CLIMACTERIC 2015;18:1–8

Hormones, herbal preparations and nutriceuticals for a better life after the menopause: part II

*F. H. Comhaire and H. T. Depypere**

Department of Endocrinology, and *Menopause and Breast Clinic, University Hospital, Gent, Belgium

Key words: MENOPAUSE, HORMONES, HERBAL PREPARATIONS, NUTRICEUTICALS

ABSTRACT

Long-term estrogen replacement therapy with estrogen has benefits for many postmenopausal women. However, some women prefer non-steroidal substitution with herbal preparations. The effectivity against vasomotor symptoms has been evidenced for the extracts of pine bark, of linseed and of *Lepidium meyenii* (Maca), whereas there is controversy about the effectiveness of genistein-rich soy extract. The extracts of cruciferous vegetables such as Broccoli and of linseed induce changes in the metabolism of estrogens, and antioxidants may reverse altered epigenetic DNA methylation, possibly reducing the risk of breast cancer or its recurrence. Indirect evidence from the literature and from clinical trials supports that a nutriceutical composed of plant extracts, low-dose vitamins and minerals may improve the quality of life by delaying certain age-related diseases. On the basis of epidemiologic studies, physiopathological considerations and controlled prospectieve trials, it is suggested that transdermal substitution therapy with estradiol together with nutriceutical food supplementation may increase the number of quality-adjusted life years of postmenopausal women, but complementary, large-scale, prospective trials are still needed.

INTRODUCTION

There is no doubt that hormone replacement therapy (HRT) with estrogen is successful in alleviating menopausal symptoms and preventing menopause-associated health problems. A decade ago, the Women's Health Initiative (WHI) published an alarming report regarding possible health risks of HRT[1]. Although this conclusion has repeatedly been questioned from the methodological point of view[2–4] and overturned by the outcome of follow-up studies[5,6], many women request 'natural products' to alleviate their symptoms. Several herbal preparations and over-the-counter nutriceuticals have been recommended because of their phytoestrogenic and alleged health-promoting properties[7].

The present article aims to quantify and critically review the benefits and undesired side-effects, the capacity to prevent cancer and to delay age-related diseases, and the potential health risks of commonly used phytoestrogens and of nutriceutical food supplementation.

MATERIALS AND METHODS

An extensive literature search was performed using the Pubmed system, Google scientific and derived articles. The papers were critically reviewed by analyzing the evidence of possible effectiveness regarding menopausal symptoms and women's health. Whenever possible, the number needed to treat (NNT) was calculated based on the result of controlled trials (see Part I[8]). This review examines present knowledge on the use of herbal preparations and of a specific nutriceutical.

RESULTS

Phytotherapy

Soy isoflavones

Soy extracts are the most commonly used plant extracts and contain the isoflavones genistein and daidzein. These bind to

Correspondence: Professor Emeritus F. H. Comhaire, Brakelmeersstraat 18, 9830 Sint Martens-Latem, Belgium; E-mail: Frank@comhaire.com

REVIEW

DOI: 10.3109/13697137.2014.985646

Received 27-05-2014
Revised 25-09-2014
Accepted 05-11-2014

the human estrogen receptors, but their estrogenic effect is weak.

In meta-analyses[9,10], it was concluded that extracted soy isoflavones, particularly genistein, were effective in treating vasomotor complaints, although the results of some placebo-controlled trials are contradictory[11,12]. However, no significant correlation was found between the intake of food rich in fibers and phytoestrogens, on the one hand, and the prevention of menopausal complaints, on the other hand[13].

In a study of 150 postmenopausal women, we have evidenced important inter-individual variations regarding the intestinal uptake and metabolization of the soy isoflavones. The gut bacterial flora and dietary factors[14] play an important role in the metabolization of the soy isoflavones into S-equol[15]. Women with an adequate intestinal bacterial flora, and who were able sufficiently to produce S-equol, were found to experience significant benefit from soy isoflavone intake with reduced vasomotor symptoms. Among women who were poor equol-producers, a favorable effect was detected when taking a fermented dadzein derivative rich in natural S-equol[16]. It remains to be proven whether the addition of certain probiotics to soy extract does enhance the intestinal metabolization to S-equol *in vivo*, similar to what was observed in the SHIME model (Simulator of the Gastrointestinal Microbial Ecosystem)[17].

During the reproductive period of life, the isoflavones may reduce the risk of breast cancer, since they inhibit the binding of the more potent estrogen 17α-estradiol to the estrogen receptor, thus exerting an effect that has been compared with that of pharmacological selective estrogen receptor modulators (SERMs)[18]. Protection against breast cancer has indeed been observed in Asian women[19], and the protective effect of long-term soy isoflavone supplementation in the western population is claimed in some publications[20,21] but rejected by others[22]. After menopause, the concentration of endogenously produced estrogens is extremely low, and the soy isoflavones exert an intrinsic estrogenic action. However, the long-term intake of extracted soy isoflavones did not increase the mammographic density[23].

Whether it is safe to give soy isoflavones to women who have been treated for estrogen-sensitive breast cancer remains a topic of debate[21,24]. Contradictory results have been published regarding the concentration of genistein, daidzein and equol in breast tissue of women who had taken soy supplementation during 5 days. *In vitro* studies suggest that genistein may counteract the antiestrogenic effect of tamoxifen[25], but no such effect was observed *in vivo*[26].

Estradiol is converted by the enzymes cytochrome-oxidase in the liver, primarily into 2-hydroxy, 4-hydroxy-en and 16α-hydroxy-estrogens (Figure 1). The 2-OH-estrogens exert virtually no hormonal effect. In contrast, the 16α-OH-estrogens are highly estrogenic and are considered oncogenic, since they may increase the risk of estrogen-dependent breast cancer. Also, one of the oxidized metabolites of 4-OH-estrogens, namely the 3,4-O-quinone, can interact with DNA, inducing depurination and mutagenesis[27,28].

