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# NAMS 2011 Isoflavones Report

# The role of soy isoflavones in menopausal health: report of The North American Menopause Society/Wulf H. Utian Translational Science Symposium in Chicago, IL (October 2010)

**Abstract**

***Objectives:*** If and to what extent soy protein, soy isoflavones, and their metabolites, including S(–)-equol, have beneficial effects on women's health is currently unclear. The North American Menopause Society (NAMS)/Utian Translational Science Symposium on Soy and Soy Isoflavones convened October 9-10, 2010, to clarify basic and clinical research findings as they relate to the risk and benefits of soy products for peri- and postmenopausal women.

***Methods:*** A working group of faculty and panelists composed of clinical and research experts in the fields of women's health and botanicals met during a 2-day translational symposium to cover the latest evidence-based science on isoflavones as they affect menopausal symptoms, breast and endometrial cancer, atherosclerosis, bone loss, and cognition. Full descriptions of the bioavailability and pharmacokinetics of isoflavones were also presented. Subspecialty groups then broke off with the goal of translating the information into a report for general medical practice and identifying further research areas. All faculty and panelists reviewed the final report, which was then approved by the NAMS Board of Trustees.

***Results:*** From the hundreds of studies reviewed in this report, there are mixed results of the effects on midlife women. Soy-based isoflavones are modestly effective in relieving menopausal symptoms; supplements providing higher proportions of genistein or increased in S(–)-equol may provide more benefits. Soy food consumption is associated with lower risk of breast and endometrial cancer in observational studies. The efficacy of isoflavones on bone has not been proven, and the clinical picture of whether soy has cardiovascular benefits is still evolving. Preliminary findings on cognitive benefit from isoflavone therapy support a "critical window" hypothesis wherein younger postmenopausal women derive more than older women.

***Conclusions:*** Several areas for further research have been identified on soy and midlife women. More clinical studies are needed that compare outcomes among women whose intestinal bacteria have the ability to convert daidzein to equol (equol producers) with those that lack that ability (equol nonproducers) in order to determine if equol producers derive greater benefits from soy supplementation. Larger studies are needed in younger postmenopausal women, and more research is needed to understand the modes of use of soy isoflavone supplements in women. The interrelations of other dietary components on soy isoflavones consumed as a part of diet or by supplement on equol production also require further study, as do potential interactions with prescription and over-the-counter medications. And finally, greater standardization and documentation of clinical trial data of soy are needed.

***Key Words:*** Aglycone – Daidzein – Equol – Equol nonproducer – Equol producer – Estrogen receptor-α – Estrogen receptor-β – Genistein – Glycitein – Glycoside – R(+)-equol – S(–)-equol – Phytoestrogen – Soy – Soy germ – Soy isoflavone – Soy protein – Soy supplement.

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The report follows this numbered outline, and the number(s) adjoining each author's name above designates which section they contributed to:

1. Terminology, Mechanisms, Bioavailability & Pharmacokinetics
2. Prevalence of Use
3. Menopausal Symptom Effects
4. Breast & Uterus Effects
5. Bone Effects
6. Cardiovascular System Effects
7. Cognitive Effects

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The NAMS/Utian Translational Science Symposium on Soy and Soy Isoflavones was convened October 9-10, 2010, by The North American Menopause Society (NAMS) in Chicago, IL. The official topic of this closed workshop was "Basic and clinical considerations of the peri- and postmenopausal effects of soy, soy isoflavones, and their metabolites, including S(–)-equol," and included evidence-based presentations on such topics as the prevalence of use of soy and soy isoflavones; the molecular, cellular, and physiologic effects of isoflavones; and the effects of soy and soy isoflavones on menopausal symptoms, breast and endometrial cancer, atherosclerosis, bone loss, and cognition.

The round table, composed of 22 clinicians and researchers acknowledged to be experts in the field, met as a full working group of Faculty (presentations) and Panelists (discussions) and was then divided into specialty groups who worked amongst themselves to describe the latest evidence-based science topic by topic. The goal was to develop a report that essentially summarized the current state of knowledge with the objective of translating that information into a report that could identify those areas ready for introduction into general medical practice and those still in need of further research. The specialty groups, under the lead of the symposium Faculty, developed individual reports that were then reviewed and commented on by the entire team.

This resultant composite manuscript centers on the issues most relevant to clinical practice—the therapeutic role of soy isoflavones in menopausal women, either in relieving short-term symptoms or preventing disease later in life. Whenever possible, conclusions and recommendations were drawn from the evidence focused specifically on clinical trials assessing health effects of isoflavones in peri- and postmenopausal women. Most isoflavone clinical trials have used soy foods or soy supplements, so the focus of this document is soy and its isoflavones.

**TABLE 1.** *Isoflavone terminology used in this paper*

| | |
|---|---|
| Aglycone | The actual isoflavone without a sugar attached |
| Daidzein | A diphenolic biochemical, one of three designated as an isoflavone found in high amounts in soy and red clover; the relative amounts of genistein and daidzein are thought to be determinants of therapeutic efficacy of soy supplementation |
| Equol | A nonsteroidal isoflavone metabolite that is produced from daidzein by intestinal bacteria |
| Equol nonproducer | A woman or man who cannot convert daidzein to equol |
| Equol producer | A woman or man who can metabolize daidzein to equol, which enables them to benefit from soy and soy isoflavone products |
| Estrogen receptors | A group of receptors within cells activated by the hormone 17β-estradiol and other structurally similar compounds such as isoflavones; generally, the isoflavones have more binding affinity for ER-β than for ER-α |
| Genistein | A biochemical, one of three designated as an isoflavone found in high amounts in soy and red clover; the relative amounts of genistein and daidzein are thought to be determinants of the therapeutic efficacy of soy supplementation |
| Glycitein | One of three isoflavones found in soy protein and the protein of other legumes in relatively minor amounts. |
| Glycoside | A sugar attached to the aglycone portion of an isoflavone |
| Isoflavone | A plant-derived compound, one of three classes of phytoestrogens, with estrogen-like biologic activity and a chemical structure similar to that of estradiol |
| Phytoestrogen | Broad term for a plant-derived compound with estrogen-like activity |
| S(–)-equol | An isomer in the plasma of equol producers and is a metabolite of daidzein |
| Soy | The most widely used isoflavone-containing food; usually refers to a product derived from the whole soybean |
| Soy germ | The part of the soybean that has a high concentration of isoflavones, with much more daidzein than genistein and high concentrations of glycitein |
| Soy isoflavone | Isoflavone derived from soy (as opposed to from red clover, kudzu, American groundnut, or other plant foods) |
| Soy protein | A product derived by extracting the protein out of the whole bean; usually rich source of isoflavones |

## TERMINOLOGY, MECHANISMS, BIOAVAILABILITY & PHARMACOKINETICS

The most studied of the botanicals for menopause-related conditions are *isoflavones*, sometimes called "phytoestrogens." They are plant-derived compounds with estrogen-like biologic activity and a chemical structure similar to that of estradiol. See Table 1 for definitions of terminology used in this paper.

