolic syndrome is defined as a cluster of conditions characterized by impaired glucose metabolism, high blood pressure, central obesity, and low LDL-C and high triglyceride levels [32]. Recent studies indicate that metabolic syndrome is more prevalent among postmenopausal women than premenopausal women [33]. Importantly, half of the postmenopausal, Iranian women have been reported to suffer from metabolic syndrome [34]. Therefore, preventive strategies must be devised for combating the impaired metabolic changes in postmenopausal women.

2.2. Menopause-Associated Changes in Fat-Free Mass (FFM) and Basal Metabolism

One of the noticeable changes in body composition that is associated with the age of women is the loss of FFM, or lean body mass (LBM). Generally, there is increased intra-abdominal adipose tissue and decreased fat in the hip–thigh area among women [31]. Many cross-sectional studies using dual-energy X-ray absorptiometry (DEXA) have reported that postmenopausal women exhibit lower FFM or LBM in the whole body, trunk, and lower extremity regions than premenopausal women [35]. The loss of FFM or LBM is likely to coincide with menopausal transition, which suggests that sarcopenia may be related to the menopausal status [36]. Sarcopenia refers to the degenerative loss of skeletal muscle that occurs at a rate of 3%–8% every 10 years after the age of 30 years, and accelerates with age. This condition is associated with increased risk of functional disability, falls, fracture, and overall mortality among the elderly [37]. Women develop sarcopenia earlier than men, and the incidence of sarcopenia increases rapidly during the menopausal period [38]. Interestingly, a six-year follow-up study by Poehlman et al. reported that menopausal women lost more FFM than age-matched premenopausal women [36]. Recently, Kim et al. reported that one out of five relatively healthy Korean menopausal women aged over 65 years exhibited a decrease in muscle mass, and 7.6% of the women exhibited a decrease in muscle mass and strength. The study also reported that sarcopenia also intensified with aging [39].

The loss of FFM or skeletal muscle induces an age-related decline in the basal metabolic rate (BMR) [40]. The loss of FFM or LBM and gain of adipose tissue can alter the BMR. The BMR or resting energy expenditure (REE)—which is the product of energy exchanges occurring in all human cells—is the amount of energy—in calories—required to maintain biological life functions, including the regulation of body temperature, contraction and relaxation of muscles, breathing, blood circulation, cell growth, and functions of the brain and neurons [16]. Generally, BMR constitutes approximately 60%–75% of the diurnal calorie expenditure. However, BMR can vary highly among individuals [41]. FFM or skeletal muscle accounts for approximately 60%–85% of the body mass, which is regarded as an energy consumer and a major determinant of REE [42], whereas fat mass acts as a storage site for surplus energy. Therefore, changes in the body composition in women—around the menopausal period—such as the loss of FFM leads to a decrease in the BMR. Thus, the physical body of menopausal women expends less energy to maintain the basic life processes [43].

Although the causality between the incidence of sarcopenia and menopausal status is still controversial [44], it is generally accepted that natural age-related menopause is associated with an accelerated loss of FFM or skeletal muscle, which is associated with decreased energy expenditure during rest and physical activity [45]. These changes may indicate alterations in energy metabolism.

3. Menopause-Associated Changes in Lipid Metabolism

3.1. Lipids: Lipoproteins, Apolipoproteins, LDL-C, High-Density Lipoprotein (HDL) Cholesterol (HDL-C), and TGs (Triglycerides)

3.1.1. Lipoproteins

Lipoproteins, which comprise proteins and lipids, play a major role in transporting the endogenous lipids. These are circulating lipids that are not directly absorbed from the intestine but are processed through various tissues, especially the liver. The lipoprotein fractions are classified as follows, based on increasing density: chylomicrons, very-low-density lipoproteins (VLDLs), LDLs, and HDLs [46,47].

3.1.2. Apolipoproteins

Apolipoproteins are the protein components of lipoproteins that stabilize the lipoproteins when they pass through the blood. Additionally, apolipoproteins are involved in several important functions, such as conferring specificity to the lipoprotein complexes, which are recognized by specific receptors expressed on cell surfaces.

3.1.3. LDL-C

LDL fraction—a major carrier of cholesterol—binds about 60% of the total serum cholesterol. The function of LDL is to transport cholesterol to the tissues. Additionally, LDL-C forms a part of the plasma membrane or can be converted into various metabolites, including steroid hormones [28]. Therefore, the function of LDL is not restricted to depositing cholesterol and other lipids in the peripheral cells. Furthermore, peripheral cells also express the LDL receptor, apoB-100. As LDL-targeted cells include cells of the vascular endothelium, high concentration and activity of LDL have serious implications in the etiology of CVDs [48]. LDL contributes to the formation of plaque, which is a thick, hard deposit that can clog the arteries, thereby compromising the flexibility of arteries, resulting in atherosclerosis [49]. Patients with type 2 diabetes exhibit dyslipidemia characterized by low HDL and high TG levels due to insulin resistance and increased levels of LDL [50].

3.1.4. HDL-C

The HDL fraction of serum lipoproteins inhibits the deposition of LDL-C. The major functions of HDL are removing unesterified cholesterol (free cholesterol) from cells and removing cholesterol from other lipoproteins. HDL delivers the cholesterol accumulated in various cells and lipoproteins to the liver, where it is excreted via bile [51]. This is accomplished via two key properties of HDL. The first is that HDL can bind to the receptors expressed on both hepatic and extrahepatic cells. These cells contain receptors specific for HDL or LDL that can be bound by HDL through its apoE component. The second property of HDL is by virtue of its apoA-1 component, which activates LCAT (lecithin-cholesterol acyltransferase, also called phosphatidylcholine-sterol O-acyltransferase) [51], an enzyme that converts free cholesterol into cholesteryl esters. The free cholesterol (recipient) substrate is derived from the plasma membrane of cells or surfaces of other lipoproteins. Subsequently, the cholesteryl esters can be exchanged between the plasma lipoproteins, which is mediated by a transfer protein, cholesterol ester transfer protein (CETP). LCAT-mediated conversion of free cholesterol to cholesteryl esters facilitates the delivery of cholesterol from nonhepatic cells as well as from other lipoproteins [51]. The cholesteryl esters can be directly transported to the liver by HDL, or indirectly by LDL after the ester is transferred from HDL to LDL by CETP. The esters accumulated in the liver cells can be hydrolyzed by cholesterol esterase to release free cholesterol, which is excreted as a bile salt in the bile through a process called reverse cholesterol transport. This is the main pathway for cholesterol excretion from the body [52]. HDL decreases the levels of accumulated cholesterol in the endothelium of blood vessels, which is beneficial for the cardiovascular system and aids in preventing the formation of fatty plaques and atherosclerosis [52]. One-fourth to one-third of blood cholesterol can be transported by HDL. Thus, an

optimal HDL level may protect against heart attack and stroke, while low HDL levels are reported to increase the incidence of coronary heart disease (CHD) [53].

3.1.5. Triglycerides

TGs (Triglycerides) are another type of fats that differ from lipoproteins in that TGs are involved in energy metabolism. Most stored body fat is in the form of TGs, which represent a highly concentrated form of energy and account for nearly 95% of dietary fat. To generate energy, fatty acids are released in nonesterified form, which is the FFAs, from TGs in the adipose tissue. The FFAs are then transported by binding albumin to various tissues for oxidation. Elevated blood TG levels are reported to be associated with CVDs, type 2 diabetes, and atherosclerosis [54].

3.2. Menopause-Associated Alterations in Various Lipids

Menopausal women are at high risk for developing CVDs due to dysregulated lipid metabolism and estrogen deficiency [55]. The SWAN study involved 2659 women with baseline ages ranging from 42 to 52 years who were in the premenopausal or menopausal transition states. The study reported that the alterations in various body fats were related to the changes in the menopausal status and concentrations of E2 and follicle-stimulating hormone (FSH). The alterations in various fats were initiated during the late phases of menopause. The levels of total cholesterol, LDL, triglycerides, and lipoprotein peaked during menopausal transition and the early postmenopausal stage [22,25]. The SWAN study also reported that the middle-aged women with the highest quartile E2 levels had the lowest levels of total cholesterol and LDL, whereas women with the lowest quartile FSH levels had the highest levels of total cholesterol and LDL-C. Lower levels of E2 and sex hormone-binding globulin were associated with medium to low LDL concentrations, while higher levels of FAI (free androgen index) were associated with higher cholesterol, TG, and LDL levels and lower HDL levels in middle-aged premenopausal women [56]. The changes in sex hormone levels and lipid metabolism may also increase the risk of CHD in women [23].

Moreover, several studies have reported that there is a positive correlation between menopausal status and high levels of total cholesterol, LDL, apo-B, and high total cholesterol-to-HDL ratio [57,58]. Recently, Anagnostis et al. reported that the total cholesterol-to-HDL ratio also increased with menopause. The total cholesterol-to-HDL ratio is a better indicator of CVD than total cholesterol itself [59]. A community-based cohort study explored the changes in serum lipid profile during menopausal transition in Chinese women. In total, 593 healthy women aged 35–64 years were followed-up annually for up to 3 years. Menopausal transition in these women exhibited a correlation with the serum lipid profile. The total cholesterol, TG levels, and total cholesterol-to-HDL ratio peaked in women exhibiting menopausal transition compared with those in the premenopausal women. Contrastingly, there was no significant change in HDL-C among the different menopausal status groups [60]. LDL particle size and density also changed with the menopausal status. The levels of small and dense LDL particles increased from 10%–13% in premenopausal women to 30%–49% in postmenopausal women [16]. The small and dense LDL is reported to be an important determinant of atherosclerosis and CVDs in postmenopausal women [61].

3.3. Correlation between Menopausal Status and HDL-C

Some studies have reported that HDL-C levels increase after menopause. The longitudinal SWAN study reported that the HDL-C levels increase gradually from premenopause and peak during menopausal transition. Subsequently, HDL-C levels gradually decline till late postmenopause [57]. However, some studies have indicated a decrease in the levels of plasma HDL-C. A cohort study on 541 healthy middle-aged women followed up through menopause reported that postmenopausal women exhibit a decline in HDL-C levels (mean, 0.53–0.43 mmol/L) and a gradual increase in LDL-C levels (mean, 3.14–3.33 mmol/L) [62]. Similarly, the Women's Health in the Lund Area (WHILA) study involving 6908 women reported that postmenopausal women exhibit significantly lower levels of

plasma HDL-C than premenopausal women [63]. Additionally, follow-up data from the Healthy Women Study demonstrated that menopausal transition was associated with decreased HDL-C levels and increased LDL-C and TG levels relative to that in the postmenopausal stage [64]. The changes in HDL-C accompanying the menopausal status may be more complex than that indicated by the measurement of plasma levels of total HDL-C. Recent studies have suggested that the changes in the proportion of HDL subclasses are more strongly associated with the menopausal status than the changes in the total HDL-C. Most of the available evidence suggests that menopausal transition period and menopause are characterized by increased levels of small HDL subfraction 3 cholesterol (HDL 3-C) and decreased levels (by 25%) of large, buoyant, cardioprotective HDL subfraction 2 cholesterol (HDL 2-C). Additionally, the levels of small dense LDL particles increased up to 30%–49% in menopause [65]. Some studies suggest that HDL 2-C is more active in reverse cholesterol transport and has better antiatherogenic properties than HDL 3-C [52].

3.4. Adiposity Associated with Menopause

The degree of accumulation of body fat is referred to as adiposity, which is commonly used to indicate excess body fat [66]. A well-documented metabolic phenotypic feature observed after menopause is an increased tendency for body fat deposition in the abdominal region with greater waist circumference [43]. The rapid decline in ovarian function and the subsequent decline in the production of circulating steroid-based sex hormones—such as estrogen—associated with menopause results in increased overall adiposity, especially the abdominal visceral adiposity [67,68]. Increased adiposity in postmenopausal women is significantly associated with hyperinsulinemia, which suggests that insulin resistance may be responsible for the development of the key features of postmenopausal dyslipidemia [69], obesity [70], metabolic syndrome [71], and type 2 diabetes [72].

Age-related loss of ovarian hormones was examined in human (menopause) and animal models. Menopause induces the loss of ovarian hormones that contributes to alterations in the distribution of adipose tissue. The significant decrease in both estrogen and progesterone due to follicular depletion results in a more androgenic pattern of fat distribution (central or abdominal adiposity) [20]. OVX mice—a surgical menopause model—exhibit a significant increase in body weight due to reduced energy expenditure and increased food consumption. Additionally, several clinical disorders, such as increased peripheral inflammation, augmented insulin resistance, and magnified adipose tissue cells, have also been observed in the OVX mice [73,74]. However, the OVX mice recovered from these clinical features upon being supplemented with estrogen (E2). Estrogen is also reported to exhibit a strong inhibitory effect on key adipogenic genes, such as PPAR- γ , CEBP β (CCAAT-enhancer-binding proteins), and lipin1 in non-OVX female and OVX female mice supplemented with estrogen [74].

The function of estrogens can be transduced by nuclear receptors—which belong to the superfamily of nuclear receptors—that also act as transcription factors. There are two types of estrogen receptors: estrogen alpha (ER α) and estrogen beta (ER β) receptors [75]. Both ER α and ER β are expressed in the human subcutaneous and visceral adipose tissues [76]. The specificity of ER α lies in its ability to regulate the activity of adipose tissue cells and fat distribution underlying sexual dimorphism. Studies on male and female ER α whole-body knockout mice reported that the absence of ER α causes a near doubling of adipocyte hyperplasia and hypertrophy, insulin resistance, hyperlipidemia with smaller LDL particles, and glucose intolerance in both sexes, which suggests that estrogens play a protective role against these phenomenon [77,78]. The knockdown of adipocyte-specific ER α (Adipo ER α) is reported to increase the body weight, adipose tissue mass, and adipocyte size in females. This indicated that adipocyte ER α regulates body fat distribution, adipose tissue inflammation, and fibrosis. Thus, adipocyte ER α is necessary for mediating the beneficial effects of estrogen on metabolism [79].

Adipokines such as leptin, adiponectin, resistin, and ghrelin can modulate the size and number of adipocytes and angiogenesis through the paracrine system, and are thus involved in regulating the fat mass [80]. Moreover, adipokines are also involved in the regulation of blood pressure, blood clot formation, lipoprotein metabolism, immune function, and inflammatory reactions [81]. Menopause is

associated with marked elevation in the levels of leptin and resistin and downregulation of ghrelin and adiponectin. Postmenopausal women with a history of hypertension exhibited increased blood leptin levels [82]. Further, postmenopausal women with low bone mineral density exhibited significantly high circulatory levels of adipocyte-secreted hormones (adiponectin, resistin, PAI-1, and adipsin) [83]. This indicated that altered adipokine levels may play a crucial role in the pathogenesis of metabolic disorders, such as hypertension, CVD, and osteoporosis. High leptin levels along with low adiponectin levels exert a positive effect on insulin resistance markers. Thus, adipocytokine levels may account for postmenopausal dyslipidemia resulting from insulin resistance [84].

3.5. Obesity Associated with Menopause

Obesity is mostly defined as per the established classification metrics with respect to the BMI [66,85] and as an abnormal or excess total body fat that presents a health risk [86]. BMI is a simple index of weight-to-height (kg/m^2), and the World Health Organization (WHO) defines obesity as a BMI $\geq 30 \text{ kg/m}^2$, overweight as BMI 25–29.9 kg/m^2 , and underweight as BMI $< 18.5 \text{ kg/m}^2$ [86]. Obesity is a precursor condition for various disorders, such as CVD, metabolic syndrome, and type 2 diabetes [87,88]. It is caused by a combination of both hereditary and environmental factors [89]. Women in midlife exhibit continuous weight gain. However, the reasons for increased obesity among menopausal women are not fully understood and are contradictory. There are several inconsistencies among studies that have reported a correlation between body mass and age among postmenopausal women due to differences in study design, analysis, or the verifying control of confounding variables [12].

One of the major reasons for increased obesity among menopausal women is the decreased level of estrogens. Obesity is associated with a higher probability of surgical menopause, where the ovaries are surgically removed by bilateral oophorectomy [2]. Clegg et al. demonstrated that the absence of estrogens may be an important obesity-triggering factor [90]. Moreover, some studies have reported that estrogen deficiency enhances metabolic dysfunction and predisposes the individual to type 2 diabetes, metabolic syndrome, and CVD [16]. Aromatase (estrogen synthase) knockout mice—that cannot synthesize E2—are obese and insulin-resistant [91]. As described previously, ER α appears to be a key regulator of obesity because ER α knockout mice exhibit increased obesity and glucose intolerance compared with wild-type female mice [77,92]. A recent study reported that postmenopausal women who have received aromatase inhibitors for the treatment of breast cancer exhibited higher body fat percentage and insulin resistance than control subjects without a history of breast cancer [93].

A reduction in energy expenditure during midlife can also cause obesity during menopause. According to a four-year follow-up study, women undergoing menopause exhibited a greater decrease in energy expenditure than premenopausal women [94], which may be attributed to the reduction in physical activity. The loss of LBM leads to a reduction in basal energy metabolism with aging [95]. A population-based cross-sectional study involving 292 Brazilian women reported that a higher risk of overweight/obesity was associated with (odds ratio (OR), 2.1; 95% confidence interval (CI) 1.233–3.622; $p = 0.006$) a waist circumference $>88 \text{ cm}$ (OR, 1.7; 95% CI 1.054–2.942; $p = 0.03$) for inactive women. Even after adjustment for age, menopause status, smoking, and hormone therapy, inactive women were associated with a higher risk of diabetes mellitus (OR, 2.7; 95% CI 1.233–6.295; $p = 0.014$) and metabolic syndrome (OR, 2.5; 95% CI 1.443–4.294; $p = 0.001$). These results suggest that the reduction in energy expenditure due to a sedentary lifestyle before menopause may be associated with an increased risk of developing obesity [96].

4. Menopause-Associated Alleviation of Dysregulated Lipid Metabolism

Dysregulation of lipid metabolism and accumulation of visceral adipose tissue after menopause can be caused by estrogen deficiency. However, the effect of diet on obesity and metabolic syndrome is still unclear. In this section, we would discuss diets that would help postmenopausal obese women introduce important nutrients and beneficial compounds in their food.

4.1. Dietary Recommendations

Hormonal and metabolic changes in women undergoing menopause are associated with increased body weight and visceral fat accumulation, which results in the prevalence of high abdominal obesity. As mentioned previously, insulin, total cholesterol, LDL-C, HDL-C, and total cholesterol-to-HDL ratio are usually altered in postmenopausal women with a 50% risk of having metabolic syndrome [33]. In this section, we will discuss the recommended diets for obese postmenopausal women. According to the North American reference standards for nutrients, macronutrient portions should consist of approximately 10%–35% protein-derived energy, 45%–65% carbohydrate-derived energy, and 20%–35% fat-derived energy [97]. The requirements of several key nutrients, such as protein, vitamin D, vitamin B-12, fiber, and fluid, must be met in a hypocaloric diet for older obese adults [98]. The 2013 American Heart Association (AHA)/American College of Cardiology (ACC) Guideline for Obesity prescribed the following methods to reduce food and calorie intake for weight loss: (a) 1200–1500 kcal/day for women (kilocalorie levels are usually adjusted for the individual's body weight), (b) a 500 kcal/day or 750 kcal/day energy deficit, (c) one of the evidence-based diets that restricts certain food types (such as high-carbohydrate foods, low-fiber foods, or high-fat foods) to create an energy deficit and reduce food intake. One of the above methods can be used to reduce food and calorie intake [99].

The current recommended dietary allowance (RDA) for dietary protein intake is 0.8 g/kg/day for adults [100]. As per dietary reference intakes for Koreans 2015, the current dietary recommendation for protein is 40–45 g/day for Korean women [101]. However, the prevalence of sarcopenia was recently observed among postmenopausal women. Women develop sarcopenia earlier than men, and the incidence of sarcopenia increases rapidly during the menopausal period [102]. Therefore, the loss of skeletal muscle can lead to significant changes in amino acid metabolism as the skeletal muscle contains 53%–75% of all proteins present in the human body. Thus, low dietary protein intake may limit weight loss and result in sarcopenia. However, this can be acutely reversed using nutritional adjustments and exercise [103]. Moreover, several intervention trials have reported that the partitioning of weight loss between fat and lean mass with a greater fat-to-lean mass ratio is present in middle-aged and older women on a high-protein hypocaloric diet. Therefore, higher protein intake (1.0–1.2 g/kg/day) may compensate for some loss of muscle mass during weight loss [104–106].

Low-energy diet is also recommended for postmenopausal women to prevent metabolic alterations. A cross-sectional study involving 4984 Korean women reported that the traditional healthy diet involving high consumption of sea fish, seaweeds, dairy products, cereals, fresh vegetables, and fruits and low consumption of fast foods, animal fat-rich foods, sweets, and fried foods has a protective effect against the dysregulation of lipid metabolism (lower TG and higher HDL-C). A healthy diet exhibits a significant protective effect against metabolic syndrome among postmenopausal women relative to that of a Western diet, which is unbalanced and has high energy, and abundant carbohydrates and saturated fats (OR (95% CI) for highest vs. lowest quartile = 0.60 (0.41–0.86); *p* for trend = 0.004). Additionally, a healthy diet exerts protective effects against blood pressure and TG levels—during menopausal transition—and obesity and HDL-C among postmenopausal women [107].

4.2. Beneficial Foods and Their Sources for Postmenopausal Women

4.2.1. Vitamin D

Vitamin D is a unique nutrient as it can be synthesized by the body with the help of sunlight. Therefore, vitamin D is not an essential nutrient. Most people do not require dietary vitamin D if they are exposed to sufficient sunlight every day. Vitamin D—synthesized from sunlight or obtained from food—is activated via chemical transformations in the liver and kidneys [108]. Although vitamin D can be easily obtained, recent surveys indicate that many people, particularly African Americans and Mexican Americans, suffer from vitamin D insufficiency [109]. According to a Korean National Health and Nutrition Examination Survey 2010–2011, 65.9% of Korean men and 77.7% of Korean women (age ≥ 10 years) exhibited below optimal levels of blood serum vitamin D (25-hydroxyvitamin

D; 25(OH)D; 20 ng/mL) [110]. The prevalence of 25(OH)D deficiency (<10 ng/mL) was significantly higher among women than that among men [111].

