PRN OPINION PAPER

Phytoestrogens as Therapeutic Alternatives to Traditional Hormone Replacement in Postmenopausal Women

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This paper represents the opinion of the Women's Health Practice and Research Network (PRN) of the American College of Clinical Pharmacy. It does not necessarily represent an official ACCP commentary, guideline, or statement of policy or position.

Epidemiologic data illustrate strong associations between diets high in foods containing phytoestrogens and a reduction in coronary heart disease (CHD), breast and other cancers, and menopausal symptoms. However, controlled clinical trials that encourage health care providers to endorse phytoestrogens as an alternative to conventional hormone replacement therapy (HRT) in postmenopausal women are lacking. Most data that support or refute widespread use of phytoestrogens come from animal and epidemiologic human studies. In comparison, controlled clinical trials have assessed the effects of conventional HRT on CHD risk factors and osteoporosis by changes in bone mineral density (BMD).[1, 2] Cohort studies in humans identified the role of HRT in breast cancer risk.[3] Epidemiologic studies further illustrated the relationship with breast cancer[4] as well as effects of HRT on overall mortality.[5]

In addition to limited clinical trial data on the efficacy of phytoestrogens, the following questions are unanswered:

- What differences in biologic activity, if any, exist among phytoestrogens?
- Does each individual phytoestrogen induce its own specific therapeutic effects?
- What are the therapeutic dosages of each subclass of the compounds?
- What are the long-term side effects of the agents?
- Do phytoestrogens, in therapeutic dosages, induce endometrial hyperplasia similarly to unopposed estrogen therapy when taken by a woman with an intact uterus?
- Do therapeutic dosages confer the same cardiovascular and osteoporosis protection and risk reduction as conventional HRT?

Background

As the baby-boomer population ages and the number of women entering menopause increases, the need for traditional HRT with agents such as estradiol or conjugated equine estrogen (CEE) is expanding. This generation has indicated its dissatisfaction with the traditional health care system and created a consumer-driven environment. Given this discontent, it is not surprising that less than 25% of women receive HRT.[6, 7] Many who are prescribed HRT either stop taking it or never start due to fear of associations with malignancy, unacceptable bleeding, or side effects.[8] Instead, they search for alternative ways to self-manage symptoms of menopause and its long-term consequences such

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Table 1. Available Oral Formulations Containing Phytoestrogens[a]

Product Name	Strength	Source	Company	Dosage	Cost ($)/Unit
Black cohosh	40 mg	Standard extract[b]	Vitanica	2–4 caps q.d.	10–12/100
Black cohosh	200 mg	Standard extract[b]	Natures Plus	1 cap q.d.	19/30
Extended-release black cohosh	525 mg	Whole root[b]	Nature's Sunshine	1–2 caps b.i.d.	10–12/100
Black cohosh	545 mg	Root and root extract[b]	Solaray	1–4 caps q.d.	15/120
Dong quai	520 mg	Root	Nature's Sunshine	1 cap q.d.	20/100
Dong quai	125 mg/ml	Root extract	Basic Organics	1 ml b.i.d.	16/oz.
Dong quai	None	Root extract	Gaia Herbs	30–40 drops b.i.d–q.i.d.	12/oz.
Femtone	None	Dong quai, vitex, licorice, black cohosh	PhytoPharmica	1–2 caps b.i.d.	17/90
Meno-fem	None	Rice bran oil, dong quai, chaste tree, licorice, wild yam, ginseng	Prevail	2–6 caps q.d.	19/90
Phyto-est	45 mg/3 caps	Isoflavones	BioTechnologies	3–6 caps q.d.	20/100
Phyto-Soy	36 mg 23 mg/9 caps	Isoflavones Genistein	Natures Sunshine	3 caps t.i.d.	21/100
Remifemin	20 mg	Standard extract[b]	Enzymatic Therapy PhytoPharmica	1–2 tabs b.i.d.	30/120 18/60
Women's Phase II	None	Burdock root, licorice root, wild yam root, dong quai, motherwort	Vitanica	2–6 caps q.d.	20/100

[a]Gathered from several East Coast retail establishments.
[b]Contains a standard extract of black cohosh, 2.5% triterpene glycosides 1 mg, and traditional dried whole root 85 mg/capsule.

as increased risk of CHD and osteoporosis.

