

CONSENSUS OPINION

The Role of Isoflavones in Menopausal Health: Consensus Opinion of The North American Menopause Society

ABSTRACT

Objective: Given the increasing interest in the effect of isoflavones on menopause-related symptoms and diseases related to menopause/aging combined with the growing body of published literature on isoflavones, much of which presents conflicting data, The North American Menopause Society (NAMS) established a goal to develop an evidence-based consensus opinion on the role of isoflavones in menopausal health.

Design: NAMS appointed a panel of clinicians and researchers acknowledged to be experts in the field of isoflavones. Their advice was used to assist the NAMS Board of Trustees in developing this consensus opinion.

Results: Many animal and human studies have evaluated the health effects of isoflavones on menopause-related symptoms and diseases related to menopause/aging. However, data are inconclusive regarding whether the observed health effects in humans are attributable to isoflavones alone or to isoflavones plus other components in whole foods. The most convincing health effects have been attributed to the actions of isoflavones on lipids. Studies have associated isoflavones with statistically significant reductions in low-density lipoproteins and triglycerides as well as increases in high-density lipoproteins. Although some data seem to support the efficacy of isoflavones in reducing the incidence and severity of hot flashes, many studies have not found any difference between the isoflavone recipients and the controls. Inadequate data exist to evaluate the effect of isoflavones on breast and other female-related cancers, bone mass, and vaginal dryness.

Conclusions: Although the observed health effects in humans cannot be clearly attributed to isoflavones alone, it is clear that foods or supplements that contain isoflavones have some physiologic effects. Clinicians may wish to recommend that menopausal women consume whole foods that contain isoflavones, especially for the cardiovascular benefits of these foods; however, a level of caution needs to be observed in making these recommendations. Additional clinical trials are needed before specific recommendations can be made regarding increased consumption of foods or supplements that contain high amounts of isoflavones. (*Menopause* 2000;7:215–229. © 2000, The North American Menopause Society.)

Key Words: Cancer – Hot flashes – Isoflavones – Lipids – Menopause – Soy.

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The North American Menopause Society (NAMS) appointed a panel of advisors to assist the NAMS Board of Trustees in developing an evidence-based consensus opinion on the therapeutic role of isoflavones in menopausal women, either in relieving short-term symptoms or in preventing disease later in life. The advisory panel was composed of clinicians and researchers acknowledged to be experts regarding isoflavones. Their advice assisted the NAMS

Board of Trustees in developing this consensus opinion of the Society. The NAMS consensus-building process was described in a previous issue.¹

This article concentrates on the issues that are most relevant to clinical practice. Whenever possible, conclusions are drawn from scientific evidence focused specifically on isoflavones, especially trials that assessed health effects in peri- and postmenopausal women. Most clinical trials have used soy foods or isoflavones derived from soy or red clover.

Although most of the cited scientific research has been published in peer-reviewed journals, some data have been published only in a journal supplement or as an abstract. Debate exists regarding the scientific rigor of some studies, the conclusions drawn, and the clinical implications. Differing opinions of the experts are noted in the text.

Phytochemicals are plant-derived compounds. Among these phytochemicals are phytoestrogens, a broad group of nonsteroidal compounds of diverse structure that have been shown to bind to estrogen receptors (ERs) in animals and humans. Functionally, they can exert both estrogenic and antiestrogenic effects, depending on their concentration and the concentration of endogenous sex hormones and the specific end-organ involved. However, many of the effects of these molecules may result from interactions with pathways of cellular activity that do not involve the ER.²⁻⁵

Several classes of phytoestrogens exist. When considering health effects, the major types of phytoestrogens of current interest are the lignans and isoflavones. This article focuses on isoflavones.

The isoflavones include the biochemicals genistein, daidzein, glycitein, biochanin A, and formononetin. Genistein and daidzein are found in rich supply in soybeans and soy products as well as in red clover.⁶⁻⁸ A wide variety of soy products are available in the United States (see Table 1). However, relying on soybeans or soy products as the source of dietary isoflavones is complicated by the large variability in isoflavone concentration and composition among different soybeans and soy-protein products⁷⁻⁹—a function of growing conditions and differences in cultivars.¹⁰ The type of industrial processing is another variable.^{7,8,11,12} For example, processed soy products such as soy hot dogs and tofu yogurt may contain only one tenth the isoflavone content of whole soybeans (see Table 2).⁹ Moreover, to obtain taste- and color-free protein preparations, some soy processors remove almost all of the phytochemicals in soy.¹¹

TABLE 1. *Soy food products*

Canned black soybeans	Milder, sweeter flavor than yellow form; creamy texture.
Green soybeans	Available only in frozen form; sweet, mild tasting.
Roasted soybeans	Nutrient dense; available salted, unsalted, flavored.
Soy flour	Processed from ground, roasted soybeans. Contains twice the protein of wheat flour but is gluten-free and cannot be used alone for baking.
Soy isolate powder	Almost tasteless white powder containing approximately 90% protein. It is the most refined form of soy protein and is available in flavors.
Soy milk	Made from pureed soybeans and water; available in flavors and regular, low-fat, and nonfat versions. Average regular 1-cup serving = 130 calories, 4 g fat, no cholesterol, 10 g protein.
Tempeh	Pressed, fermented soybean cake, similar to tofu but stronger in taste.
Tofu	Made from soy milk using a process similar to that used to make cheese. Available in regular and low-fat versions, in soft, firm, extra-firm, and silken textures.

PHYSIOLOGIC EFFECTS

The structure of the isoflavone molecule resembles those of many estrogenic and antiestrogenic compounds—including the physiologic estrogen 17 β -estradiol and the synthetic antiestrogen tamoxifen—prompting investigation of its mechanisms of action according to estrogenic and antiestrogenic activities.²⁻⁵ The isoflavones have a common phenolic structure that seems to be a prerequisite for binding to ERs. Isoflavones, and genistein in particular, seem to have more binding affinity for ER- β than for ER- α ¹³; therefore, given the different tissue distribution of the α and β receptors,¹³ there is a clear potential that isoflavones could exhibit tissue-selective effects.