It is important to understand the role of vitamin D to examine the deficiency of vitamin D. Vitamin D is the best-known nutrient among the various micronutrients and hormones which interact to regulate the balance between blood calcium and phosphorus levels. Therefore, vitamin D is essential for maintaining bone integrity [112]. Calcium is indispensable for the proper functioning of cells in all body tissues, including muscles, nerves, and glands, which obtain calcium from the blood. Vitamin D acts at three sites in the body to replenish the blood calcium levels [113]. The skeleton serves as a vast warehouse of stored calcium that can be utilized when the blood calcium levels decrease. Only two other organs can increase blood calcium, the digestive tract, which provides dietary calcium and kidneys, which recycle calcium that would otherwise be excreted in the urine. Vitamin D functions as a hormone as it is synthesized by one organ and acts on others [114]. In addition to regulation of the bone mass, vitamin D functions at the genetic level to regulate cell growth, multiplication, and specialization. Moreover, vitamin D affects over 30 body tissues, including hair follicles, reproductive system cells, and immune cells [115].

Studies on the role of vitamin D suggest that vitamin D deficiency may cause several diseases, including high blood pressure, CVD [116], some common cancers [117], infections (such as tuberculosis and flu) [118], inflammatory conditions, autoimmune diseases (such as type 1 diabetes and rheumatoid arthritis) [119], psoriasis [120], and multiple sclerosis [121], and that it is even associated with a higher risk of mortality [122].

A recent study reported the correlation between serum 25(OH)D levels and the alteration of lipid profile among postmenopausal women [123]. Vitamin D is a fat-soluble vitamin that can be stored in the SATs. Interestingly, Holick et al. reported that the bioavailability of vitamin D decreases in obese individuals. Thus, obesity—a major outcome of lipid metabolism dysregulation—is one of the causes of vitamin D deficiency in obesity-related diseases [109]. A cross-sectional analysis of 292 postmenopausal women aged 50–79 years in the Women's Health Initiative Calcium–Vitamin D trial revealed that higher serum 25(OH)D levels were inversely correlated with adiposity, BMI, and waist–hip ratio. This indicated that postmenopausal obese women are susceptible to vitamin D deficiency [124]. Vitamin D deficiency is also associated with high TG levels in the blood. A cross-sectional study involving 778 postmenopausal Korean women reported that women in the lowest quartile 25(OH)D level (4.2–9.7 ng/mL) group were associated with a significantly increased risk of metabolic syndrome (OR = 2.44, 95% CI: 1.32–4.48, $p < 0.05$) compared with that in women in the highest quartile 25(OH)D level (19.9–55.9 ng/mL) group. Low serum 25(OH)D levels were also associated with some metabolic disorders, especially elevated blood pressure (95% CI, 1.15–2.85) and high TG level (95% CI, 1.64–4.57) [125]. The Korean National Health and Nutrition Examination Survey (KNHANES 2008–2010) revealed that postmenopausal women in the highest quartile serum 25(OH)D level group exhibited a significant decrease in the prevalence of elevated blood pressure, elevated TGs, and reduced HDL-C when compared with the postmenopausal women in the lowest quartile serum 25(OH)D level ($p = 0.02$, 0.014, and 0.002, respectively) group. This suggests that serum levels of 25(OH)D are significantly associated with a decrease in elevated blood pressure, elevated TG, and reduced HDL-C levels in postmenopausal women [126].

In adults, inadequate vitamin D intake may lead to poor mineralization of the bone, which results in osteomalacia, a painful bone disease in which the bones become increasingly soft, flexible, brittle, and deformed. Low vitamin D levels among elderly individuals may result in painful joints and muscles. Vitamin D deficiency also causes loss of calcium from the bones, which can result in osteoporotic fractures [127]. Vitamin D supplementation is indispensable for menopausal women as it promotes bone metabolism and prevents osteomalacia and osteoporosis [123].

A randomized controlled trial involving 76 healthy postmenopausal women with vitamin D deficiency (defined as a 25(OH)D level < 75 nmol/L)—in which the vitamin D deficiency group randomly received vitamin D 2000 IU once daily for 12 weeks—revealed that the lipid profile and

blood pressure of the vitamin D deficiency group were not significantly different from those of the control groups [128]. Various studies have demonstrated that low serum vitamin D levels are correlated to several metabolic conditions, such as elevated TG, low HDL-C, and high blood pressure, in postmenopausal women [129].

Interestingly, vitamin D has been reported to be important not only in alleviating metabolic diseases, but also in improving the quality of life in postmenopausal women [130]. The intake of calcium, vitamin D, and insulin along with isoflavones is reported to have a positive effect on the quality of life parameters (hot flashes, anxiety, and depressive symptoms), sexual life [131], body composition, and metabolic parameters in menopausal women. The ingestion of equal and resveratrol is also recommended to improve the quality of life parameters, such as dryness of vagina, heart discomfort, and sexual problems, in healthy postmenopausal women [132].

However, the role of vitamin D supplementation in preventing the alteration of lipid metabolism in menopausal transition and postmenopausal women needs to be investigated further.

4.2.2. Dietary Reference Intake (DRI) and Food Sources of Vitamin D

DRI recommendations for vitamin D among adults are as follows: 5 µg/day (19–50 years), 10 µg/day (51–70 years), 15 µg/day (>70 years). The tolerable upper vitamin D intake for adults is 50 µg/day [133]. In the USA and Canada, milk (fluid, dried, or evaporated) is fortified with vitamin D. Furthermore, 3/4 cup of enriched cereal, 3 oz (85 g) of sardines, 3 oz of salmon, 3 oz of tuna (light, canned), 1 tablespoon of cod liver oil, and 1 cup of fortified milk contain 1, 5.8, 15.3, 5.7, 11, and 3.2 µg of vitamin D, respectively. Additionally, exposure to sunlight promotes vitamin D synthesis in the skin [134].

4.2.3. Omega-3 Fatty Acids

The human body can use carbohydrate, fat, or protein to synthesize almost all fatty acids, except linoleic acid and linolenic acid [135]. The human cells cannot synthesize these two polyunsaturated fatty acids (PUFAs) from starch. Moreover, the cells cannot convert linoleic acid to linolenic acid and vice versa [135]. Therefore, linoleic and linolenic acids are essential fatty acids that must be obtained from the diet.

Linoleic acid is a polyunsaturated omega-6 fatty acid family member that belongs to one of the two families of essential fatty acids. Thus, the human body cannot synthesize linoleic acid from other nutrients. If linoleic acid is consumed as a part of the diet, the human body can synthesize other necessary omega-6 fatty acids, such as arachidonic acid, prostaglandins, leukotrienes, and thromboxane [136]. Among them, arachidonic acid is the starting material for the synthesis of several eicosanoids. Omega-6 fatty acids are abundant in various nuts and fatty seeds, such as flax seeds, poppy seeds, sesame seeds, and their derived vegetable oils [137].

Linolenic acid is a polyunsaturated omega-3 fatty acid family member. Linolenic acid is an essential fatty acid and thus must be acquired through dietary intake. Both linoleic and linolenic acid fatty acids are members of a family of fatty acids, namely eicosapentaenoic acids (EPAs; 20:5 omega-3 fatty acid) and docosahexaenoic acid (DHA; 22:6 omega-3 fatty acid), that are of great interest to scientists in relation to CVDs and human development. Although the human body can synthesize a very small amount of these omega-3 fatty acids, they can be supplied through the consumption of oily fishes (cod liver, herring, mackerel, salmon, menhaden, and sardine) or fish oils. These essential fatty acids serve as raw materials for the synthesis of eicosanoids that function as hormones. EPA is a PUFA that acts as a precursor for prostaglandin-3 (that inhibits platelet aggregation), thromboxane-3, and leukotriene-5 eicosanoids. Eicosanoids affect multiple human body functions, such as the contraction and expansion of blood vessels or muscles, blood coagulation, regulation of blood lipid metabolism, and immune response to injury and infections, such as fever, inflammation, and pain, by slowing the synthesis [138].

Omega-3 fatty acids exert anti-inflammatory, cardioprotective, and insulin-sensitizing effects, whereas omega-6 fatty acids exert proinflammatory effects and increase the risk of cardiometabolic disorders and cancer [139]. A 25 year follow-up study reported that the supplementation of long-chain omega-3 fatty acid and non-fried fish in young adulthood is inversely associated with the incidence of metabolic syndromes later in life [140]. A 16 year follow-up study involving 84,688 female nurses reported that women with a higher intake of omega-3 fatty acids had a lower risk of CHD. Moreover, the inverse correlation of omega-3 fatty acid intake via fish consumption with CHD deaths (multivariable relative risks for fish consumption five times per week, 0.55 (95% CI, 0.33–0.90) for CHD deaths vs. 0.73 (0.51–1.04) was stronger than that for nonfatal myocardial infarction [141]. Western diets are insufficient in omega-3 fatty acids and contain excessive amounts of omega-6 fatty acids. Additionally, the ratio of omega-6/omega-3 fatty acids in the Western diet ranges from 15/1 to 16.7/1. An omega-6/omega-3 fatty acids ratio of 4/1 is associated with a 70% decrease in total mortality. A lower omega-6/omega-3 ratio was associated with decreased risk of breast cancer in women, while the ratio of 2–3/1 suppressed inflammation in patients with rheumatoid arthritis [135]. Therefore, supplementation of low omega-6/omega-3 fatty acids can be useful in the prevention of CVD, cancer, and inflammation [135]. Postmenopausal women are reported to exhibit lower omega-6/omega-3 fatty acid ratio than premenopausal women. Healthy postmenopausal women exhibited higher omega-3 fatty acid levels than women with diabetes or CHD. A significant shift in increased omega-6 and decreased omega-3 was observed in diabetes and CHD [142]. Recently, omega-3 fatty acids were reported to protect against obesity and metabolic syndrome, including the lipid alteration. Tardivo et al. reported that oral supplementation of 900 mg omega-3 per day significantly decreased BMI, waist circumference, blood pressure, serum triglyceride, interleukin (IL)-6, and insulin resistance in a randomized controlled trial involving 87 postmenopausal Brazilian women [143].

4.2.4. Recommendations for Omega-3 Fatty Acid Intake

The consumption of optimal amounts of omega-3 and omega-6 fatty acids is required for obtaining health benefits from essential fatty acids. In the cells, both types compete for the same metabolic enzymes. Thus, excess omega-6 fatty acids prevent omega-3 fatty acids from interacting with the enzymes that convert them to the required compounds [135]. A previous study suggested that rationing between omega-3 and omega-6 fatty acids can promote health, but this is still controversial [144]. The AHA recommends including two fatty fish servings totaling 8 oz (227 g) a week for a heart-healthy diet [145].

4.2.5. Food Sources of Omega-6 and Omega-3 Fatty Acids

The food sources of linoleic acid, an omega-6 fatty acid, are seeds, nuts, vegetable oils (corn, cottonseed, safflower, sesame, soybean, and sunflower), and poultry fat. Linolenic acid, an omega-3 fatty acid, is abundant in oils (canola, flaxseed, soybean, walnut, wheat germ; liquid or soft margarine made from canola or soybean oil), nuts, and seeds (flaxseeds, walnuts, soybeans). The food sources of omega-3 EPA and DHA are human milk, fish, and seafood (>500 mg per 3.5 oz serving), European seabass (branzini), herring (Atlantic and Pacific), mackerel, oyster (Pacific wild), salmon (wild and farmed), sardines, toothfish (includes Chilean seabass), and trout (wild and farmed). Black bass, catfish (wild and farmed), clam, cod (Atlantic), crab (Alaskan king), croakers, flounder, haddock, hake, halibut, oyster (eastern and farmed), perch, scallop, shrimp (mixed varieties), sole, swordfish, and tilapia (farmed) provide 150–500 mg omega-3 EPA and DHA per 3.5 oz serving. Cod (Pacific), grouper, lobster, mahimahi, monkfish, red snapper, skate, triggerfish, tuna, and wahoo provide less than 150 mg per 3.5 oz serving [135].

4.2.6. Antioxidants and Their Food Sources

Antioxidants are compounds that protect biomolecules from oxidation-mediated damage. Oxidation can potentially damage the chemical components of the normal cell. Oxidative stress

is defined as a derangement in the balance between oxidants and antioxidants, which damages biological structures and contributes to disease progression [146]. Oxidative stress is reported to cause damages to the cellular components, such as proteins, lipids, and DNA [147], and increases the levels of proinflammatory cytokines, such as tumor necrosis factor and IL-1 β [146]. Moreover, oxidative stress is associated with various diseases, such as obesity, diabetes, metabolic syndrome, insulin resistance, atherosclerosis, and CVD, as it is closely associated with chronic inflammation and endocrine function of adipocyte [148]. Most recent studies have reported that estrogens exert antioxidant activity by modulating the expression of antioxidant enzymes [6]. Sack et al. reported that endocrine disruption in systemic oxidative stress at menopause could be attributed to a change in the levels of E2, which exerts antioxidant activity [149]. Postmenopausal women administered with E2 exhibited increased resistance to LDL oxidative modification and pro-atherogenic transformation. However, this antioxidant action was reversed upon discontinuation of E2 administration [149]. Several studies have reported that E2 dose-dependently inhibits the oxidation of LDL in various ex vivo models [150]. Furthermore, E2 is reported to decrease the concentration of atherogenic plasma lipids, ameliorate endothelial function and inflammatory homeostasis, and increases neuronal survival at the metabolic level [151]. Therefore, postmenopausal women usually have less effective antioxidative defense due to the loss of estrogens. Postmenopausal women are recommended to increase their intake of exogenous antioxidants, such as vitamins (vitamin A, β -carotene, vitamin C, and vitamin E), plant flavonoids, and soy isoflavones. These nutrients are commonly found in fruits, vegetables, soybean, cocoa, and tea leaf extracts. The highest antioxidant content is found in almonds [152], artichokes, blackberries, blueberries, cherries (sour), chokeberry [153], chocolate (dark, unsweetened), cloves, ground cranberry juice, coffee, cranberries, grape juice, cranberry juice, pomegranate juice, pecans, raspberries, spinach, strawberries, walnuts [154], and red wine [134]. Recently, the administration of olive leaf extract [155] and Hawthorn fruit extract [156] was reported to improve serum lipid profiles in OVX mice via elevating mRNA expression of adiponectin and PPAR- α . Generally, antioxidants have characteristic molecular structures with multiple bonds and aromatic rings. This enables antioxidants to function as reducing agents and to terminate oxidative chain reactions by binding free radicals and inhibiting various oxidative reactions, such as lipid peroxidation [6]. Free radicals are atoms or molecules with one or more unpaired electrons that render the atom or molecule unstable and highly reactive [157]. Lipid peroxidation is initiated on the double bonds of plasma membrane fatty acids via free radical attack. The lipid peroxidation products are highly reactive and dose-dependently affect cell signaling, protein, and DNA [146]. Therefore, the oxidative stress-related diseases in postmenopausal women can be prevented by oral administration of antioxidants. The oral administration of vitamin C (ascorbic acid) and vitamin E (alpha-tocopherol) decreases TC, LDL-C, TG, lipid peroxide, glucose, and glycated hemoglobin levels in diabetic and nondiabetic postmenopausal women receiving hormone therapy [158]. This indicated that the co-administration of both vitamins can further enhance the defense against oxidative stress, not only in the aqueous phase, but also in the lipid phase of the mitochondrial membranes [159]. Supplementation with antioxidative vitamins A, C, E, and omega-3 fatty acids significantly decreased serum lipid peroxide levels in Korean postmenopausal women with hypercholesterolemia. Furthermore, the dietary intervention might be more effective than hormone replacement therapy (HRT). Additionally, the group subjected to a combination of HRT and dietary intervention exhibited reduced levels of thromboxane B2 (TBX2), which is a strong prothrombotic factor [160].

4.2.7. Phytochemicals and Probiotics

Phytochemicals are the nutrient components of plants that contain bioactive components, which can alter the physiology of an organism. They have been used in foods for their sensory properties, such as taste, color, texture, and aroma. However, the ability of phytochemicals to regulate physiology is recently gaining attention. Several phytochemicals function as antioxidants, which protect DNA, lipids, and other cellular components from oxidative stress. Some phytochemicals bind to gene sequences

to regulate protein synthesis, mimic hormones, and affect blood chemistry [161]. One of the largest phytochemical groups is the flavonoids. These are detected in several plant foods, such as whole grains, vegetables, fruits, nuts, red wine, spices, and even dark chocolate.

Probiotics are microorganisms that can be used as dietary supplements. Probiotics aid in digesting food, producing vitamins [162], or preventing intestinal infections. Interestingly, recent studies have demonstrated that multistrain probiotic uptake improved iron uptake [163] and positively modified vascular endothelial function among postmenopausal obese women by decreasing arterial stiffness [164]. Here, we summarize various phytochemicals and probiotics among the natural food sources that can exert antioxidant and anticancer effects, attenuate alterations in lipid profile, and protect DNA [165].

1. Alkylresorcinols (phenolic lipids) in grains may exert a protective effect by decreasing the risks of CVD, diabetes, and several cancers. The food sources of alkylresorcinols are whole grain, wheat, and rye [166].
2. Allicin (organosulfur compound) exerts antimicrobial effects that may reduce ulcers and lower blood cholesterol. The food sources of allicin are chives, leeks, garlic, and onions [167].
3. Carotenoids (including beta-carotene, lycopene, lutein, and hundreds of related compounds) act as antioxidants and may reduce the risks of cancer and other diseases. The food sources of carotenoids are highly pigmented fruits and vegetables (apricot, broccoli, cantaloupe, carrots, pumpkin, spinach, sweet potatoes, and tomatoes) [168].
4. Capsaicin inhibits blood coagulation by decreasing the risk of fatal clots in the heart and arteries. The food sources of capsaicin are peppers [169].
5. Curcumin acts as an antioxidant and anti-inflammatory agent, which may decrease blood coagulation and suppress enzymes that activate carcinogens. The food source of curcumin is turmeric, a yellow-colored spice [170].
6. Flavonoids (including flavones, flavanols, isoflavones, and catechins) exert an antioxidant effect. They scavenge reactive oxygen species and carcinogens and bind to nitrates in the stomach, which prevents the conversion to nitrosamines. Additionally, flavonoids inhibit cell proliferation. The food sources of flavonoids are berries, black tea, celery, citrus fruits, green tea, olives, onions, oregano, purple grapes, purple grape juice, soybean, soy products, vegetables, whole wheat, and wine [165].
7. Genistein and daidzein (isoflavones) are phytoestrogens that are structurally similar to the female sex hormone, estrogen and weakly mimic or modulate estrogen in the human body [171]. They inhibit cell proliferation in the gastrointestinal (GI) tract, which may reduce the risk of breast, colon, ovarian, prostate, and other estrogen-sensitive cancers and osteoporosis [172]. The food sources of genistein and daidzein are soybeans, soy flour, soymilk, tofu, textured vegetable protein, and other legume products.
8. Indoles (organosulfur compound) may trigger the production of enzymes that inhibit carcinogen-mediated DNA damage and estrogen activity. The food sources of indoles are cruciferous vegetables, such as broccoli, Brussels sprouts, cabbage, cauliflower, horseradish, mustard greens, and kale [173].
9. Isothiocyanates (organosulfur compounds that include sulforaphane) function as antioxidants, inhibit enzymes that activate carcinogens [174], and activate enzymes that detoxify carcinogens, and thus reduce the risk of breast cancer and prostate cancer. The food sources of isothiocyanates are cruciferous vegetables, such as broccoli, Brussels sprouts, cabbage, cauliflower, horseradish, mustard greens, and kale.
10. Monoterpenes (including limonene) may trigger enzyme production to detoxify carcinogens and inhibit cancer progression and cell proliferation. The food sources of monoterpenes are citrus fruit peels and oils [175].

11. Phenolic acids may promote enzyme production that increases the water solubility and excretion of carcinogens. The food sources of phenolic acids are coffee beans, fruits (apples, blueberries, cherries, grapes, oranges, pears, and prunes), oats, potatoes, and soybeans [176].
12. Resveratrol functions as an antioxidant and can suppress cancer cell growth. Additionally, resveratrol reduces inflammation, LDL oxidation, and blood clot formation. The food sources of resveratrol are red wine, peanuts, grapes, and raspberries [177].
13. Saponins (glucosides) may interfere with DNA replication and prevent cancer cell [178] proliferation and stimulate immune response [179]. The food sources of saponins are soybean, alfalfa sprouts, other sprouts, green vegetables, potatoes, and tomatoes.
14. Tannins act as antioxidants that may inhibit carcinogen activation and cancer progression [180]. The food sources of tannins are black-eyed peas, grapes, lentils, red and white wine, and tea [134].
15. Probiotics promote the digestion of food, produce vitamins [162], or protect probiotic bacteria from intestinal infections. The food sources for probiotics are yogurt and other fermented foods. The multi-strain probiotics also improve insulin resistance, endothelial dysfunction, and iron uptake [164].

4.2.8. Whole Foods: Best Antioxidant Supplements

Generally, the body absorbs nutrients from a complex mixture in foods [181]. If the nutrients are consumed in a purified and concentrated form, they are likely to interfere with the absorption of other nutrients or with the absorption of other nutrients from foods consumed at the same time. These effects are especially well-documented in case of minerals. For instance, zinc disrupts absorption of copper and calcium, iron disrupts absorption of zinc, and calcium disrupts absorption of magnesium and iron [182]. Among vitamins, vitamin C supplements enhance the absorption of iron, which may result in enhanced iron absorption in susceptible individuals. A high dose of vitamin E interferes with the functions of vitamin K, which delays blood clotting and increases the risk of brain hemorrhage [182] and blocks the entry of useful vitamin E isomers into the bone [183]. The vitamin A precursor, beta-carotene, impairs vitamin E metabolism [182]. These interactions serve as a drawback for the use of purified supplements. Therefore, natural, whole foods are the best source of nutrients.

5. Conclusions

Women experience menopause with aging. Menopause may lead to various changes in lipid metabolism due to reduced estrogen secretion. These changes include enhanced fat mass and decreased FFM, which affects the BMR. Additionally, these changes increase the susceptibility to adiposity and obesity. As menopause is associated with altered lipid levels and increased risk of metabolic disorders, including CVD, postmenopausal women must consume recommended diets and beneficial foods. The strength of this study is that the lipid metabolic disorders associated with menopause were comprehensively summarized right from the basic physiological modifications, including alterations in E2 to dysregulated lipid metabolism along with references. However, the limitation of this review is that physical activity, which is a prominent factor affecting metabolic changes in menopausal women, was not reviewed.