Approximately 5000 Internet sites contain information on traditional and alternative treatments for menopause.[9–11] Popular lay literature promotes phytoestrogens as a means of reducing menopausal symptoms and overall breast cancer risk.[12–14] To date, however, no clinical trials have directly compared the compounds with traditional HRT relative to their impact on these two postmenopausal health issues. Foods containing phytoestrogens are soy products such as tofu, tempeh, soy flour, and soy milk (Table 1). Other sources are alfalfa, apples, green tea, sesame, and wheat. The abundance of food products promoted as beneficial to menopausal symptoms, however, pales in comparison with the enormous number of herbal, nutritional, and supplement products marketed to postmenopausal women.

Much of the information included with these products is tempered by qualifiers. For example, dosages required to prevent CHD and osteoporosis are not established,[12] and the Food and Drug Administration lacks quality control over these and other pure soy protein extract products.[13] Many of these products are marketed as powder formulations containing isoflavones (primarily genistein and daidzein) and isolated soy protein. Others contain high lignan flaxseed oil, flaxseed, wild yam, and roasted soy nuts (containing isoflavones).

To date, much of the rationale for phytoestrogens in treating menopause is derived from observational studies. One study noted that the frequency of breast, colon, endometrial, and ovarian cancer is lower in Asia and eastern European countries than in all Western countries.[15] A comparison of Japanese and Caucasian women living in Western society reported a lower frequency of CHD, breast cancer, and endometrial cancer among the Japanese, perhaps secondary to maintaining a diet high in phytoestrogen content.[16] A third study showed an increased risk of developing cancers and CHD in Asian immigrants, both men and women, after they adopted a "Western" diet.[17] Overall, these lower frequencies in Asian and eastern European subjects are believed to be related to increased dietary intake of phytoestrogens compared with Western society.

The frequency of hot flashes among different cultures also was studied. Among menopausal women in China and Singapore, 18%[18] and 14%,[19] respectively, experienced hot flashes, compared with 70–80% of menopausal women in Europe.[20] Such differences in disease development and menopausal symptoms largely are attributed to dietary differences, specifically soy and vegetable content. It is these foods that are high in phytoestrogens. Therefore, are phytoestrogens

responsible, entirely or in part, for differences in the frequency of menopausal symptoms and cancer and heart disease risk among these populations? Answering this requires detailed discussion of phytoestrogens as therapeutic entities and a look at available literature and clinical trial data establishing their risks and benefits.

Phytoestrogens

Clinical Properties

Phytoestrogens are plant compounds that are structurally and functionally similar to 17,β-estradiol and bind to estradiol receptors. Whereas their affinity for binding is only 1/500–1/1000 of that of estradiol, they compete with estradiol for receptor sites.[21, 22] As a result, phytoestrogens are thought to act as both agonists and antagonists.

Several plants are purported to possess phytoestrogens, such as black cohosh, dong quai, ginseng, licorice, and wild Mexican yam. Phytoestrogens are divided into various classes that include isoflavones, lignans, coumestans, resorcylic acid lactones, and mycotoxins.[16, 23–25] Significant in vitro estrogenic responses were seen with two isoflavones, daidzein and genistein, and their respective precursors, formononetin and biochanin A.[26] Exertion of their hormone-like activity occurs in the concentration range of 0.1–10 μM.[23] However, specific dosages equivalent to dosages of ethinyl estradiol (EE) and CEE are not uniformly established. Other classes are not thought to have as much estrogenic activity. For example, by observing changes in sex hormone-binding globulin (SHBG), it was discovered that genistein, a phytoestrogen in the isoflavone class, stimulated the synthesis of SHBG. However, zearalenone, an agent in the mycotoxin class, actually had a very low affinity for SHBG and thus had little to no effect on stimulating further SHBG production. It is postulated that it is stimulation of SHBG by isoflavones that inhibits cancer by reducing available 17,β-estradiol.[25]

Actions of compounds with estrogenic activity are species specific and complex. For example, tamoxifen is estrogenic in mice, agonist-antagonist in humans, and antiestrogenic in frogs and chickens.[27] Within species, age differences also affect the type of estrogenic activity of different compounds, perhaps due to circulating levels of endogenous estrogens.[28] For these reasons caution must be exercised when extrapolating animal data for phytoestrogens and estrogens to humans, or when comparing human data from different age groups.