### Terminology

Isoflavones are a class of phytochemicals, a broad group of nonsteroidal compounds of diverse structures derived from plants that bind to estrogen receptors (ERs) in animals and human beings. Isoflavones have greater affinity for ER-β than for ER-α and possess both estrogen-agonist and estrogen-antagonist properties. The isoflavones include the biochemicals genistein, daidzein, glycitein, biochanin A, and formononetin. *Genistein* and *daidzein* are found in high amounts in soybeans and soy products as well as in red clover, kudzu, and the American groundnut.

*Soy* is the most widely used isoflavone-containing food. The term soy usually refers to a product derived from the whole soybean (or soya bean). *Soy protein* refers to a product derived by extracting the protein out of the whole bean. Soy protein is usually a rich source of isoflavones. The primary isoflavones of soybeans are genistein, daidzein, and *glycitein*. The relative amounts of these isoflavones vary depending on the portion of the soybean from which the material is obtained. The whole soybean contains about equal amounts of genistein and daidzein, with smaller amounts of glycitein. The germ of the soybean, however, is quite different; it has a high concentration of isoflavones, with daidzein being about four times greater than that of genistein and with relatively high concentrations of glycitein. Some soy supplements are made from *soy germ*. The relative amounts of genistein and daidzein are now thought to be determinants of the therapeutic efficacy of soy supplementation, as will be discussed later in this paper. Also to be discussed later is the possibility that

individual isoflavones, such as genistein, have different therapeutic outcomes when administered alone compared with the same amounts administered with all three isoflavones (genistein, daidzein, and glycetin) in the supplement.

About 30% of North American women have the ability to metabolize daidzein to equol. *Equol* is a nonsteroidal estrogen-like compound that binds to both ERs but with a high affinity for ER-β; thus, it is often designated as an ER-β agonist. Equol is produced from daidzen by intestinal bacteria and is thought to be a stable characteristic that is best revealed by a soy challenge of just a few days. Equol has two isomers, *S(–)-equol* and *R(+)-equol*. Only S(–)-equol is detected in the plasma of equol-producing women and thought to have any biological activity. By far the most exciting research opportunities in the area of soy isoflavone menopausal health concern the potential benefits of equol and the unanswered issue of whether equol is merely a marker for some beneficial effect of gut bacteria on steroid metabolism. More research is needed that compares equol producers with equol nonproducers.

**Mechanisms**

Isoflavones are frequently referred to as having pleiotropic properties (ie, many modes of action). It is therefore no surprise that they have been proposed to have important benefits in regard to vasomotor symptoms, the breast and uterus, cardiovascular system, bone, and cognition—all of which have relevance to women approaching and experiencing menopause. Detailed discussions of these effects can be found later in this paper.

Isoflavones were first considered to be phytoestrogens or "plant estrogens" because of early work noting their infertility effects in certain but not all species and because they bind to both of the ERs and because of their infertility effects in certain, but not all, species. However, during the 1980s, they were also demonstrated to be inhibitors of critical protein tyrosine kinases such as the epidermal growth factor receptor[1]—a risk factor for recurrence of breast cancer—and the platelet-derived growth factor receptor.[2] Subsequently, other mechanisms of action were proposed, including as antioxidants,[3,4] inhibitors of DNA topoisomerases,[5] and many enzyme systems in steroid synthesis and metabolism.[6,7]

Today, the question of which mechanisms of action of isoflavones and their metabolites are relevant to women's health remains controversial. The dose used in cell culture assays to investigate mechanisms is crucial. It has been proposed that concentrations in excess of 5 μM are unphysiologic, even in situations in which therapeutic doses are administered.[8] Many investigators have reported data from studies that used 25 to 100 μM isoflavones. These studies had to use the solvent dimethylsulfoxide to keep the isoflavones in solution, which may have introduced nonbiological variables into the results.

Another rapidly emerging area for the study of all plant-based and synthetic therapeutics is their impact on microRNA expression. MicroRNAs are targeted inhibitors of the translation of specific proteins. An important aspect of microRNA action is that they inhibit translation of multiple proteins. This may account for their pleiotropic properties. The application of microRNA concepts to the actions of isoflavones is in its infancy.[9-13]

Although most of the work on the mechanisms of action of isoflavones has been carried out on genistein and daidzein, recent studies on S(–)- and R(+)-equol have challenged earlier concepts about how this metabolite might alter biological systems. S(–)-equol is a better agonist for the ER-β, with agonist properties comparable to genistein.[14]

Overall, evidence suggests that the biological effects of isoflavones and their metabolites are mediated by many pathways, not just estrogen-dependent events. Future studies may exploit data that have been obtained in DNA microarray experiments.[15] In addition, studies have suggested that some of the benefits of dietary isoflavones observed in other populations may depend on early life exposure,[16-18] which may involve their impact on gene expression at an epigenetic level.

**Bioavailability & pharmacokinetics**

Puerarin (daidzein 8-C-glucoside) is rapidly absorbed and eliminated with a terminal elimination half-life ($t_{1/2}$) of approximately 4.3 hours in adult men.[19] It is likely that similar pharmacokinetics occur in women.

An extensive literature exists on the pharmacokinetics of soy isoflavones administered as natural components of soy foods,[20-28] as isolated isoflavone extracts or supplements,[27,29-37] as pure compounds,[38,39] and also as stable-isotope labeled analogs.[40,41] Overall, apparent bioavailability of these isoflavones are similar. Nonetheless, the rates of absorption of the isoflavones daidzein and genistein as glycosides are distinctly different from those of daidzein and genistein in their aglycone form and this has recently been suggested to be an important difference that could influence the ultimate efficacy of isoflavones.[42] Aglycones demonstrate rapid absorption and peak plasma concentrations are attained within 1 to 3 hours, depending on whether the isoflavones are taken with a meal or without a meal.[37] The effect of a meal is to delay absorption and shift the $T_{max}$ value. In contrast, for the β-glycoside conjugates, peak plasma concentrations of isoflavones typically occur 4 to 10 hours later due to a requirement for prior hydrolysis by intestinal brush border β-glycosidases,[43] which is a rate-limiting and time-dependent process.

The $t_{1/2}$ of all isoflavones is similar and typically 6 to 12 hours although it is significantly longer in patients with renal disease.[44] The clearance rate of genistein is significantly slower than that of daidzein, explaining why the plasma genistein concentrations are typically 1.5 to 2.0 times higher than daidzein concentrations. For supplements containing soy germ, daidzein and glycitein become the predominant isoflavones in plasma because they are enriched in soy germ.[38] Glycitein has relatively poor affinity for the ERs when compared with daidzein or genistein.[45] The plasma appearance/disappearance concentration profile of glycitein is similar to that of other isoflavones. These differences in plasma profiles of isoflavones indicate that not all soy foods or supplements

are created equal and therefore may not be expected to have the same efficacy.