The correlation between the menopausal state and alterations in lipid metabolism should be understood to prevent growing problems of obesity-related disorders, including metabolic syndrome. To protect the cellular components from oxidative stress caused due to estrogen deficiency, further studies are needed to develop energy-balanced dietary interventions, antioxidative foods—and their sources—depending on the menopausal status. Menopause is associated with various alterations in lipid metabolism due to estrogen deficiency. Therefore, supplying beneficial foods such as those described in this review will enable postmenopausal women to counteract these changes.

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Abbreviations

E2	estrogen
E3	estriol
PPAR α	peroxisome proliferator-activated receptor α
PPAR β	peroxisome proliferator-activated receptor β
PGC1 α	peroxisome proliferator-activated receptor gamma coactivator 1 α
PGC1 β	peroxisome proliferator-activated receptor gamma coactivator 1 β
ERR1	estrogen-related receptor 1
BMI	body mass index
SWAN	Study of Women's Health Across the Nation
SAT	subcutaneous adipose tissue
TG	triacylglycerol
TC	total cholesterol
FFA	free fatty acid
AT-LPL	adipose tissue lipoprotein lipase
FFM	fat-free mass
LBM	lean body mass
DEXA	dual-energy X-ray absorptiometry
BMR	basal metabolic rate
REE	resting energy expenditure
VLDLs	very-low-density lipoproteins
LDLs	low-density lipoproteins
HDLs	high-density lipoproteins
LCAT	lecithin-cholesterol acyltransferase
CETP	cholesterol ester transfer protein
CHD	coronary heart disease
FSH	follicle-stimulating hormone
WHILA	Women's Health in the Lund Area
OVX	ovariectomized
CEBPb	CCAAT-enhancer-binding proteins
ER α	estrogen receptor α
ER β	estrogen receptor β
Adipo ER α	adipocyte-specific ER α
AHA	American Heart Association
ACC	American College of Cardiology
RDA	Recommended dietary allowance
CVD	cardiovascular disease
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
PUFA	polyunsaturated fatty acid
CHD	coronary heart disease
HRT	hormone replaces therapy
TBX2	thromboxane B2

References

- Landgren, B.M.; Collins, A.; Csemiczky, G.; Burger, H.G.; Baksheev, L.; Robertson, D.M. Menopause transition: Annual changes in serum hormonal patterns over the menstrual cycle in women during a nine-year period prior to menopause. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2763–2769. [[CrossRef](#)] [[PubMed](#)]
- Takahashi, T.A.; Johnson, K.M. Menopause. *Med. Clin. N. Am.* **2015**, *99*, 521–534. [[CrossRef](#)] [[PubMed](#)]
- Potter, B.; Schrager, S.; Dalby, J.; Torell, E.; Hampton, A. Menopause. *Prim. Care* **2018**, *45*, 625–641. [[CrossRef](#)] [[PubMed](#)]
- Patel, S. Disruption of aromatase homeostasis as the cause of a multiplicity of ailments: A comprehensive review. *J. Steroid Biochem. Mol. Biol.* **2017**, *168*, 19–25. [[CrossRef](#)] [[PubMed](#)]
- Dewailly, D.; Robin, G.; Peigne, M.; Decanter, C.; Pigny, P.; Catteau-Jonard, S. Interactions between androgens, FSH, anti-Müllerian hormone and estradiol during folliculogenesis in the human normal and polycystic ovary. *Hum. Reprod. Update* **2016**, *22*, 709–724. [[CrossRef](#)] [[PubMed](#)]
- Cervellati, C.; Bergamini, C.M. Oxidative damage and the pathogenesis of menopause related disturbances and diseases. *Clin. Chem. Lab. Med.* **2016**, *54*, 739–753. [[CrossRef](#)]
- Lobo, R.A.; Davis, S.R.; De Villiers, T.J.; Gompel, A.; Henderson, V.W.; Hodis, H.N.; Lumsden, M.A.; Mack, W.J.; Shapiro, S.; Baber, R.J. Prevention of diseases after menopause. *Climacteric* **2014**, *17*, 540–556. [[CrossRef](#)]
- Sobenin, I.A.; Myasoedova, V.A.; Orekhov, A.N. Phytoestrogen-Rich Dietary Supplements in Anti-Atherosclerotic Therapy in Postmenopausal Women. *Curr. Pharm. Des.* **2016**, *22*, 152–163. [[CrossRef](#)]
- Thaung Zaw, J.J.; Howe, P.R.C.; Wong, R.H.X. Postmenopausal health interventions: Time to move on from the Women's Health Initiative? *Ageing Res. Rev.* **2018**, *48*, 79–86. [[CrossRef](#)]
- Toda, K.; Takeda, K.; Akira, S.; Saibara, T.; Okada, T.; Onishi, S.; Shizuta, Y. Alternations in hepatic expression of fatty-acid metabolizing enzymes in ArKO mice and their reversal by the treatment with 17 β -estradiol or a peroxisome proliferator. *J. Steroid Biochem. Mol. Biol.* **2001**, *79*, 11–17. [[CrossRef](#)]
- Kamei, Y.; Suzuki, M.; Miyazaki, H.; Tsuboyama-Kasaoka, N.; Wu, J.; Ishimi, Y.; Ezaki, O. Ovariectomy in mice decreases lipid metabolism-related gene expression in adipose tissue and skeletal muscle with increased body fat. *J. Nutr. Sci. Vitaminol.* **2005**, *51*, 110–117. [[CrossRef](#)] [[PubMed](#)]
- Al-Safi, Z.A.; Polotsky, A.J. Obesity and menopause. *Best Pract. Res. Clin. Obstet. Gynaecol.* **2015**, *29*, 548–553. [[CrossRef](#)] [[PubMed](#)]
- Russell, N.; Grossmann, M. Management of bone and metabolic effects of androgen deprivation therapy. *Urol. Oncol.* **2018**. [[CrossRef](#)] [[PubMed](#)]
- Spangenberg, A.; Maghsoodi, N.; Dulnoan, D.; Moore, A.E.; Edwards, S.; Frost, M.L.; Hampson, G. Bone Mineral Density and Body Composition are Associated with Circulating Angiogenic Factors in Post-menopausal Women. *Calcif. Tissue Int.* **2016**, *99*, 608–615. [[CrossRef](#)]
- Marchand, G.B.; Carreau, A.M.; Weisnagel, S.J.; Bergeron, J.; Labrie, F.; Lemieux, S.; Tchernof, A. Increased body fat mass explains the positive association between circulating estradiol and insulin resistance in postmenopausal women. *Am. J. Physiol. Endocrinol. Metab.* **2018**, *314*, E448–E456. [[CrossRef](#)]
- Razmjou, S.; Abdunour, J.; Bastard, J.P.; Fellahi, S.; Doucet, E.; Brochu, M.; Lavoie, J.M.; Rabasa-Lhoret, R.; Prud'homme, D. Body composition, cardiometabolic risk factors, physical activity, and inflammatory markers in premenopausal women after a 10-year follow-up: A MONET study. *Menopause* **2018**, *25*, 89–97. [[CrossRef](#)]
- Kim, H.M.; Park, J.; Ryu, S.Y.; Kim, J. The effect of menopause on the metabolic syndrome among Korean women: The Korean National Health and Nutrition Examination Survey, 2001. *Diabetes Care* **2007**, *30*, 701–706. [[CrossRef](#)]
- Kozakowski, J.; Gietka-Czernel, M.; Leszczynska, D.; Majos, A. Obesity in menopause—our negligence or an unfortunate inevitability? *Prz. Menopauzalny Menop. Rev.* **2017**, *16*, 61–65. [[CrossRef](#)]
- Schubert, C.M.; Rogers, N.L.; Remsberg, K.E.; Sun, S.S.; Chumlea, W.C.; Demerath, E.W.; Czerwinski, S.A.; Towne, B.; Siervogel, R.M. Lipids, lipoproteins, lifestyle, adiposity and fat-free mass during middle age: The Fels Longitudinal Study. *Int. J. Obes.* **2006**, *30*, 251–260. [[CrossRef](#)]
- Liedtke, S.; Schmidt, M.E.; Vrieling, A.; Lukanova, A.; Becker, S.; Kaaks, R.; Zaineddin, A.K.; Buck, K.; Benner, A.; Chang-Claude, J.; et al. Postmenopausal sex hormones in relation to body fat distribution. *Obesity* **2012**, *20*, 1088–1095. [[CrossRef](#)]

21. Stefanska, A.; Bergmann, K.; Sypniewska, G. Metabolic Syndrome and Menopause: Pathophysiology, Clinical and Diagnostic Significance. *Adv. Clin. Chem.* **2015**, *72*, 1–75. [[CrossRef](#)]
22. Torrens, J.I.; Sutton-Tyrrell, K.; Zhao, X.; Matthews, K.; Brockwell, S.; Sowers, M.; Santoro, N. Relative androgen excess during the menopausal transition predicts incident metabolic syndrome in midlife women: Study of Women's Health Across the Nation. *Menopause* **2009**, *16*, 257–264. [[CrossRef](#)]
23. Perry, A.; Wang, X.; Goldberg, R.; Ross, R.; Jackson, L. Androgenic sex steroids contribute to metabolic risk beyond intra-abdominal fat in overweight/obese black and white women. *Obesity* **2013**, *21*, 1618–1624. [[CrossRef](#)]
24. Laforest, S.; Pelletier, M.; Denver, N.; Poirier, B.; Nguyen, S.; Walker, B.R.; Durocher, F.; Homer, N.Z.M.; Diorio, C.; Tchernof, A.; et al. Simultaneous quantification of estrogens and glucocorticoids in human adipose tissue by liquid-chromatography-tandem mass spectrometry. *J. Steroid Biochem. Mol. Biol.* **2019**, *195*, 105476. [[CrossRef](#)]
25. Janssen, I.; Powell, L.H.; Kazlauskaitė, R.; Dugan, S.A. Testosterone and visceral fat in midlife women: The Study of Women's Health Across the Nation (SWAN) fat patterning study. *Obesity* **2010**, *18*, 604–610. [[CrossRef](#)]
26. Ziaei, S.; Mohseni, H. Correlation between Hormonal Statuses and Metabolic Syndrome in Postmenopausal Women. *J. Fam. Rep. Health* **2013**, *7*, 63–66.
27. Blouin, K.; Boivin, A.; Tchernof, A. Androgens and body fat distribution. *J. Steroid Biochem. Mol. Biol.* **2008**, *108*, 272–280. [[CrossRef](#)]
28. Sareen, S.; Gropper, J.L.S.; James, L. Groff. In *Advanced Nutrition and Human Metabolism*, 4th ed.; Gropper, S.S., Smith, J.L., Groff, J.L., Eds.; Wadsworth, Inc.: Belmont, CA, USA, 2005; pp. 159–161.
29. Dmitruk, A.; Czelewska, J.; Czelewska, E.; Golach, J.; Parnicka, U. Body composition and fatty tissue distribution in women with various menstrual status. *Rocz. Panstw. Zakl. Hig.* **2018**, *69*, 95–101.
30. Yamatani, H.; Takahashi, K.; Yoshida, T.; Soga, T.; Kurachi, H. Differences in the fatty acid metabolism of visceral adipose tissue in postmenopausal women. *Menopause* **2014**, *21*, 170–176. [[CrossRef](#)] [[PubMed](#)]
31. Greendale, G.A.; Sternfeld, B.; Huang, M.; Han, W.; Karvonen-Gutierrez, C.; Ruppert, K.; Cauley, J.A.; Finkelstein, J.S.; Jiang, S.F.; Karlamangla, A.S. Changes in body composition and weight during the menopause transition. *JCI Insight* **2019**, *4*. [[CrossRef](#)] [[PubMed](#)]
32. Prasad, H.; Ryan, D.A.; Celzo, M.F.; Stapleton, D. Metabolic syndrome: Definition and therapeutic implications. *Postgrad. Med.* **2012**, *124*, 21–30. [[CrossRef](#)] [[PubMed](#)]
33. Ebtekar, F.; Dalvand, S.; Gheshlagh, R.G. The prevalence of metabolic syndrome in postmenopausal women: A systematic review and meta-analysis in Iran. *Diabetes Metab. Syndr.* **2018**, *12*, 955–960. [[CrossRef](#)] [[PubMed](#)]
34. Amirkalali, B.; Fakhrzadeh, H.; Sharifi, F.; Kelishadi, R.; Zamani, F.; Asayesh, H.; Safiri, S.; Samavat, T.; Qorbani, M. Prevalence of Metabolic Syndrome and Its Components in the Iranian Adult Population: A Systematic Review and Meta-Analysis. *Iran. Red Crescent Med. J.* **2015**, *17*, e24723. [[CrossRef](#)] [[PubMed](#)]
35. Douchi, T.; Kuwahata, R.; Yamasaki, H.; Yamamoto, S.; Oki, T.; Nakae, M.; Nagata, Y. Inverse relationship between the changes in trunk lean and fat mass during gonadotropin-releasing hormone agonist therapy. *Maturitas* **2002**, *42*, 31–35. [[CrossRef](#)]
36. Poehlman, E.T.; Toth, M.J.; Gardner, A.W. Changes in energy balance and body composition at menopause: A controlled longitudinal study. *Ann. Intern. Med.* **1995**, *123*, 673–675. [[CrossRef](#)]
37. Collins, B.C.; Laakkonen, E.K.; Lowe, D.A. Aging of the musculoskeletal system: How the loss of estrogen impacts muscle strength. *Bone* **2019**, *123*, 137–144. [[CrossRef](#)]
38. Moreira, M.A.; Zunzunegui, M.V.; Vafaei, A.; da Camara, S.M.; Oliveira, T.S.; Maciel, A.C. Sarcopenic obesity and physical performance in middle aged women: A cross-sectional study in Northeast Brazil. *BMC Public Health* **2016**, *16*, 43. [[CrossRef](#)]
39. Lee, E.S.; Park, H.M. Prevalence of Sarcopenia in Healthy Korean Elderly Women. *J. Bone Metab.* **2015**, *22*, 191–195. [[CrossRef](#)]
40. Lazzer, S.; Bedogni, G.; Lafortuna, C.L.; Marazzi, N.; Busti, C.; Galli, R.; De Col, A.; Agosti, F.; Sartorio, A. Relationship between basal metabolic rate, gender, age, and body composition in 8780 white obese subjects. *Obesity* **2010**, *18*, 71–78. [[CrossRef](#)]
41. McMurray, R.G.; Soares, J.; Caspersen, C.J.; McCurdy, T. Examining variations of resting metabolic rate of adults: A public health perspective. *Med. Sci. Sports Exerc.* **2014**, *46*, 1352–1358. [[CrossRef](#)]

42. Geisler, C.; Braun, W.; Pourhassan, M.; Schweitzer, L.; Gluer, C.C.; Bosy-Westphal, A.; Muller, M.J. Gender-Specific Associations in Age-Related Changes in Resting Energy Expenditure (REE) and MRI Measured Body Composition in Healthy Caucasians. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2016**, *71*, 941–946. [[CrossRef](#)] [[PubMed](#)]
43. Dube, M.C.; Lemieux, S.; Piche, M.E.; Corneau, L.; Bergeron, J.; Riou, M.E.; Weisnagel, S.J. The contribution of visceral adiposity and mid-thigh fat-rich muscle to the metabolic profile in postmenopausal women. *Obesity* **2011**, *19*, 953–959. [[CrossRef](#)] [[PubMed](#)]
44. Lee, J.Y.; Lee, D.C. Muscle strength and quality are associated with severity of menopausal symptoms in peri- and post-menopausal women. *Maturitas* **2013**, *76*, 88–94. [[CrossRef](#)] [[PubMed](#)]
45. Greco, E.A.; Pietschmann, P.; Migliaccio, S. Osteoporosis and Sarcopenia Increase Frailty Syndrome in the Elderly. *Front. Endocrinol.* **2019**, *10*, 255. [[CrossRef](#)] [[PubMed](#)]
46. Ahotupa, M. Oxidized lipoprotein lipids and atherosclerosis. *Free Radic. Res.* **2017**, *51*, 439–447. [[CrossRef](#)] [[PubMed](#)]
47. Ooi, E.M.; Watts, G.F.; Ng, T.W.; Barrett, P.H. Effect of dietary Fatty acids on human lipoprotein metabolism: A comprehensive update. *Nutrients* **2015**, *7*, 4416–4425. [[CrossRef](#)] [[PubMed](#)]
48. Cannon, C.P.; de Lemos, J.A.; Rosenson, R.S.; Ballantyne, C.M.; Liu, Y.; Yazdi, D.; Elliott-Davey, M.; Mues, K.E.; Bhatt, D.L.; Kosiborod, M.N. Getting to an ImprOved Understanding of Low-Density Lipoprotein-Cholesterol and Dyslipidemia Management (GOULD): Methods and baseline data of a registry of high cardiovascular risk patients in the United States. *Am. Heart J.* **2019**, *219*, 70–77. [[CrossRef](#)]
49. Nichols, T.C. Bad cholesterol breaking really bad. *Blood* **2013**, *122*, 3551–3553. [[CrossRef](#)]
50. Lim, Y.; Yoo, S.; Lee, S.A.; Chin, S.O.; Heo, D.; Moon, J.C.; Moon, S.; Boo, K.; Kim, S.T.; Seo, H.M.; et al. Apolipoprotein B Is Related to Metabolic Syndrome Independently of Low Density Lipoprotein Cholesterol in Patients with Type 2 Diabetes. *Endocrinol. Metab.* **2015**, *30*, 208–215. [[CrossRef](#)]
51. Dobiasova, M. Atherogenic impact of lecithin-cholesterol acyltransferase and its relation to cholesterol esterification rate in HDL (FER(HDL)) and AIP [log(TG/HDL-C)] biomarkers: The butterfly effect? *Physiol. Res.* **2017**, *66*, 193–203. [[CrossRef](#)]
52. Rosales, C.; Gillard, B.K.; Xu, B.; Gotto, A.M., Jr.; Pownall, H.J. Revisiting Reverse Cholesterol Transport in the Context of High-Density Lipoprotein Free Cholesterol Bioavailability. *Methodist DeBakey Cardiovasc. J.* **2019**, *15*, 47–54. [[CrossRef](#)] [[PubMed](#)]
53. Silva, I.T.; Almeida-Pititto, B.; Ferreira, S.R. Reassessing lipid metabolism and its potentialities in the prediction of cardiovascular risk. *Arch. Endocrinol. Metab.* **2015**, *59*, 171–180. [[CrossRef](#)] [[PubMed](#)]
54. Reiner, Z. Managing the residual cardiovascular disease risk associated with HDL-cholesterol and triglycerides in statin-treated patients: A clinical update. *Nutr. Metab. Cardiovasc. Dis.* **2013**, *23*, 799–807. [[CrossRef](#)] [[PubMed](#)]
55. Rhee, Y.; Paik, M.J.; Kim, K.R.; Ko, Y.G.; Kang, E.S.; Cha, B.S.; Lee, H.C.; Lim, S.K. Plasma free fatty acid level patterns according to cardiovascular risk status in postmenopausal women. *Clin. Chim. Acta* **2008**, *392*, 11–16. [[CrossRef](#)] [[PubMed](#)]
56. Sutton-Tyrrell, K.; Wildman, R.P.; Matthews, K.A.; Chae, C.; Lasley, B.L.; Brockwell, S.; Pasternak, R.C.; Lloyd-Jones, D.; Sowers, M.F.; Torrens, J.I.; et al. Sex-hormone-binding globulin and the free androgen index are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women enrolled in the Study of Women Across the Nation (SWAN). *Circulation* **2005**, *111*, 1242–1249. [[CrossRef](#)] [[PubMed](#)]
57. Derby, C.A.; Crawford, S.L.; Pasternak, R.C.; Sowers, M.; Sternfeld, B.; Matthews, K.A. Lipid changes during the menopause transition in relation to age and weight: The Study of Women's Health Across the Nation. *Am. J. Epidemiol.* **2009**, *169*, 1352–1361. [[CrossRef](#)]
58. Zago, V.; Sanguinetti, S.; Brites, F.; Berg, G.; Verona, J.; Basilio, F.; Wikinski, R.; Schreier, L. Impaired high density lipoprotein antioxidant activity in healthy postmenopausal women. *Atherosclerosis* **2004**, *177*, 203–210. [[CrossRef](#)]
59. Anagnostis, P.; Stevenson, J.C.; Crook, D.; Johnston, D.G.; Godsland, I.F. Effects of menopause, gender and age on lipids and high-density lipoprotein cholesterol subfractions. *Maturitas* **2015**, *81*, 62–68. [[CrossRef](#)]
60. Zhou, J.L.; Lin, S.Q.; Shen, Y.; Chen, Y.; Zhang, Y.; Chen, F.L. Serum lipid profile changes during the menopausal transition in Chinese women: A community-based cohort study. *Menopause* **2010**, *17*, 997–1003. [[CrossRef](#)]