Despite relatively weak estrogen receptor-binding potencies, phytoestrogens have bioactivity similar to that of estradiol and reach concentrations sufficient to elicit response.[24] This similarity of bioactivity suggests functional equivalency between estradiol- and isoflavinoid-receptor complexes. In addition, genistein can achieve circulating concentrations 1000-fold greater than those of endogenous estradiol in premenopausal women,[29] suggesting the potential for a significant dose-response relationship.

Research reveals two types of estrogen receptors, α and β. Expression of the latter occurs in a wide range of adult tissues including the vasculature, bone, brain, heart, gonads, and genital tract. It has a distinct pattern from estrogen receptor-α in some of these tissues.[30] Estrogen receptor-β appears to be expressed preferentially in normal breast[31] and ovarian tissue.[32] Estrogen receptor-α is expressed in hypertrophic epiphyseal chondrocytes that are involved in cartilage and bone formation.[33] However, it is preferentially expressed in ovarian cancer lines and estrogen receptor node-positive breast cancers.[31, 32, 34] Tissue-specific phyto-estrogen activity, including tumor induction or inhibition, is likely related to receptor-binding specificity. Relative in vitro binding affinity for the estrogen receptor-α is estradiol >> coumestrol > genistein > daidzein > biochanin A > fomononetin. In vitro binding affinity for the estrogen receptor-β is estradiol >> genistein = coumestrol > daidzein > biochanin A > formononetin.[35]

In two estrogen receptor-positive breast cancer cell lines (MCF7, T47D), genistein has biphasic effects on proliferation, with low concentrations stimulating cell growth and higher concentrations inhibiting growth (< 10 μM and > 20 μM, respectively). These findings are comparable with the limited estradiol data that are available.[36] In estrogen receptor-negative and normal breast epithelial cells, low-dose genistein was slightly inhibitory, whereas higher concentrations (> 1 μM) significantly increased growth. By comparison, equol, an isoflavinoid that is a metabolite of daidzein, was stimulatory at low concentrations and only slightly inhibitory at higher concentrations (> 1 μM). Extensive damage to chromatin structure suggests apoptosis as the mechanism for growth inhibition with genistein. Because genistein and equol act

Table 2. Phytoestrogen Studies in Women

Subjects	Agent and Dose/Day	Effects Assessed
104 postmenopausal women	Isoflavone 76 mg or placebo	Reduction in mean number of moderate and severe hot flashes/mo
91 postmenopausal women	Isoflavones 165 mg	Vaginal epithelium, LH, FSH, SHBG
25 postmenopausal women	Soya flour 45 g, red clover sprouts (dry seed), 10 g, linseed 25 g each treatment for 2 wks	Vaginal epithelium, LH, FSH
288 women	No intervention; urine assessed for excretion of equol and enterolactone	Association between excretion rates and breast cancer risk compared with controls
21 peri- and postmenopausal women	Isoflavones 80 mg (45 mg genistein)	Systemic arterial compliance, LDL
17 postmenopausal women	Isoflavone 40 and 800 mg	Systemic arterial compliance; fasting cholesterol panels
66 postmenopausal women	Isolated soy protein 40 mg with either moderate or high concentrations of isoflavones	Fasting cholesterol panels; BMD of total body, proximal femur, lumbar spine (L1–L4)
80 postmenopausal women	Ipriflavone 600 mg or ipriflavone 400 mg +CEE 0.3 mg	BMD of lumber spine (L2–L4), urinary excretion of hydroproline, vaginal epithelium maturity
105 postmenopausal women	Ipriflavone or low-dose HRT[d] or ipriflavone 600 mg + low-dose HRT or sequential HRT[e] or calcium 500 mg	BMD of spine (L2–L4)
56 postmenopausal women	Ipriflavone 600 mg + calcium 1000 mg or calcium only	BMD of lumber spine (L2–L4)