Much interest has focused on S(–)-equol, the intestinal bacterial metabolite and end product of daidzin/daidzein[42,46] because this biologically active isoflavone is not produced by all adults who consume soy foods.[47-49] Only 20% to 30% of Western adults will produce S(–)-equol when fed soy isoflavones, which is significantly lower than the 50% to 60% frequency of equol producers reported in adults living in Asia and consuming soy foods.[49] The equol hypothesis of Setchell et al proposed that the ability to produce S(–)-equol may explain the greater efficacy of soy in studies of Asians when compared with those reported for Western adults.[50] S(–)-equol has a high systemic bioavailability and relatively slow plasma clearance. Peak plasma concentrations occur 1 to 2 hours after oral administration, consistent with the behavior of other isoflavone aglycones. High circulating plasma concentrations can be achieved in most adults with low oral doses (5-10 mg). S(–)-equol undergoes little biotransformation, apart from phase II metabolism, and consequently its fractional absorption is high. It is cleared from plasma with a $t_{1/2}$ of 6 to 8 hours and excreted almost exclusively in urine.[37,41] The optimal clinical efficacy is more likely to occur with twice-daily administration rather than single-dose administration. This is important to consider when designing clinical studies of isoflavones.[51]

Species differ in the extent of isoflavone conjugation; the athymic mouse and several transgenic species of mice show significantly higher proportions of unconjugated isoflavones in plasma than the rat or human. This may be due to differences in expression of individual UDP-glucuronosyltransferases. While conjugation can take place in both the liver and the enterocyte,[52] it appears that extensive conjugation of isoflavones occurs by intestinal UDP-glucuronyltransferase on first-pass uptake.

Unlike endogenous estrogens, which are extensively bound to sex hormone-binding globulin and albumin, genistein and equol have both been reported to be only 45% to 50% protein bound.[53] In contrast, biochanin A and formononetin exhibit strong protein binding.

## PREVALENCE OF USE

Peri- and postmenopausal women can consume soy and soy isoflavones via two sources: their diet (in soy-containing foods, soy milk, and foods containing soy flour or soy oil) and dietary soy and isoflavone dietary supplements.[54] Isoflavones are contained in many edible plants but among foods consumed in the United States, only in soybeans is their concentration sufficient to be physiologically relevant.[55] Other nonfood sources (such as red clover) also contain notable amounts.[56] Most soy and isoflavone supplements are derived from soybeans, and dozens of types of such supplements are marketed, sometimes in combination with vitamin and mineral supplements. Soy and isoflavone supplements are regulated in the United States under the Dietary Supplement Health and Education Act, under which their marketers cannot make health claims related to disease risk reduction but may make claims that they support the structure or function of the body.[54] While the US government (Food and Drug Administration and Federal Trade Commission) has the power to inspect, they have insufficient personnel to effectively implement the regulations involving quality control or health claims.

### Dietary intake of soy and soy isoflavones

Frequently used soy foods and their isoflavone content are listed in Table 2. The isoflavone content of each soy food can vary considerably depending on growing conditions and processing. In Southeast Asia, many soy foods are manufactured from fermentation of soy beans (eg, miso and tempeh). This process tends to concentrate the isoflavones prior to consumption and produces metabolites not formed in the human body. Other processing that removes fats, taste, and color tends to remove isoflavones.

Functionally, in theory, isoflavones can exert both estrogenic and antiestrogenic effects, depending on their concentration, the concentration of endogenous sex hormones, and the specific end organ involved. Some effects of these molecules may result from interactions with pathways of cellular activity that do not involve the ERs. In addition, it is not clear whether the putative health effects in human beings are attributable to isoflavones alone or to isoflavones plus other components in whole foods.

#### *US intake*

Although soy-containing foods have been consumed by Asian populations for centuries, the best-known soy food, tofu, was only introduced on a large scale in US markets in the 1970s.[58]

As more and more scientific publications over the last few decades suggested potential health benefits of dietary soy and isoflavones, US soy food sales increased—from $1 billion in 1996 to $4.5 billion in 2009.[59] The most dramatic increase occurred between 1996 and 2003, with the biggest gains occurring in sales of soy milk and energy bars. Recently introduced categories of soy foods include soy-based drinks, drinkable cultured soy, soy dairy-free frozen desserts, and energy bars, all of which have shown strong and steady growth in sales. US food manufacturers introduced more than 2,700 new foods with soy as an ingredient from 2000 to 2007. Previously, most sales of soy food and drinks occurred in health food stores, but now 75% of these sales are from supermarkets.[60]

**TABLE 2.** *Isoflavone content of foods*[57]

| Food | Mean mg isoflavones per 100 g of food |
|---|---|
| Soybeans (green, raw, edamame) | 48.95 |
| Soy flour (textured) | 172.55 |
| Soy protein isolate | 91.05 |
| Miso soup (mix, dry) | 69.84 |
| Tempeh | 60.61 |
| Soybeans (mature seeds, sprouted, raw) | 34.39 |
| Tofu (silken) | 18.04 |
| Tofu yogurt | 16.30 |
| Soy hot dog (frozen, unprepared) | 1.00 |
| Soy milk (original, vanilla) | 10.73 |
| Soy sauce (soy + wheat; shoyu) | 1.18 |

From 2006 through 2009, approximately one third of Americans consumed soy foods or beverages once a month or more frequently. In addition, soy flour and soy oil are used in baked goods, entrees, cereal, pasta, meal replacements, powdered soy beverages, chips, snack foods, and low-carbohydrate foods, comprising about one third of total soy food sales in recent years.[59] Soy isoflavones may also appear unexpectedly in many products in which soy protein is used for its textural properties.

Soy food intake has been assessed specifically among midlife US women. A 2002 telephone survey of 886 women ages 45 to 65 who were members of the Group Health Cooperative in Washington State found that 22.9% used dietary soy.[61] Breast cancer survivors were six times as likely as the overall survey respondents to report use of dietary soy, while women taking hormone therapy (HT) were half as likely to report use of dietary soy. The Study of Women's Health Across the Nation (SWAN), a multisite, longitudinal US cohort study, found substantial variation in dietary isoflavone intake by race/ethnicity among 3,133 women who were premenopausal or early perimenopausal at baseline.[62] Considerably lower isoflavone intakes were found at baseline in Caucasian, Hispanic, and African women (averaging <0.5 mg, a level of questionable biologic significance) than in Asian women (averaging >9.7 mg/d in women of Chinese ethnicity and >18 mg/d in women of Japanese ethnicity). Approximately 40% of non-Asian US women in SWAN consumed no daidzein or genistein, the most prevalent isoflavones.