61. Ruiz-Cabello, P.; Coll-Risco, I.; Acosta-Manzano, P.; Borges-Cosic, M.; Gallo-Vallejo, F.J.; Aranda, P.; Lopez-Jurado, M.; Aparicio, V.A. Influence of the degree of adherence to the Mediterranean diet on the cardiometabolic risk in peri and menopausal women. The Flamenco project. *Nutr. Metab. Cardiovasc. Dis. NMCD* **2017**, *27*, 217–224. [\[CrossRef\]](#)
62. Matthews, K.A.; Wing, R.R.; Kuller, L.H.; Meilahn, E.N.; Plantinga, P. Influence of the perimenopause on cardiovascular risk factors and symptoms of middle-aged healthy women. *Arch. Intern. Med.* **1994**, *154*, 2349–2355. [\[CrossRef\]](#)
63. Nerbrand, C.; Lidfeldt, J.; Nyberg, P.; Schersten, B.; Samsioe, G. Serum lipids and lipoproteins in relation to endogenous and exogenous female sex steroids and age. The Women's Health in the Lund Area (WHILA) study. *Maturitas* **2004**, *48*, 161–169. [\[CrossRef\]](#)
64. Hall, G.; Collins, A.; Csemiczky, G.; Landgren, B.M. Lipoproteins and BMI: A comparison between women during transition to menopause and regularly menstruating healthy women. *Maturitas* **2002**, *41*, 177–185. [\[CrossRef\]](#)
65. Mogarekar, M.R.; Kulkarni, S.K. Small Dense Low Density Lipoprotein Cholesterol, Paraoxonase 1 and Lipid Profile in Postmenopausal Women: Quality or Quantity? *Arch. Med. Res.* **2015**, *46*, 534–538. [\[CrossRef\]](#)
66. Bays, H.E.; Toth, P.P.; Kris-Etherton, P.M.; Abate, N.; Aronne, L.J.; Brown, W.V.; Gonzalez-Campoy, J.M.; Jones, S.R.; Kumar, R.; La Forge, R.; et al. Obesity, adiposity, and dyslipidemia: A consensus statement from the National Lipid Association. *J. Clin. Lipidol.* **2013**, *7*, 304–383. [\[CrossRef\]](#)
67. Tchernof, A.; Desmeules, A.; Richard, C.; Laberge, P.; Daris, M.; Mailloux, J.; Rheume, C.; Dupont, P. Ovarian hormone status and abdominal visceral adipose tissue metabolism. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 3425–3430. [\[CrossRef\]](#)
68. Wildman, R.P.; Tepper, P.G.; Crawford, S.; Finkelstein, J.S.; Sutton-Tyrrell, K.; Thurston, R.C.; Santoro, N.; Sternfeld, B.; Greendale, G.A. Do changes in sex steroid hormones precede or follow increases in body weight during the menopause transition? Results from the Study of Women's Health Across the Nation. *J. Clin. Endocrinol. Metab.* **2012**, *97*, E1695–E1704. [\[CrossRef\]](#)
69. Lovegrove, J.A.; Silva, K.D.; Wright, J.W.; Williams, C.M. Adiposity, insulin and lipid metabolism in post-menopausal women. *Int. J. Obes. Relat. Metab. Disord.* **2002**, *26*, 475–486. [\[CrossRef\]](#)
70. Rachon, D.; Teede, H. Ovarian function and obesity-interrelationship, impact on women's reproductive lifespan and treatment options. *Mol. Cell. Endocrinol.* **2010**, *316*, 172–179. [\[CrossRef\]](#)
71. Carr, M.C. The emergence of the metabolic syndrome with menopause. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 2404–2411. [\[CrossRef\]](#)
72. Wajchenberg, B.L. Subcutaneous and visceral adipose tissue: Their relation to the metabolic syndrome. *Endocr. Rev.* **2000**, *21*, 697–738. [\[CrossRef\]](#)
73. Rogers, N.H.; Perfield, J.W., 2nd; Strissel, K.J.; Obin, M.S.; Greenberg, A.S. Reduced energy expenditure and increased inflammation are early events in the development of ovariectomy-induced obesity. *Endocrinology* **2009**, *150*, 2161–2168. [\[CrossRef\]](#)
74. Stubbins, R.E.; Holcomb, V.B.; Hong, J.; Nunez, N.P. Estrogen modulates abdominal adiposity and protects female mice from obesity and impaired glucose tolerance. *Eur. J. Nutr.* **2012**, *51*, 861–870. [\[CrossRef\]](#)
75. Simons, S.S., Jr.; Edwards, D.P.; Kumar, R. Minireview: Dynamic structures of nuclear hormone receptors: New promises and challenges. *Mol. Endocrinol.* **2014**, *28*, 173–182. [\[CrossRef\]](#)
76. Miller, W.L.; Auchus, R.J. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr. Rev.* **2011**, *32*, 81–151. [\[CrossRef\]](#)
77. Heine, P.A.; Taylor, J.A.; Iwamoto, G.A.; Lubahn, D.B.; Cooke, P.S. Increased adipose tissue in male and female estrogen receptor- α knockout mice. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12729–12734. [\[CrossRef\]](#)
78. Cooke, P.S.; Heine, P.A.; Taylor, J.A.; Lubahn, D.B. The role of estrogen and estrogen receptor- α in male adipose tissue. *Mol. Cell. Endocrinol.* **2001**, *178*, 147–154. [\[CrossRef\]](#)
79. Davis, K.E.; Neinast, M.D.; Sun, K.; Skiles, W.M.; Bills, J.D.; Zehr, J.A.; Zeve, D.; Hahner, L.D.; Cox, D.W.; Gent, L.M.; et al. The sexually dimorphic role of adipose and adipocyte estrogen receptors in modulating adipose tissue expansion, inflammation, and fibrosis. *Mol. Metab.* **2013**, *2*, 227–242. [\[CrossRef\]](#)
80. Waki, H.; Tontonoz, P. Endocrine functions of adipose tissue. *Annu. Rev. Pathol.* **2007**, *2*, 31–56. [\[CrossRef\]](#)
81. Reaven, G.; Abbasi, F.; McLaughlin, T. Obesity, insulin resistance, and cardiovascular disease. *Recent Prog. Horm. Res.* **2004**, *59*, 207–223. [\[CrossRef\]](#)

82. Olszanecka, A.; Posnik-Urbanska, A.; Kawecka-Jaszcz, K.; Czarnecka, D.; Fedak, D. Adipocytokines and blood pressure, lipids and glucose metabolism in hypertensive perimenopausal women. *Kardiol. Pol.* **2010**, *68*, 753–760.
83. Azizieh, F.Y.; Shehab, D.; Al Jarallah, K.; Mojiminiyi, O.; Gupta, R.; Raghupathy, R. Circulatory pattern of cytokines, adipokines and bone markers in postmenopausal women with low BMD. *J. Inflamm. Res.* **2019**, *12*, 99–108. [\[CrossRef\]](#)
84. Matsuzawa, Y. Therapy Insight: Adipocytokines in metabolic syndrome and related cardiovascular disease. *Nat. Clin. Pract. Cardiovasc. Med.* **2006**, *3*, 35–42. [\[CrossRef\]](#)
85. National Institutes of Health, National Heart, Lung, and Blood Institute in cooperation with the National Institute of Diabetes and Digestive and Kidney Diseases. *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report*; Bethesda (MD); National Heart, Lung, and Blood Institute: Bethesda, MD, USA, 1998.
86. WHO. Obesity and Overweight. Available online: <https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight> (accessed on 16 December 2019).
87. Kim, B.; Feldman, E.L. Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. *Exp. Mol. Med.* **2015**, *47*, e149. [\[CrossRef\]](#)
88. Bonomini, F.; Rodella, L.F.; Rezzani, R. Metabolic syndrome, aging and involvement of oxidative stress. *Aging Dis.* **2015**, *6*, 109–120. [\[CrossRef\]](#)
89. Damcott, C.M.; Sack, P.; Shuldiner, A.R. The genetics of obesity. *Endocrinol. Metab. Clin. N. Am.* **2003**, *32*, 761–786. [\[CrossRef\]](#)
90. Clegg, D.J. Minireview: The year in review of estrogen regulation of metabolism. *Mol. Endocrinol.* **2012**, *26*, 1957–1960. [\[CrossRef\]](#)
91. Jones, M.E.; Thorburn, A.W.; Britt, K.L.; Hewitt, K.N.; Wreford, N.G.; Proietto, J.; Oz, O.K.; Leury, B.J.; Robertson, K.M.; Yao, S.; et al. Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12735–12740. [\[CrossRef\]](#)
92. Bryzgalova, G.; Gao, H.; Ahren, B.; Zierath, J.R.; Galuska, D.; Steiler, T.L.; Dahlman-Wright, K.; Nilsson, S.; Gustafsson, J.A.; Efendic, S.; et al. Evidence that oestrogen receptor-alpha plays an important role in the regulation of glucose homeostasis in mice: Insulin sensitivity in the liver. *Diabetologia* **2006**, *49*, 588–597. [\[CrossRef\]](#)
93. Gibb, F.W.; Dixon, J.M.; Clarke, C.; Homer, N.Z.; Faqehi, A.M.M.; Andrew, R.; Walker, B.R. Higher Insulin Resistance and Adiposity in Postmenopausal Women With Breast Cancer Treated With Aromatase Inhibitors. *J. Clin. Endocrinol. Metab.* **2019**, *104*, 3670–3678. [\[CrossRef\]](#)
94. Lovejoy, J.C.; Champagne, C.M.; de Jonge, L.; Xie, H.; Smith, S.R. Increased visceral fat and decreased energy expenditure during the menopausal transition. *Int. J. Obes.* **2008**, *32*, 949–958. [\[CrossRef\]](#)
95. Polotsky, H.N.; Polotsky, A.J. Metabolic implications of menopause. *Semin. Reprod. Med.* **2010**, *28*, 426–434. [\[CrossRef\]](#)
96. Colpani, V.; Oppermann, K.; Spritzer, P.M. Association between habitual physical activity and lower cardiovascular risk in premenopausal, perimenopausal, and postmenopausal women: A population-based study. *Menopause* **2013**, *20*, 525–531. [\[CrossRef\]](#)
97. Zello, G.A. Dietary Reference Intakes for the macronutrients and energy: Considerations for physical activity. *Appl. Physiol. Nutr. Metab. Physiol. Appl. Nutr. Metab.* **2006**, *31*, 74–79. [\[CrossRef\]](#)
98. Weaver, A.A.; Houston, D.K.; Shapses, S.A.; Lyles, M.F.; Henderson, R.M.; Beavers, D.P.; Baker, A.C.; Beavers, K.M. Effect of a hypocaloric, nutritionally complete, higher-protein meal plan on bone density and quality in older adults with obesity: A randomized trial. *Am. J. Clin. Nutr.* **2019**, *109*, 478–486. [\[CrossRef\]](#)
99. Jensen, M.D.; Ryan, D.H.; Apovian, C.M.; Ard, J.D.; Comuzzie, A.G.; Donato, K.A.; Hu, F.B.; Hubbard, V.S.; Jakicic, J.M.; Kushner, R.F.; et al. 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *J. Am. Coll. Cardiol.* **2014**, *63*, 2985–3023. [\[CrossRef\]](#)
100. Groenendijk, I.; den Boeft, L.; van Loon, L.J.C.; de Groot, L. High Versus low Dietary Protein Intake and Bone Health in Older Adults: A Systematic Review and Meta-Analysis. *Comput. Struct. Biotechnol. J.* **2019**, *17*, 1101–1112. [\[CrossRef\]](#)
101. Ministry of Health and Welfare. *Dietary Reference Intakes for Koreans*; Sejong: Seoul, Korea, 2015.

102. Janssen, I.; Heymsfield, S.B.; Ross, R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J. Am. Geriatr. Soc.* **2002**, *50*, 889–896. [\[CrossRef\]](#)
103. Timmerman, K.L.; Volpi, E. Amino acid metabolism and regulatory effects in aging. *Curr. Opin. Clin. Nutr. Metab. Care* **2008**, *11*, 45–49. [\[CrossRef\]](#)
104. Layman, D.K.; Evans, E.; Baum, J.I.; Seyler, J.; Erickson, D.J.; Boileau, R.A. Dietary protein and exercise have additive effects on body composition during weight loss in adult women. *J. Nutr.* **2005**, *135*, 1903–1910. [\[CrossRef\]](#)
105. Bopp, M.J.; Houston, D.K.; Lenchik, L.; Easter, L.; Kritchevsky, S.B.; Nicklas, B.J. Lean mass loss is associated with low protein intake during dietary-induced weight loss in postmenopausal women. *J. Am. Diet. Assoc.* **2008**, *108*, 1216–1220. [\[CrossRef\]](#) [\[PubMed\]](#)
106. Gordon, M.M.; Bopp, M.J.; Easter, L.; Miller, G.D.; Lyles, M.F.; Houston, D.K.; Nicklas, B.J.; Kritchevsky, S.B. Effects of dietary protein on the composition of weight loss in post-menopausal women. *J. Nutr. Health Aging* **2008**, *12*, 505–509. [\[CrossRef\]](#) [\[PubMed\]](#)
107. Cho, Y.A.; Kim, J.; Cho, E.R.; Shin, A. Dietary patterns and the prevalence of metabolic syndrome in Korean women. *Nutr. Metab. Cardiovasc. Dis.* **2011**, *21*, 893–900. [\[CrossRef\]](#) [\[PubMed\]](#)
108. Lips, P. Vitamin D physiology. *Prog. Biophys. Mol. Biol.* **2006**, *92*, 4–8. [\[CrossRef\]](#)
109. Holick, M.F. Vitamin D deficiency. *N. Engl. J. Med.* **2007**, *357*, 266–281. [\[CrossRef\]](#)
110. Jung, I.K. Prevalence of vitamin D deficiency in Korea: Results from KNHANES 2010 to 2011. *J. Nutr. Health* **2013**, *46*, 540–551. [\[CrossRef\]](#)
111. Nah, E.H.; Kim, S.; Cho, H.-I. Vitamin D Levels and Prevalence of Vitamin D Deficiency Associated with Sex, Age, Region, and Season in Koreans. *Lab. Med. Online* **2015**, *5*, 84–91. [\[CrossRef\]](#)
112. Bronner, F. Recent developments in intestinal calcium absorption. *Nutr. Rev.* **2009**, *67*, 109–113. [\[CrossRef\]](#)
113. Heaney, R.P. Vitamin D and calcium interactions: Functional outcomes. *Am. J. Clin. Nutr.* **2008**, *88*, 541S–544S. [\[CrossRef\]](#)
114. Haussler, M.R.; Haussler, C.A.; Bartik, L.; Whitfield, G.K.; Hsieh, J.C.; Slater, S.; Jurutka, P.W. Vitamin D receptor: Molecular signaling and actions of nutritional ligands in disease prevention. *Nutr. Rev.* **2008**, *66*, S98–S112. [\[CrossRef\]](#)
115. Van Etten, E.; Stoffels, K.; Gysemans, C.; Mathieu, C.; Overbergh, L. Regulation of vitamin D homeostasis: Implications for the immune system. *Nutr. Rev.* **2008**, *66*, S125–S134. [\[CrossRef\]](#) [\[PubMed\]](#)
116. Kilkkinen, A.; Knekt, P.; Aro, A.; Rissanen, H.; Marniemi, J.; Heliovaara, M.; Impivaara, O.; Reunanen, A. Vitamin D status and the risk of cardiovascular disease death. *Am. J. Epidemiol.* **2009**, *170*, 1032–1039. [\[CrossRef\]](#) [\[PubMed\]](#)
117. Gombart, A.F.; Luong, Q.T.; Koeffler, H.P. Vitamin D compounds: Activity against microbes and cancer. *Anticancer Res.* **2006**, *26*, 2531–2542. [\[PubMed\]](#)
118. Ginde, A.A.; Mansbach, J.M.; Camargo, C.A., Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch. Intern. Med.* **2009**, *169*, 384–390. [\[CrossRef\]](#)
119. Mullin, G.E.; Dobs, A. Vitamin d and its role in cancer and immunity: A prescription for sunlight. *Nutr. Clin. Pract.* **2007**, *22*, 305–322. [\[CrossRef\]](#)
120. Holick, M.F. Vitamin D: A D-Lightful health perspective. *Nutr. Rev.* **2008**, *66*, S182–S194. [\[CrossRef\]](#)
121. Cantorna, M.T. Vitamin D and multiple sclerosis: An update. *Nutr. Rev.* **2008**, *66*, S135–S138. [\[CrossRef\]](#)
122. Zittermann, A.; Gummert, J.F.; Bergermann, J. Vitamin D deficiency and mortality. *Curr. Opin. Clin. Nutr. Metab. Care* **2009**, *12*, 634–639. [\[CrossRef\]](#)
123. Lee, C.J.; Kim, S.S. The Effect of Education and Vitamin D Supplementation on the Achievement of Optimal Vitamin D Level in Korean Postmenopausal Women. *J. Bone Metab.* **2019**, *26*, 193–199. [\[CrossRef\]](#)
124. Chacko, S.A.; Song, Y.; Manson, J.E.; Van Horn, L.; Eaton, C.; Martin, L.W.; McTiernan, A.; Curb, J.D.; Wylie-Rosett, J.; Phillips, L.S.; et al. Serum 25-hydroxyvitamin D concentrations in relation to cardiometabolic risk factors and metabolic syndrome in postmenopausal women. *Am. J. Clin. Nutr.* **2011**, *94*, 209–217. [\[CrossRef\]](#)
125. Song, H.R.; Park, C.H. Low serum vitamin D level is associated with high risk of metabolic syndrome in post-menopausal women. *J. Endocrinol. Investig.* **2013**, *36*, 791–796. [\[CrossRef\]](#) [\[PubMed\]](#)

126. Chon, S.J.; Yun, B.H.; Jung, Y.S.; Cho, S.H.; Choi, Y.S.; Kim, S.Y.; Lee, B.S.; Seo, S.K. Association between vitamin D status and risk of metabolic syndrome among Korean postmenopausal women. *PLoS ONE* **2014**, *9*, e89721. [[CrossRef](#)] [[PubMed](#)]
127. Dadra, A.; Aggarwal, S.; Kumar, P.; Kumar, V.; Dibar, D.P.; Bhadada, S.K. High prevalence of vitamin D deficiency and osteoporosis in patients with fragility fractures of hip: A pilot study. *J. Clin. Orthop. Trauma* **2019**, *10*, 1097–1100. [[CrossRef](#)] [[PubMed](#)]
128. Moghassemi, S.; Marjani, A. The effect of short-term vitamin D supplementation on lipid profile and blood pressure in post-menopausal women: A randomized controlled trial. *Iran. J. Nurs. Midwifery Res.* **2014**, *19*, 517–521. [[PubMed](#)]
129. Branco, J.; Smoraog, D.C.; Bentes, C.M.; Netto, C.C.; Marinheiro, L.P.F. Association between vitamin D status and glycemic profile in postmenopausal women with type 2 diabetes. *Diabetes Metab. Syndr.* **2019**, *13*, 1685–1688. [[CrossRef](#)] [[PubMed](#)]
130. Vitale, S.G.; Caruso, S.; Rapisarda, A.M.C.; Cianci, S.; Cianci, A. Isoflavones, calcium, vitamin D and inulin improve quality of life, sexual function, body composition and metabolic parameters in menopausal women: Result from a prospective, randomized, placebo-controlled, parallel-group study. *Prz. Menopauzalny Menop. Rev.* **2018**, *17*, 32–38. [[CrossRef](#)]
131. Lagana, A.S.; Vitale, S.G.; Stojanovska, L.; Lambrinoudaki, I.; Apostolopoulos, V.; Chiofalo, B.; Rizzo, L.; Basile, F. Preliminary results of a single-arm pilot study to assess the safety and efficacy of visnadine, prenylflavonoids and bovine colostrum in postmenopausal sexually active women affected by vulvovaginal atrophy. *Maturitas* **2018**, *109*, 78–80. [[CrossRef](#)]
132. Davinelli, S.; Scapagnini, G.; Marzatico, F.; Nobile, V.; Ferrara, N.; Corbi, G. Influence of equol and resveratrol supplementation on health-related quality of life in menopausal women: A randomized, placebo-controlled study. *Maturitas* **2017**, *96*, 77–83. [[CrossRef](#)]
133. Newberry, S.J.; Chung, M.; Shekelle, P.G.; Booth, M.S.; Liu, J.L.; Maher, A.R.; Motala, A.; Cui, M.; Perry, T.; Shanman, R.; et al. Vitamin D and Calcium: A Systematic Review of Health Outcomes (Update). *Evid. Rep. Technol. Assess.* **2014**, 1–929. [[CrossRef](#)]
- 134.Sizer, F.; Whitney, E.N. *Nutrition Concepts and Controversies*, 12th ed.; Rose, N., Ed.; Cengage Learning: Boston, MA, USA, 2011.
135. Saini, R.K.; Keum, Y.S. Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance—A review. *Life Sci.* **2018**, *203*, 255–267. [[CrossRef](#)]
136. Shearer, G.C.; Walker, R.E. An overview of the biologic effects of omega-6 oxylipins in humans. *Prostaglandins Leukot. Essent. Fatty Acids* **2018**, *137*, 26–38. [[CrossRef](#)] [[PubMed](#)]
137. Ahuja, J.K.C.; Moshfegh, A.J.; Holden, J.M.; Harris, E. USDA Food and Nutrient Databases Provide the Infrastructure for Food and Nutrition Research, Policy, and Practice. *J. Nutr.* **2012**, *143*, 241S–249S. [[CrossRef](#)] [[PubMed](#)]
138. Wiktorowska-Owczarek, A.; Berezinska, M.; Nowak, J.Z. PUFAs: Structures, Metabolism and Functions. *Adv. Clin. Exp. Med.* **2015**, *24*, 931–941. [[CrossRef](#)] [[PubMed](#)]
139. Kris-Etherton, P.M.; Richter, C.K.; Bowen, K.J.; Skulas-Ray, A.C.; Jackson, K.H.; Petersen, K.S.; Harris, W.S. Recent Clinical Trials Shed New Light on the Cardiovascular Benefits of Omega-3 Fatty Acids. *Methodist DeBakey Cardiovasc. J.* **2019**, *15*, 171–178. [[CrossRef](#)] [[PubMed](#)]
140. Kim, Y.S.; Xun, P.; Iribarren, C.; Van Horn, L.; Steffen, L.; Davi, M.L.; Siscovick, D.; Liu, K.; He, K. Intake of fish and long-chain omega-3 polyunsaturated fatty acids and incidence of metabolic syndrome among American young adults: A 25-year follow-up study. *Eur. J. Nutr.* **2016**, *55*, 1707–1716. [[CrossRef](#)]
141. Hu, F.B.; Bronner, L.; Willett, W.C.; Stampfer, M.J.; Rexrode, K.M.; Albert, C.M.; Hunter, D.; Manson, J.E. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* **2002**, *287*, 1815–1821. [[CrossRef](#)]
142. Simopoulos, A.P. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients* **2016**, *8*, 128. [[CrossRef](#)]
143. Tardivo, A.P.; Nahas-Neto, J.; Orsatti, C.L.; Dias, F.B.; Poloni, P.F.; Schmitt, E.B.; Nahas, E.A.P. Effects of omega-3 on metabolic markers in postmenopausal women with metabolic syndrome. *Climacteric* **2015**, *18*, 290–298. [[CrossRef](#)]