Many of the possible benefits of isoflavones and other phytoestrogens may be attributable to metabolic activities that involve systems other than ERs, including an influence on enzymes, such as adenosine triphosphatase; inhibition of DNA topoisomerases; antioxidant effects on lipids, lipoproteins, and DNA; effects on glucose transport and a variety of ion transport systems; and specific actions on protein synthesis, cell proliferation, angiogenesis, growth factor action, vascular smooth muscle cells, and cell differentiation.¹⁴

Further complicating the efforts to determine the effects of these compounds in animal and human studies is that the chemical methods used to isolate isoflavones (e.g., ethanol extraction) may alter their physiologic effects.¹⁴ In addition, striking compositional differences are found between the types of soy foods

TABLE 2. Isoflavone (daidzein plus genistein) content of foods

Description	Mean (mg isoflavone/100 g food)
Soybeans, green, raw	151.17
Soy flour	148.61
Soy protein concentrate (water-washed)	102.07
Soy protein isolate	97.43
Miso soup, dry	60.39
Tempeh	43.52
Soybeans, sprouted, raw	40.71
Soybean curd (fermented)	39.00
Soy cheese, unspecified	31.32
Tofu (Mori-Nu) silken, firm	27.91
Tofu (Azumaya) extra firm, steamed	22.70
Tofu yogurt	16.30
Soy hot dog, unprepared	15.00
Soy protein concentrate (alcohol extraction)	12.47
Soy milk	9.65
Soy noodles, flat	8.50
Vegetable burgers, prepared (Green Giant Harvest Burger)	8.22
Soylinks, cooked (Morning Star Breakfast)	3.75
Frankfurters, canned, meatless (Worthington Foods, Loma Linda, Big Franks)	3.35
Split peas, raw	2.42
Soy sauce (shoyu, made from soy and wheat)	1.64
Pinto beans, raw	0.27
Peanuts, all types, raw	0.26
Granola bar, snack	0.13
Chickpeas (garbanzos)	0.10
Soy sauce (made from hydrolyzed vegetable protein)	0.10
Tea, green, Japan	0.05
Beans, kidney, red, raw	0.01
Lentils, mature, raw	0.01
Beans, kidney, red, boiled	0.00
Green snap beans, raw or boiled	0.00
Lima beans, boiled	0.00

Source: United States Department of Agriculture—Iowa State University.

commonly used in Southeast Asia and those used in Western countries.¹⁵ In the United States, soy has been used mostly for the production of vegetable oil, although other soy products (e.g., soy milk, tofu, soy flour, soy protein preparations) are becoming more popular. In Southeast Asia, many of the soy foods arise from fermentation (e.g., miso, tempeh); the microorganisms used in their preparation are capable of hydrolyzing the inactive glycoside conjugates to active aglycones^{12,15,16}—differences that may be important with regard to metabolism and bioavailability.¹⁷

Isoflavones, like all phytoestrogens, are extensively biotransformed in the intestine by the action of bacterial enzymes. The extent of intestinal bacterial metabolism influences the bioavailability of phytoestrogens and, there-

fore, their potential for physiologic effects, although the degree of influence varies among individuals.^{17,18}

In soybeans, genistein and daidzein are present as β -glycosides. Isoflavones are inactive in their conjugated form as glycosides; they become activated in the aglycone form when the sugar residue is removed.^{12,15,16} In a proprietary isoflavone supplement derived from red clover, a substantial portion of the methylated isoflavones is converted into genistein and daidzein in vivo, although small amounts of the methylated compounds remain in circulation. The health effects of methylated isoflavones have not been evaluated in definitive studies.

Genistein has been shown to exert both proliferative and antiproliferative effects in human cell lines (Table 3)^{5,19,20}; its antiproliferative actions occur in both ER-positive and ER-negative cell lines and seem not to be mediated by the ER. Some experts have proposed that isoflavones may inhibit tumor cell growth by interfering with the tyrosine kinase activities that are essential for mitogenic signals,²⁰ but this concept has been challenged by some experts.

Two primary confounding factors when attempting to determine physiologic effects of isoflavones are (1) that many trials evaluating isoflavones fail to clarify the studied concentration of the bioavailable isoflavones and (2) the nonspecific use of terms when reporting results from clinical studies. Terms such as *phytoestrogens* (or *plant estrogens*), *soy*, *soy protein*, *isoflavones*, and others often are used interchangeably, although they are not synonymous. *Phytoestrogens* are plant compounds that have estrogen-like activity but are not necessarily isoflavones; *isoflavones* are a subclass of the bioflavonoids but are not necessarily phytoestrogens. The term *soy* usually is used to refer to a product derived from the whole soy (soya) bean; *soy (soya) protein* refers to a product derived by extracting the protein out of the whole bean. Both soy and soy protein contain isoflavones as well as other phytochemicals.

TABLE 3. Mechanisms of action of genistein

Estrogen-mediated effects
Partial estrogen agonist/antagonist
Binding to estrogen receptor β
Decrease in estrogen biosynthesis
Increase in estrogen metabolism
Estrogen-independent effects
Protein tyrosine kinase inhibition
As an antioxidant and related pathways
Ion transport systems (both up- and downregulation)
Inhibition of glucose uptake via the GLUT transporter
Inhibition of DNA topoisomerases
Inhibition of angiogenesis

Adapted from Barnes et al.¹⁹

The question of whether the isoflavones alone are responsible for health benefits has not been answered definitively. For conditions in which isoflavones have presumptive health benefit, there may be no reason to differentiate among the various sources for obtaining isoflavones (e.g., whole foods, second-generation foods made from soy, isoflavone-enriched foods, supplements in capsule form). However, a considerable number of reported benefits from isoflavones are based on studies with whole soy foods; in some studies, the clinical and biologic effects of soy were lost when isoflavones were removed. Moreover, isoflavone content varied considerably among the products studied. Clinical trials using standardized preparations studied in a controlled setting are the only means to define positively the active components.

CLINICAL TRIALS

The effects of isoflavones have been studied in many clinical trials, using both animal and human subjects, although the scientific rigor of some of those studies and their clinical implications have been challenged. Some data seem to be sufficient to conclude that the physiologic effects of isoflavones are beneficial, but much debate exists regarding the clinical implications of those effects. Difficulties in obtaining definitive data include variations in response due to the populations studied, the soy products used, and the duration of exposure, as well as the nonresponse of some individuals to isoflavone or phytoestrogen supplementation. Following is an analysis of the published data on isoflavones for menopause-related conditions.