### *Comparative Asian intake*

To put these US dietary intakes into context, data from large Japanese cohort studies included in a comprehensive review published in 2006[63] indicated that soy protein intake among older Japanese adults was approximately 10 g/day and was nearly the same in women and men.[64-68] In these studies, soy foods contributed from 6.5%[68] to 12.8%[66] of total protein intake. Mean estimates of isoflavone intake (expressed as aglycone equivalents) ranged from about 30 to 50 mg/day.[63,69,70] In a prospective cohort study in Japanese adults ages 45 to 74, the mean fourth quartile isoflavone (excluding glycitein) intake was 78 mg/day in men (n = 9,044) and 77 mg/day in women (n = 10,121).[71] According to food disappearance data from the United Nations' Food and Agriculture Organization, per capita soy protein intake in Japan has remained relatively constant during the past 40 years, although soy's share of total protein intake has decreased from about 13% to 10% because of the increased protein content (mostly from animal sources) of the Japanese diet.[63]

Additional context for US isoflavone intake comes from mainland China, where diet is more heterogeneous than in Japan. The Shanghai Men's Health Study and the Shanghai Women's Health Study, prospective epidemiologic studies involving approximately 50,000 participants each between ages 40 and 70,[72-74] have indicated that daily mean soy protein and isoflavone intakes in Shanghai are similar to[72,73] or somewhat higher than[74] those in Japan.

### Soy supplement use

Studies on the prevalence of soy or isoflavone supplement use in the United States are very limited. The only data from a national sample, the 2002 National Health Interview Survey (NHIS),[75] indicate that 9.4% of US adults reported use of soy supplements in the prior 12 months, but this survey report did not specifically address use among midlife women.

In the absence of further data specific to soy supplement use, it may be reasonable to look to use of complementary and alternative medicine (CAM) as a surrogate. Among all gender and age groups in the 2002 NHIS, midlife women had the highest prevalence of reported CAM use.[75,76] Approximately 45% of women ages 40 to 59 in the 2002 NHIS reported any CAM use in the prior 12 months, and 28.4% reported use of biologically based CAM therapies.[77] The latter percentage was up from 16.2% from the 1999 NHIS.[78] In both the 1999 and 2002 NHIS, use of biologically based CAM therapies was highest among Asian and Caucasian women. Use of medicinal herbs, which include soy, comprised the largest proportion of these therapies, with 18.6% of women overall reporting such use in the 2002 NHIS.[79] A similar level of use of medicinal herbs and teas was reported in a separate nationally representative sample of US women conducted in 2001, with complex and nuanced differences in use by race and ethnicity.[80]

### Conclusions

US dietary consumption of soy has increased severalfold over the past 15 years, with one third of Americans consuming soy food or beverages at least once a month. Despite this increase, dietary consumption of soy in the United States remains far below that in Asia. Although nearly 1 in 10 US adults reported use of soy supplements in a 2002 nationwide survey, further studies are limited. More research is needed to understand the health reasons for soy use among midlife women—specifically of soy isoflavone supplements. In addition, the interrelations of dietary intake and supplement use with equol production in terms of effects on health outcomes in midlife women require further study, as do potential interactions with prescription and over-the-counter medications.

## MENOPAUSAL SYMPTOM EFFECTS

### Key recommendations

- In postmenopausal women with distressing vasomotor symptoms, initial treatment with isoflavones is reasonable.
- The starting isoflavone dose should be 50 mg/day or higher, and therapy should be given for at least 12 weeks.
- Studies of women who do not benefit from soy isoflavones should be undertaken to monitor longer-term beneficial or possible adverse effects.
- If a woman responds to isoflavone supplementation, treatment can continue with monitoring for side effects; if a woman does not respond after 12 weeks, other treatment options should be discussed.

- A supplement containing natural S(–)-equol may be effective for some women who do not have the capacity to produce equol.

**Vasomotor symptoms**

In the past decade, a major effort has been made to determine if and to what extent soy or soy isoflavones can control menopause-related vasomotor symptoms (ie, hot flashes). The original basis for that effort was the observation that only 10% to 20% of Asian women report hot flashes whereas 70% to 80% of North American women report experiencing them. The speculation was that the isoflavones present in the high soy diets of Asian women were providing some protection by binding to ERs and thus might be comparable to the well-known benefits of prescription HT.

***Clinical outcomes of soy foods and soy supplements***

The studies summarized below were selected as good examples of more recent randomized controlled trials (RCTs) evaluating the efficacy of isoflavones in the treatment of postmenopausal vasomotor symptoms. The majority focused specifically on soy isoflavones, while a few evaluated red clover isoflavones. Finding alternatives to HT has become a priority for midlife women since the Women's Health Initiative reported adverse cardiovascular and cancer outcomes in the estrogen-progestin group.[81] Despite the fact that several RCTs have now been conducted, most have had some notable limitations, including:

1. Lack of comparability of agents (eg, soy and red clover isoflavones)
2. Individual versus combination isoflavones (eg, genistein alone vs a combination of genistein, daidzein, and glycitein)
3. Use of glycosides versus aglycones
4. Variations in dose and duration of therapy
5. Relatively small sample sizes
6. Use of a variety of tools to evaluate symptoms
7. Lack of measurement of compliance
8. Lack of identification of women who could convert daidzein to equol
9. Lack of control for concurrent use of medications (especially antibiotics, which may alter intestinal bacteria, hence decrease equol production)
10. Lack of control for dietary sources of isoflavones (possibly accounting for the observed placebo effect)
11. Lack of identifying potential modifiers of soy's effectiveness (eg, menopausal status, ability to produce equol, previous breast cancer, race/ethnicity)
12. Differences in definitions of menopause for inclusion purposes (>6 mo of amenorrhea vs >12 mo of amenorrhea)

The following 14 studies were selected by the panel because they all included the following: dose of soy isoflavones, mean age of the study participants; prevalence of hot flashes at baseline and also the magnitude of improvement, treatment duration of at least 12 weeks, and patient population who had experienced natural (not induced) menopause.[82-95]

*Combined statistics of the 14 trials:*

- Total number of women in the trials was 1,422 (761 in the isoflavone arms and 661 in the placebo arms).
- Dose of isoflavones ranged from 40 to 160 mg/day.
- Mean age was 53.
- Duration of trials ranged from 12 to 96 weeks.
- Majority of women were Caucasian and within 5 years of their final menstrual period.
- Daily prevalence of hot flashes at baseline ranged from 3 to 11 episodes.

*Combined results of the 14 trials:*

- A total of 11 showed significant improvement of vasomotor symptoms in the isoflavone arms compared to placebo, while three trials failed to show any benefit.
- The percentage of decrease in daily frequency of hot flashes ranged from 24% to 60%.
- The dose of 50 to 60 mg/day was sufficient for significant symptom improvement over placebo in many of the studies.
- Although some studies using higher doses of soy isoflavones also reported significant benefit, no linear dose-response relationship was observed.
- It appeared that women who benefitted from isoflavones experienced at least four episodes per day at baseline, which generally agrees with previously published data.[96]
- Women experiencing more than the four daily hot flashes did not necessarily show greater improvement over placebo.
- Trial duration of 12 weeks was sufficient to see a benefit in the isoflavone group over placebo; trials of longer duration did not necessarily result in a greater improvement in symptoms.