LH = luteinizing hormone; FSH = follicle-stimulating hormone; SHBG = sex hormone-binding globulin; BMD = bone mineral density; CEE = conjugated equine estrogen; LDL = low-density lipoprotein; HDL = high-density lipoprotein.
[a]$p<0.01$.
[b]$p<0.05$.
[c]Not statistically significant.
[d]Transdermal 17,β-estradiol 25 μg/day plus medroxyprogesterone acetate 5 mg 12 days of the month.
[e]Transdermal 17,β-estradiol 50 μg/day + medroxyprogesterone acetate 5 mg 12 days of the month.

as pure estrogen agonists at low dosages, this rules out competitive inhibition with higher dosages of estradiol. In addition, the low concentrations are comparable with reported in vivo ranges of circulating genistein levels in Asians consuming a soy-rich diet.[29] This suggests that low dosages of genistein and equol may be sufficient to provide therapeutic effects.

Numerous antiproliferative properties are suggested for phytoestrogens.[27] Genistein inhibits tyrosine protein kinases, which are coded by protooncogenes and play a key role in tumorigenesis. It also inhibits deoxyribonucleic acid (DNA) topoisomerases I and II, and may prevent cell mutations by stabilizing cell DNA. Another mechanism may involve reduction of cell oxidants. Genistein reportedly inhibits formation of tumor promoter-induced hydrogen peroxide and superoxide anion, and it scavenges hydrogen peroxide that is added exogenously to cultured human cells. Daidzein appears to have similar antioxidant activities.[27] Genistein also induces apoptosis; inhibits angiogenesis, subsequent tumor growth, and cell differentiation; and may reduce malignant cell metastasis as a result.[27, 29] These phytoestrogen effects probably are related to relative tissue concentrations. Estrogen concentrations are 40-fold higher in breast fluid aspirates than in serum concentrations; if phytoestrogens can achieve similar relative concentrations, this may account for their protective effects by estrogen receptor antagonism.[29] However, relative to their inhibitory effects on angiogenesis, the question of

Table 2. Phytoestrogen Studies in Women (continued)

Findings
45% reduction from baseline[a, 39]
↑ vaginal maturation; no changes in LH, FSH, SHBG[40]
↑ vaginal maturation,[a] ↓ FSH,[a] no change in LH[41]
64% risk reduction in patients with highest enterolactone excretion; 73% risk reduction in patients with highest equol excretion[42]
26% improvement in systemic arterial compliance,[b] no changes in LDL oxidizability[43]
Improvement in systemic arterial compliance vs placebo,[b] reduction in LDL, increase in HDL[c, 44]
Increase in HDL,[b] reduction in LDL,[b] BMD of L1–L4 reduced with moderate concentration of isoflavones[c] and increased with high concentration of isoflavones,[b] lack of BMD changes at other sites[45]
1.2% increase in BMD in 600-mg and 400-mg + CEE 0.3 mg groups at 24 mo compared with baseline,[b] reduction in hydroxyproline excretion in both groups after 24 mo,[d] lack of vaginal cytology changes in ipriflavone-only group, maturation noted in ipriflavone-CEE group[46]
0.11% increase in BMD in ipriflavone-only group at 12 mo compared with calcium-only group,[b] 0.22% reduction in BMD in ipriviflavone + HRT group compared with calcium-only group who experienced 3.41% mean reduction in BMD[b, 47]
1.2% and 3.8% reductions in BMD in ipriflavone and calcium groups, respectively, at 24 mo[a, 48]

the potential negative impact on cardiovascular disease may be raised. To date, the only exploration of this compared the effects of 17,β-estradiol, genistein, and daidzein on the walls of the aorta after vessel injury in male and female rabbits.[37] This study suggests deleterious effects in rabbits. Extrapolating these data to humans is difficult, and further study is warranted.