Menopausal symptoms

The following sections review the clinical trial data for the effects of isoflavones and soy on the most common menopausal symptoms in the United States—hot flashes and vaginal dryness.

Hot flashes

Epidemiologic data suggest that diets rich in isoflavone-containing foods reduce the incidence of menopausal hot flashes. Although women in Western countries have approximately an 80% incidence of hot flashes, Asian women living in China (where there is high consumption of soy foods) have an incidence of only 20%.²¹

Randomized, controlled clinical trials, however, have shown that, in general, hot flashes are only slightly reduced in women who consume soy or isoflavones as compared with control subjects.^{22–27} In the three

12-week studies ($N = 276$) in which soy/isoflavones were more effective than the controls,^{24–26} the incidences of hot flashes were reduced by 40%, 54%, and 45% versus 25%, 35%, and 30% for the controls, respectively. The differences between the two groups were statistically significant only in the third study.

In an open study of 23 postmenopausal women who were receiving 40 mg/day of isoflavones derived from red clover, the severity and frequency of hot flashes were reduced from baseline by 56% and 43%, respectively, after 2–3 months.²⁸ A placebo-controlled, crossover study of the same preparation in 51 postmenopausal women found no significant differences in the incidence of hot flashes between the isoflavone and placebo recipients after the 12-week treatment period.²⁹

When the effect of soy protein on the incidence of hot flashes was investigated in a short-term, crossover trial, soy protein supplementation reduced the number of hot flashes in postmenopausal women who had severe vasomotor symptoms.³⁰ One double-blind, placebo-controlled trial using isoflavones extracted from soy showed a significant decrease in hot flash severity but not frequency.²⁷

Vaginal dryness

The effect of diets that are high in phytoestrogens (soy or linseed vs. wheat control) on vaginal cytology was evaluated in a double-blind, randomized, crossover study of 44 postmenopausal women.³¹ This study found that phytoestrogens alter the vaginal cytology maturation index to a more estrogenic epithelial pattern. However, in a study of isoflavones derived from red clover,²³ 40 or 160 mg/day had neither an impact on vaginal cytology nor any effect on serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), or sex hormone binding globulin (SHBG) in 37 postmenopausal women.

In another randomized study designed to test the hypothesis that a 4-week soy-supplemented diet (165 mg/day of isoflavones) would have estrogenic effects on the liver and pituitary in 97 postmenopausal women,³² a small estrogenic effect on vaginal cytology was documented, but the overall maturation index of superficial cells from the vaginal epithelium did not differ between the treatment and control groups. No estrogenic differences were seen in endogenous 17β -estradiol concentration, SHBG, FSH, or LH.

CONCLUSIONS

Some data support the efficacy of isoflavones in reducing the incidence and severity of hot flashes, although many studies failed to find any difference between the

isoflavone recipients and the control group. Inadequate data also exist to evaluate the effect, if any, of isoflavones on vaginal dryness. Clearly, more research is needed.

Cardiovascular disease

Epidemiologic evidence from Asia indicates that soy foods may reduce the risk of coronary heart disease (CHD).³³ This conclusion is speculative, however, because the lower serum cholesterol levels in that region also could be a result of the lower saturated fat intake and/or other factors. Nevertheless, the discovery of ERs in blood vessels^{34–36}—particularly ER- β —is compatible with a role for all estrogenic substances in cardiovascular disease. The isoflavone genistein has shown at least a sixfold higher affinity for ER- β than for ER- α .¹³

Cholesterol

Clinical trials^{37,38} and animal studies^{39,40} have demonstrated a beneficial effect of soy protein on improving plasma cholesterol levels. This effect is highly variable—ranging from modest benefits to activity comparable with that of the 3-hydroxy-3-methylglutaryl coenzyme A reductase-inhibitor drugs. It is unclear whether it is the isoflavones or other soy components that are responsible for the hypocholesterolemic effect. For example, two studies found that when purified isoflavones were added to a casein-based diet, they had no effect on plasma cholesterol levels.^{39,41}

Most of the evidence suggests that soy protein lowers serum cholesterol levels. A meta-analysis⁴² of 38 published controlled clinical trials of soy protein consumption (47 g/day on average) concluded that soy protein was associated with a mean 9.3% reduction in total cholesterol, 12.9% reduction in low-density lipoprotein cholesterol (LDL-C), and 10.5% reduction in triglycerides, with no change in high-density lipoprotein cholesterol (HDL-C). The hypocholesterolemic effect was seen in studies of normocholesterolemic and hypercholesterolemic women, and it was statistically signif-

icant related to baseline levels. On average, those who had hypercholesterolemia achieved a 10% reduction in cholesterol levels in response to approximately 25 g/day of soy protein. As most studies were conducted for short periods of time, no conclusions can be drawn regarding any cholesterol effects of soy protein over the long term.

The extent to which isoflavones influence the hypocholesterolemic effects of soy protein has yet to be determined. As described previously, some data suggest that although isoflavone-rich soy protein is effective, isoflavones alone are ineffective. Some experts believe that the isoflavones of soy protein account for much of the beneficial effects on lipoprotein metabolism, with recent observations suggesting that the LDL-C receptor is necessary for isoflavone action.⁴³

Although it seems that isoflavones do not independently lower serum cholesterol, there are data (particularly from nonhuman primate models) suggesting that isoflavones can cause a modest increase in HDL-C levels. A red clover isoflavone preparation studied in a 12-week, placebo-controlled trial of 37 postmenopausal women increased HDL-C by 18% (40 mg/day), although 160 mg/day produced no significant difference versus placebo.²³ Another study using the same red clover isoflavone formulation also found that HDL-C levels were not significantly improved in 17 postmenopausal women who were receiving either 40 or 80 mg/day of isoflavones.⁴⁴ In a separate study, a different formulation of red clover-derived isoflavones was tested in 50 postmenopausal women.⁴⁵ After 6 months of 50 mg/day of isoflavones, HDL-C increased by 28% compared with placebo. Other studies in postmenopausal women⁴¹ (as well as in monkeys³⁹) found that the administration of purified soy isoflavones without concomitant soy protein did not improve plasma lipid profiles (Figs. 1 and 2).