144. Zanoaga, O.; Jurj, A.; Raduly, L.; Cojocneanu-Petric, R.; Fuentes-Mattei, E.; Wu, O.; Braicu, C.; Gherman, C.D.; Berindan-Neagoe, I. Implications of dietary omega-3 and omega-6 polyunsaturated fatty acids in breast cancer. *Exp. Ther. Med.* **2018**, *15*, 1167–1176. [\[CrossRef\]](#)
145. Siscovick, D.S.; Barringer, T.A.; Fretts, A.M.; Wu, J.H.; Lichtenstein, A.H.; Costello, R.B.; Kris-Etherton, P.M.; Jacobson, T.A.; Engler, M.B.; Alger, H.M.; et al. Omega-3 Polyunsaturated Fatty Acid (Fish Oil) Supplementation and the Prevention of Clinical Cardiovascular Disease: A Science Advisory From the American Heart Association. *Circulation* **2017**, *135*, e867–e884. [\[CrossRef\]](#)
146. Solleiro-Villavicencio, H.; Rivas-Arancibia, S. Effect of Chronic Oxidative Stress on Neuroinflammatory Response Mediated by CD4(+)T Cells in Neurodegenerative Diseases. *Front. Cell. Neurosci.* **2018**, *12*, 114. [\[CrossRef\]](#) [\[PubMed\]](#)
147. Ko, S.H.; Lee, J.K.; Lee, H.J.; Ye, S.K.; Kim, H.S.; Chung, M.H. 8-Oxo-2'-deoxyguanosine ameliorates features of metabolic syndrome in obese mice. *Biochem. Biophys. Res. Commun.* **2014**, *443*, 610–616. [\[CrossRef\]](#) [\[PubMed\]](#)
148. Bonomini, F.; Tengattini, S.; Fabiano, A.; Bianchi, R.; Rezzani, R. Atherosclerosis and oxidative stress. *Histol. Histopathol.* **2008**, *23*, 381–390. [\[PubMed\]](#)
149. Sack, M.N.; Rader, D.J.; Cannon, R.O., 3rd. Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopausal women. *Lancet* **1994**, *343*, 269–270. [\[CrossRef\]](#)
150. Bhavnani, B.R.; Cecutti, A.; Gerulath, A.; Woolever, A.C.; Berco, M. Comparison of the antioxidant effects of equine estrogens, red wine components, vitamin E, and probucol on low-density lipoprotein oxidation in postmenopausal women. *Menopause* **2018**, *25*, 1214–1223. [\[CrossRef\]](#)
151. Weitzmann, M.N.; Pacifici, R. Estrogen deficiency and bone loss: An inflammatory tale. *J. Clin. Investig.* **2006**, *116*, 1186–1194. [\[CrossRef\]](#)
152. Liu, Y.; Hwang, H.J.; Ryu, H.; Lee, Y.S.; Kim, H.S.; Park, H. The effects of daily intake timing of almond on the body composition and blood lipid profile of healthy adults. *Nutr. Res. Pract.* **2017**, *11*, 479–486. [\[CrossRef\]](#)
153. Park, H.; Liu, Y.; Kim, H.S.; Shin, J.H. Chokeberry attenuates the expression of genes related to de novo lipogenesis in the hepatocytes of mice with nonalcoholic fatty liver disease. *Nutr. Res.* **2016**, *36*, 57–64. [\[CrossRef\]](#)
154. Hwang, H.J.; Liu, Y.; Kim, H.S.; Lee, H.; Lim, Y.; Park, H. Daily walnut intake improves metabolic syndrome status and increases circulating adiponectin levels: Randomized controlled crossover trial. *Nutr. Res. Pract.* **2019**, *13*, 105–114. [\[CrossRef\]](#)
155. Yoon, L.; Liu, Y.N.; Park, H.; Kim, H.S. Olive Leaf Extract Elevates Hepatic PPAR alpha mRNA Expression and Improves Serum Lipid Profiles in Ovariectomized Rats. *J. Med. Food* **2015**, *18*, 738–744. [\[CrossRef\]](#)
156. Yoo, J.H.; Liu, Y.; Kim, H.S. Hawthorn Fruit Extract Elevates Expression of Nrf2/HO-1 and Improves Lipid Profiles in Ovariectomized Rats. *Nutrients* **2016**, *8*, 283. [\[CrossRef\]](#) [\[PubMed\]](#)
157. Mattson, M.P. Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. *Exp. Gerontol.* **2009**, *44*, 625–633. [\[CrossRef\]](#) [\[PubMed\]](#)
158. Naziroglu, M.; Simsek, M.; Simsek, H.; Aydilek, N.; Ozcan, Z.; Atilgan, R. The effects of hormone replacement therapy combined with vitamins C and E on antioxidants levels and lipid profiles in postmenopausal women with Type 2 diabetes. *Clin. Chim. Acta* **2004**, *344*, 63–71. [\[CrossRef\]](#) [\[PubMed\]](#)
159. Miquel, J.; Ramírez-Boscá, A.; Ramírez-Bosca, J.V.; Alperi, J.D. Menopause: A review on the role of oxygen stress and favorable effects of dietary antioxidants. *Arch. Gerontol. Geriatr.* **2006**, *42*, 289–306. [\[CrossRef\]](#)
160. Jung, K.; Kim, S.; Woo, J.; Chang, Y. The Effect of Dietary Intervention Through the Modification of Fatty Acids Composition and Antioxidant Vitamin Intake on Plasma TXB Level in Korean Postmenopausal Women with Hypercholesterolemia. *J. Korean Med. Sci.* **2002**, *17*, 307–315. [\[CrossRef\]](#)
161. Hasler, C.M.; Brown, A.C.; American Dietetic, A. Position of the American Dietetic Association: Functional foods. *J. Am. Diet. Assoc.* **2009**, *109*, 735–746.
162. Hatti-Kaul, R.; Chen, L.; Dishisha, T.; Enshasy, H.E. Lactic acid bacteria: From starter cultures to producers of chemicals. *FEMS Microbiol. Lett.* **2018**, *365*. [\[CrossRef\]](#)
163. Skrypnik, K.; Bogdanski, P.; Sobieska, M.; Suliburska, J. The effect of multistrain probiotic supplementation in two doses on iron metabolism in obese postmenopausal women: A randomized trial. *Food Funct.* **2019**, *10*, 5228–5238. [\[CrossRef\]](#)

164. Szulinska, M.; Loniewski, I.; Skrypnik, K. Multispecies Probiotic Supplementation Favorably Affects Vascular Function and Reduces Arterial Stiffness in Obese Postmenopausal Women—A 12-Week Placebo-Controlled and Randomized Clinical Study. *Nutrients* **2018**, *10*, 1672. [\[CrossRef\]](#)
165. Yeon, J.Y.; Kim, H.S.; Sung, M.K. Diets rich in fruits and vegetables suppress blood biomarkers of metabolic stress in overweight women. *Prev. Med.* **2012**, *54*, S109–S115. [\[CrossRef\]](#)
166. Oishi, K.; Yamamoto, S.; Itoh, N.; Nakao, R.; Yasumoto, Y.; Tanaka, K.; Kikuchi, Y.; Fukudome, S.; Okita, K.; Takano-Ishikawa, Y. Wheat alkylresorcinols suppress high-fat, high-sucrose diet-induced obesity and glucose intolerance by increasing insulin sensitivity and cholesterol excretion in male mice. *J. Nutr.* **2015**, *145*, 199–206. [\[CrossRef\]](#) [\[PubMed\]](#)
167. Lee, B.; Jung, J.H.; Kim, H.S. Assessment of red onion on antioxidant activity in rat. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* **2012**, *50*, 3912–3919. [\[CrossRef\]](#) [\[PubMed\]](#)
168. Bai, C.; Zheng, J.; Zhao, L.; Chen, L.; Xiong, H.; McClements, D.J. Development of Oral Delivery Systems with Enhanced Antioxidant and Anticancer Activity: Coix Seed Oil and beta-Carotene Coloaded Liposomes. *J. Agric. Food Chem.* **2019**, *67*, 406–414. [\[CrossRef\]](#) [\[PubMed\]](#)
169. Srinivasan, K. Biological Activities of Red Pepper (*Capsicum annuum*) and Its Pungent Principle Capsaicin: A Review. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1488–1500. [\[CrossRef\]](#)
170. Keihanian, F.; Saeidinia, A. Curcumin, hemostasis, thrombosis, and coagulation. *J. Cell. Physiol.* **2018**, *233*, 4497–4511. [\[CrossRef\]](#)
171. Hong, J.; Kim, S.; Kim, H.S. Hepatoprotective Effects of Soybean Embryo by Enhancing Adiponectin-Mediated AMP-Activated Protein Kinase α Pathway in High-Fat and High-Cholesterol Diet-Induced Nonalcoholic Fatty Liver Disease. *J. Med. Food* **2016**, *19*, 549–559. [\[CrossRef\]](#)
172. Basu, P.; Maier, C. Phytoestrogens and breast cancer: In vitro anticancer activities of isoflavones, lignans, coumestans, stilbenes and their analogs and derivatives. *Biomed. Pharmacother.* **2018**, *107*, 1648–1666. [\[CrossRef\]](#)
173. Wang, X.; Yan, M.; Wang, Q.; Wang, H.; Wang, Z.; Zhao, J.; Li, J.; Zhang, Z. In Vitro DNA-Binding, Anti-Oxidant and Anticancer Activity of Indole-2-Carboxylic Acid Dinuclear Copper(II) Complexes. *Molecules* **2017**, *22*, 171. [\[CrossRef\]](#)
174. Esfandiari, A.; Saei, A.; McKenzie, M.J.; Matich, A.J.; Babalar, M.; Hunter, D.A. Preferentially enhancing anti-cancer isothiocyanates over glucosinolates in broccoli sprouts: How NaCl and salicylic acid affect their formation. *Plant Physiol. Biochem. PPB* **2017**, *115*, 343–353. [\[CrossRef\]](#)
175. Nair, S.A.; Sr, R.K.; Nair, A.S.; Baby, S. Citrus peels prevent cancer. *Phytomed. Int. J. Phytother. Phytopharmacol.* **2018**, *50*, 231–237. [\[CrossRef\]](#)
176. Hazafa, A.; Rehman, K.U.; Jahan, N.; Jabeen, Z. The Role of Polyphenol (Flavonoids) Compounds in the Treatment of Cancer Cells. *Nutr. Cancer* **2019**, 1–12. [\[CrossRef\]](#) [\[PubMed\]](#)
177. Buhrmann, C.; Yazdi, M.; Popper, B. Resveratrol Chemosensitizes TNF-beta-Induced Survival of 5-FU-Treated Colorectal Cancer Cells. *Nutrients* **2018**, *10*, 888. [\[CrossRef\]](#) [\[PubMed\]](#)
178. Kim, H.Y.; Yu, R.; Kim, J.S.; Kim, Y.K.; Sung, M.K. Antiproliferative crude soy saponin extract modulates the expression of IkappaB α , protein kinase C, and cyclooxygenase-2 in human colon cancer cells. *Cancer Lett.* **2004**, *210*, 1–6. [\[CrossRef\]](#) [\[PubMed\]](#)
179. Jun, H.S.; Kim, S.E.; Sung, M.K. Protective effect of soybean saponins and major antioxidants against aflatoxin B1-induced mutagenicity and DNA-adduct formation. *J. Med. Food* **2002**, *5*, 235–240. [\[CrossRef\]](#)
180. Smeriglio, A.; Barreca, D.; Bellocchio, E.; Trombetta, D. Proanthocyanidins and hydrolysable tannins: Occurrence, dietary intake and pharmacological effects. *Br. J. Pharmacol.* **2017**, *174*, 1244–1262. [\[CrossRef\]](#)
181. Jacobs, D.R., Jr.; Gross, M.D.; Tapsell, L.C. Food synergy: An operational concept for understanding nutrition. *Am. J. Clin. Nutr.* **2009**, *89*, 1543S–1548S. [\[CrossRef\]](#)
182. Corte-Real, J.; Bohn, T. Interaction of divalent minerals with liposoluble nutrients and phytochemicals during digestion and influences on their bioavailability—A review. *Food Chem.* **2018**, *252*, 285–293. [\[CrossRef\]](#)
183. Chin, K.Y.; Ima-Nirwana, S. The effects of alpha-tocopherol on bone: A double-edged sword? *Nutrients* **2014**, *6*, 1424–1441. [\[CrossRef\]](#)



The effect of *Salvia officinalis* extract on symptoms of flushing, night sweat, sleep disorders, and score of forgetfulness in postmenopausal women

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ABSTRACT

Background: Complications of hormone therapy (as replacement) during menopause prompted us to research on alternative therapies including herbal therapy in this regard. **Objectives:** The effect of *Salvia officinalis* extract on symptoms of flushing, night sweat, sleep disorders, and score of forgetfulness in postmenopausal women on Namazi Hospital Bone Density Center in Shiraz 2015. **Methods:** In a double-blind randomized controlled clinical trial, 66 postmenopausal women complaining of menopausal symptoms were divided into two groups of intervention and control, respectively. The intervention group received *S. officinalis* tablets (containing 100 mg *S. officinalis* extract), with a dose of three tablets a day for 3 months, while the control group received placebo tablets with the same prescription order. MRS (Menopause Rating Scale) and PSQI (Pittsburgh Sleep Quality Index) questionnaires were completed at the beginning and end of the study. The checklists of hot flushing and night sweating were completed a week before the intervention and at weeks 2, 4, 6, 8, 10, 12 during the intervention. Finally, the data were analyzed through SPSS₁₈ software, using paired *t*-test, ANOVA. A significant level of 5% was considered. **Results:** According to the paired *t*-test, the mean score of flushing, palpitation, sleeping disorders, muscle and joint aches, depression, nervousness, anxiety, and sexual desire and satisfaction significantly decreased by 1.6, 0.4, 1.6, 2.1, 1.4, 1.2, 1.6, and 0.8 units, respectively, in the intervention group compared to the control group ($P < 0.001$). Therefore, the mean score of PSQI significantly decreased by 3.8 units in the intervention group after the intervention (9.4 ± 3.7 vs 5.6 ± 1.9 ($P < 0.05$)). **Conclusions:** *Salvia* extract improved menopausal symptoms such as flushing, night sweat, heart palpitations, muscle and joint pain, depression, anxiety, sleep disorders, and sexual desire.

Keywords: Flushing, menopause, *Salvia officinalis* extract, sleep, sweat

Introduction

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Reduced level of estrogen in the menopause women leads to a wide range of symptoms. Hot flushes are the most common symptom of menopause.^[1] Racial and cultural differences play an

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important role in the incidence of flushing in eastern and western societies.^[2] In the study, the prevalence of flushing was reported 46.4% and 41.2% in Spanish and American women and the prevalence of night sweat was 34.7% and 36.9%, respectively.^[3] In a study done in Tehran, the prevalence of flushing and night sweat was reported 59.5% and 38.2%, respectively.^[4] A study in gynecologic clinics at the Royal Women's Hospital, Melbourne has been shown in postmenopausal women forgetfulness and less satisfying memory than women before menopause.^[5] Other studies have also referred to sleep problems, hot flashes, mood disorders, sexual dysfunction, weight gain, and cognitive decline in postmenopausal women.^[6-8] Over the past decade, the use of complementary and alternative medicine (CAM) to treat female problems such as menopause, premenstrual syndrome, sexual disease has increased.^[9-14] The standard treatment for menopause symptoms is a hormonal replacement.^[15] The standard treatment for menopausal symptoms is hormone replacement therapy (7), which temporarily improves women's quality of life, but can pose serious risks over the years.^[16] There are many options for managing menopausal symptoms when they affect the quality of life.^[17] The researchers reported that other interventions other than HT should be considered.^[18] Phytoestrogen is one of the alternative and supplement options. Phytoestrogens are plant compounds that have estrogenic activity.^[19] Phytoestrogens can be found in a wide range of herbal products such as valerian, Black cohosh, chamomile, Hypericum, Licorice, Fennel, Soya, Red clover, and *Salvia*. *Salvia officinalis* has numerous common names. Some of the best-known are sage, common sage, garden sage, golden sage, kitchen sage, true sage, culinary sage, Dalmatian sage, and broadleaf sage. The specific epithet officinal refers to plants with a well-established medicinal or culinary value.^[20] The phytoestrogen flavonoids present in this plant is used to reduce menopause symptoms.^[21] There is a limitation of studies on the effectiveness of *salvia* in managing menopausal symptoms, as there are various studies on herbal medicine effects other than *salvia* on menopausal symptoms like black cohosh. Therefore, menopause is a turning point in the life of women, and if it is a physiological process and a general event, reducing estrogen secretion can cause many health problems. This physiological process has a number of effects that are harmful to one's life. As one of the potential effects of menopause and lowering sex hormones, are Complications of Menopausal symptoms and reduced quality of for women.^[22,23]

Thus, obtaining accurate and reliable information from the practice of primary care physicians is one of the primary and essential needs of women in the premenopausal phase. Women need to know what awaits them and whether their experiences are normal or not. Proper lifestyle is the cornerstone of middle-aged women's health management programs. It can provide a basis for improving the quality of physical and mental performance during the following years of life.^[24,25] Sanchez *et al.* found that as many as 80% of women aged 18–55 have lifestyle-related risk behaviors that are preventable.^[26] It is also predicted that in 2020 noncommunicable lifestyle-related diseases will cause 7 out of 10 deaths in developing countries.^[27] The present study aimed

to know the effect of *Salvia officinalis* extract on symptoms of flushing, night sweat, sleep disorders, and score of forgetfulness in postmenopausal women at Namazi Hospital Bone Density Center in Shiraz 2015.

Materials and Methods

The study is a double-blind randomized controlled clinical trial was carried out from July to November 2015, that approved by the ethics committee of shiraz university of medical sciences and proposal No is: 94-10255, ethic code: IR.SUMS.REC.1394.185). The population of this study consisted of all menopause women who referred to Namazi Hospital Bone Density Center affiliated to Shiraz University of Medical Sciences. Based on the Sadeghi *et al.* study in 2013 and the formula below, the sample size for each group is calculated 38. But, due to a financial constraint on the purchase of a new kit for 5 people, the sample size for each group was reduced to 33 people.^[28] However, during the study, 2 participants in the interventional group and 5 patients in the placebo group were excluded from the study due to incomplete use of the pills, and finally, 59 patients completed the study [Figure 1]. Totally, 66 individuals were selected through purposive sampling technique and were divided into two groups using block permutation method. For example; group A, B as AABB, and another group of BABA, were named. Sampling continued until samples were completed.

The inclusion criteria were postmenopausal women who were willing to participate in the study, no history of taking any hormonal medicine, and no history of allergy to herbal medicines. The exclusion criteria were an allergic reaction to medication and a lack of interest in continuing participation.

$$n_1 = n_2 = 2 \frac{\left(\bar{z}_{1-\frac{\alpha}{2}} + \bar{z}_{1-\beta} \right)^2 (\sigma_1^2 + \sigma_2^2)}{d^2}$$

$$\sigma_1 = 4.7$$

$$\sigma_2 = 3.84$$

$$d = \mu_1 - \mu_2 = 2.78$$

$$\alpha = 0.05$$

$$\beta = 0.2$$

Before the intervention, obtaining the informed consent was done. The intervention group received *S. officinalis* as tablets (containing 100 mg *S. officinalis* extract produced by Gol Daroo company in Isfahan), which was prescribed three tablets a day for 3 months, while the control group received placebo tablets (containing 100 mg starch produced by Shiraz Pharmacy University of Medical Science) with the same prescription order and size in a packed in black cover with a

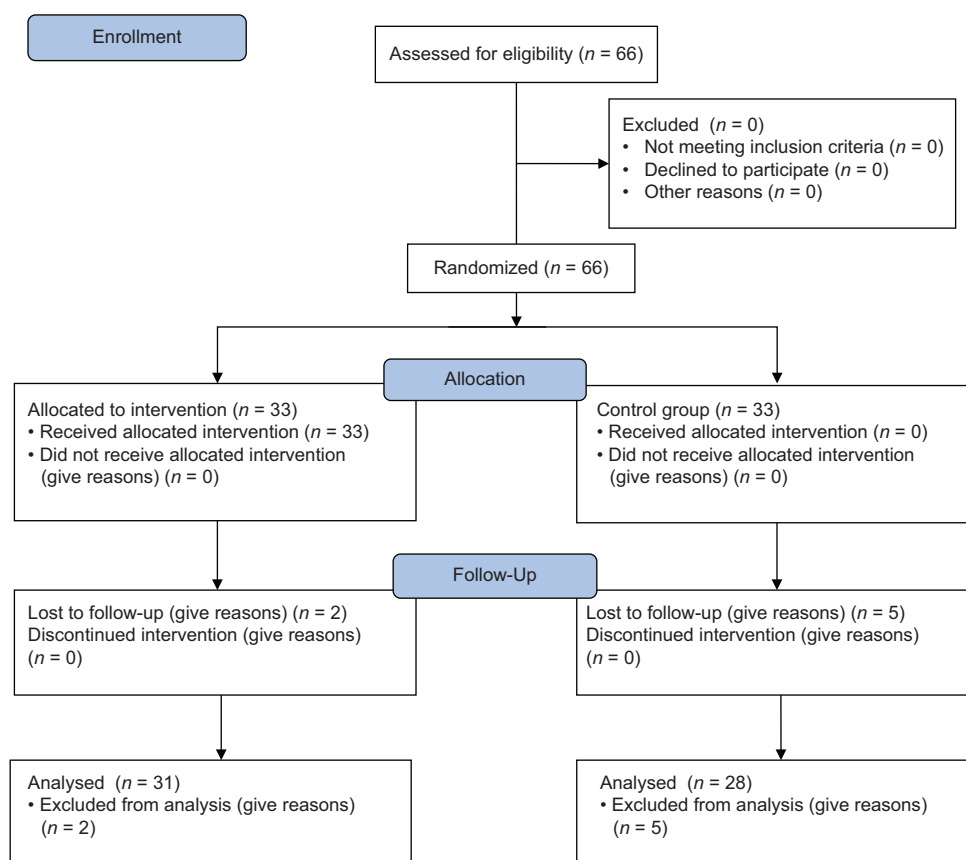


Figure 1: Consort Flow Diagram for sampling

code. The study was conducted as a double-blind study both researcher and patient. The following sections describe the questionnaires were completed by the women, and if needed, the research was made clear. Then, women completed an informed consent, demographic, MRS questionnaire, and Pittsburgh Sleep Quality Index (PSQI) questionnaire initially. Then, they were asked to complete the checklists regarding the symptoms of flushing and sweating (frequency, severity, duration) daily for about a week before the intervention and at weeks 2, 4, 6, 8, 10, 12 during the intervention. MRS and PSQI were refilled 3 months after the intervention. Menopause rating scale (MRS) consists of 11 menopause symptoms in three domains: Physical (4 items), psychological (4 questions), and genitourinary (3 questions). Questions are measured with a 5-point Likert scale from rating 1 (I do not have symptom) to 5 points (severe symptoms).