Endocrine-disrupting compounds, particularly man-made xeno-estrogens present in the environment, such as phthalates, alkylphenols, methylparabene, bisphenol-A, polychlorinated bisphenyls, and certain pesticides influence the metabolic pathway of endogenous estrogens favoring the production of the oncogenic 16α-OH estrogens[29]. Several prospective controlled trials have shown soy isoflavones to correct this process by redirecting the pathway to the hormonally inactive 2-OH-estrogens. Also, in mice, genistein was shown to counteract the hypomethylating effect of bisphenol-A on metastable epialleles during development[30]. Both the findings on estrogen metabolism and on epigenetic effects may explain the alleged protective effects of soy isoflavones against breast cancer.

There are data to sustain that soy intake may temporarily reduce or delay the occurrence of osteoporosis[31], but controlled studies on the prevalence of bone fractures are lacking. The dosage and duration of consumption probably determine the alleged effect of soy consumption on cognition[32]. Though genistein induces epigenic modifications that stabilize genes involved with colon carcinogenesis[33] and estrogen intake was shown to reduce age-related DNA-methylation in colon mucosa[34], the possible role of soy isoflavones in the prevention of colon cancer and its metastasis awaits confirmation[35].

Cimicifuga racemosa

Extracts of *Cimicifuga racemosa* ('black cohosh') are commonly recommended for the phyto-therapy of menopausal symptoms. Randomized, placebo-controlled trials have not revealed any statistically significant effect on vasomotor complaints compared to placebo[36]. Recently, contradictory reports have been published regarding possible hepatotoxic damage in relation to the use of black cohosh[37,38]. After having received a large number of case reports, the Medicines and Healthcare Products Regulatory Agency has obliged the distributers of black cohosh to include a warning that liver damage may occur, such as abnormal liver enzymes in blood, jaundice and hepatitis[39]. In addition, black cohosh has been suspected to increase the risk of cardiovascular disease[40].

Vitex agnus-castus

The fruit-oil of the *Vitex agnus-castus* has been used in natural medicine for the treatment of the premenstrual syndrome. Its binding to the estrogen receptor is attributed to the presence of linoleic acid which induces certain estrogen-dependent genes. This substance seems to exert some dopaminergic action, but a favorable effect on menopausal symptoms could not be evidenced in a randomized, controlled trial, also when combined with the extract of *Hypericum perforatum* (St John's Wort)[41].

Hopflavonoides

The most important flavonoid extracted from hop (*Humulus lupulus*) is 8-pregnylnaringenin (8-PN). *In vitro* this molecule

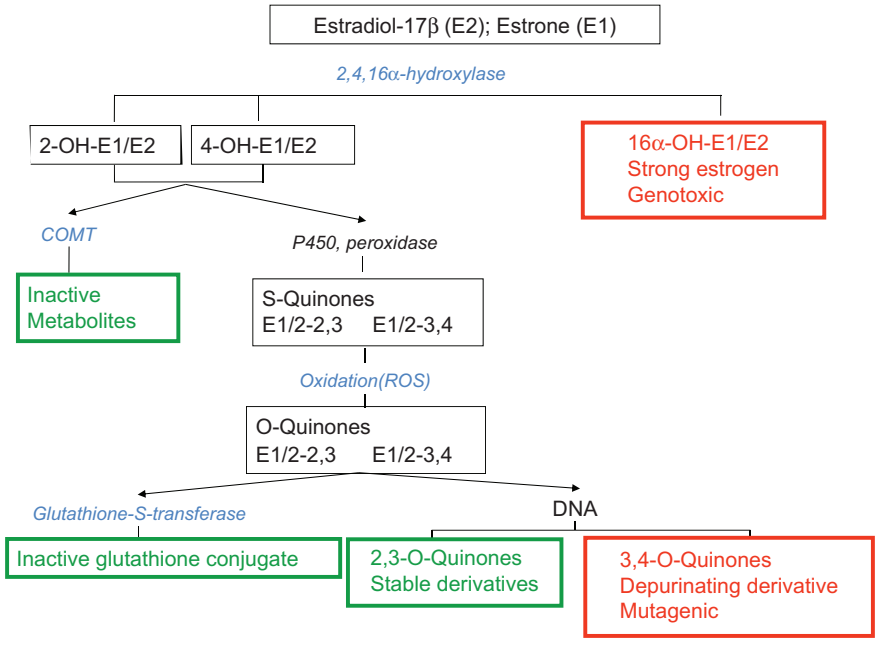

Figure 1 The hydroxylase iso-enzymes metabolize estradiol (E2) and estrone (E1) into water-soluble hydroxy-estradiol and hydroxy-estrone. There are three different *hydroxylases* that interact at either the position 2, or 4, or 16α of the estradiol/estrone. The 16α-hydroxy-estradiol/estrones (16α-OH-E1/E2) exert a strong estrogenic action. They induce cell division and are genotoxic, hence are considered cancerogenic. The 2- and 4-hydroxy-estrogens (2-OH-E1/E2, 4-OH-E1/E2) can either be metabolized by the enzyme *Cathechol-O-methyltransferase* (COMT) into inactive metabolites, or by *Cytochrome P450* or *peroxidase* being converted into their respective S-quinones: the 2,3 S-quinone for the 2-hydroxy E1/E2, and the 3,4 S-quinone for the 4-hydroxy E1/E2. The S-quinones are non-enzymatically oxidized by *reactive oxygen species* (ROS) into their respective O-quinones. Both these O-quinones can be inactivated by the enzyme *Glutathione-S-transferase* into inactive glutathione conjugates which are devoid of estrogenic effect. However, both O-quinones can also bind to DNA. The binding of the 2,3-O-quinone to DNA generates stable adducts that do not induce genetic risks. The binding of the 3,4-O-quinones of estradiol and estrone to DNA induces depurination of particular DNA-nucleotides. These derivatives can cause strand breaks of the DNA and mutations in the daughter cells after replication, which may be cancerogenic