***Clinical outcomes of supplements high in genistein***

A 2006 review of papers that characterized the isoflavone composition of the supplements used concluded that isoflavone supplements containing predominately genistein reduced hot flashes.[97] In the five studies (177 treated patients) that provided more than 15 mg/day of genistein (in aglycone equivalents), a significant reduction was observed in hot flash symptoms. Whereas, in six studies (201 treated patients) that provided less than 15 mg genistein/day, only one reported any decrease in vasomotor symptoms.

Genistein given alone has been reported to have beneficial effects on vasomotor symptoms. One study[86] used pure genistein (54 mg/d) with significantly beneficial results in reducing symptom frequency. Another study[87] used the same product and replicated the results with a larger sample size (genistein, n = 119; placebo, n = 117). The women in the treatment group were given two tablets per day containing 27 mg total isoflavone, 98% of which was reported to be

genistein. In addition, the tablets contained 400 IU of vitamin D and 500 mg calcium carbonate. At 12 and 24 months, the number of episodes experienced by the women in the placebo group was about four per day and the number in the treated group was about two per day (with both groups having at baseline about four episodes a day).

More than 12 studies have been conducted in which the genistein content of the supplement is known. Further analysis is needed to confirm the 2006 conclusion[97] that more than 15 mg genistein/day results in a significant reduction in hot flashes.

### *Does equol production predict the effectiveness of soy isoflavone treatment?*

There is increasing evidence that women whose gut bacteria have little or no capacity to convert daidzein to equol may continue to suffer from severe hot flashes despite soy isoflavone supplementation.[98] A 6-month proof-of-concept trial studied the importance of intestinal equol generation on improvement in hot flashes.[99] Healthy menopausal women (n = 96) were randomized to the isoflavone or placebo group. The isoflavone group was further divided into equol producers (n = 34) and nonproducers (n = 32) based on urinary equol levels after consuming 135 mg isoflavones/day for a week. In both the equol producers and nonproducers, women ingested 3 g soy germ extract powder twice a day for 6 months (equivalent to 135 mg/d). Equol producers showed significantly greater reduction in some categories of Kupperman menopausal symptom scores than the placebo group, while no such benefit was observed in nonproducers.

Because the majority of trials of soy isoflavones did not measure serum or urinary levels of equol, the status of the study participants as equol producers or nonproducers is not known. Furthermore, as discussed previously, many factors influence the conversion of equol from daidzein. Recently, some attention has been directed toward using a natural equol supplement to treat menopausal symptoms in which the majority of daidzein is converted to S(–)-equol. A few clinical trials have been completed with such a product.

### *Is equol treatment alone effective?*

The first RCT using a natural equol supplement in which the daidzein had been converted to S(–)-equol to evaluate its efficacy in relieving menopausal symptoms was conducted with Japanese women. In that 12-week trial,[100] 134 healthy Japanese women ages 40 to 59 were divided into three groups: 10 mg equol/day (n = 44), 10 mg equol three times a day (n = 46), and placebo (n = 44). The self-reported menopausal symptom and mood scores before and after 12 weeks of intervention were evaluated. No benefit was seen in equol producers; improvement was seen only in the 10 mg equol thrice daily group of equol nonproducers in menopausal symptom and mood scores (decrease in somatic symptoms, anxiety, depression, tension, vigor, and fatigue). To confirm these findings, another 12-week RCT parallel trial in Japanese equol nonproducing peri- and postmenopausal women was performed.[98] During the placebo run-in phase, equol nonproducers who showed a greater than 50% reduction of symptoms were excluded from the trial. The remaining participants, who were randomized to 10 mg S(–)-equol/day (n = 66), experienced a significant reduction in the frequency and severity of hot flashes compared with the placebo group (n = 60). Women in the equol group also showed a greater reduction in the severity of neck and shoulder stiffness (frequently reported menopausal symptoms in Japan) compared to the placebo group.

Another trial, conducted in the United States, compared the efficacy of a supplement containing primarily S(–)-equol to a supplement that combined genistein, daidzein, and glycetin.[101] A total of 102 postmenopausal women (ages 45-65) with moderate to severe hot flashes were randomized to the S(–)-equol supplement at doses of 10, 20, or 40 mg/day or the combination supplement with a total isoflavone dose of 50 mg/day for 8 weeks. Reduction in hot flash frequency was similar in the 10 mg S(–)-equol and combination isoflavone groups. Women in the 20- and 40-mg equol groups, however, showed greater reduction in symptoms than those receiving the combination supplement.

## Vaginal dryness

Only two studies have explored the potential benefits of isoflavones for the treatment of postmenopausal vaginal dryness. In one double-blind, crossover RCT,[102] the isoflavone treatment consisted of 114 mg isoflavones/day for 3 months. The investigators concluded that the isoflavones had no effect on subjective perception of vaginal dryness or on objective findings in the vagina.

A year later, in a second crossover RCT, the peri- and postmenopausal women were given either a daily placebo or 25 g soy. The authors concluded that a soy-rich diet did not relieve urogenital symptoms, restore vaginal epithelium, or improve vaginal health.

## Adverse effects

The majority of RCTs have reported mild adverse events with isoflavone use, mainly centered on gastrointestinal tolerability or taste issues. Although the studies discussed above were designed to evaluate the efficacy of soy isoflavones, some evaluated—and found no increase in—endometrial thickness, vaginal cytology, or breast density associated with soy isoflavone intake. While these data are reassuring, most of the trials were short-term. Long-term studies adequately powered to conclusively evaluate safety issues of soy supplementation are needed. Note that safety has not been discerned for breast cancer survivors.

## Conclusions

Soy-based isoflavones are modestly effective in controlling hot flashes, as demonstrated to date in predominantly Caucasian women in early postmenopause who have at least four hot flashes a day. The minimal dose at which significant benefit has been seen is 50 mg total isoflavones/day, which could be considered the starting dose. A trial of 12 weeks is generally sufficient to evaluate response to therapy. Supplements

providing pure or higher proportions of genistein have shown particular benefit. Initial trials of supplements containing primarily natural S(–)-equol also look promising.

Further research is needed to evaluate efficacy of isoflavones in racially and ethnically diverse populations and in younger perimenopausal and older postmenopausal women who can continue to have symptoms 10 to 20 years postmenopause. The role of isoflavones in equol producers versus nonproducers also needs to be evaluated.