The total score is 11–55, and the higher the score, the more severe the symptoms. This tool was designed by Garage, and According to Heinemann, the MRS has internal consistency and a reliability coefficient 0.92 and Cronbach's alpha 0.81.^[29] In Iranian studies, instrument validity was determined by the content validity method and Cronbach's alpha coefficient for measuring psychological symptoms (0.81) and physical signs (79.8%). Urinary symptoms were reported to be 78%, which is the basis of our study.^[30] Pittsburgh Sleep Disorders Questionnaire (PQRS) was created in 1989 by Buysse *et al.* at the Pittsburgh Psychiatric

Institute.^[31] The questionnaire has nine questions in its original form, but since the 5th question contains 10 subquestions, the entire questionnaire has 19 items which are graded on a 4-point Likert scale from 0 to 3. It can effectively evaluate sleeping by measuring seven domains: Subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction over the last month. Scoring of the answers is based on a 0 to 3 scale, where 3 reflects the negative extreme on the Likert Scale. A global sum of "5" or greater indicates a "poor" sleeper. The sensitivity of the PSQI questionnaire was 89.6%, the specificity 86.5%, the validity of 88%, and the scientific reliability with Cronbach's score was 83%.^[32] The validity of the Iranian version of the questionnaire was determined in the study of Hossein Ebadi, according to Montazeri and colleageuse in 2007 with a sensitivity of 90%, the specificity 87%, and Cronbach's score of 88%, which is the basis of this study.^[33]

Statistical analysis

The data were analyzed through SPSS software. Before the intervention, ANOVA was applied to compare the mean score of MRS and PQRS between both groups and a paired *t*-test was used to compare the mean score before and after the intervention. Repeated measures ANOVA test was used to compare the frequency and severity of flushing and night sweating in both groups. Besides, $P < 0.05$ was considered statistically significant.

Results

Based on ANOVA test, mean age, menopause age, duration of menopause, body mass index (BMI), duration of marriage, number of parity, pregnancy, and children, abortion were $P = 0.747$, $P = 0.409$, $P = 0.419$, $P = 0.151$, $P = 0.069$, $P = 0.054$, $P = 0.079$, $P = 0.054$, and $P = 0.719$ in the intervention and control groups; therefore, they were homogeneous with no significant statistical difference ($P > 0.05$). Demographic characteristics are shown in [Table 1]. Before the intervention, mean score of PSQI ($P = 0.384$) and MRS ($P = 0.842$) showed no significant difference between both groups. The mean score of severity of flushing and night sweats a week before intervention ($P = 0.33$) and from 2 weeks (33.0) to 8 weeks after intervention ($P = 0.225$) showed no significant difference; it was significant at 10 ($P = 0.003$) and 12 ($P \leq 0.001$) weeks after the intervention compared to the control group. According to the paired t -test, the mean score of flushing, palpitation, sleeping disorders, muscle and joint aches, depression, nervousness, anxiety, and sexual desire and satisfaction significantly decreased by 1.6, 0.4, 1.6, 2.1, 1.4, 1.2, 1.6, and 0.8 units, respectively, in the intervention group compared to the control group. The most and least decrease in menopausal symptoms has belonged to joint and muscular discomfort, which decreased by about 1.2 units (2.8 ± 1.3 before and 0.7 ± 0.6 after intervention). Besides, using a paired t -test, the mean of forgetfulness score after intervention was reduced in comparison with before intervention in the salivary group, which was statistically significant ($P < 0.001$), but in the control group, there was no significant difference ($P = 0.326$). [Tables 2 and 3]. Repeated measures ANOVA revealed no significant difference in flushing frequency and severity between both groups before and at 2, 4, 6, 8 weeks after the intervention. However, the difference was significant at 10 and 12 weeks after the intervention. No significant difference was reported in night sweating between both groups before and at 2, 4, 6, 8 weeks after the intervention. However, the difference was significant at 10 and 12 weeks after the intervention ($P < 0.001$) [Table 4]. Regarding the paired t -test, the mean score of PSQI significantly decreased by 3.8 units in the intervention group after the intervention ($P < 0.05$) [Table 5].

Table 1: Demographic characteristics of the society research

Variable	n (%)		PV
	Salvia officinalis extract	Control	
Education			0.783
Illiterate	6 (19.4)	4 (14.8)	
Elementary	12 (38.7)	13 (48.1)	
Secondary school	4 (12.9)	4 (14.8)	
Diploma	8 (25.8)	5 (18.5)	
Academic	1 (3.2)	1 (3.7)	
Total	31 (100)	27 (100)	
Occupation			0.604
Employed	2 (6.5)	2 (7.4)	
Housewife	29 (93.5)	25 (92.6)	

Discussion

The effect of Salvia was studied on physical, psychological, and urogenital symptoms for 12 weeks. Finally, it was shown that Salvia significantly improved these symptoms (except dementia, urinary incontinence, and vaginal dryness) compared to the control group. Plants containing phytoestrogens (estrogen-like compounds) have a special place among plants, which are advised to treat menopausal symptoms. Phytoestrogens (plant-based compounds) are non-steroidal plants^[34,35] containing polyphenolic compounds with 1-3 hydroxyl group (OH) similar to the hydroxyl group in phenolic estrogen circle.^[36] Thus, a low level of estrogen after menopause is relatively compensated by phytoestrogens to improve the symptoms of menopause. Accurate pathophysiology of hot flushes is complicated and unknown. However, there is the premise that the region of temperature regulation is narrowed in women suffering from hot flushes which causes the increase of sensitivity for temperature change and hot flushes.^[37] Noradrenaline plays a leading role through adrenergic α_2 receptors in regulating the temperature which causes vascular vasodilation and loss of heat and decline of central temperature. Gonadal steroids regulate the central noradrenaline^[38]; there is the hypothesis that the decline of sexual hormones causes an increase in hypothalamus noradrenaline and stimulation of LHRH secretion for gonadotropins secretion. At the same time, increase in hypothalamus noradrenaline (not its peripheral metabolites) at the vicinity of the center of temperature regulation in LHRH neurons gives rise to hot flushes.^[39] In the study is effective and another study is not effective in declining the menopause symptoms.^[40] In the study in Iran, Perforate St John's-wort and chasteberry flower, which contain phytoestrogen, decrease the menopausal symptoms and hot flushes.^[2] In this study, the post-treatment score of forgetting after the intervention as compared with pre-treatment has decreased 1 unit in common sage groups and this reduction of forgetting score was statistically significant. However, it was not significant when compared with the control group. This has been known well that stopping of ovary estrogen can affect the cognitive performance in women; in contrast, treatment with hormone and phytoestrogen can minimize the cognitive changes in menopausal women. The role of estrogen in cognitive performance is associated with the expansion of estrogen receptors in some regions of the brain related to learning and memory such as the hippocampus and basal forebrain.

In the study, common sage increased the memory and temper and this effect was applied through inhibition of acetylcholinesterase.^[41] Similarly, Salvia has a protecting effect against toxic effects of $A\beta$ in the brain neurons. Rosmarinic acid in the Salvia has antioxidant and anti-apoptosis effects and protects the neurons against the toxicity of $A\beta$.^[42] Also, in the study, Salvia enhanced the memory.^[43] Study findings showed that the average of post-intervention sleep score in common sage groups has been decreased 3.8 unit when compared with preintervention and this decline of sleep score was statistically significant. However, in the control group, after treatment,

Table 2: Comparison of the average menopausal rating scale (MRS) before and after between the intervention and control groups

Variable		Mean±SD		P**
		Intervention Group	Group control	
Hot flashes - night sweats	Before	1.46±1.84	1.62±1.64	0.588
	After	0.37±0.16	1.58±1.17	<0.001
P*		<0.001	0.326	
Heart discomfort	Before	1.13±4.07	1.36±2.13	0.197
	After	0.58±0.26	1.38±1.29	<0.001
P*		0.001	0.326	
Sleep problems	Before	1.49±0.19	1.10±2.39	0.344
	After	0.53±0.29	1.06±6.23	<0.001
P*		<0.001	0.326	
Joint and muscular discomfort	Before	1.31±2.77	1.04±2.96	0.763
	After	0.63±0.74	1.07±2.96	<0.001
P*		<0.001	0.998	
Depressive mood	Before	1.37±2.00	1.29±2.04	0.953
	After	0.71±5.06	1.23±2.04	<0.001
P*		<0.001	1	
Irritability	Before	1.32±1.84	1.18±1.86	0.382
	After	0.67±0.58	1.19±1.82	<0.001
P*		<0.001	0.573	
Anxiety	Before	1.36±2.13	1.31±2.00	0.925
	After	0.72±0.52	1.35±1.96	<0.001
P*		<0.001	0.573	
Physical and mental exhaustion	Before	1.41±2.23	1.43±1.46	0.088
	After	1.16±0.89	1.43±1.39	0.634
P*		<0.001	0.326	
Sexual problems	Before	1.19±1.81	1.58±2.14	0.619
	After	0.97±0.87	2.11±1.57	0.002
P*		<0.001	0.326	
Urinary incontinence	Before	1.31±1.13	1.49±1.07	0.501
	After	0.81±1.11	1.07±1.49	0.369
P*		0.016	1	
Dryness of vagina	Before	1.25±1.19	1.47±1.36	0.83
	After	1.06±1.21	1.36±1.47	0.7
P*		0.103	1	

*Paired sample t-test, **Independent t-test

Table 3: Comparison of the impact of sleep before and after the intervention in the intervention and control groups based on PSQI questionnaires

Group	Mean±SD		P*
	Intervention Group	Group control	
Before intervention	3.71±9.35	4.19±10.64	0.384
After intervention	1.94±5.61	4.16±10.57	<0.001
P**	<0.001	0.573	

*Independent t test, **Paired sample t-test

the sleep score average remained the same. The studies show that isoflavonoids decrease insomnia among menopausal women.^[44] In a clinical trial in the Switzerland research on menopausal symptoms was showed the intensity and frequency of hot flushes for two months was evaluated. Other variables including symptom in menopause scale (MRS) were evaluated at the beginning and after 2 months. The results showed that the average total number of flushing and symptoms of MRS scale was decreased weekly from 1 to 8 weeks significantly. A study

concluded that Salvia has clinical value in the treatment of hot flushes and associated menopausal symptoms. The results of this study are similar to ours, but there is a difference in the duration and dosage of treatment.^[28] In another study, the common sage had no effect on the inhibition of the reuptake of serotonin and acetylcholinesterase^[37]; therefore, understanding the impact of mechanisms of Salvia calls for further studies.

The limitation of this study was the preparation of Salvia tablets (Salvia extract) and placebo in exactly the same color as possible. Since Salvia was used in herbal supplement, a comparative study of herbal supplement and synthetic drugs is suggested to improve the symptoms of menopause.

Conclusion

The study showed the influence of Salvia extract on menopausal symptoms such as flushing, night sweats, palpitations, muscle and joint pain, depression, anxiety, sleep disorders, and sexual

Table 4: Mean number and severity of hot flashes before and after the intervention between two groups

The number and severity of hot flashes Time	Intervention Group		Group control		P*	
	Mean±SD		Mean±SD		Mean±SD	
	Frequency	Severity	Frequency	Severity	Frequency	Severity
A week before the intervention	2.04±2.79	1.21±2.17	1.81±2.00	1.37±2.46	0.243	0.669
Week 2 intervention	2.02±2.71	1.19±2.20	1.81±2.00	1.37±2.46	0.292	0.716
Week 4 intervention	1.75±2.43	1.09±2.33	1.81±2.00	1.35±2.50	0.597	0.839
Week 6 intervention	1.47±2.04	1.04±2.53	1.72±1.86	1.26±2.61	0.873	0.899
Week 8 intervention	0.95±1.21	0.89±2.97	1.73±1.89	1.26±2.61	0.195	0.401
Week 10 intervention	0.58±0.50	0.81±3.40	1.68±1.82	1.25±2.68	<0.001	0.007
Week 12 intervention	0.55±0.32	0.81±3.60	1.68±1.82	1.25±2.64	<0.001	<0.001
P**	<0.001	0.001	0.075	0.115		

*Independent t test, **Paired sample t-test

Table 5: Comparison of the severity of night sweats before and after the intervention between two groups

Group	Mean±SD		P*
	Intervention Group	Group control	
A week before the intervention	1.17±1.57	1.17±1.11	0.313
Week 2	1.17±1.57	1.17±1.11	0.313
Week 4	1.07±0.14	1.12±1.07	0.55
Week 6	0.93±1.03	1.17±1.04	0.991
Week 8	0.77±0.57	1.12±1.00	0.225
Week 10	0.55±0.20	1.11±0.96	0.003
Week 12	0.37±0.07	1.17±1.04	<0.001
P**	<0.001	0.427	

*Independent t test, **Paired sample t-test

desire. Expansive use of this plant can be suggested in case it is confirmed in further investigations. Regarding the effect of Salvia extract on some menopausal symptoms, it is suggested that multiple chemical compounds of this plant can be isolated and its active compounds are investigated in studies similar to the present study.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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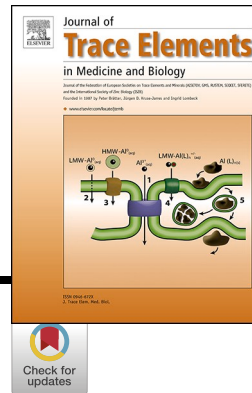
Conflicts of interest

There are no conflicts of interest.

References

1. Ibrahim ZM, Ghoneim HM, Madny EH, Kishk EA, Lotfy M, Bahaa A, *et al.* The effect of menopausal symptoms on the quality of life among postmenopausal Egyptian women. *Climacteric* 2019;1-8. doi: 10.1080/13697137.2019.1656185. [Epub ahead of print]
2. Ghazanfarpour M, Sadeghi R, Abdollahian S, Roudsari RL. The efficacy of Iranian herbal medicines in alleviating hot flashes: A systematic review. *Int J Reprod Biomed (Yazd)* 2016;14:155-66.
3. Pérez-Alcalá I, Sievert LL, Obermeyer CM, Reher DS. Cross cultural analysis of determinants of hot flashes and night sweats: Latin-American immigrants to Madrid and their Spanish neighbors. *Menopause* 2013;20:1111-9.
4. Asadi M, Jouyandeh Z, Nayebzadeh F. Prevalence of menopause symptoms among Iranian women. *J Fam Plann Reprod Health* 2012;6:1-3.
5. Unkenstein AE, Bryant CA, Judd FK, Ong B, Kinsella GJ. Understanding women's experience of memory over the menopausal transition: Subjective and objective memory in pre-, peri-, and postmenopausal women. *Menopause* 2016;23:1319-29.
6. Johnson A, Roberts L, Elkins G. Complementary and alternative medicine for menopause. *J Evid Based Integr Med* 2019;24:2515690X19829380.
7. Bruyneel M. Sleep disturbances in menopausal women: Aetiology and practical aspects. *Maturitas* 2015;81:406-9.
8. Caretto M, Giannini A, Simoncini T. An integrated approach to diagnosing and managing sleep disorders in menopausal women. *Maturitas* 2019;128:1-3.
9. Akbarzadeh M, Moshfeghy Z, Dehghani M, Emamghoreishi M, Tavakoli P, Zare N. Comparison of the effect of melissa officinalis capsule and care educational programs on the intensity of physical, mental and social symptoms of premenstrual syndrome in high school female students. *Int J Women's Health Reprod Sci* 2018;6:18-26.
10. Zeinalzadeh S, Akbarzadeh M, Faridi P, Mohagheghzadeh AA, Sayadi M. Effect of sildenafil citrate on women affected by sexual dysfunction referred to health clinics. *Fam Med Prim Care Rev* 2017;19:167-72.
11. Heydari N, Dehghani M, Emamghoreishi M, Akbarzadeh M. Effect of melissa officinalis capsule on the mental health of female adolescents with premenstrual syndrome: A clinical

- trial study. *Int J Adolesc Med Health* 2018;31.doi: 10.1515/ijamh-2017-0015.
12. Akbarzadeh M, Zeinalzadeh S, Zolghadri J, Mohagheghzadeh A, Faridi P, Sayadi M. Comparison of *elaegnus angustifolia* extract and sildenafil citrate on female orgasmic disorders: A randomized clinical trial. *J Reprod Infertil* 2014;15:190-8.
 13. Akbarzadeh M, Dehghani M, Moshfeghy Z, Emamghoreishi M, Tavakoli P, Zare N. Effect of melissa officinalis capsule on the intensity of premenstrual syndrome symptoms in high school girl students. *Nurs Midwifery Stud* 2015;4:e27001.
 14. Heidary M, Yazdanpanahi Z, Dabbaghmanesh MH, Parsanezhad ME, Emamghoreishi M, Akbarzadeh M. Effect of chamomile capsule on lipid- and hormonal-related parameters among women of reproductive age with polycystic ovary syndrome. *J Res Med Sci* 2018;26;23:33.
 15. Zhang J-M, Li J, Liu E-W, Wang H, Fan G-W, Wang Y-F, *et al.* Danshen enhanced the estrogenic effects of Qing E formula in ovariectomized rats. *BMC Complement Altern Med* 2016;16:181.
 16. Remport J, Blázovics A. [Phytoestrogens in the treatment of menopause]. *Orv Hetil* 2017;158:1243-51.
 17. Potter B, Schrager S, Dalby J, Torell E, Hampton A. Menopause. *Prim Care* 2018;45:625-41.
 18. Javed AA, Mayhew AJ, Shea AK, Raina P. Association between hormone therapy and muscle mass in postmenopausal women: A systematic review and meta-analysis. *JAMA Netw Open* 2019;2:e1910154.
 19. Simbalista RL, Sauerbronn AV, Aldrighi JM, Arêas JA. Consumption of a flaxseed-rich food is not more effective than a placebo in alleviating the climacteric symptoms of postmenopausal women. *J Nutr* 2010;140:293-7.
 20. Harrison, L. *RHS Latin for Gardeners*. United Kingdom: Mitchell Beazley; 2012. p. 224.
 21. Abdallah ZA, Khattab AH, Sawiress FA, EL-Banna RA. Effect of *Salvia officinalis* L. (sage) herbs on osteoporotic changes in aged non-cycling female rats. *Med J Cairo Univ* 2010;78:1-9.
 22. Dotlic J, Nicevic S, Kurtagic I, Radovanovic S, Rancic B, Markovic N, *et al.* Hormonal therapy in menopausal transition: Implications for improvement of health-related quality of life. *Gynecol Endocrinol* 2019;1-6. doi: 10.1080/09513590.2019.1676409. [Epub ahead of print]
 23. Rathnayake N, Lenora J, Alwis G, Lekamwasam S. Prevalence and severity of menopausal symptoms and the quality of life in middle-aged women: A study from Sri Lanka. *Nurs Res Pract* 2019;2019:2081507.
 24. van Dam RM, Li T, Spiegelman D, Franco OH, Hu FB. Combined impact of lifestyle factors on mortality: Prospective cohort study in us women. *BMJ* 2008;337:a1440.
 25. Chiuve SE, Rexrode KM, Spiegelman D, Logroscino G, Manson JE, Rimm EB. Primary prevention of stroke by health lifestyle. *Circulation* 2008;118:947-54.
 26. Sanchez A, Norman GJ, Sallis JF, Calfas KJ, Rock C, Patrick K. Patterns and correlates of multiple risk behaviors in overweight women. *Prev Med* 2008;46:169-202.
 27. Habib SH, Saha S. Burden of non- communicable disease: Global overview. *Diab Met Syndr Clin Res Rev* 2010;4:41-7.
 28. Sadeghi AH, Bakhshi M, Behboodi Z, Goodarzi S, Haghani H. Effect of sage extract on hot flashes in postmenopausal women. *CMJA* 2013;2:324-35.
 29. Heinemann K, Ruebig A, Potthoff P, Schneider HP, Strelow F, Heinemann LA. The Menopause rating scale (MRS) scale: A methodological review. *Health Qual Life Out* 2004;2:45.
 30. Taavoni S, Nazem Ekbatani N, Haghani H. [Effect of afrodit on sexual satisfaction in menopausal women]. *Arak Medical University Journal* 2014;17:10-8.
 31. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Res* 1989;28:193-213.
 32. Ghasab SM, Raeesi DZ, Alidoosti M, Raei M, Del Aram MA, Mirmohammad AM, *et al.* Comparison of maternal sleep quality in postpartum period between vaginal delivery and cesarean section. *J Res Dev Nurs Midwifery* 2013;10:69-76.
 33. Montazeri A, Torkan B, Omidvari S. The Edinburgh postnatal depression scale (EPDS): Translation and validation study of the Iranian version. *BMC Psychiatry* 2007;7:11.
 34. Simbalista RL, Sauerbronn AV, Aldrighi JM, Areas JA. Consumption of a flaxseed-rich food is not more effective than a placebo in alleviating the climacteric symptoms of postmenopausal women. *J Nutr* 2010;140:293-7.
 35. Ciotta L, Stracquadanio M, Pagano I, Andò A, Valenti O, Roccasalva L. [Clinical effects of treatment with phytoestrogens in postmenopausal women]. *Minerva Ginecol* 2012;64:15-22.
 36. Elkomy MM, Elsaid FG. Anti-osteoporotic effect of medical herbs and calcium supplementation on ovariectomized rats. *J Basic Appl Zool* 2015;72:81-8.
 37. Rahte S, Evans R, Eugster PJ, Marcourt L, Wolfender J-L, Kortenkamp A, *et al.* *Salvia officinalis* for hot flushes: Towards determination of mechanism of activity and active principles. *Planta Med* 2013;79:753-60.
 38. Albertazzi P. Noradrenergic and serotonergic modulation to treat vasomotor symptoms. *J Br Menopause Soc.* 2006;12:7-11.
 39. Vandecasteele K, Ost P, Oosterlinck W, Fonteyne V, De Neve W, De Meerleer G. Evaluation of the efficacy and safety of *Salvia officinalis* in controlling hot flashes in prostate cancer patients treated with androgen deprivation. *Phytother Res* 2012;26:208-13.
 40. Hur M-H, Yang YS, Lee MS. Aromatherapy massage affects menopausal symptoms in Korean climacteric women: A pilot-controlled clinical trial. *Evid Based Complement Alternat Med* 2008;5:325-8.
 41. Moss L, Rouse M, Wesnes KA, Moss M. Differential effects of the aromas of *Salvia* species on memory and mood. *Hum Psychopharmacol* 2010;25:388-96.
 42. Hamidpour M, Hamidpour R, Hamidpour S, Shahlari M. Chemistry, pharmacology, and medicinal property of Sage (*Salvia*) to prevent and cure illnesses such as obesity, diabetes, depression, dementia, lupus, autism, heart disease, and cancer. *J Tradit Complement Med* 2014;4:82-8.
 43. Scholey AB, Tildesley NT, Ballard CG, Wesnes KA, Tasker A, Perry EK, *et al.* An extract of *Salvia* (sage) with anticholinesterase properties improves memory and attention in healthy older volunteers. *Psychopharmacology* 2008;198:127-39.
 44. Hachul H, Monson C, Kozasa EH, Oliveira DS, Goto V, Afonso R, *et al.* Complementary and alternative therapies for treatment of insomnia in women in postmenopause. *Climacteric* 2014;17:645-53.