has an up to 100-fold stronger estrogenic action than the soy isoflavones[42]. There is some evidence that 8-PN may be efficacious against vasomotor complaints. However, two randomized, controlled trials come to contradictory results. Indeed, Heyerick and colleagues[43] recorded a significant benefit of 8-PN during the first 12 weeks of intake. Though this benefit remained present after 12 weeks, it was not significantly better than that of placebo. In contrast, Erkkola and colleagues[44] did not find a significant advantage of 8-PN over placebo in the first 16 weeks of treatment, while after this time period the difference became significant as a result of the decreasing effect of placebo.

In vitro 8-NP reduced the malignant transformation of MCF-10A cells and the production of the oncogenic estrogen-O-quinone[45]. However, it is questionable whether the concentration of 8-PN in breast tissue, after the daily intake of 100 µg[46], is sufficient to exert any local chemoprotective effect against breast cancer[47].

Dioscorea villosa

Diosgenin is the saponine extracted from the *Dioscorea villosa* (wild yam). We have shown that diosgenin does not bind to the human estrogen receptor *in vitro*[48], and it cannot be converted into progesterone. Dioscorea may be given to women who have been treated for breast cancer. A significant favorable effect of Dioscorea extract on menopausal symptoms has been observed, but it seems to be rather weak[49]. Dioscorea extract decreases the estrogen metabolization into the genotoxic metabolite 16α-OH-estrone.

In animal experiments, diosgenin was found to exert an anti-osteoporotic effect by appositional growth to the long bones[50], possibly through the inhibition of osteoclast formation. However, studies on a possible bone-protecting effect in humans are lacking.

Linum usitatissimum

Linseed or flaxseed (*Linum usitatissimum*) extract is a rich source of lignans, which are converted into enterodiol and enterolacton by the gut bacteria. These lignans exert a weak estrogenic effect. Linseed extract was moderately effective on menopausal vasomotor symptoms[51], and its effectiveness compared to placebo was confirmed in one randomized trial, but not in another trial[52].

In a controlled, parallel-design trial in 46 postmenopausal women, linseed extract increased the 2-OH/16-OH-estrogen ratio significantly more effectively than the soy isoflavones[53]. In addition, enterolacton inhibits the aromatase enzyme, so that less estradiol is produced intracellularly from the precursors testosterone and dehydroepiandrosterone[54]. Several publications suggest that the intake of linseed extracts may protect against hormone-dependent breast cancer[55], but this could not be confirmed in a recent population study[56]. Lignans were reported to increase the time of survival of a cohort of breast cancer patients[57]. Also, dietary lignans may have the potential to protect against cardiovascular risk[58].

Pine bark extract

The extract of the bark of the (Mediterranean) pine tree is rich in polyphenols, namely anthocyanidins with antioxidant effect, and it reduces inflammatory reaction through the inhibition of the cyclo-oxygenase enzymes 1 and 2[59] and of the nuclear factor kappa B[60]. In several randomized, double-blind trials, pine bark extract was found to significantly reduce vasomotor symptoms and to improve the quality of life of menopausal women[61]. Pine bark extract is a rich source of polyphenols[62], the higher intake of which was found to be associated with a 46% decreased risk of atherosclerotic vascular disease[63] and to reduced overall mortality (odds ratio 0.63, 95% confidence interval 0.41–0.97) after an average of 4.8 years follow-up in 7447 participants of a parallel-group, randomized, multicenter, controlled trial[64]. On the basis of the life-table survival curve published in this paper, it can be derived that the persons belonging to the highest quartile of polyphenol intake survived 1.5 years longer than those belonging to the group with the lowest intake[64].

Cruciferous vegetable

The extract of several plants of the *Brassica* species, including Broccoli, Brussels sprouts, cabbage and savoy, contains glycosylates which are hydrolyzed in the body into indol-3-carbinol and di-indolmethane. These constituents are under investigation in clinical trials for their chemoprotective effects against gynecological cancers[65]. They activate the P450 enzyme and promote estrogen metabolism toward the 2-OH-estrogens[66]. Also, Broccoli extract is an important source of sulforafane that has a demethylating effect, favorably influencing the epigenoma[67]. Meta-analysis of epidemiologic studies concluded that the consumption of cruciferous vegetables was inversely associated with the risk of (breast) cancer[68]. The extract contains phylloquinone, also called vitamin K1, which has been reported to reduce the risk of bone fractures in aging women and to slow the progression of coronary artery calcium deposits[69]. Although Brassica extract does not reduce menopausal symptoms, the benefit of its moderate consumption is considered to outweigh the potential risks.

Lepidium meyenii

Lepidium meyenii, also called Maca, is another plant belonging to the cruciferous (Brassica) family and grows in the Peruvian Andes mountains. It is considered to be a phyto-adaptogen which increases the production of the heat shock protein P 72, thus reducing the negative impact of stress[70] on protein conformation and cell death. *Lepidium meyenii* has been tested against menopausal symptoms and was found to reduce the Kupperman menopausal index and the Greene Climacteric Score in a controlled trial[49]. Tolerability is excellent and no serious adverse effects have been noted.