No study has directly compared the lipid effects of estrogen replacement therapy (ERT) and isoflavones/soy protein/soy in postmenopausal women. ERT has been shown to produce positive lipid effects. One study, the

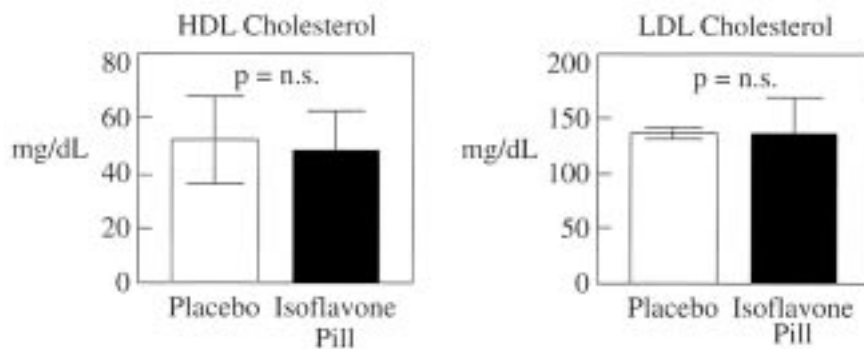
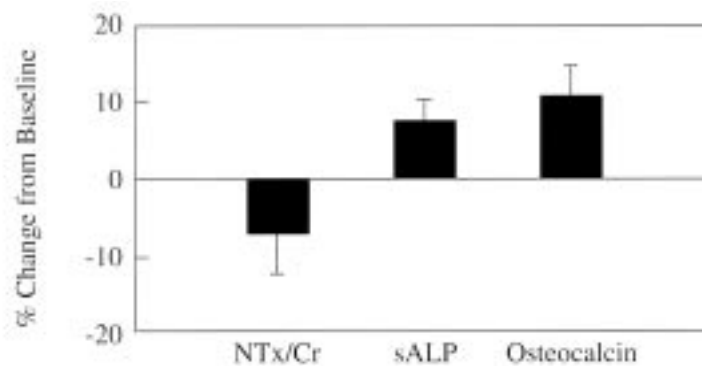


FIG. 1. No effect on plasma lipids was found when isoflavones alone were administered to postmenopausal women. Adapted from Nestel et al.⁴¹

FIG. 2. Effect on lipids and lipoproteins of a diet containing casein-lactalbumin (C/L), C/L plus isoflavone-rich soy extract (C/L + Iso), or isoflavone-containing soy protein (Soy +). TPC, total plasma cholesterol; V + IDL-C, very low-density lipoproteins plus intermediate-density lipoproteins. Adapted from Greaves et al.³⁹



PEPI Trial (Postmenopausal Estrogen/Progestin Interventions Trial),⁴⁶ evaluated the effects on lipid levels of conjugated equine estrogens, with or without micronized progesterone or medroxyprogesterone acetate, in 875 postmenopausal women. Taking all active treatment groups as a whole, triglyceride levels increased from baseline by 12–25% after 6 months and by 20–25% after 3 years, which was statistically significant compared with placebo recipients. Cholesterol differences in active treatment groups at 6 months and 3 years, respectively, were as follows: LDL-C decreased by 11–14% at both time points, HDL-C increased by 0–12% and 0–8%, and total cholesterol decreased by 3–7% and 4–7%.

Blood pressure

Recent studies demonstrated that soy lowers high blood pressure in salt-loaded hypertensive rats.⁴⁷ The results of these studies, although impressive in the animal model, were not duplicated in a randomized controlled trial of human subjects with high-normal blood pressure.⁴⁸ A similar lack of effect was found in peri- and postmenopausal women who were receiving an isoflavone-enriched extract of soy.⁴¹ However, a significant decrease in diastolic blood pressure was noted when a soy protein supplement was given twice daily to 51 nonhypertensive perimenopausal women in a placebo-controlled trial.⁴⁹ The same study showed that the recipients had significantly improved lipid and lipoprotein levels, as well as significant reductions in the perceived severity of vasomotor symptoms.

Atherosclerotic plaque

Long-term (3-year) studies of ovariectomized cynomolgus monkeys have shown that soy protein with

isoflavones is associated with a significant reduction in atherosclerotic plaque progression when compared with soy protein isolate without isoflavones.⁵⁰ Studies of cultured vascular cells have demonstrated that isoflavones alter cellular processes associated with atherosclerotic lesion development.⁵¹ In addition, experiments have shown that the isoflavone genistein reduces production of proteolytic enzymes and migration of endothelial cells as well as inhibits tyrosine kinase activity, all of which produce antiangiogenesis and antithrombotic effects.^{52,53}

Arterial compliance

In a placebo-controlled crossover trial of 21 women aged 46–67 years,⁴¹ 80 mg/day of soy isoflavones (45 mg genistein) given for 5- to 10-week periods resulted in a statistically significant 26% improvement in systemic arterial compliance (elasticity) versus placebo. A trial using red clover-derived isoflavones (40 and 80 mg/day) found approximately a 23% improvement in arterial compliance in isoflavone recipients compared with placebo recipients.⁴⁵ A more recent report in postmenopausal women who were treated with long-term ERT showed an almost identical effect.⁵⁴ Some experts believe that the effects of isoflavones on increasing arterial compliance will be shown to be of much greater relevance in the reduction of coronary heart disease than any cholesterol-lowering effects.

Oxidation

Isoflavones seem to protect LDL-C from oxidation.^{55,56} These antioxidant effects were observed with relatively low levels of isoflavones. In general, oxidation is important in protecting the arterial wall from atheroma.

Conclusions

It seems clear that whole soy foods are associated with favorable effects on lipid profiles. Some isoflavone supplements have shown efficacy in improving HDL-C and arterial compliance. In October 1999, the US Food and Drug Administration (FDA) allowed the marketing claim that 25 g/day of soy protein, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. The FDA ruling was based on its finding that the scientific literature demonstrates a consistent, clinically significant effect of this amount of daily soy protein on total cholesterol and LDL-C levels.