Effect of zinc supplementation on circulating concentrations of homocysteine, vitamin B₁₂, and folate in a postmenopausal population

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ABSTRACT

Introduction: The decrease in estrogen levels associated with menopause increases the risk of deficiencies of key micronutrients such as zinc and of disturbances in methylation cycle-related markers. The present study assesses the effect of 8-week Zn supplementation upon circulating concentrations of Hcy, B₁₂, and Fol levels in a population of postmenopausal women.

Methods: Fifty-one postmenopausal women aged between 44 and 76 years took part in the study. Two randomized groups (placebo and zinc [50 mg/day]) were treated during 8 weeks. Nutrient intake was assessed based on the 72-hour recall method. Zinc was analyzed by flame atomic absorption spectrophotometry. Clinical-nutritional parameters were determined by enzyme immunoassay techniques.

Results: Folate levels increased significantly ($p < 0.05$) in the zinc group on comparing the baseline versus follow-up values. Homocysteine decreased in the inter-group analysis ($p < 0.05$) after the intervention. Furthermore, higher folate ($r = -0.632$; $p = 0.005$) and vitamin B₁₂ ($r = -0.512$; $p = 0.030$) levels were correlated to low homocysteine levels in the zinc group after the intervention, although the zinc intervention had the same effect on B₁₂ levels in both groups.

Conclusion: Zinc supplementation enhanced circulating folate and homocysteine by improving the folate values in the zinc-supplemented group and decreasing homocysteine levels inter-groups. Further studies involving larger samples and optimizing the doses and intervention period are needed to reinforce our main findings.

1. Introduction

Menopause is the time in life when menstrual cycles cease due to a diminished secretion of ovarian hormones [1]. The decrease in estrogen production could lead to changes in lipid profile, which together with certain menopausal physiological disorders, could disturb clinical-nutritional cardiovascular health related parameters, deriving in cardiovascular diseases [2,3]. Moreover, if this menopausal situation is not monitored, the risk of deficiencies in the nutritional status of numerous key micronutrients, such as minerals, is seen to increase [4].

Zinc (Zn) is one of the most important trace elements, and its deficiency is a major health problem worldwide [5]. Zn is a cofactor of more than 300 enzymes, carrying out its cardiovascular protective functions by combating oxidative stress and inflammation, which are risk factors for cardiovascular diseases [6]. Abnormalities in Zn homeostasis have

been reported in patients with metabolic disorders [7,8], since Zn deficiency may cause multiple systemic disturbances, including cardiovascular disease and dyslipidemia [9].

Supplementation of trace elements such as Zn with the aim of improving cardiovascular health is becoming increasingly popular [10]. Although several trials support the beneficial effects of Zn supplementation upon regulating the cardiovascular profile, there is a lack of clarity in the data reported on the impact of Zn supplementation upon human health, especially in postmenopausal women [11]. In this regard, Zn status may also influence the circulating amounts of clinically relevant cardiovascular biomarkers such as homocysteine (Hcy), by increasing folate (Fol) and vitamin B₁₂ (B₁₂), which are inversely related to Hcy levels [12]. It is well documented that Fol and B₁₂ deficiencies result in increased Hcy levels in aged populations such as postmenopausal women [13,14]. Recently, this relationship has been

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explained, albeit in a Zn-supplemented younger population, by the fact that Zn, Hcy, Fol and B₁₂ play an important role in the methylation cycle. In the case of Zn deficiency, Hcy concentrations seem to be increased due to disturbances in the methylation cycle [15]. In this line, two methionine synthase (MS) and betaine Hcy methyltransferase (BHMT), which are enzymes in Hcy metabolism, are Zn-dependent, being Zn deficiency an important factor in the increase in Hcy levels [16]. It therefore would be interesting to assess the possible influence of Zn upon Fol and B₁₂ levels in order to elucidate possible alterations in Hcy metabolism in the postmenopausal population.

To our knowledge, few data are available on the relationship between Zn supplementation and Hcy cycle-related parameters in postmenopausal women. The present study assesses the effect of 8-week Zn supplementation upon circulating concentrations of Hcy, B₁₂, and Fol levels, in a population of postmenopausal women.

2. Materials and methodology

2.1. Study design and intervention

This is an eight-week, double-blinded, placebo-controlled, randomized intervention trial. Participants were randomly assigned to one of two treatment groups: Zinc Group – 50 mg/day of Zn (ZG: 26 women) and Placebo group (PG: 25 women). Zn capsules were provided by SM Natural Solutions, Sabadell, Spain (Number 0B62713821). Placebo capsules were made of the same color and size as Zn supplements for identical taste and appearance. The manufacturer recommended a period of eight weeks in order to ensure the supplementation effects. The supplementation period took place in winter from January 15th to March 15th.

Adherence to both Zn and Placebo interventions was checked as the percentage of capsules ingested during the intervention period. In order to verify the adverse effects, the safety and the efficacy of the product, two biochemical analytics were performed at baseline and follow-up. Written informed consent was obtained from all patients. The intervention was conducted according to the principles of the Declaration of Helsinki and the approval by the Ethics Committee of the University of Granada (149/CEIH/2016), in accordance with the International Conference on Harmonization/Good Clinical Practice Standards. The study was registered at the US National Institutes of Health (ClinicalTrials.gov) NCT03672513.

2.2. Study participants

51 healthy postmenopausal women volunteers from the province of Granada, Spain, aged between 44 and 76 years, were recruited once informed consent was signed. All participants are derived from a larger cohort of postmenopausal women [17]. Inclusion criteria were (i) to present postmenopausal status (with at least 12 months of amenorrhea), (ii) to present baseline plasma Zn (one to five days before the intervention) determined by flame atomic absorption spectrophotometry (FAAS), (iii) to be non-sedentary, (iv) to smoke and drink alcohol in occasional and moderate quantities, (v) to present normal parameters of a routine hospital laboratory analysis. Exclusion criteria were (i) not to accept the randomization procedure, (ii) to take vitamin and mineral supplements, (iii) to present any pathology that could affect their nutritional status (i.e., the main components of metabolic syndrome, celiac disease, bulimia and anorexia), (iv) to be subjected to hormone replacement therapy, (v) not to present systemic inflammatory status (C-reactive protein was included as a reference biomarker to assess inflammation status of the participants at baseline).

Randomization was performed in a 1:1 ratio using a table of random numbers, prepared by a researcher who did not participate in the data collection. Allocation concealment was ensured by not releasing the randomization code until the participants were recruited into the trial after all baseline measurements were completed. Women were

randomly assigned (simple randomization) to study groups (parallel design). Both study participants and investigators were blinded to the group allocation.

2.3. Body composition analysis

We measured the participants height with a stadiometer (Seca, model 213, range 85–200 cm; precision: 1 mm; Hamburg, Germany). Body weight was assessed with bioelectrical impedance (Tanita MC-980 Body Composition Analyzer MA Multifrequency Segmental, Barcelona, Spain). The analyzer complies with the applicable European standards (93/42EEC, 90/384EEC) for use in the medical industry. BMI was obtained as weight (kg)/ height (m²). Participants were informed in advance of the required conditions prior to the measurement: (i) no alcohol intake the previous 24 h, (ii) no vigorous exercise 12 h before the measurement, (iii) no food or drink intake less than 3 h prior to the measurement, and (iv) no urination immediately before the measurement.

2.4. Nutrient intake

Dietary nutrient intake was assessed and administered by a professional dietitian before and after intervention using a manual 72 h-recall, including one weekend day. Recall accuracy was recorded with a set of photographs of prepared foods and dishes that are frequently consumed in the region where the study was performed. Dietwin software (7.1. version, Barcelona, Spain) was employed to quantitatively convert food intake to both energy and micronutrients, determining their adequacy according to the Recommended Dietary Allowance (RDA) for the menopausal Spanish population [18].

2.5. Sample treatment

Plasma samples were obtained through a blood extraction which was centrifuged at 4 °C during 15 min at 3000 rpm in the morning in fasting conditions before and after intervention. To obtain erythrocyte aliquots, 3 mL of 0.9% sodium chloride solution was employed during 4 erythrocyte washes, centrifuging for 15 min at 3000 rpm after each wash. The samples were stored at –80 °C for further analysis. All samples were measured in one run, in the same assay batch and blinded quality control samples were included in the assay batches.

2.5.1. Measurement of biochemical parameters

Biochemical parameters such as glucose, urea, uric acid, albumin, prealbumin, creatinine, total bilirubin, transferrin, total proteins, triglycerides, Hcy, C-reactive protein (CRP), lactate dehydrogenase (LDH), triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol, were determined in the Analysis Unit at the Virgen de las Nieves Hospital, Granada (ECLIA, Elecsys 2010 and Modular Analytics E170, Roche Diagnostics, Mannheim, Germany). All reference values were provided by the Analysis Unit. Zn content was analysed by FAAS (Perkin Elmer A. Analyst'300 Norwalk, CT, USA), previous wet-mineralized way, in the Scientific Instrumentation Center (SIC) from the University of Granada. Accuracy of the method was evaluated by analysis of a Certified Reference Material (SeronomTM Trace Elements ref. MI0181 SERO AS, Billingstad, Norway), at different optimal wavelengths (slit 0.7 nm), using a flow rate (Air/C₂H₂) of 10/1.9 L min^{–1}, and a five-point calibration curves ($r^2 = 0.9997$). The reliability was established by setting cut-off scores and considering a sample to be valid and reliable if it showed a coefficient of variation (CV) of less than 5% and an inter-class correlation coefficient (ICC) above 0.90. Fol and B₁₂ were measured in SIC using a DxI® Autoanalyzer (Beckman Coulter, CA, USA) employing a competitive electrochemiluminescence immunoassay for quantitative determinations. The reference values of Zn, Fol and B₁₂ were provided by the SIC.

2.6. Statistical analysis

All calculations were performed using the SPSS 22.0 Software for MAC (SPSS Inc. Chicago, IL, USA). Previously, a sample size calculation was estimated to determine the effect of a 50 mg/day Zn intervention upon Hcy levels in menopausal women. To our knowledge, no information is available on differences in means and standard deviations in Hcy response to Zn treatment compared to a PG. The sample size calculation was determined by applying a two-factor repeated measures ANOVA test being considered an effect size of 0.20 (effect size *f*, moderate), an alpha error of 0.05 and a power of 90%. Based on sample size calculations it was determined that a total sample size of 46 participants would be needed (G*Power, version 3.1.9.6; Universität Kiel, Germany). Descriptive analysis was presented as mean and standard deviation ($\bar{X} \pm \text{SD}$). The hypothesis of normal distribution was accepted using the Kolmogorov-Smirnov test as a previous step to the execution of a parametric model or not. For the comparative analysis based intra-group and inter-group, the paired and unpaired *t*-tests for parametric samples were used, respectively. Correlation analyses and partial correlation coefficients were performed with Pearson test. Significance was set at *p*-value < 0.05.

3. Results

All participants self-reported 100% adherence to the intervention in both PG and ZG. The baseline characteristics of the postmenopausal population by group are presented in Table 1. In relation to the anthropometric parameters, the total population presented type I overweight on average. In terms of nutrient intake, energy consumption was seen to be below the reference values. With regard to the intake of micronutrients, Zn intake was below 50% of RDA, and reached over 100% of RDA after the Zn intervention in ZG. Moreover, two-thirds of Fol intake were not covered by 66% of the total population, and B₁₂ intake was above the reference values in both groups. The biochemical parameters did not show mean differences at baseline.

The supplementation protocol has been published elsewhere. In summary, Zn status was assessed prior to the intervention, evidencing a deficient plasma and erythrocyte Zn status in 58.3% and 54.2% of all postmenopausal women, respectively. After Zn supplementation in ZG, the deficient plasma Zn levels were corrected by 50% of baseline Zn deficient intervened women (*p* < 0.001), and erythrocyte Zn deficiency was almost completely corrected (*p* < 0.001) [19]. Fig. 1 shows effect of placebo and zinc interventions upon main parameters of the study by groups. Fol values were within the reference ranges, increasing significantly (percentage change: 14.9% in ZG versus −6.48% in PG) after Zn supplementation in ZG (*p* < 0.05). In relation to B₁₂, although the levels were within normal limits in both PG and ZG, this parameter increased significantly after the intervention in both groups (*p* < 0.05). The Hcy levels in turn were above the reference values in PG, and significantly higher than in ZG after the intervention (percentage change: −5.41% in ZG versus 9.09% in PG) (*p* < 0.05).

Fig. 2 shows the bivariate Pearson correlation coefficients between Hcy-related parameters of the study by groups. With regard to the relationship between erythrocyte Zn and Hcy (Fig. 2A and B), no significant associations were observed by groups (all *p* > 0.05). When relating B₁₂ and Hcy (Fig. 2C and D), a significant inverse correlation was found for ZG (*p* = 0.030). In case of the Fol and Hcy (Fig. 2E and F), the indirect significant correlation only was observed too for ZG (*p* = 0.005).

4. Discussion

The current study, which to our knowledge is the first of its kind in a postmenopausal population, was designed to investigate the effectiveness of an 8-week Zn intervention upon circulating concentrations of Hcy, vitamin B₁₂, and Fol levels in a population of postmenopausal

Table 1

Baseline characteristics of the study population.

Features	Reference values	Total population (n = 51)	ZG (n = 26)	PG (n = 25)	P-value
		Baseline ($\bar{X} \pm \text{SD}$)	Baseline ($\bar{X} \pm \text{SD}$)	Baseline ($\bar{X} \pm \text{SD}$)	
Age (Years)	–	58.3 ± 8.67	57.1 ± 8.34	59.7 ± 9.15	0.167
Weight (Kg)	–	68.1 ± 12.8	67.7 ± 14.4	69.2 ± 11.0	0.687
Height (cm)	–	158.8 ± 6.24	160.3 ± 6.31	157.2 ± 6.01	0.086
BMI (Kg / m ²)	22.0–25.0	26.9 ± 4.54	26.2 ± 4.56	28.0 ± 4.31	0.150
Energy intake (Kcal)	2000.0	1414.9 ± 341.8	1487.9 ± 385.5	1339.5 ± 283.1	0.130
CHO intake (g/day)	275.0	149.8 ± 39.7	154.1 ± 39.8	146.6 ± 40.3	0.520
Protein intake (g/day)	50.0	61.8 ± 14.2	63.8 ± 14.4	59.7 ± 14.2	0.316
Fat intake (g/day)	70.0	62.2 ± 22.6	67.7 ± 26.1	56.1 ± 17.2	0.073
Zn intake (mg/day)	12.0	6.24 ± 3.67	5.82 ± 1.39	6.74 ± 5.16	0.383
Folic acid intake (μg/day)	400.0	240.8 ± 85.4	246.1 ± 80.8	238.2 ± 92.2	0.750
Vitamin B ₁₂ intake (μg/day)	2.40	5.29 ± 4.79	5.75 ± 5.45	4.80 ± 4.14	0.496
Glucose (mg/dL)	70.0–110.0	93.2 ± 17.9	90.6 ± 16.0	96.0 ± 19.8	0.295
Urea (mg/dL)	10.0–50.0	34.7 ± 9.23	33.3 ± 8.20	36.3 ± 10.2	0.262
Uric acid (mg/dL)	2.40–5.70	4.39 ± 0.99	4.28 ± 1.00	4.50 ± 0.98	0.436
Albumin (mg/dL)	3.50–5.20	4.42 ± 0.22	4.37 ± 0.22	4.48 ± 0.21	0.070
Prealbumin (mg/dL)	20.0–40.0	25.4 ± 4.23	24.6 ± 3.50	26.6 ± 5.02	0.133
Total bilirubin (mg/dL)	0.10–1.20	0.48 ± 0.15	0.47 ± 0.17	0.49 ± 0.13	0.533
Transferrin (mg/dL)	200.0–360.0	278.0 ± 45.3	273.0 ± 48.6	285.8 ± 39.6	0.381
Total proteins (g/dL)	6.60–8.80	7.08 ± 0.53	7.05 ± 0.62	7.11 ± 0.43	0.705
CRP (mg/L)	< 5.00	0.29 ± 0.33	0.29 ± 0.39	0.29 ± 0.24	0.994
LDH (U/L)	110.0–295.0	183.0 ± 54.2	183.2 ± 69.8	182.8 ± 29.3	0.975
Triglycerides (mg/dL)	50.0–200.0	106.6 ± 76.1	98.2 ± 82.7	115.8 ± 68.9	0.419
HDL (mg/dL)	40.0–60.0	66.7 ± 16.2	70.4 ± 19.3	62.6 ± 11.2	0.089
LDL (mg/dL)	70.0–190.0	126.8 ± 33.8	119.6 ± 31.3	134.4 ± 35.3	0.123
Total cholesterol (mg/dL)	110.0–200.0	218.3 ± 36.5	212.8 ± 33.1	224.1 ± 39.7	0.279

n = 51. PG = Placebo Group. ZG = Zinc Group. BMI = Body Mass Index. CHO = Carbohydrates. Zn = Zinc. CRP = C-reactive protein. LDH = Lactate dehydrogenase. HDL = High density lipoprotein. LDL = Low density lipoprotein. Baseline values are expressed as mean ($\bar{X}$) ± standard deviation (SD). For inter-groups *p*-value, unpaired *t*-student test was used. Intake reference values were obtained from the Reference Dietary Intakes (RDI) for the Spanish Population [18]. Reference values from biochemical parameters were provided from the Virgen de las Nieves Hospital and the Scientific Instrumental Center, Granada.

women. The results of our study showed a decrease of Hcy levels at the end of the intervention inter-groups. Moreover, the postmenopausal women supplemented with Zn were seen to increase their Fol levels to within the reference ranges, compared to the baseline values. Interestingly, this improvement in Fol levels was correlated to a decrease in plasma Hcy in this group. Likewise, B₁₂ was inversely related with Hcy

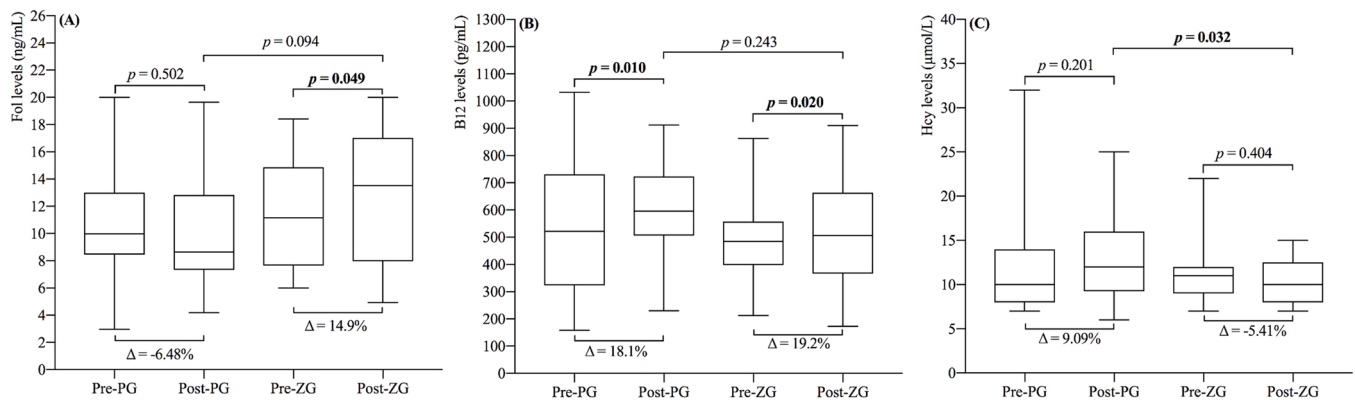


Fig. 1. Effect of placebo and zinc interventions upon main parameters of the study by groups. $n = 51$. Baseline (Pre) and follow-up (Post) values are expressed as mean (X) $\pm$ standard deviation (SD). Mean changes intra-group are expressed as mean change (%). Both for intra-group and inter-groups p -value, paired and unpaired t -student test was used. Reference values were provided from the Virgen de las Nieves Hospital and the Scientific Instrumental Center, Granada: Fol = 2.70–17.0 ng/mL; B₁₂ = 190.0–900.0 pg/mL; Hcy = < 13 μ mol/L. Abbreviations: Zn = Zinc. PG = Placebo Group. ZG = Zinc Group. Fol = Folate. B₁₂ = Vitamin B₁₂. Hcy = Homocysteine.