Food supplementation and nutraceuticals

Nutraceuticals are over-the-counter food supplements composed of natural substances such as minerals, vitamins and herbal extracts. The amount of multivitamins may not significantly exceed the recommended daily intake, and the combination of substances with complementary antioxidant effects is expected to act in synergism because some are lipophilic (such as the tocopherols), others are hydrophilic (such as vitamin C), some act as reducing agents and others as oxygen-radical scavengers[71]. Neither vitamins nor nutritional food supplementation had immediate or long-term risk of adverse effects[72,73]. Their role and importance have been acknowledged by the World Health Organization[74]. Nutraceuticals may delay the effects of aging and this will cause substantial economic returns and improve public health[75].

The nutraceutical discussed below has been formulated specifically for menopausal women and aims at attenuating the signs and symptoms of the menopause and at preventing or delaying aging and the occurrence of age-related diseases as well as of certain cancers.

The nutraceutical food supplement is composed of the following constituents (Nutriphyt Ltd, Oostkamp, Belgium): Vitamin B6 0.5 mg; vitamin B9 100 μg; vitamin B12 1.5 μg; vitamin C 15 mg; vitamin E 15 mg; lipoic acid 25 mg; glutathione 7.5 mg; selenium methionine 10 mg (= 50 μg Se); co-enzyme Q10 7.5 mg; zinc glycinate 50 mg (= 10 mg Zn); phosphatidyl choline/serine 50 mg; Pycnogenol® 10 mg; *Lepidium meyenii* extract (root) 75 mg; Linum extract (seeds) 20 mg.

Low doses of several antioxidants are presumed to act in synergism, e.g. natural vitamin E is composed of the eight isomers of tocopherol and tocotrienol. It is a lipophylic scavenger and is regenerated by vitamin C, which is a hydrophylic reducing agent. Both vitamin E[76] and alpha lipoic acid, which is both lipo- and hydrophylic, may mitigate bone loss in osteopenic postmenopausal women[77], and the latter also exerts a neuroprotective effect[78]. The anti-oxidant ubiquinone Q10 is active at the level of the mitochondria, improving energy production, and it may be beneficial in preventing coronary heart disease[79]. Glutathione is known for its complementary detoxifying effect via glutathione-S-transferase and glutathione peroxidase. Thus, glutathione may facilitate the elimination of xeno-estrogens, and it favorably influences estrogen

metabolism (Figure 1). Selenium methionine acts as a protein antioxidant, and higher selenium levels have been associated with higher bone mineral density in population studies[80]. Zinc is part of many enzymes playing a pivotal role in cell function. In association with multivitamin supplementation, long-term supplementation with zinc was found to protect women from developing breast cancer[81].

The herbal preparations from *Lepidium meyenii* (Maca), pine bark, linseed, Broccoli, and *Discorea villosa* have been described above.

The nebulized extract of *Vinca minor* has been reported to increase blood circulation, glucose uptake, and metabolism of the brain. This extract, together with phosphatidyl serine/choline, which is incorporated in the outer cell membrane of Schwann cells, improves brain function[82] by optimizing the composition of the axonemal myelin sheath[83].

The association of vitamins B6 (pyridoxin), B9 (folic acid), and B12 (cyanocobalamine) reduces the production of homocysteine[84] and DNA methylation, but there are contradictory reports regarding the protection against cognitive decline[85,86] or vascular disease[87,88]. Recently, it has been documented that the regular intake of these vitamins B prevents brain shrinkage and the resulting dementia among women with a high homocysteine concentration in blood[89]. The association of vitamins B may also reduce the incidence of bone fractures.

It is generally recommended to complement nutrition with long-chain polyunsaturated fatty acids of the ω-3 group (docosahexaënoic acid or cervonic acid, DHA, and eicosapentaënoic acid, EPA) present in fish oil and krill oil. These fatty acids improve cell membrane fluidity and function, may possibly protect against cardiovascular diseases[90,91], reduce the risk of thromboembolism[92], improve cognitive performance and the metabolic syndrome[93] and general health[94], and reduce total mortality[95], though perhaps not in patients suffering from diabetes.

Nutraceuticals and multivitamins may exert a chemoprotective effect against cancer through epigenetic mechanisms[96] as well as against certain age-related diseases[97], whilst being devoid of any toxic side-effects. In fact, a significant decrease by up to 12% of cancers, other than prostate cancer, was observed in a double-blind, prospective trial in general practitioners, after an average of 11.2 years' use of a low-dose multivitamin supplement[98] (NNT: 8.3). From published life-table curves[64,98], it can be calculated that between 1 and 1.5 quality-adjusted life years can be gained after 5–10 years' intake of polyvitamins or polyphenols.

It should be emphasized that the impeccable quality of food supplements must be carefully monitored. Also, certain food supplements containing high doses of artificial vitamins and analogs, such as alfa-tocoferol succinate[99], or ascorbic acid[100], or retinol[101,102], or pro-oxidant metals such as iron[103] have been documented not to cause any beneficial effects, and may even exert a negative influence on general health and increase mortality[104].

CONCLUSION

The best treatment of menopausal complaints, and to prevent osteoporosis, is with estradiol given via the transdermal route. Food supplements containing phytoestrogens from plant extracts improve vasomotor symptoms in between one- and two-thirds of women, but some controlled trials suggest that a similar effect is obtained with placebo. Women with equol-producing gut bacterial flora seem to benefit from soy isoflavones. Also, the extracts of *Lepidium meyenii*, of (Mediterranian) pine bark or linseed were proven effective. Whereas *Cimicifuga racemosa* (black cohosh) extract may be toxic for the liver, the extracts of hop, wild yam, *Dioscorea villosa*, pine bark, and soy are safe. Whether some of these extracts and lignanes from linseed can protect against breast cancer and osteoporosis remains to be firmly established.