Metabolism

Enterolactone and enterodiol, both mammalian lignans, are produced by colonic bacterial metabolism of plant lignans matairesinol and secoisolariciresinol.[38] Most isoflavones occur in plants as glycoside-bound complexes that are biologically inactive.[27] Active forms most likely are produced by colonic bacterial metabolism to remove the sugar moiety.[24] Dietary aglycones may be absorbed directly.[24] Recent data suggest a high degree of variability in the metabolic pathways involved.[24, 27] Isoflavones undergo enterohepatic circulation after absorption, and

they are conjugated to glucuronic acid in the liver and excreted in urine.[27] Even smaller amounts are excreted as sulfates and sulfo-glucuronides. The isoflavone daidzein is formed from formononetin and is metabolized by intermediates into equol and the less estrogenic *O*-desmethylangolensin (*O*-DMA).[27] Metabolic variability, possibly due to differences in intestinal microflora, contribute to the relative amounts of equol and *O*-DMA produced, and therefore influences the degree of estrogenic activity.[24] Genistein is formed from biochanin A and metabolized to estrogenically inactive p-ethylphenol.[27] Genistein, daidzein, and equol have been detected in human urine,[38] plasma, saliva, breast aspirate or cyst fluid, and prostatic fluid.[24] Lignans enterolactone and enterodiol have been detected in human urine,[38] serum, feces, semen, and bile.[24] Therefore, it appears that drug interactions would be minimal, and significant dosage adjustments for renal or hepatic impairment would not be necessary. However, given lack of clinical experience with these agents, certainty relative to these dosing issues does not exist.

Phytoestrogen Research

Each of the several classes of phytoestrogens contains different substances that appear to occur in various concentrations in different anatomic areas. Although limited, research is beginning to address the gynecologic effects of these agents, including effects on menopausal symptoms, vaginal and endometrial tissues, breast cancer risk, CHD risk, and osteoporosis. Tables 2[39–48] and 3[49–54] summarize the studies relative to subjects (animal and human), agent and daily dose, outcome measure(s), and results.

Menopausal Symptoms

A randomized, double-blind, placebo-controlled, parallel, multicenter study enrolled 104 postmenopausal women to assess the effects of the isoflavone class of phytoestrogens on hot flashes.[39] The primary efficacy criterion was change in mean number of moderate and severe daily hot flashes in each month of treatment compared with baseline. Fifty-one women received isolated soy protein 60 g/day that contained 76 mg isoflavones (approximately equivalent to 0.15 mg CEE/day); 53 women received placebo protein 69 g/day. The average number of hot flashes/24 hours at baseline was 11/patient. Compared with the placebo group,

Table 3. Phytoestrogen Studies in Animals

Subjects	Agent and Dose/Day	Effects Assessed	Findings
8 ovariectomized ewes	17,β-estradiol 45 μg or formononetin 3.5 g	Changes in vulva, breast, uterine tissue	Enlargement of vulva, uterus[a]; color of vulva changed to pink[a]; enlargement of teats, udders[a, 49]
Female rats	Diet enriched with flavinoid and coumestrol[b] followed by estradiol 0.1 μg	Changes in uterine weight	Coumestrol not antiestrogenic, 228% ↑ in uterine cell proliferation[e, 50]
40 ovariectomized cynomolgus monkeys	Genistein 26.6 mg or CEE 0.166 mg	Vaginal cytology	No statistically significant changes in genistein group, significant maturation in CEE group[d, 51]
14 female rhesus monkeys	Genistein 1.27 mg + daidzein 0.42 mg/g soy protein or genistein 0.121 mg + daidzein 0.052 mg/g soy protein	Total cholesterol, LDL, HDL, triglycerides, lipoprotein(a)	Statistically significant ↓ in total,[d] LDL,[d] lipoprotein(a)[d]; significant ↑ in HDL[d]; no significant change in triglycerides[52]
32 ovariectomized Sprague-Dawley rats	Soy protein diet or 17,β-estradiol 10 μg/kg	BMD of right femur and L4	Soy-treated subjects with greater BMD at both sites vs placebo group,[d] BMD of right femur less in soy group compared with estrogen group,[d] no difference in L4 BMD between estrogen- and soy-treated groups[53]
41 ovariectomized cynomolgus monkeys	Soy protein diet, isoflavone 148 mg/day ± 17,β-estradiol 1 mg/day	Histomorphometric bone changes at periosteal, osteonal, endocortical surfaces	Increased bone turnover in soy-fed subjects, bone turnover prevented in estrogen-treated group[d, 54]

[a]No statistically significant difference compared with changes in ewes receiving 17,β-estradiol.
[b]Dose not specified.
[c]Statistics not performed.
[d]p≤0.05.

women who received isoflavones experienced a statistically significant (p<0.01) reduction in hot flashes every week from weeks 3–12 of the study. One exception was the eighth week when no statistically significant difference was noted. After 12 weeks, the treatment group had 45% and the placebo group 25% less hot flashes than at baseline (p<0.01).