To date, study results indicate that purified isoflavones have less effect than soy foods and soy protein on lipid levels; other components of soy seem to contribute to reducing cardiovascular disease risk factors. No studies have evaluated the effects of soy or soy components on cardiac events.

Bone metabolism

The low hip fracture rate among Asians (living in Asia) is often cited as support for a protective effect of soy/isoflavones. It is interesting that the spinal fracture rate among Asians is very high, and the average bone mineral density (BMD) among Asians is equivalent to or lower than that of Caucasians. It seems from rather limited epidemiologic data that the lower hip fracture rate in Asia may not be related to the consumption of soy but rather to the short hip axis length of Asians.⁵⁷

Indirect evidence for the potential benefits of isoflavones on bone metabolism comes from the large number of studies of ipriflavone,⁵⁸ a synthetic isoflavone that undergoes extensive intestinal bacterial biotransformation to many metabolites, including daidzein.⁵⁹ A review of randomized, controlled clinical trials revealed that ipriflavone (400–600 mg/day) has a bone-sparing effect in postmenopausal women.⁵⁸ However, even if the effects of ipriflavone and dietary isoflavones were dose equivalent, corresponding high doses of dietary isoflavones could not be realistically achieved; the average consumption among Asian women is 20–30 mg/day of isoflavones.^{60,61}

Several research groups have shown that isoflavones can preserve or increase BMD in ovariectomized rat models. One study found genistein to be more effective than conjugated equine estrogens (CEE) in preventing bone loss.⁶² Another found that daidzein and genistein were equal to estrone but better than ipriflavone.⁶³ Soy protein also has been shown to prevent bone loss in ovariectomized rats,⁶⁴ although the magnitude of the effect was lower, perhaps because of the inability to deliver high enough doses of isoflavones in a soy pro-

tein product or because the soy isoflavones were in the conjugated form. In another study, genistein was shown to be three times more effective in preventing bone loss than its glycoside genistin.⁶⁵

In contrast, two long-term studies of ovariectomized cynomolgus monkeys that were fed low-calcium diets failed to demonstrate an effect of soy protein either low or high in isoflavones on preserving BMD or decreasing bone turnover, although estrogen was effective in these studies.^{66,67}

The efficacy of isoflavones on human bone was studied in a double-blind trial in 66 postmenopausal, hypercholesterolemic women aged 49–73 years.⁶⁸ This 6-month study found that daily consumption of 40 g isolated soy protein (90 mg isoflavones) significantly increased BMD and bone mineral content in the spine compared with the control group that consumed 40 g/day of protein from casein and nonfat dry milk. No significant bone changes were observed for the group that consumed 40 g/day of soy protein (56 mg of isoflavones). Neither group exhibited changes in hip BMD.

Experts often ascribe soy's mechanisms of action on bone to the isoflavones' mimicry of the effects of estrogen. The more pronounced effect on trabecular rather than cortical bone is consistent with the action of ERT.^{69,70} Moreover, there are high levels of ER- β in bone.^{71,72} Nevertheless, other mechanisms may be involved, based on findings that genistein directly inhibits osteoclast activity.⁶⁵ This inhibition suggests that a significant part of genistein's action on bone is a decrease in the spurt of osteoclast-caused bone loss during menopause, rather than enhancement of bone mass.

Although two published human studies^{73,74} support the hypothesis that soy protein favorably influences bone health, the only documented effects were in the spine; moreover, those trials have been criticized for poor study design.

Two unpublished studies also are of interest. One of these studies⁴⁶ evaluated the effects of red clover-derived isoflavones (25, 50, or 75 mg/day) for 6 months followed by a 2-month placebo washout in 50 postmenopausal women. As measured by dual-energy x-ray absorptiometry (DXA), all treatment groups exhibited a significant increase in bone density at the proximal radius and ulna. There was no significant change in bone density at the 8-mm point of the distal radius and ulna. In another study⁷⁵ in which 50 postmenopausal women consumed a diet of 60–70 mg/day of isoflavones, a significant reduction in bone turnover after 12 weeks was observed, as measured by specific markers of osteoclast and osteoblast activity (Fig. 3); there was no control group in this study.

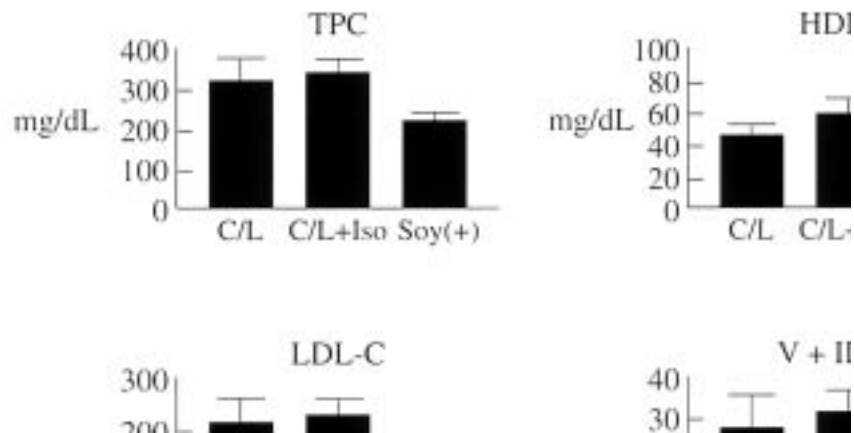


FIG. 3. Change in bone turnover markers in 43 postmenopausal women after 12 weeks of supplementation with dietary soy (60–70 mg/day total isoflavones). Adapted from Scheiber.⁷⁵

In contrast, a 9-month study of postmenopausal women found that isoflavone-rich soy protein had no effect in the spine or hip.⁷⁶ However, in these women, there was no effect of soy on their plasma cholesterol. Another study demonstrated that soy protein and high doses (100–160 mg/day) of an isoflavone extract derived from red clover had no effect on measures of bone turnover after 12 weeks of treatment.⁷⁷

Conclusions

Although some data suggest that isoflavones may favorably affect bone health, few human studies have been conducted and all involved small numbers of subjects in trials of short duration. Future clinical studies therefore are justified in order to demonstrate whether isoflavones play a role in limiting the extent of osteoporosis.