The long-term intake of a judiciously composed nutriceutical containing low-dose vitamins, antioxidants, minerals and particular herbal preparations seems justified for older persons who take medication[105], or who consume an unbalanced diet[106], or who are exposed to environmental toxins. Recent reports suggest these nutriceuticals may delay age-related diseases[107], reduce mortality, and lower the risk of cancer. It is suggested for postmenopausal women to combine transdermal estrogen substitution with the regular intake of the nutriceutical, which should result in an increase of at least one quality-adjusted life-year.

Conflict of interest The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

Source of funding Nil.

References

1. Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women. Pricipal results from the Women's Health Initiative randomized controlled trial. *JAMA* 2002;288:321–33
2. Burger HG, Maclennan AH, Huang KE, Castelo-Branco C. Evidence-based assessment of the impact of the WHI on women's health. *Climacteric* 2012;15:381–7
3. Gompel A, Santen RJ. Hormone therapy and breast cancer risk 10 years after the WHI. *Climacteric* 2012;15:241–9
4. Sood R, Faubion SS, Kuhle CL, Thiele JM, Shuster LT. Prescribing menopausal hormone therapy: an evidence-base approach. *Int J Womens Health* 2014;6:47–57
5. Anderson GL, Chlebowski RT, Aragaki AK, *et al*. Conjugated equine oestrogen and breast cancer incidence and mortality in

postmenopausal women with hysterectomy: extended follow-up of Women's Health Initiative randomised placebo-controlled trial. *Lancet Oncol* 2012;13:476–86
6. LaCroix AZ, Chlebowski RT, Manson JE, *et al*. Health outcomes after stopping conjugated estrogens among postmenopausal women with prior hysterectomy: a randomized controlled trial. *JAMA* 2011;305:1305–14
7. Depypere H, Comhaire F. Herbal preparations for the menopause: Beyond isoflavones and black cohosh. *Maturitas* 2014;77:191–4
8. Comhaire FH, Depypere HT. Hormones, herbal preparations and nutriceuticals for a better life after the menopause: part I. *Climacteric* 2015 Epub ahead of print
9. Taku K, Melby MK, Kronenberg F, Kurzer MS, Messina M. Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: systematic review and meta-analysis of randomized controlled trials. *Menopause* 2012;19:776–90
10. Lethaby A, Marjoribanks J, Kronenberg F, *et al*. Phytoestrogens for menopausal vasomotor symptoms. *Cochrane Database Syst Rev* 2013 dec 10;12:CD 001395.pub4
11. Nikander E, Kikkinen A, Metsä-Heikkilä M, *et al.* A randomized placebo-controlled crossover trial with phytoestrogens in the treatment of menopause in breast cancer patients. *Obstet Gynecol* 2003;101:1213–20
12. Cancelo Hidalgo MJ, Castelo Branco C. Optimizing soy isoflavones effect in postmenopausal women: the impact of timing on climacteric symptoms. *Gynecol Endocrinol* 2011;27:696–700
13. Gold EB, Leung K, Crawford SL, *et al*. Phytoestrogen and fiber intakes in relation to incident vasomotor symptoms: results from the Study of Women's Health Across the Nation. *Menopause* 2013;20:305–14
14. Bolca S, Possemiers S, Herregat A, *et al*. Microbial and dietary factors are associated with the equol producer phenotype in healthy postmenopausal women. *J Nutr* 2007;137:2242–6
15. Setchell KD, Clerici C, Lephart ED, *et al*. S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am J Clin Nutr* 2005;81:1072–9
16. Aso T, Uchiyama S, Matsumura Y, *et al*. A natural S-(-) equol supplement alleviates hot flushes and other menopausal symptoms in equol nonproducing postmenopausal japanese women. *J Womens Health (Larchmt)* 2012;21:92–100
17. Decroos K, Eeckhaut E, Possemiers S, Verstraete W. Administration of equol-producing bacteria alters the equol production status in the Simulator of the Gastrointestinal Microbial Ecosystem (SHIME). *J Nutr* 2006;136:946–52
18. Qin W, Zhu W, Shi H, *et al*. Soy isoflavones have antiestrogenic effect and alter mammary promoter hypermethylation in healthy premenopausal women. *Nutr Cancer* 2009;61:238–44
19. Wada K, Nakamura K, Tamai Y, *et al*. Soy isoflavone intake and breast cancer risk in Japan: from the Takayama study. *Int J Cancer* 2013;133:952–60