## BREAST & UTERUS EFFECTS

### Key recommendations

- Soy foods, in populations that typically consume them, appear to be breast cancer protective. Therefore, moderate lifelong dietary soy consumption is recommended as part of a healthy lifestyle. The best evidence indicates that there is no adverse effect of this dietary pattern, and that there is potential for prevention of breast and endometrial cancer.
- Dietary soy and isolated isoflavones should not be considered equivalent.
- Studies of endometrial risk should focus on long-term, postmenopausal exposures.
- Specific recommendations regarding soy or isoflavone consumption by breast cancer survivors cannot be made at this time; studies in human subjects indicate a null or protective effect, whereas cell culture and rodent studies indicate potential for risk. Further studies are needed.

### Mechanisms active on the breast and uterus

Soy isoflavones as estrogen-like compounds[103] but are much less potent than estradiol[14] and are ER-β-selective ligands.[14] They can alter metabolism[104,105] of endogenous estrogens, potentially producing indirect effecs on estrogenic pathways.

There are many other mechanisms by which soy isoflavones may be cancer protective, including antiproliferative effects,[106] tyrosine kinase inhibition,[1] modulation of steroid hormone metabolizing enzyme activity,[104] induction of apoptosis,[107] and inhibition of angiogenesis.[108-110] Dietary isoflavones reduce circulating[111] and intra-breast[112] estradiol concentrations in monkeys, with a corresponding decrease in uterine and breast tissue proliferation.

Genistein administered to young animals causes both cell proliferation and cell differentiation in the mammary gland.[113] These animals, when challenged with a carcinogen, have fewer mammary tumors than controls. Daidzein, on the other hand, had no chemopreventive effects.[114] Similar experiments carried out using equol revealed that the estrogenic S(–)-equol had no chemopreventive effect (consistent with the lack of effect of daidzein), whereas the unnatural diastereoisomer R(+)-equol caused a 43% reduction of palpable mammary tumors.[115] And yet, both equol isomers caused precocial mammary gland development and differentiation.[116]

### Clinical outcomes on the breast

In vitro, mammary cancer cells expressing ER-α grow in response to genistein at doses in the low micromolar range, whereas ER-negative cells do not.[117] In these cells, gene expression patterns are similar to those of estradiol.[118] However, the majority of rodent studies[119] clearly indicate a preventive effect of early-life soy isoflavone exposure for mammary cancer.[113,120] In contrast to this early protective effect, once neoplasms are present, genistein exposure can increase the proliferation of mammary cancers in rodents.[121,122] Thus, timing of exposure may be a critical determinant of the balance between benefit and risk for the breast. In contrast to the stimulatory effects on the mammary glands in cell culture and rodent models, one study showed that in nonhuman primates, soy isoflavones did not stimulate proliferation of the mammary glands, but rather diminished the mammary gland proliferation induced by exogenous estrogens.[111]

The observation of subpopulations of human equol producers and nonproducers has led to recent interest in this metabolite of daidzein, which is produced by most nonhuman species but not by most humans. Data suggest that S(–)-equol is not a strong antioxidant nor a chemopreventive agent in animal and cellular models.[115,116,123] In observational studies of Western populations, little association of breast cancer risk in relation to equol has been suggested.[124] In contrast, in Asian populations who have higher prevalence of soy consumption and equol producers,[125] equol may be associated with lower breast cancer risk.[124]

A meta-analysis of soy intake and breast cancer risk evaluated 18 epidemiological studies (12 case-control and six cohort) published between 1978 and 2004.[126] High soy intake was associated with modestly reduced breast cancer risk. Among the 10 studies that stratified by menopausal status, the inverse association between soy exposure and breast cancer risk was stronger in premenopausal women. In these studies, risk estimate levels, measures of soy exposure and controls for confounding factors varied considerably. The authors concluded that soy intake may be associated with a small reduction in breast cancer. A more recent meta-analysis showed a significant trend of decreasing breast risk with increasing soy food consumption among Asian women.[127] Compared to the lowest soy food intake (<5 mg isoflavones/d), risk was intermediate with moderate intake (~10 mg isoflavone/d) and lowest with high intake (>20 mg/d). Soy intake was unrelated to breast cancer in low-consuming Western populations, whose average highest and lowest isoflavone intake levels were around 0.8 to 15 mg per day.

Evidence has been continuing to emerge since 2007 suggesting that soy food intake may reduce the risk of breast cancer, particularly among Asian women:

- In Dutch women, high circulating genistein levels were associated with reduced breast cancer risk.[128]
- The Japan Collaborative Cohort of over 30,000 women, however, found null association between soy food intake and breast cancer risk.[129]
- In another large Japanese study in which serum isoflavones were measured, a benefit was seen for genistein but not daidzein.[130]

- In a prospective study of 35,303 Singapore Chinese women followed from 1993 to 1998, the breast cancer risk was reduced significantly among women with high soy intake; the association was predominantly seen in postmenopausal women.[131]
- The Shanghai Women's Health Study, a population-based cohort study of 75,000 women, reported an almost 50% lower breast cancer risk in premenopausal women at the highest quintile of soy consumption.[132]
- A multiethnic cohort study including 36,458 women reported a significant reduction in breast cancer risk in Japanese-American women in association with high excretion of urinary daidzein. The breast cancer risk was reduced among Caucasian women with the highest compared with the lowest quartile excretion of urine daidzein.[133]
- Several recent case-control studies from Asia also reported an inverse association between soy food consumption and breast cancer risk; the association did not appear to depend on ER status.[134-137]
- Although limited, studies have found an equal or stronger inverse association between breast cancer and soy intake during adolescence than in adulthood, suggesting a life-stage-specific benefit for soy food consumption.[16,17,132,138]
- A case-control study examining childhood intake (defined as ages 5-11) showed a profound inverse risk association among Asian-American women, an effect that was not altered by later adult intake.[18]

### *In postmenopausal women*

Intervention studies have been recently reviewed[139]; selected studies are summarized in Table 3.