Cognitive function

Data are sparse regarding the influence of isoflavones on brain activities such as memory and cognitive function. It is known that both ER- α and ER- β are present in the brain in different distributions in the animals evaluated⁷⁸; however, it is not known whether these mediate actions of steroidal estrogens or isoflavones.

Some animal studies have suggested potential benefits of isoflavones on cognitive function. In one study,⁷⁹ ovariectomized rats were given oral 17 β -estradiol, soy phytoestrogens, or no treatment for 8 weeks. The soy phytoestrogen effects were found to be equivalent to those of 17 β -estradiol in upregulating two chemical factors—choline acetyltransferase and nerve growth factor—believed to be essential for learning and memory. In a study on ovariectomized cynomolgus monkeys, soy treatment for 3 years was shown to sharply decrease phosphorylation of the brain protein tau, an important biochemical parameter associated with the development of Alzheimer's disease.⁸⁰

In humans, however, a short-term (12-week) study in 11 postmenopausal women found that isoflavone supplementation had no effect on verbal learning.⁸¹ Two observational studies (published as abstracts) are of interest:

- In a report from the Honolulu-Asia Aging Study, an association was seen in midlife men between consistently higher levels of tofu consumption (at least twice weekly compared with rarely or never) and increased risk of dementia, low brain weight, and poor cognitive function.⁸²
- In a study among Japanese-American women in King County (Seattle), Washington, estrogen users who consumed tofu more than three times per week were not protected from cognitive impairment, whereas estrogen users with lower tofu consumption were protected.⁸³ However, the 2-year follow-up data revealed that tofu had no effect on the rate of cognitive change and did not oppose the beneficial effect of estrogen.⁸⁴

Conclusions

Few data have been published in this area. The two unpublished epidemiologic studies are conflicting in whether high consumption of one type of soy food (tofu) may have potentially harmful effects on cognitive function, whereas the limited research on animals does not suggest any adverse effects of soy, rather the possibility of improved cognitive function. Only one small study on isoflavones has been conducted.

Cancer

It has been suggested that isoflavones do not increase the risk of cancer and may be anticarcinogenic. The incidence of hormone-related cancers (endometrium, breast, and ovary) vary among different populations, with the lowest rates found in Asian

women; some experts attribute these low rates to a diet that is high in foods derived from soybeans.⁸⁵ It is important to note, however, that soybeans contain four known anticarcinogenic compounds unrelated to isoflavones: saponins, phytates, protease inhibitors, and phytosterols.⁸⁶ In vitro, high concentrations of the isoflavone genistein inhibit most types of cancer cells and some animal studies suggest that genistein inhibits metastases.^{87,88}

Breast cancer

Hargreaves et al.,⁸⁹ in a study of 84 premenopausal women who were taking 60 g/day of dietary soy supplementation (45 mg isoflavones) for 14 days, observed a weak estrogenic effect on normal breast tissue, as measured by nipple aspirate apolipoprotein D and pS2 expression. No effect was noted in breast epithelial cell proliferation, estrogen and progesterone receptor status, apoptosis, or mitosis.

In a case-control study,⁹⁰ a significant reduction in breast cancer risk was observed among both premenopausal and postmenopausal women who demonstrated increased excretion of phytoestrogen urinary metabolites. In a comment published later,⁹¹ it was noted that the urinary findings indicated a very low level of soy consumption. Further research is needed to determine any possible breast cancer protective effects of phytoestrogens and the lignan enterolactone.

In a study of Asian Americans, increased tofu consumption was significantly associated with decreased breast cancer risk.⁹² In a follow-up analysis of the data, soy intake was found to be protective only in Asian-born Japanese.⁹³ A case-control study of 200 breast cancer patients and 213 controls in Singapore found that soy intake was inversely associated with breast cancer; the findings were significant only among premenopausal and not postmenopausal women.⁹⁴

It has been shown that isoflavones cause measurable alterations of the menstrual cycle, including prolongation of cycle length and suppression of midcycle surges of LH and FSH,^{95,96} characteristics that are inversely related to breast cancer risk.⁹⁵ Another study, however, did not find an increase in menstrual cycle length with soy.⁹⁷

Ongoing studies are evaluating the effect of soy and isoflavones on breast cancer, including a phase I trial at the University of North Carolina in women with advanced breast cancer and another at the University of California at Los Angeles examining bilateral cancer recurrence in patients who have had a mastectomy. Although these clinical trial data are unavailable, experiments in animal models have provided some insight:

- There is evidence suggesting that genistein can alter cancer cell growth in vitro, with differing effects depending on the concentration. In one study, low concentrations (10–100 nM) of genistein stimulated in vitro the growth of cultured human estrogen-dependent breast cancer (MCF-7) cells and enhanced expression of an estrogen-responsive gene.⁹⁸ Conversely, high concentrations (>20 μM) inhibited the growth of the cells.
- A rat study demonstrated that early administration of genistein to the mother (during the gestational and neonatal period—before day 15) was protective against chemically induced mammary cancer in the offspring,⁹⁹ possibly because of enhanced mammary gland differentiation. This may be considered analogous to the protective effect of diethylstilbestrol in the same model. Some investigators suggest that studies such as these indicate that early exposure to phytoestrogens may confer benefit in later life. Such evidence has been used to support the epidemiologic evidence suggesting that consumption of genistein from soy by Asian women is protective against breast cancer.
- There is also animal research showing a stimulatory effect of genistein on breast cancer in vivo. In one study using ovariectomized athymic mice implanted with the estrogen-dependent human breast cancer (MCF-7) cells,⁹⁸ genistein acted as an estrogen agonist, leading to enhanced growth of MCF-7 cell tumors (17β-estradiol had a much greater effect than the genistein in this model). The genistein effect in this study may not be identical to that of genistein in postmenopausal women who have intact ovaries and, therefore, produce some steroid hormones; nevertheless, it suggests that there is the potential for dietary genistein to stimulate the growth of estrogen-dependent tumors in women who have low-circulating endogenous estrogen levels, such as those found in postmenopausal women.
- Conversely, soy has been shown to prevent cancer in some breast cancer models,^{100–102} and genistein alone has prevented breast cancer in animals, primarily in young rats (perinatally and prepubertally).^{103,104} However, it is unclear what the effects of genistein are in older animals or postmenopausal women, or in animals or women with preexisting tumors. Soy also has been found to inhibit the appearance of N-nitrosomethyl urea-induced mammary tumors that appear after the first one is resected.¹⁰⁵