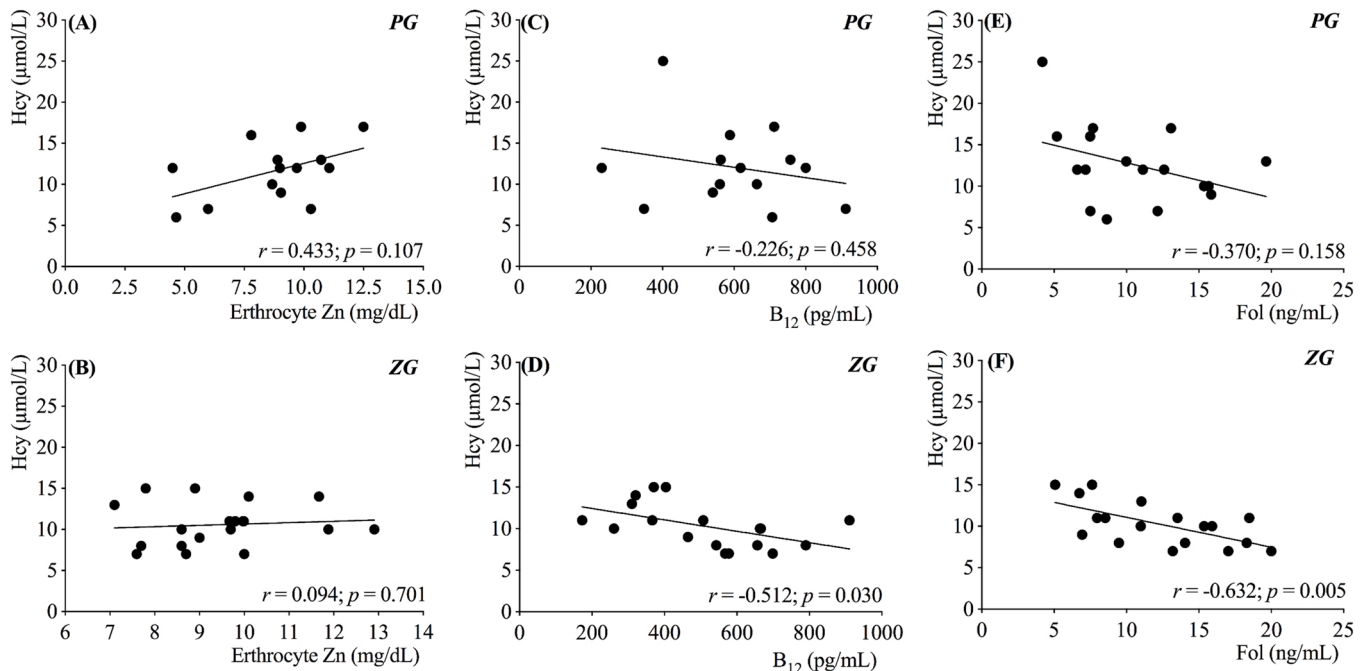


Fig. 2. Bivariate Pearson's correlation coefficients between homocysteine-related parameters of the study by groups. (A) Erythrocyte Zn and Hcy in PG. (B) Erythrocyte Zn and Hcy in ZG. (C) B₁₂ and Hcy in PG. (D) B₁₂ and Hcy in ZG. (E) Fol and Hcy in PG. (F) Fol and Hcy in ZG. Abbreviations: Hcy = Homocysteine. Fol = Folic Acid. B₁₂ = Vitamin B₁₂. PG = Placebo Group. ZG = Zinc Group.

levels, but, regrettably, the intervention had the same effect upon B₁₂ in both groups.

Zn status in humans depends on multiple factors (e.g., gender, age, physiological condition and diet), with Zn intake being the main contributor to Zn levels [20]. In our study, low Zn intake (below 50% of RDA) before the intervention was reported. The main cause of Zn deficiency is inadequate dietary intake, which is common in many parts of the world and is further increased in populations at risk, such as postmenopausal women [21]. Folic acid intake was found to be below two-thirds of RDA in 66% of our postmenopausal population. Some authors have found Fol intake to decrease with age, with inadequate nutrient intake being most notorious in postmenopausal women, with the RDA for Fol not being covered in 75% of the cases [22]. Some studies have reported lower rates of inadequate Fol intake in other European countries, where deficiency was found to range from 17.6% to 45.9% in women older than 64 years [23]. With regard to B₁₂ intake, our results

evidenced sufficient intake, which is not consistent with the above-mentioned study conducted in postmenopausal women, where lower B₁₂ intakes were recorded [22].

Approximately 60% of the postmenopausal population in our study presented Zn deficiency in both the plasma and erythrocyte compartments. In this line, recent studies have found high rates of hypozincemia in elderly women [24]. This tendency seems to be less manifest in women of childbearing age, where lower deficiency rates (30%) have been found compared with the present study [25]. Moreover, our results showed a significant increase in both erythrocyte and plasma Zn levels in ZG after the Zn intervention, reversing most of the initial deficiency seen in both compartments. In this sense, a previous study reported that plasma Zn levels were significantly lower than when postmenopausal subjects were intervened with Zn [26]. Humans respond better to a Zn intervention when Zn status is low, as seen in a study performed in a cohort of Zn-intervened postmenopausal women who were adapted to a

Zn intervention within 8 weeks [27]. It should be noted that plasma Zn is characterized by rapid dynamics, increasing the risk of suffering several pathophysiological alterations in response to numerous conditions [28]. In this line, the regulation of cellular Zn is complex, and may involve several mechanisms to take into account for regulating cellular Zn turnover besides plasma and erythrocyte Zn [29]. Thus, cellular biomarkers such as erythrocyte Zn may not be a sensitive and a reliable marker of Zn status as previously described [30]. Accordingly, the observed increase in plasma and erythrocyte Zn levels, that could reflect genuine improvement of Zn status after Zn supplementation, should be interpreted with caution.

In our study, the plasma Fol values increased significantly in the Zn-supplemented group after the intervention. Published information on the effect of Zn supplementation upon Fol levels is scarce and controversial. The proposed mechanism underlying this increase was described by Manger et al. [31], who suggested that a Zn-deficient state reduces the activity of Fol Zn-dependent conjugase, which is necessary for the absorption of Fol; accordingly, an adequate Zn status could improve the Fol levels. To the best of our knowledge, there have been no Zn supplementation studies in postmenopausal populations assessing changes in Fol levels. A study carried out in an elderly Australian population (65–85 years of age) observed the same significant increase in plasma Fol levels after a 12-week Zn intervention [32]. However, in another study carried out in an elderly population supplemented with Zn during 6 months, the increase in plasma Fol was not accompanied by an increase in erythrocyte Fol. This would suggest that a Zn intervention would only be effective in correcting low plasma Fol levels, which reflect short-term Fol deficiency [33]. On the other hand, it is known that Zn could affect B₁₂ through its effect upon Zn-dependent methionine synthase and therefore on the Hcy cycle also mediated by Fol [31]. In this line, we found the B₁₂ levels to be within the reference ranges, but, regrettably, they increased significantly over follow-up in both ZG and PG in our postmenopausal population. Unfortunately, the limitations of 72-hour recall did not allow us to determine whether the regular diet was responsible for the increase observed in PG [34].

Studies that jointly examine Zn, Hcy, Fol and B₁₂ status in humans are limited. In our study, a negative correlation was found between Hcy and both Fol and B₁₂ levels after the intervention in our Zn-supplemented women. Previous studies involving animal models have found Zn-deficient status in rats to be related to increased Hcy levels and decreased Fol values [35]. Other studies in children and adolescents, involving a 15 mg/day Zn-intervention during three months, found the Hcy and B₁₂ levels to decrease in the Zn-supplemented group, while the Fol values showed no significant changes [15]. Similar findings have been reported by other authors in diabetic Zn-supplemented individuals, with Zn supplementation leading to a significant rise in serum Fol and B₁₂ levels, associated to a decrease in Hcy concentrations [36]. These findings moreover are in agreement with a previous study that evidenced no effect upon the plasma Hcy, B₁₂ and Fol levels after 6 months of Zn intervention (15 mg/day and 30 mg/day) in a population of French healthy elderly individuals [37]. Since we observed these associations in menopausal women, we believe that the lack of significance in the abovementioned studies could be due to the administration of lower Zn supplementation doses in the general older population.

Some limitations must be considered in the interpretation of our findings. Firstly, the sample size, while appropriate for the present study, was small. Future studies involving larger samples are needed to confirm the validity of our results. Secondly, other biomarkers for identifying Zn homeostatic mechanisms triggered by Zn supplementation, such as Zn transporters, should be considered in order to elucidate the underlying processes. Thirdly, more 72-hour recalls should have been performed during the intervention, as this could have helped to elucidate the observed B₁₂ increase in PG. Fourth, the present study included menopausal women with a wide age range, which makes it necessary to interpret the results with caution. Lastly, the Zn intervention period may have been insufficient to evidence more significant

changes in cardiovascular risk factors. We thus suggest the need for Zn supplementation studies involving longer follow-up periods among postmenopausal women.

5. Conclusions

An 8-week Zn intervention reduced the initial high prevalence of Zn deficiency and improved Fol in the postmenopausal Zn-intervened group, together with a decrease in Hcy levels when comparing inter-both intervened groups. The previous mentioned findings, and in accordance to the inverse correlations between the Hcy and Fol levels, could reflect an enhancement of some Hcy cycle-related parameters in the studied population after the Zn intervention, improving circulating Fol and Hcy. Regrettably, the intervention had the same effect in B₁₂ levels for both intervened groups. Further studies involving larger sample sizes and long-term Zn interventions are needed to clarify the possible influence of a prolonged Zn supplementation upon circulating Hcy, B₁₂ and Fol levels in a population at risk of Hcy cycle-related parameters disturbances, such as postmenopausal women.

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CRediT authorship contribution statement

Héctor Vázquez-Lorente: Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Lourdes Herrera-Quintana:** Conceptualization, Investigation, Visualization. **Jorge Molina-López:** Methodology, Software, Data curation, Writing – review & editing, Supervision, Project administration. **Yenifer Gamarra:** Validation. **Elena Planells:** Conceptualization, Software, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Trial registration

The study was registered at the US National Institutes of Health (ClinicalTrials.gov) NCT03672513.

References

- [1] H.D. Nelson, Menopause, *Lancet* 371 (2008) 760–770, [https://doi.org/10.1016/S0140-6736\(08\)60346-3](https://doi.org/10.1016/S0140-6736(08)60346-3).
- [2] S. Paccosi, L. Pala, B. Cresci, A. Silvano, M. Cecchi, R. Caporale, C. Maria Rotella, A. Parenti, Insulin resistance and obesity affect monocyte-derived dendritic cell phenotype and function, *Diabetes Res. Clin. Pract.* 170 (2020), 108528, <https://doi.org/10.1016/j.diabres.2020.108528>.
- [3] M.-K. Sung, U.S. Lee, N.H. Ha, E. Koh, H.-J. Yang, A potential association of meditation with menopausal symptoms and blood chemistry in healthy women: a pilot cross-sectional study, *Medicine* 99 (2020), e22048, <https://doi.org/10.1097/MD.00000000000022048>.

- [4] D. Bruce, J. Rymer, Symptoms of the menopause, *Best Pract. Res. Clin. Obstet. Gynaecol.* 23 (2009) 25–32, <https://doi.org/10.1016/j.bpobgyn.2008.10.002>.
- [5] P.K. Muhamed, S. Vadstrup, Zinc is the most important trace element, *Ugeskr. Laeger.* 176 (2014).
- [6] S. Choi, X. Liu, Z. Pan, Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases, *Acta Pharmacol. Sin.* 39 (2018) 1120–1132, <https://doi.org/10.1038/aps.2018.25>.
- [7] P. Gunasekara, M. Hettiarachchi, C. Liyanage, S. Lekamwasam, Effects of zinc and multimineral vitamin supplementation on glycemic and lipid control in adult diabetes, *Diabetes Metab. Syndr. Obes. Targets Ther.* 4 (2011) 53–60, <https://doi.org/10.2147/DMSO.S16691>.
- [8] M. Foster, S. Samman, Zinc and redox signaling: perturbations associated with cardiovascular disease and diabetes mellitus, *Antioxid. Redox Signal.* 13 (2010) 1549–1573, <https://doi.org/10.1089/ars.2010.3111>.
- [9] I. Guler, O. Himmetoglu, A. Turp, A. Erdem, M. Erdem, M.A. Onan, C. Taskiran, M. Y. Taslipinar, H. Guner, Zinc and homocysteine levels in polycystic ovarian syndrome patients with insulin resistance, *Biol. Trace Elem. Res.* 158 (2014) 297–304, <https://doi.org/10.1007/s12011-014-9941-7>.
- [10] P. Gać, K. Czerwińska, P. Macek, A. Jaremków, G. Mazur, K. Pawlas, R. Poręba, The importance of selenium and zinc deficiency in cardiovascular disorders, *Environ. Toxicol. Pharmacol.* 82 (2021), 103553, <https://doi.org/10.1016/j.etap.2020.103553>.
- [11] M. Khazdouz, S. Djalalinia, S. Sarrafi Zadeh, M. Hasani, F. Shidfar, A. Ataie-Jafari, H. Asayesh, M. Zarei, A.M. Gorabi, M. Noroozi, M. Qorbani, Effects of zinc supplementation on cardiometabolic risk factors: a systematic review and meta-analysis of randomized controlled trials, *Biol. Trace Elem. Res.* 195 (2020) 373–398, <https://doi.org/10.1007/s12011-019-01870-9>.
- [12] H.I. Atasoy, G. Bugdayci, Zinc deficiency and its predictive capacity for anemia: unique model in school children, *Pediatr. Int.* 60 (2018) 703–709, <https://doi.org/10.1111/ped.13603>.
- [13] M.N. Haan, J.W. Miller, A.E. Aiello, R.A. Whitmer, W.J. Jagust, D.M. Mungas, L. H. Allen, R. Green, Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging, *Am. J. Clin. Nutr.* 85 (2007) 511–517, <https://doi.org/10.1093/ajcn/85.2.511>.
- [14] S. González, J.M. Huerta, S. Fernández, A.M. Patterson, C. Lasheras, Homocysteine increases the risk of mortality in elderly individuals, *Br. J. Nutr.* 97 (2007) 1138–1143, <https://doi.org/10.1017/S0007114507691958>.
- [15] I. Sonmez Ozkarakaya, B. Celik, C. Karakukcu, Y. Altuner Torun, Effect of zinc supplementation on hemogram parameters and circulating concentrations of homocysteine, vitamin B12, and folate in zinc-deficient children and adolescents, *J. Trace Elem. Med. Biol.* 65 (2021), 126724, <https://doi.org/10.1016/j.jtemb.2021.126724>.
- [16] K. Grüngreiff, T. Gottstein, D. Reinhold, Zinc deficiency—an independent risk factor in the pathogenesis of haemorrhagic stroke? *Nutrients* 12 (2020) 3548, <https://doi.org/10.3390/nu12113548>.
- [17] H. Vázquez-Lorente, J. Molina-López, L. Herrera-Quintana, Y. Gamarra-Morales, B. López-González, E. Planells, Association between body fatness and vitamin D3 status in a postmenopausal population, *Nutrients* 12 (2020) 667, <https://doi.org/10.3390/nu12030667>.
- [18] Ingestas Dietéticas de Referencia (IDR) para la Población Española, *Act. Diet.*, 14, 2010, pp. 196–197. ([https://doi.org/10.1016/S1138-0322\(10\)70039-0](https://doi.org/10.1016/S1138-0322(10)70039-0)).
- [19] H. Vázquez-Lorente, J. Molina-López, L. Herrera-Quintana, J. Gamarra-Morales, B. López-González, E. Planells, Effectiveness of eight-week zinc supplementation on vitamin D3 status and leptin levels in a population of postmenopausal women: a double-blind randomized trial, *J. Trace Elem. Med. Biol.* 65 (2021), 126730, <https://doi.org/10.1016/j.jtemb.2021.126730>.
- [20] Z.S. Lassi, J. Kurji, C.S. de Oliveira, A. Moin, Z.A. Bhutta, Zinc supplementation for the promotion of growth and prevention of infections in infants less than six months of age, *Cochrane Database Syst. Rev.* 2020 (2020), <https://doi.org/10.1002/14651858.CD010205.pub2>.
- [21] M. Nasiadek, J. Stragierowicz, M. Klimczak, A. Kilanowicz, The role of zinc in selected female reproductive system disorders, *Nutrients* 12 (2020) 2464, <https://doi.org/10.3390/nu12082464>.
- [22] Y. Manios, G. Moschonis, C. Mavrogianni, R. Bos, C. Singh-Povel, Micronutrient intakes among children and adults in Greece: the role of age, sex and socio-economic status, *Nutrients* 6 (2014) 4073–4092, <https://doi.org/10.3390/nu6104073>.
- [23] B. Roman Viñas, L. Ribas Barba, J. Ngo, M. Gurinovic, R. Novakovic, A. Cavelaars, L.C.P.G.M. de Groot, P. van't Veer, C. Matthys, L. Serra Majem, Projected prevalence of inadequate nutrient intakes in Europe, *Ann. Nutr. Metab.* 59 (2011) 84–95, <https://doi.org/10.1159/000332762>.
- [24] D. Asaoka, T. Takeda, Y. Inami, D. Abe, Y. Shimada, K. Matsumoto, H. Ueyama, K. Matsumoto, H. Komori, Y. Akazawa, T. Osada, M. Hojo, A. Nagahara, The association between frailty and abdominal symptoms: a hospital-based cross-sectional study, *Intern. Med.* 59 (2020) 1677–1685, <https://doi.org/10.2169/internalmedicine.4399-19>.
- [25] A.K.M. Brazier, N.M. Lowe, M. Zaman, B. Shahzad, H. Ohly, H.J. McArdle, U. Ullah, M.R. Broadley, E.H. Bailey, S.D. Young, S. Tishkovskaya, M.J. Khan, Micronutrient status and dietary diversity of women of reproductive age in rural Pakistan, *Nutrients* 12 (2020), E3407, <https://doi.org/10.3390/nu12113407>.
- [26] C.D. Davis, D.B. Milne, F.H. Nielsen, Changes in dietary zinc and copper affect zinc-status indicators of postmenopausal women, notably, extracellular superoxide dismutase and amyloid precursor proteins, *Am. J. Clin. Nutr.* 71 (2000) 781–788, <https://doi.org/10.1093/ajcn/71.3.781>.
- [27] J.M. Beiseigel, L.M. Klevay, L.K. Johnson, J.R. Hunt, Zinc absorption adapts to zinc supplementation in postmenopausal women, *J. Am. Coll. Nutr.* 28 (2009) 177–183, <https://doi.org/10.1080/07315724.2009.10719769>.
- [28] F. Ennes Dourado Ferro, V.B. de Sousa Lima, N.R. Mello Soares, S. Ma Franciscato Cozzolino, D. do Nascimento Marreiro, Biomarkers of metabolic syndrome and its relationship with the zinc nutritional status in obese women, *Nutr. Hosp.* 26 (2011) 650–654.
- [29] N.M. Lowe, K. Fekete, T. Decsi, Methods of assessment of zinc status in humans: a systematic review, *Am. J. Clin. Nutr.* 89 (2009) 2040S–2051S, <https://doi.org/10.3945/ajcn.2009.27230G>.
- [30] J.C. King, K.H. Brown, R.S. Gibson, N.F. Krebs, N.M. Lowe, J.H. Siekmann, D. J. Raiten, Biomarkers of nutrition for development (BOND)-zinc review, *J. Nutr.* 146 (2016) 1437, <https://doi.org/10.3945/jn.115.220079>.
- [31] M.S. Manger, T.A. Strand, S. Taneja, H. Refsum, P.M. Ueland, O. Nygård, J. Schneede, H. Sommerfeldt, N. Bhandari, Cobalamin status modifies the effect of zinc supplementation on the incidence of prolonged diarrhea in 6- to 30-month-old North Indian children, *J. Nutr.* 141 (2011) 1108–1113, <https://doi.org/10.3945/jn.110.127415>.
- [32] R. Sharif, P. Thomas, P. Zalewski, M. Fenech, Zinc supplementation influences genomic stability biomarkers, antioxidant activity, and zinc transporter genes in an elderly Australian population with low zinc status, *Mol. Nutr. Food Res.* 59 (2015) 1200–1212, <https://doi.org/10.1002/mnfr.201400784>.
- [33] F. Intorre, A. Polito, M. Andriollo-Sanchez, E. Azzini, A. Raguzzini, E. Toti, M. Zaccaria, G. Catasta, N. Meunier, V. Ducros, J.M. O'Connor, C. Coudray, A. M. Roussel, G. Maiani, Effect of zinc supplementation on vitamin status of middle-aged and older European adults: the ZENITH study, *Eur. J. Clin. Nutr.* 62 (2008) 1215–1223, <https://doi.org/10.1038/sj.ejcn.1602844>.
- [34] Gemma Salvador Castell, Lluís Serra Majem, Lourdes Ribas-Barba, ¿Qué y cuánto comemos? El método Recuerdo de 24 horas, *Rev. Esp. Nutr. Comunitaria* (2015) 42–44, <https://doi.org/10.14642/RENC.2015.21.sup1.5049>.
- [35] M. Jing, L. Rech, Y. Wu, D. Goltz, C.G. Taylor, J.D. House, Effects of zinc deficiency and zinc supplementation on homocysteine levels and related enzyme expression in rats, *J. Trace Elem. Med. Biol.* 30 (2015) 77–82, <https://doi.org/10.1016/j.jtemb.2014.10.013>.
- [36] E. Heidarian, M. Amini, M. Parham, A. Aminoroaya, Effect of zinc supplementation on serum homocysteine in type 2 diabetic patients with microalbuminuria, *Rev. Diabet. Stud. RDS* 6 (2009) 64–70, <https://doi.org/10.1900/RDS.2009.6.64>.
- [37] V. Ducros, M. Andriollo-Sanchez, J. Arnaud, N. Meunier, F. Laporte, I. Hininger-Favier, C. Coudray, M. Ferry, A.-M. Roussel, Zinc supplementation does not alter plasma homocysteine, vitamin B12 and red blood cell folate concentrations in French elderly subjects, *J. Trace Elem. Med. Biol.* 23 (2009) 15–20, <https://doi.org/10.1016/j.jtemb.2008.08.003>.