Gynecologic Effects

Clinical Studies

Studies of the gynecologic effects of phyto-estrogens examined a diverse number of clinical end points, including biologic markers of estrogen supplementation in the postmenopausal state, vaginal and endometrial cytology, and associations between phytoestrogen therapy and development of breast cancer.

On the most elementary level, the biologic effects of phytoestrogen supplementation were assessed by comparing isoflavone 165 mg/day (equivalent to 0.3 mg CEE on a molar basis) with placebo in 91 postmenopausal women.[40] Subjects randomly received either a diet high in

phytoestrogens or instructions to continue their regular diet for 4 weeks. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and SHBG concentrations were compared with baseline values. Both groups had no statistically significant differences from baseline; however, when vaginal cytology, a sensitive and specific indicator of estrogenicity, was assessed, the maturation index was increased in the phytoestrogen group compared with the control group. This difference was not statistically significant.

Changes in vaginal cytology also were observed in a 10-week study of 25 postmenopausal women.[41] After a 2 week run-in period, each woman's daily diet was supplemented with soya flour 45 g, red clover sprouts (dry seed) 10 g, and linseed 25 g, each for 2 weeks in turn. The equivalent dose of EE or CEE was not noted. At the end of each 2-week period, LH, FSH, and vaginal maturation values were assessed. A cumulative, significant reduction was noted for FSH concentrations, but no difference was noted for LH. Vaginal cytology revealed a statistically significant increase in maturation value after soya

flour and linseed (p<0.01) but not after red clover. This increase persisted for 2 weeks after discontinuation of all supplementation.

It was hypothesized that phytoestrogens exert their beneficial effects on breast cancer risk by competing with estradiol for estrogen receptors, thereby acting as an estrogen antagonist in breast tissue.[55] In a case-control study of 144 pairs of women matched for age and area of residence, excretion of equol and enterolactone (a lignan) was measured as an indicator of phytoestrogen intake.[42] The reduction in risk of breast cancer was 73% in the quartile of patients with the highest equol excretion (p=0.009) and 64% in the quartile with the highest enterolactone excretion (p=0.013).

Animal Studies

Animal studies illustrated positive gynecologic effects of different phytoestrogens. Some of this information dates back to the 1940s when Australian sheep, after eating large quantities of red clover, a source of lignan phytoestrogens, were noted to have extensive lesions of their reproductive organs.[55, 56] They also had cystic endometrial hyperplasia, which led to the descriptive term, clover disease.

Additional animal studies reproduced findings similar to those in clover disease. The effects of 17,β-estradiol implants 45 μg/day were compared with a diet containing formonetin 3.5 g/day in ovariectomized ewes.[49] Both groups had changes in reproductive tracts and breast tissue, such as enlargement of the vulva, uterus, udder, and teats (length and circumference). The color of the vulva changed from pale to pink-red. All changes were attributed to estrogen stimulation.

Ovariectomized rats were fed diets enriched with flavinoid or with flavinoid and coumestrol, followed by estradiol 0.1 μg/day to test for coumestrol's antagonist effects against estrogen.[50] In both arms, uterine weight, a marker for estrogenic endometrial stimulation, increased. Coumestrol did not have an antiestrogenic effect but resulted in a 228% mean increase in uterine cell proliferation.

Contradicting results were reported in a study of ovariectomized cynomolgus monkeys; it compared vaginal responses of a control group and two treatment groups.[51] One group received genistein 26.6 mg/day and the other received CEE 0.166 mg/day, equivalent to the human dosage of 0.625 mg/day. At 6 months there was no statistically significant difference between control and genistein groups. The CEE group, however, experienced a statistically significant increase from baseline in vaginal maturation (p<0.05). The authors concluded that genistein was "tissue-selective." Similar results were reported in a study of rhesus monkeys that were fed diets with isolated soy protein with or without added isoflavones (genistein 1.27 mg and daidzein 0.42 mg/day).[52] No differences in SHBG and uterine weight were detected when comparing diets with or without added isoflavones.