Isoflavones might reduce breast cancer risk through multiple pathways, including a reduction in estrogen

levels, enhancement of estrogen metabolism, and down-regulation of ERs.^{98,106} Other research suggests that genistein inhibits carcinogenesis through nonhormonal mechanisms and that it has the further capability of inhibiting angiogenesis *in vitro*, which may help reduce cancer risk through yet another mechanism.⁵³

Further evidence of alternative pathways of cell growth regulation was obtained in recent experiments that compared a fermented isoflavone preparation (miso) with the antiestrogen tamoxifen in a model of breast cancer in rats.¹⁰⁷ In this study, animals that were given a 10% miso diet and tamoxifen developed significantly fewer breast cancers than those given miso or tamoxifen alone.

However, two studies have led to significant concern about whether genistein will increase cancer risk or tumor growth. In one study, women who consumed a soy protein beverage had an increased volume of nipple aspirate and the presence of atypia in cells in the nipple aspirate,¹⁰⁸ whereas another found that markers of proliferation were increased among women who were given soy before mastectomy.¹⁰⁹ Added to the results in mice that were transplanted with MCF-7 breast cancer cells,⁹⁸ these data raise concern and need to be considered. However, the Hargreaves et al. study⁸⁹ places these data in a better context (i.e., the proliferative markers may not equal proliferation).

Conclusions

Research has shown both protective and stimulatory effects of soy and soy isoflavones on breast cancer, based on epidemiologic, *in vitro*, and *in vivo* studies. Specific clinical trials to demonstrate a preventive action by isoflavones on breast cancer development have not been completed.

Two case-control studies show support for soy consumption in reducing breast cancer risk in premenopausal, but not postmenopausal, women. As a corollary, no evidence shows that a soy diet increases breast cancer risk in postmenopausal women.

Data obtained from studies in animals and epidemiologic studies of women living in Asia suggest that prepubertal exposure to isoflavones may be required for manifestation of breast cancer protective effects. Such information should be evaluated further before making specific recommendations that Western women increase their intake of isoflavones as a preventive measure against breast cancer.

For premenopausal women who have relatively normal ovarian function after treatment for breast cancer, opinions among experts differ on whether the modest antigonadotrophic/antigonadal actions of isoflavones

would be of greater benefit than the risk of direct estrogenic stimulatory actions on undetected or new malignant cells. It has been suggested that if isoflavones, in general, and genistein, in particular, are protective against breast cancer development *in vitro*, then they will be beneficial to a woman who has estrogen-dependent cancers. However, it is also clear that isoflavones have estrogenic effects at certain dosage levels and at certain developmental stages of life. In a postmenopausal woman who has low-circulating estradiol levels and an estrogen-dependent tumor, dietary estrogens may stimulate tumor growth.

Some experts do not believe that food-derived isoflavones present a risk to women who have breast cancer. They believe that these isoflavones behave as selective estrogen-receptor modulators, providing specific target tissue beneficial effects. They would not discourage women who have breast cancer from consuming soy protein, because there is no conclusive evidence of untoward effects. These experts, however, do not recommend isoflavone supplements (e.g., pills) for these women, as they consider supplements to be pharmacologic agents, with the potential for overuse. Other experts believe that phytoestrogens from any source pose a risk to women who have breast cancer.

Thus, some clinicians and their patients who have estrogen-dependent tumors may have the same concerns about using isoflavones as they have about using ERT. One critical difference between dietary estrogens and ERT is that the dosage of ERT is somewhat standardized; moreover, with isoflavones, the use typically is self-administered and not prescribed or monitored by a healthcare provider.

Endometrial cancer

Limited data are available for the effects of isoflavones or soy on the uterine endometrium—a surprising finding given the estrogenic influences in this organ. Even large review studies that document the effects of isoflavones on cancers of the breast, liver, lung, colon/rectum, and stomach, as well as various forms of melanoma and leukemia, generally omit any mention of uterine cancer.

Uterine and vaginal epithelial cell proliferation is a typical estrogenic response that has been mimicked by isoflavones in several mammalian species. A review of studies¹¹⁰ reveals that varying oral doses of isoflavones have been shown to induce uterine growth in mice, rats, and cows, although a soy-based diet did not induce uterine growth in ovariectomized rats or rhesus macaques. In addition, continuing trials with menopausal women have suggested an absence of endometrial growth in response to isoflavone supplementation.¹¹⁰

The possible increase in the risk of uterine cancer associated with tamoxifen has led some clinicians to question whether consumption of “unopposed” dietary phytoestrogens (using terminology from prescribing ERT alone) is safe during postmenopause. Few data, however, address endometrial growth secondary to high levels of dietary isoflavone consumption. Of interest is the low incidence of endometrial cancer in countries with high consumption of isoflavones.¹¹¹

One epidemiologic case-control study in the multi-ethnic population of Hawaii found that plant-based diets low in fat, high in fiber, and rich in fruits and vegetables (primarily legumes, especially soybeans) reduced the risk of endometrial cancer. According to the authors, this is the first study to show an inverse association of soy consumption with the risk of endometrial cancer.¹¹² The association was independent of other dietary risk factors, such as total calories, fat calories, and vitamin A, as well as other nondietary risk factors, such as oral contraceptive use, ethnicity, and diabetes mellitus.

One experimental study in adult surgically induced postmenopausal female macaque monkeys that were given 17 β -estradiol, an isoflavone-containing soy isolate, or the combination found that 17 β -estradiol, but not the soy isolate, induced increases in endometrial thickness, gland area, and epithelial proliferation.¹¹³ It is interesting that the effects of 17 β -estradiol were modified by the addition of the isoflavone-containing extract, as indicated by a decrease in an epithelial proliferation marker.

Conclusions

More research is needed to clarify the relationship between soy and isoflavones and endometrial cancer.