### *In women with breast cancer*

Of particular interest to the question of isoflavone effects on preexisting breast cancer is the recent report from the Shanghai Breast Cancer Study, demonstrating a lack of tumor-promoting effect in breast cancer patients.[140] In this study, dietary soy consumption by breast cancer survivors was significantly associated with lower risk of recurrence and death ($P < 0.01$ for trend by quartile of consumption). Similar findings were reported for women in the Life After Cancer Epidemiology in western US women,[141] and for women receiving anti-estrogen (anastrozole) therapy.[142] Consumption of soy isoflavones before diagnosis was also not associated with any adverse effect on survival.[143] Few intervention studies have been done in women with breast cancer; one older study included primarily benign disease.[144] In a pilot study, isoflavone supplementation (200 mg/d) for 2 weeks did not alter breast proliferation (mitosis counts) in 14 women with invasive breast cancer.[145] One registry-based retrospective immunohistochemical study of proliferation and expression of estrogen receptor, progesterone receptor, and HER2/neu was done using cancerous tissues from a subset of the Hawaiian Multiethnic Cohort; no differences in proliferation or sex steroid receptor expression were seen with respect to either adult or childhood soy consumption; HER2/neu expression was decreased in women consuming soy as adults.[146]

### Clinical outcomes on the uterus

Genistein and daidzein induce alkaline phosphatase expression in endometrial Ishikawa cells but with one millionth the potency of estradiol.[147] The dose required for uterotrophic effects of genistein in immature mice is approximately 10,000 times the dose of estradiol,[148] and in adult rodents appears to be around 50 mg/kg/day.[149] Exposure of neonatal mice to high doses of genistein may induce uterine adenocarcinomas later in life.[150] Genistein at high doses induces a gene expression profile similar to estradiol in the mouse uterus,[151] but different patterns of ER-α and ER-β expression in the uterus have been seen in the uterus of rats given genistein, daidzein, or equol.[152] The type and ratio of specific isoflavones may produce strikingly different effects in the uterus of mice, with daidzein-rich mixtures being the most uterotrophic.[153]

Primate reproductive physiology differs markedly from that of rodents and other species. Only humans and Old-World primates menstruate. Therefore, particular significance is attached to the inability to produce endometrial hyperplasia in nonhuman primates by administration of soy isoflavones, even at doses that exceed the highest human exposures by approximately 10-fold on a caloric basis.[154]

A case-control study that included 424 cases of endometrial cancer and 398 controls suggested a decreased cancer risk in lean women taking isoflavones.[155] In another study involving 500 cases and 470 controls in non-Asian women around San Francisco, the highest quartile of total isoflavone intake was associated with lower endometrial cancer risk.[156] In a third case-control study in China with 832 cases and 842 controls, soy food consumption was inversely associated with endometrial cancer risk, particularly among women with higher

**TABLE 3.** *Intervention studies on the effect of soy products on breast tissue*

| Study | N | Sample | Control | Treatment | Results |
|---|---|---|---|---|---|
| Cheng et al, 2007[85] | 60 | Postmenopausal women | Placebo | 60 mg isoflavones per d for 3 mo | No effect on ER-α, ER-β, ER-βcx, PRA, PRB, AR, or Ki67 |
| Hargreaves et al, 1999[144] | 84 | Premenopausal women | | 45 mg isoflavones per d for 14 d | No effect on estrogen receptors, progesterone receptors, apoptosis, mitosis, or Bcl-2 expression |
| Maskarinec et al, 2009[146] | 406 | Postmenopausal women | Placebo | 80-120 mg isoflavones per d for 2 y | No effect on breast density |
| Verheuset et al, 2007[128] | 202 | Postmenopausal women | Placebo | 99 mg isoflavones per d for 12 mo | No difference in breast density between placebo and treatment |

ER-α, estrogen receptor alpha; ER-β, estrogen receptor beta; ER-βex, ER-β splice variant; PRA, progesterone receptor A; PRB, progesterone receptor B; AR, androgen receptor.

body mass index and waist-hip ratio.[157] A meta-analysis of four case-control studies estimated a summary risk of 0.73.[158]

A number of RCTs of the effect on the uterus of soy treatment for climacteric symptoms have been published:

- A 3-month study did not find any effects on endometrial thickness, estrogen, progesterone, androgen, or Ki-67 expression in postmenopausal women taking 60 mg isoflavones/day.[85]
- In a study involving 64 postmenopausal women with a history of breast cancer, treated with 114 mg isoflavones or placebo, no change in endometrial histology, expression of estrogen, or progesterone receptors or Ki-67 was observed.[102]
- In a 6-month study on the effects of 0.5 mg estradiol plus 120 mg isoflavones or 1.0 mg estradiol plus 120 mg isoflavones or placebo, no protection of the endometrium from estradiol-induced hyperplasia was seen.[159]
- In another study, 62 postmenopausal women were administered 72 mg isoflavones or placebo for 6 months.[92] Soy had no effect on endometrial thickness.
- In one follow-up study for 12 months[160] with a total of 395 postmenopausal women treated with 70 mg isoflavones, no stimulation of the endometrium was observed.
- The same result was seen in 198 women given 54 mg genistein/day compared to placebo.[161] When the same women were followed for 24 months, no significant difference was found in mean endometrial thickness and the maturation value score between the two groups.[162]
- The longest follow-up to date was 5 years.[163] A total of 298 postmenopausal women completed 5 years of treatment with 150 mg isoflavones or placebo; 70% of treated women had an atrophic or nonassessable endometrium versus 81% in the placebo group. The authors concluded that long-term treatment is associated with an increased occurrence of endometrial hyperplasia.

Evidence from published human trials reveals that soy isoflavone treatment does not stimulate proliferation in the endometrium during short-term treatment for at least 2 years. More studies on the long-term effects need to be performed.

### Conclusions

Isoflavone effects on the breast and uterus present a complex picture, with some conflicting experimental findings. These may be summarized as follows:

- The predominant soy isoflavones (genistein and daidzein), as well as the daidzein metabolite equol, are weak ER-β-selective estrogen agonists but exert other ER-independent effects as well.
- Soy isoflavones prevent mammary carcinogenesis in rodents when given prior to exposure to a carcinogen.
- Genistein promotes the growth of ER-positive mammary cancer cells in vitro and in vivo.
- Soy isoflavones are weakly uterotrophic in rodents, but there is no stimulatory effect of isoflavones on the nonhuman primate uterus.
- Soy isoflavones antagonize the effects of estradiol on the breast and uterus of nonhuman primates.
- Soy food consumption is associated with lower risk of breast and endometrial cancer in observational studies.
- Soy food consumption or intervention in women does not promote breast cancer growth or cancer recurrence.
- Short-term studies in women provide no evidence for uterotrophic effects, but a single long-term study found isoflavone supplementation was associated with a slight increase in simple endometrial hyperplasia in women.

Preclinical and clinical observations of soy isoflavone effects on the breast and uterus are confounded by differences in species evaluated, soy isoflavone type and dose, verification of exposure, timing of exposure, and experimental outcomes measured. Only in the case of the rat mammary carcinogenesis model is there a large body of experimental evidence in which prevention is the major finding. Primate studies provide a model that is genetically and physiologically more similar to the human primate, and in that model, isoflavones inhibit estrogen-induced stimulation of the breast and endometrium. The major unresolved questions relate to the applicability of rodent studies to human risk; the potential for life-stage-specific risks or benefits; the potential for differing effects of different isoflavones; and the effect of isoflavones on endogenous estrogen metabolism. As noted in a recent National Institutes of Health consensus statement, greater standardization and documentation of clinical trial data of soy are needed.[51]

## BONE EFFECTS

### Key recommendations

- Efficacy studies of soy isoflavones on bone density should be performed for a minimum of 24 months.
- More human studies on bone density need to be conducted with equol producers, soy products with a higher genistein content, and higher doses of isoflavones.