Cardiovascular Effects

Data on phytoestrogens in cardiovascular disease in women are limited by the small numbers of trials and enrolled patients. In addition, the trials lacked consistency among agents, making clinical application of the results difficult.

A small, placebo-controlled, single-blind, crossover trial of 21 women (6 perimenopausal, 1 premenopausal, 14 postmenopausal) evaluated the effects of isoflavones 80 mg/day (in a combination containing genistein, daidzein and glycetin).[43] Several women followed a "near-vegetarian diet" that was not defined. After 5–10 weeks of therapy, systemic arterial compliance was measured by two-dimensional echocardiogram and hand-held Doppler flow, and results were compared with baseline in 15 patients. In addition, low-density lipoprotein (LDL) oxidizability was measured in vitro and compared with baseline. Arterial compliance improved 26% in the treatment group compared with the placebo group (p<0.001); LDL oxidizability did not change from baseline.

Arterial compliance was further evaluated, together with specific changes in LDL and high-density lipoprotein (HDL) concentrations, in another double-blind, placebo-controlled, crossover trial of 26 postmenopausal women.[44] After a 3-week run-in period, patients were randomized to receive placebo or isoflavones 40 or 80 mg/day. Every 40 mg of isoflavones contained genistein 4 mg, daidzein 3.5 mg, formononetin 8 mg, and biochanin 24.5 mg. Treatment continued for 5 weeks, after which patients were randomized to another treatment arm for an equal amount of time, and finally to the remaining treatment arm for the final 5 weeks. Seventeen participants completed the trial with both cardiovascular end points evaluated. The large number of dropouts was

attributed to menopausal symptoms requiring traditional HRT. A nonsignificant trend toward declining LDL levels and increasing HDL levels was noted with increasing doses of isoflavones compared with placebo. Arterial compliance increased from an average of $18.5 \pm 6.4\%$ at baseline to $23.7 \pm 5.3\%$ with isoflavones 40 mg to $24.4 \pm 4.9\%$ with 80 mg (p=0.032 and p=0.021, respectively) compared with baseline.

The largest trial on the effects of phytoestrogens on cholesterol concentrations enrolled 66 women whose last menstrual period was 12 or more months earlier and who had a total cholesterol between 240 and 300 mg/dl.[45] After 2 weeks of a step 1 diet (< 30% of calories from fat, 8–10% of total calories from saturated fat, and < 300 mg/day cholesterol), subjects were randomized to one of the following arms: isolated soy protein containing moderate amounts of isoflavones/day (ISP56); isolated soy protein containing high amounts of isoflavones/day (ISP90); or protein 40 g/day through casein and nonfat dry milk (NFDM). The specific constituents of the isoflavones were not stated. At 24 weeks, HDL increased and LDL decreased significantly in both the ISP56 and ISP90 groups compared with the NFDM group (p<0.05). However, when comparing ISP56 and ISP90, no significant difference was seen in either HDL or LDL changes.

Osteoporosis

The trial that assessed the effects of phytoestrogens on cholesterol also evaluated changes in BMD.[45] After 24 weeks of therapy, vertebral BMD, measured from L1–L4, declined in both ISP56 and NFDM groups. Compared with baseline, it increased significantly from 0.892 ± 0.114 g/cm^2 to 0.912 ± 0.119 g/cm^2 in the ISP90 group (p<0.05). No significant changes were noted in total body BMD or BMD at the proximal femur, although specific values were not provided.

In a prospective trial, 80 women (aged 40–49 yrs) were required to have a BMD in the normal range for their age/reference population; their menopausal status was not stated.[46] At the end of 2 years, 52 women had completed the trial that randomized them to receive calcium 500 mg/day; ipriflavone, a synthetic isoflavone-derived compound, 600 mg/day; CEE 0.3 mg/day; or ipriflavone 400 mg plus CEE 0.3 mg/day. All women were recommended to take additional calcium 500 mg/day. The BMD at L2–L4 was measured by dual-energy x-ray absorptiometry (DEXA) scan at baseline and every 6 months. Compared with baseline, after 24 months of therapy BMD declined in the calcium-only and CEE-only groups (p<0.001 each). The ipriflavone-only group experienced increases in BMD compared with baseline at 12 and 24 months, 1.1% and 1.2%, respectively (p<0.05). The group receiving ipriflavone plus CEE had increases in BMD at 12 and 24 months of 0.5% and 1.2%, respectively (p<0.05). Statistical comparisons among groups were not performed.