20. Steinberg FM, Murray MJ, Lewis RD, *et al*. Clinical outcomes of a 2-y soy isoflavone supplementation in menopausal women. *Am J Clin Nutr* 2011;93:356–67
21. Nechuta SJ, Caan BJ, Chen WY, *et al*. Soy food intake after diagnosis of breast cancer and survival: an in-depth analysis of combined evidence from cohort studies of US and Chinese women. *Am J Clin Nutr* 2012;96:123–32
22. Cassidy A, Albertazzi P, Lise-Nielsen I, *et al*. Critical review of health effects of soybean phyto-oestrogens in post-menopausal women. *Proc Nutr Soc* 2006;65:76–92
23. Delmanto A, Nahas-Neto J, Traiman P, *et al*. Effects of soy isoflavones on mammographic density and breast parenchyma in postmenopausal women: a randomized, double blind, placebo-controlled clinical trial. *Menopause* 2013;20:1049–54
24. Khan SA, Chatterton RT, Michel N, *et al*. Soy isoflavone supplementation for breast cancer risk reduction: a randomised phase II trial. *Cancer Prev Res (Phila)* 2012;5:309–19
25. Helferich WG, Andrade JE, Hoagland MS. Phytoestrogens and breast cancer: a complex story. *Inflammopharmacology* 2008;16:219–26
26. Guha N, Kwan ML, Quesenberry CP, Weltzien EK, Castillo AL, Caan BJ. Soy isoflavones and risk of cancer recurrence in a cohort of breast cancer survivors: the Life After Cancer Epidemiology study. *Breast Cancer Res Treat* 2009;118:395–405
27. Fuhrman BJ, Schairer C, Gail MH, *et al*. Estrogen metabolism and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 2012;104:326–39
28. Falk RT, Brinton LA, Dorgan JF, *et al*. Relationship of serum estrogens and estrogen metabolites to postmenopausal breast cancer risk: a nested case-control study. *Breast Cancer Res* 2013;15:R34
29. Bradlow HL, Davis DL, Lin G, Sepkovic D, Tiwari R. Effects of pesticides on the ratio of 16 alpha/2-hydoxyestrone: a biologic marker of breast cancer risk. *Environ Health Perspect* 1995;103(Suppl 7):147–50
30. Bernal AJ, Jirtle RL. Epigenomic disruption: the effects of early development exposures. *Birth Defects Res A Clin Mol Teratol* 2010;88:938–44
31. 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; 146:839–47
32. Soni M, Rahardjo TB, Soekardi R, *et al.* Phytoestrogens and cognitive function: a review. *Maturitas* 2014;77:209–20
33. Zang Y, Li Q, Chen H. DNA methylation and histone modifications of Wny genes by genistein during colon cancer development. *Carcinogenesis* 2013;34:1756–63
34. Noreen F, Röösli M, Gaj P, *et al*. Modulation of age- and cancer-associated DNA methylation change in the healthy colon by aspirin and lifestyle. *J Natl Cancer Inst* 2014;106. Pii:dju161
35. Qi W, Weber CR, Wasland K, Savkovic SD. Genistein inhibits proliferation of colon cancer cells by attenuating a negative effect of epidermal growth factor on tumor suppressor FOX03 activity. *BMC Cancer* 2011;11:219
36. Leach MJ, Moore V. Black cohosh (Cimicifuga spp.) for menopausal symptoms. *Cochrane Database Syst Rev* 2012 Sept 12;9:CD007244
37. Guzman G, Kallwitz ER, Wojewoda C, *et al*. Liver injury with features mimicking autoimmune hepatitis following the use of Black Cohosh. *Case Report Med* 2009:918156
38. Teschke R, Schwarzenboeck A, Schmidt-Taenzer W, *et al*. Herb induced liver injury presumably caused by black cohosh: a survey of initially purported cases and herbal quality specifications. *Ann Hepatol* 2011;10:249–59
39. Medicines and Healthcare products Regulatory Agency (MHRA). Liver failure case highlights need to use Black Cohosh remedies carefully. MHRA press release; 29 october, 2012. press.office@mhra.psi.gov.uk
40. Bebenek M, Kemmler W, van Stengel S, *et al.* Effect of exercise and Cimicifuga racemosa (CR BNO 1055) on bone mineral density, 10-year coronary heart disease risk, and menopausal complaints: the randomized controlled Training and Cimicifuga racemosa Erlangen (TRACE) study. *Menopause* 2010;17:791–800
41. Laakmann E, Grajecki D, Doege K, zu Eulenburg C, Buhling KJ. Efficacy of Cimicifuga racemosa, Hypericum perforatum and Agnus castus in the treatment of climacteric complaints: a systematic review. *Gynecol Endocrinol* 2012;28:703–9