Other cancers

The clinical trial data for the effects of isoflavones or soy on other cancers in women are sparse or inconclusive. Epidemiologic data suggest that soy may reduce lung cancer risk, but similar data on colorectal cancer are variable and not impressive.

Epidemiologic studies demonstrate that bladder cancer occurs considerably more frequently in the United States than in Southeast Asia,¹¹⁴ a region with considerably higher soy consumption and where biochemical measurements demonstrate that isoflavones are secreted in the inhabitants' urine at micromolar concentrations. Recently, genistein was shown to inhibit murine bladder cancer,¹¹⁵ although there is little other published information. In two experiments, researchers coupled genistein to an epidermal recep-

tor antibody¹¹⁶ and demonstrated beneficial effects in mice with leukemias.¹¹⁷

Conclusions

No definitive statements can be made about the use of isoflavones and/or soy in moderating risks for other cancers found in women.

ISOFLAVONE INTAKE AMOUNTS

Increasing consumption of soy, soy products, and plant-based foods, in general, is supported by current recommendations to increase intake of fiber and antioxidants while lowering intake of saturated fat and cholesterol. However, it is premature to recommend specific amounts of dietary isoflavones to prevent specific chronic diseases. On the basis of the studies cited in this article, there is some evidence, albeit limited, to suggest the following:

- Optimal cholesterol reduction seems to require approximately 50 mg/day of isoflavones; this amount would be found in approximately 25 g/day of soy protein, which corresponds to the health claim allowed by the FDA. There is some evidence that amounts of 40–80 mg/day of isoflavones are needed for effects on arterial compliance, and for antioxidant effects on lipids, as little as 10 mg/day may be effective. Studies are inadequate to recommend an amount to prevent CHD.
- In limited studies, a minimum of 50 mg/day of isoflavones may benefit bone health. As with CHD, there are inadequate data to recommend an amount to prevent osteoporosis.
- Most studies on hot flashes have used isoflavone amounts of 40–80 mg/day, and these amount may benefit vasomotor symptoms.

A sizeable variation in plasma levels is seen in individuals receiving the same amount of isoflavones, an important issue as to whether a therapeutic level will be reached. Moreover, the safety of isoflavones at specific amounts has not been established. It is not clear, for example, whether the same product (e.g., a serving of isoflavone-fortified protein shake) should be consumed by a woman who has osteoporosis and a woman who has an estrogen-dependent cancer. Even in apparently healthy women, questions exist regarding possible overuse of isoflavones.

To receive potential health benefits, it seems to be preferable to obtain isoflavones from whole foods. However, there is little quality control of food storage,

time in stores, preservation, preparation, and sizes—resulting in a wide variation in the isoflavone content in foods.

Foods made from soybeans and soy-protein formulations may provide other phytochemicals that enhance the effect of isoflavones; these effects could be lost when isoflavones are given as additives alone. Isoflavone-fortified foods eventually may be an appropriate vehicle for obtaining isoflavones, although they should not be added to foods in an indiscriminate manner or on a widespread scale. The ready availability of supplements may lead the public to consume larger amounts than are advisable. If isoflavones are added to foods, they should be regulated for purity, standardization of amount, and safety.

It is unlikely that the isoflavones present in the available pills and powders all will have equivalent pharmacologic effects, as each product contains different amounts and varying concentrations. Until standardization of isoflavone extracts is achieved, it will not be possible to predict outcomes after consumption of the different supplements. In addition, the matrix in which the isoflavones are delivered will have an impact on the release of the agents for absorption and, therefore, will produce differential effects on intestinal metabolism and microflora. Like isoflavone-fortified foods, isoflavone-containing pills and powders need standardization and regulation.

Until more studies documenting benefits and safety are conducted, it is prudent for clinicians to advise their patients that whole soy foods may be a better choice than such products as supplements or soy-enriched or soy-fortified foods. Many of these products are available, and many more undoubtedly will be marketed soon, all containing differing amounts of isoflavones. When assessing patient-specific conditions, the clinician needs to be aware of this variability and to remind the patient that it is important to read labels to determine isoflavone content and to warn that, at least in the United States, there are no regulations to ensure content of such products.

FUTURE STUDY TOPICS

Many issues remain to be clarified regarding the role of isoflavones in menopausal health, and several are mentioned throughout this article. Other suggested study topics include the following:

- Establish the efficacy and safety of isoflavones at different stages of life and, if effective, the amounts needed to produce benefits and avoid side effects.
- Assess possible gender differences regarding response to isoflavones.
- Determine whether possible health benefits are attributed to isoflavones or to other components of soy and phytoestrogen-rich foods.
- Assess the effects of the concomitant use of isoflavones and ERT or hormone replacement therapy (i.e., estrogen plus progestogen), as well as selective estrogen-receptor modulators, on all of the normal and pathologic conditions addressed in this article.

SUMMARY

Although many studies have evaluated the effects of isoflavones, the scientific literature is conflicting because of inconsistencies in the populations studied, lack of use of an appropriate control group, selection of end points, and type of study. It is clear, however, that isoflavones may exert their actions through the ERs α and β (depending on the concentration and the end-organ involved) as well as directly on enzyme systems.

Nevertheless, the role of isoflavones in the management of short-term menopausal symptoms as well as diseases related to menopause/aging is still uncertain, although there is a growing database of information suggesting that isoflavones exhibit a wide range of diverse physiologic actions in humans, including effects on menopausal symptoms, lipids and lipoproteins, vascular function, bone, and a number of cancers.

The addition of even small amounts of isoflavone-containing foods to the Western diet may reduce the risk of heart disease through beneficial effects on cholesterol levels and increased arterial compliance. Specific controlled clinical trials are needed before definitive recommendations can be made about increasing the consumption of isoflavones in large populations of women approaching menopause and beyond. However, a suggestion to eat more fruits and vegetables (specifically legumes) and less high-fat animal food is an appropriate one.

The side effects of soy may arise if soy is overconsumed. Some experts argue that it might be difficult for adult women to consume sufficiently large quantities of isoflavones from traditional soy foods to cause adverse effects but that uncontrolled use of more potent isoflavone supplements might lead to negative outcomes, as estrogenic activities are dose dependent. In this regard, some level of caution needs to be maintained until more conclusive data become available.

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