Treatment arms were similar in a prospective, comparative study of 105 healthy, Caucasian, early postmenopausal women.[47] The BMD of L2–L4 was measured by DEXA scan at baseline and 12 months in women randomized to receive one of the following: calcium only 500 mg/day; low-dose HRT 25 µg/day (transdermal 17,β-estradiol plus medroxyprogesterone acetate 5 mg for 12 days/mo); ipriflavone 600 mg/day; low-dose HRT plus ipriflavone; or sequential HRT (50 µg/day transdermal 17,β-estradiol plus medroxyprogesterone acetate 5 mg for 12 days/mo). The BMD declined in the calcium-only, low-dose HRT, and ipriflavone plus low-dose HRT groups, 3.41%, 0.55%, and 0.22%, respectively. Compared with the calcium-only group BMD increased significantly in the ipriflavone-only and sequential HRT groups (p<0.05), 0.11% and 1.84%, respectively. Compared with baseline, changes in BMD were not statistically significant in any treatment group.

Another clinical trial evaluating phytoestrogens and their effects on BMD enrolled 56 Caucasian women who were postmenopausal for 5 years or less, who had at least two osteoporosis risk factors, and whose vertebral BMD was at least 1 standard deviation (SD) below normal.[48] After randomization to ipriflavone 600 mg plus calcium 1000 mg or placebo plus calcium 1000 mg, BMD of L2–L4 was measured at baseline and every 6 months. At 24 months, BMD had declined compared with baseline in both groups. The ipriflavone group experienced a mean reduction of 1.2% (NS), and in the calcium-only group it declined 3.8% (p=0.001). These reductions differed significantly (p=0.045).

Discussion

Epidemiologic data regarding phytoestrogens appear positive, but two major points must be addressed before recommending the compounds

as an alternative to conventional HRT. Insufficient clinical trials exist to support routine, long-term therapy with unopposed phyto-estrogens, and differences among classes of phytoestrogens must be identified clearly, including dosing and biologic activity. In addition, results of animal trials must be interpreted cautiously as they do not appear to be fully applicable to humans.

Preliminary clinical data regarding the effects of ipriflavone on BMD appear to be inconsistent,[45–48] and long-term study is required. In contrast, the effects of phytoestrogens on menopausal symptoms appear to be promising.[39] Despite conflicting information regarding the effect of isoflavones on FSH concentrations, clinical studies illustrate that they increase vaginal maturation.[40, 41] Based on these findings, one can question whether they would produce the same endometrial changes in women as occurred in Australian sheep with clover disease.[56, 57] In addition, the question remains whether progesterone should be given to, or routine endometrial biopsies be performed in, women with an intact uterus who take phytoestrogens. Dosing is also a concern. What are the normal and high dosages of any of the phytoestrogens? Do differences exist between diets high in phytoestrogen-containing foods and supplementation with isoflavone-fortified soy protein? Are benefits and/or risks associated with combining traditional HRT with these diets or supplements? These questions are crucial to providing sound pharmacotherapy, and they are unanswered at this time.

Conclusion

Evidence is lacking that phytoestrogens reduce cardiovascular disease (especially given inconsistent findings relative to effects on lipoproteins) and osteoporosis risk in a manner comparable with HRT. No long-term studies have evaluated potential side effects of prolonged phytoestrogen therapy. Therefore, routine administration of the compounds is not recommended as an alternative to traditional HRT. Recommendations regarding short-term treatment of menopausal symptoms should be made with caution, if at all.

Human data regarding the hormones' ability to alleviate menopausal symptoms, their potential reduction in breast cancer risk,[39, 42] and potential increase in BMD[45–48] are positive. These compelling data, in conjunction with absence of information regarding dosing and long-term effects, should serve as stepping stones for further research evaluating phytoestrogens as alternatives or adjuncts to conventional HRT.

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