42. Overk CR, Yao P, Chadwick LR, *et al*. Comparison of the in vitro estrogenic activities of compounds from hops (Humulus lupulus) and red clover (Trifolium pratense). *J Agric Chem* 2005;53:6246–53
43. Heyerick A, Vervarcke S, Depypere H, Bracke M, De Keukeleire D. A first prospective, randomized, double-blind, placebo-controlled study on the use of a standardized hop extract to alleviate menopausal discomforts. *Maturitas* 2006;54:164–75
44. Erkkola R, Vervarcke S, Vansteelandt S, *et al*. A randomized, double-blind, placebo-controlled, cross-over pilot study on the use of a standardized hop extract to alleviate menopausal discomforts. *Phytomedicine* 2010;17:389–96
45. Hemachandra LP, Madhubhani P, Chandrasena R, *et al*. Hops (Humulus lupulus) inhibits oxidative estrogen metabolism and estrogen-induced malignant transformation in human mammary epithelial cells (MCF-10A). *Cancer Prev Res (Phila)* 2012;5:73–81
46. Bolca S, Li J, Nikoloic D, *et al*. Disposition of hop pregnyl flavonoids in human breast tissue. *Mol Nutr Food Res* 2010;54(Suppl 2):S284–94
47. Bulzomi P, Bolli A, Galluzzo P, *et al*. The naringenin-induced proapoptotic effect in breast cancer cell lines holds out against high bisphenol a background. *IUBMB Life* 2012;64:690–6
48. Dhooge W, Eertmans F, Comhaire F. Estrogen receptor based in vitro essays for the evaluation of endocrine disrupters. *Folia Histochem Cytobiol* 2001;39(Suppl 2):44–5
49. Lee MS, Shin BC, Yang EJ, Lim HJ, Ernst E. Maca (Lepidium meyenii) for treatment of menopausal symptoms: a systematic review. *Maturitas* 2011;70:227–33
50. Chiang SS, Chang SP, Pan TM. Osteoprotective effect of monascus-fermented Dioscorea in ovariectomized rat model of postmenopausal osteoporosis. *J Agric Food Chem* 2011;59:9150–7
51. Colli MC, Bracht A, Soares AA, *et al*. Evaluation of the efficacy of flaxseed meal and flaxseed extract in reducing menopausal symptoms. *J Med Food* 2012;15:840–5
52. Pruthi S, Qin R, Terstreip SA, *et al*. A phase III, randomized, placebo-controlled, double-blind trial of flaxseed for the treatment of hot flashes: North Central Cancer Treatment Group N08C7. *Menopause* 2011;19:48–53
53. Brooks JD, Ward WE, Lewis JE, *et al*. Supplementation with flaxseed alters estrogen metabolism in postmenopausal women to a greater extent than does supplementation with an equal amount of soy. *Am J Clin Nutr* 2004;79:318–25
54. Wang C, Mäkelä T, Hase T, Adlercreutz H, Kurzer MS. Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. *J Steroid Biochem Mol Biol* 1994;50:205–12
55. Sonestedt E, Ivarsson MI, Harlid S, *et al*. The protective association of high plasma enterolactone with breast cancer is reasonably robust in women with polymorphisms in the estrogen receptor alpha and beta genes. *J Nutr* 2009;139:993–1001
56. Zamora-Ros R, Ferrari P, Gonzáles CA, *et al*. Dietary flavonoid and lignan intake and breast cancer risk according to menopause and hormone receptor status in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *Breast Cancer Res Treat* 2013;139:163–76
57. Buck K, Zaineddin AK, Vrieling A, *et al*. Estimated enterolignans, lignan-rich foods, and fibre in relation to survival after postmenopausal breast cancer. *Br J Cancer* 2011;105:1151–7
58. Peterson J, Dwyer J, Adlercreutz H, Scalbert A, Jacques P, McCullough ML. Dietary lignans: physiology and potential for cardiovascular disease risk reduction. *Nutr Rev* 2010;68:571–603
59. Schäfer A, Chovanova Z, Muchova J, *et al*. Inhibition of COX-1 and COX-2 activity by plasma of human volunteers after ingestion of French maritime pine bark extract (Pycnogenol). *Biomed Pharmacother* 2006;60:5–9
60. Grimm T, Chovanova Z, Michova J, *et al*. Inhibition of NF-kB activation and MMP-9 secretion by plasma of human volunteers after ingestion of maritime pine bark extract (Pycnogenol). *J Inflamm* 2006;3:1
61. Kohama T, Negami M. Effect of low-dose french maritime pine bark extract on climacteric syndrome in 170 perimenopausal women. *J Reprod Med* 2013;58:39–46
62. Devaraj S, Vega-Lopez S, Kaul N, *et al*. Supplementation with pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters plasma lipoprotein profile. *Lipids* 2002;37:931–4
63. Tresserra-Rimbau A, Rimm EB, Medina-Remon A, *et al*. Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study. *Nutr Metab Cardiovasc Dis* 2014;24:639–47
64. Treserra-Rimbau A, Rimm EB, Medina-Remón A, *et al*. Polyphemol intake and mortality risk: a re-analysis of the PREDIMED trial. *BMC Med* 2014;12:77
65. Acharya A, Das I, Singh S, Saha T. Chemopreventive properties of indole-3-carbinol, diindolylmethane and other constituents of cardamom against carcinogenesis. *Recent Pat Food Nutrit Agric* 2010;2:166–7
66. Morrison J, Multell D, Pollock TA, *et al*. Effects of dried cruciferous powder on raising 2/16 hydroxyestrogen ratios in premenopausal women. *Altern Ther Health Med* 2009;15:52–3
67. Thomson CA, Rock CL, Caan BJ, *et al*. Increase in cruciferous vegetable intake in women previously treated for breast cancer participating in a dietary intervention trial. *Nutr Cancer* 2007;57:11–19
68. Liu X, Lv K. Cruciferous vegetables intake is inversely associated with risk of breast cancer: a meta-analysis. *Breast* 2013;22:309–13
69. Shea MK, O'Donnell CJ, Hoffmann U, *et al*. Vitamin K supplementation and progression of coronary artery calcium in older men and women. *Am J Clin Nutr* 2009;89:1799–807
70. Panossian A, Wikman G, Kaur P, Asea A. Adaptogens stimulate neuropeptide y and hsp72 expression and release in neuroglia cells. *Front Neurosci* 2012;6:6
71. Scott G. *Antioxidants in Science, Technology, Medicine and Nutrition*. Chichester, UK: Albion Publishing Ltd, 1997
72. Kalra EK. Nutraceutical – definition and introduction. AAPS PharmSci 2003;5(3): article 25 (http://www.pharmsci.org)
73. Wang L, Sesso HD, Glynn RJ, *et al*. Vitamin E and C supplementation and risk of cancer in men: posttrial follow-up in Physicians' Health Study II randomized trial. *Am J Clin Nutr* 2014;100:915–23
74. World Health Organization. WHO traditional medicine strategy 2014–2023. http://www.who.int/iris/handle/10665/92455
75. Goldman DP, Cutler D, Rowe JW, *et al*. Substantial health and economic returns from delayed aging may warrant new focus for medical research. *Health Aff (Millwood)* 2013;32:1698–705
76. Michaëlsson K, Wolk A, Byberg L, Arnlöv J, Melhus H. Intake and serum concentrations of α-tocopherol in relation to fractures in elderly women and men: 2 cohort studies. *Am J Clin Nutr* 2014;99:107–14
77. Mainini G, Rotondi M, Di Nola K, *et al*. Oral supplement with antioxidant agents containing alpha lipoic acid: effects on postmenopausal bone mass. *Clin Exp Obstet Gynecol* 2012;39:489–93
78. Hager K, Kenklies M, McAfoose J, Engel J, Münch G. Alpha-lipoic acid as a new treatment option for Alzheimer's disease – a 48 months follow-up analysis. *J Neural Transm* 2007; Suppl 72: 189–193.
79. Lee BJ, Huang YC, Chen SJ, Lin PT. Coenzyme Q10 supplementation reduces oxidative stress and increases antioxidant

enzyme activity in patients with coronary artery disease. *Nutrition* 2012;28:250–5
80. Hoeg A, Gogakos A, Murphy E, *et al*. Bone turnover and bone mineral density are independently related to selenium status in healthy euthyroid postmenopausal women. *J Clin Endocrinol Metab* 2012;97:4061–70
81. Pan SY, Zhou J, Gibbons L, *et al*. Antioxidants and breast cancer risk – a population-based case-control study in Canada. *BMC Cancer* 2011;11:372
82. Schaefer EJ, Bongard V, Beiser AS, *et al*. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. *Arch Neurol* 2006;63:1545–50
83. Vakhapova V, Cohen T, Richter Y, Herzog Y, Corczyn AD. Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in non-demented elderly with memory complaints: a double-blind placebo-controlled trial. *Dement Geriatr Cogn Disord* 2010;29:467–74
84. Gariballa SE, Forster SJ, Powers HJ. Effects of mixed dietary supplements on total plasma homocysteine concentration (tHcy): a randomized, double-blind, placebo-controlled trial. *Int J Vitam Nutr Res* 2012;82:260–6
85. De Jager CA, Oulhaj A, Jacoby R, *et al*. Cognitive and clinical outcomes of homocystein-lowering B-vitamin treatment in mild cognitive impairment: a randomized controlled trial. *Int J Geriatr Psychiatry* 2012;27:592–600
86. Nachum-Biala Y, Troen AM. B-vitamins for neuroprotection: narrowing the evidence gap. *Biofactors* 2012;38:145–50
87. Saposnik G, Ray JG, Sheridan P, *et al*. Homocysteine-lowering therapy and stroke risk, severity, and disability: additional findings from the HOPE 2 trial. *Stroke* 2009;40:1365–72
88. Marti-Carvajal AJ, Solà I, Lathyris D, Karakitsiou DE, Simancas-Racines D. Homocysteine-lowering interventions for preventing cardiovascular events. *Cochrane Database Syst Rev* 2013 Jan 31;1:CD006612
89. Douaud G, Refsum H, de Jager CA, *et al*. Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment. *Proc Natl Acad Sci USA* 2013;110:9523–8
90. Marik PE, Varon J. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. *Clin Cardiol* 2009;32:365–72
91. Kotwal S, Jun M, Sullivan D, Perkavic V, Neal B. Omega 3 fatty acids and cardiovascular outcomes: systematic review and meta-analysis. *Circ Cardiovasc Qual Outcomes* 2012;5:808–18
92. Hansen-Krone IJ, Enga KF, Südduth-Klinger JM, *et al*. High fish plus oil intake is associated with slightly reduced risk of venous thromboembolism: The Tromsö Study. *J Nutr* 2014;144:861–7
93. Tierney AC, McMonagle J, Shaw DI, *et al*. Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome – LIPGENE: a European randomized dietary intervention study. *Int J Obes* 2011;35:800–9
94. Kiecolt-Glaser JK, Belury MA, Andridge R, *et al*. Omega-3 supplementation lowers inflammation in healthy middle-aged and older adults: a randomized controlled trial. *Brain Behav Immun* 2012;26:988–95
95. Mozaffarian D, Lemaitre RN, King IB, *et al*. Plasma phospholipid long-chain ω3 fatty acids and total and cause-specific mortality in older adults: a cohort study. *Ann Intern Med* 2013;158:515–25
96. Gerhauser C. Cancer chemoprevention and nutriepigenetics: state of the art and future challenges. *Top Curr Chem* 2013;329:73–132
97. Park LK, Friso S, Choi SW. Nutritional influences on epigenics and age related disease. *Proc Nutr Soc* 2012;71:75–83
98. Gaziano JM, Howard D, Sesso HD, *et al*. Multivitamins in the prevention of cancer in men: The Physicians' Health Study III randomized controlled trial. *JAMA* 2012;308:1751–60
99. Chaumontet C, Bex V, Véran F, Martel P. The vitamin E analog tocopherol succinate strongly inhibits gap junctional intercellular communication in rat liver epithelial cells (IAR203). *J Nutr Biochem* 2008;19:263–8
100. Gaziano JM, Glynn RJ, Christen WG, *et al*. Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. *JAMA* 2009;301:52–62
101. Omenn GS, Goodman GE, Thornquist MD, *et al*. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Cartotene and Retinol Efficacy Trial. *J Natl Cancer Inst* 1996;88:1550–9
102. Neuhouser ML, Barnett MJ, Kristal AR, *et al*. Dietary supplement use and prostate cancer risk in the Carotene and Retinol Efficacy Trial. *Cancer Epidemiol Biomarkers Prev* 2009;18:2202–6
103. Bernard D, Christophe A, Delanghe J, *et al*. The effect of supplementation with an antioxidant preparation on LDL-oxidation is determined by haptoglobin polymorphism. *Redox Rep* 2003;8:41–6
104. Mursu J, Robien K, Harnack LJ, *et al*. Dietary supplements and mortality in older women: the Iowa Women's Health Study. *Arch Intern Med* 2011;171:1625–33
105. Fabian E, Bogner M, Kickinger A, *et al*. Intake of medication and vitamin status in the elderly. *Ann Nutr Metab* 2011;58:118–25
106. Ulbricht C. The top five nutritional deficiencies in the United States. *Alternat Compl Ther* 2013;19:119–22
107. Ames BN. Low micronutrient intake amy accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc Natl Acad Sci USA* 2006;103:17589–94