### Mechanisms active on bone

Genistein may activate ER-β in osteoblasts. Because it is also a tyrosine kinase inhibitor, it inhibits cell growth and acid transport in bone cells leading to inhibition of osteoclast activity.[164] Therefore, soy isoflavones may suppress bone resorption and minimize bone loss under some conditions.

### Clinical outcomes in animals

The ovariectomized rat model is classically used for studying osteoporosis. Combination isoflavones have generally provided little benefit to bone in ovariectomized rats. In one study, 6-month-old ovariectomized and sham-operated rats were studied with an isolated combination of isoflavones at levels of 0.3 and 0.8 mg/g of diet compared to estrogen.[165] Estrogen, but not soy isoflavones, was found to prevent trabecular bone loss in these rats, and no advantage was found in combining estrogen and soy isoflavones. The study also demonstrated that estrogen, but not soy isoflavones, suppressed bone remodeling as determined by dynamic histomorphometry and kinetic

modeling. Calcium and bone balance (bone formation minus bone resorption) were unaffected by soy isoflavones. Another 3-year longitudinal study in 181 ovariectomized female monkeys compared the effect of a diet with or without soy isoflavones to estrogen treatment on spine and whole body bone mineral content and bone mineral density (BMD); the results showed that estrogen, but not soy isoflavones, was found to protect against menopausal bone loss.[166]

Equol was found to be as effective as estradiol in preventing bone loss but without stimulating the uterus.[167] Genistein has also had similar effects in preventing bone loss in several other studies.[166-170] One of these studies suggested that genistein increased bone formation.[168] The amounts of soy isoflavones used in these animal studies, however, were severalfold greater than those used in human studies.

**Clinical outcomes in humans**

Studies of Asian populations who regularly consume much larger amounts of soy than Caucasians have suggested that a high-soy diet is related to a much lower incidence of osteoporotic fracture.[171] For instance, genistein intake was twofold higher in Japanese than Chinese women and 2,000 times higher than in Caucasian women.

In the US SWAN study, genistein intake was correlated with bone density in premenopausal Japanese-American women. In the top tertile of genistein intake, the adjusted spine bone density was 7.7% higher and femoral neck bone density was 12% higher compared to the lowest tertile of intake.[172]

There have now been 23 human studies on the effect of soy isoflavones on bone density. In general, a decrease in bone density at 12 months is probably a valid indicator of no effect. A positive result within 12 to 18 months, however, may represent the transient remodeling effect of an antiresorptive agent. Therefore, efficacy studies should be performed for a minimum of 24 months. Only four of the 23 studies were properly designed to address the potential efficacy of soy isoflavones to prevent bone loss.[173-176]

- In the first study, there was a very significant increase in bone density at all bone sites of approximately 5% compared to a 5% loss on placebo in a study comparing pure 54 mg genistein/day to placebo. The results from this study are completely at variance with the above studies that used comparable doses of genistein.[176]
- The second study used soy protein isolate with 90 mg isoflavones/day (48 mg genistein; 38 mg daidzein; 6 mg glycetin) compared to soy protein isolate without isoflavones and milk protein as a control. There was no significant effect on bone density amongst groups.[173]
- A third study compared tablets containing 80 mg soy hypocotyl isoflavones/day (10 mg genistein; 44 mg daidzein; 27 mg glycetin) or 120 mg/day (15 mg genistein; 66 mg daidzein; 40 mg glycetin) to placebo. There was significantly less bone loss on total-body BMD with 120 mg/day and there was no effect on spine and hip. All three groups lost bone over the 2-year study period.[174]
- And, the fourth study also compared tablets containing 80 mg soy protein isolate isoflavones/day (40 mg genistein; 31 mg daidzein; 9 mg glycetin) or 120 mg/day (60 mg genistein; 46 mg daidzein; 14 mg glycetin) to placebo. There was no significant effect on BMD among groups in the intent-to-treat analysis. After adjusting for age, body fat, and bone resorption, there was a significant effect of the 120-mg dose on femoral neck density although all three groups lost bone over the 3-year study period.[175]

It is possible that the failure of isoflavones to prevent bone loss in the human studies is due to the low number of equol producers in the study groups.[49]

- In a recent 1-year Japanese study of 75 mg isoflavones/day (38 mg daidzin; 0.6 mg daidzein; 8.6 mg genistin; 0.2 mg genistein; 24 mg glycitin with glycitein) compared to placebo, the results were analyzed according to equol status.[177] There was no effect on spine BMD comparing equol producers and nonproducers but there was a significant benefit on total hip and femoral neck BMD in the equol producers.
- A 1-year double-blind RCT with 10 mg natural S(–)-equol supplements/day for 93 equol-nonproducing postmenopausal Japanese women resulted in some inhibition of urine bone resorption markers and significantly less bone loss at the hip though there was no effect on the spine.[178] However, the effects are clearly less than those reported with low-dose estrogen. The potential bone benefits reported with natural S(–)-equol in Japanese women need confirmation in a Caucasian population.

Differences in soy products may also have different effects on bone metabolism. In a small study, the effect of soy cotyledon, soy germ, red clover, and kudzu were compared on calcium-41 excretion as markers of bone resorption and 1 mg estradiol/day was used as the active control.[179] Serum genistein levels were four times higher on soy cotyledon than soy germ. Soy cotyledon and soy germ had a mild antiresorptive effect resulting in a decrease in bone resorption markers of 10% compared to 25% on estradiol.

There are no prospective intervention studies of soy isoflavones on the incidence of bone fracture. In an observational study of Chinese women over a 4.5-year period, women in the highest quintile of soy intake had a lower fracture rate.[180] And there is some epidemiologic evidence that soy may reduce risk of osteoporotic fracture in women.[181]

**Conclusions**

Despite the theoretical considerations suggesting that isoflavones might have efficacy on bone in human studies, the long-term studies are mainly negative, especially in view of the well-known beneficial effects of low-dose estrogen on bone. However, the possibility remains that the isoflavone content was not sufficiently high to bind to ER-β and inhibit bone resorption. An ongoing study in North America—the

Women's Isoflavone Soy Health (WISH) Trial—is evaluating 25 g soy protein/day in postmenopausal women. The results from this study should be useful in the context of previous studies. Another limiting factor in soy studies might be that only 25% to 35% of women living in Western countries are equol producers.

Because there is currently no compelling evidence for the beneficial effect of soy isoflavones on bone density in postmenopausal women, more human studies need to be conducted with equol producers, with soy products with a higher genistein content, and with higher doses of isoflavones (>120 mg/d).

## CARDIOVASCULAR SYSTEM EFFECTS

### Key recommendations

- Independent of the effect of soy and soy isoflavones on cardiovascular disease (CVD), replacement of some dietary animal protein with soy protein should improve cardiovascular health.
- To better understand the impact of equol and equol production capacity on cardiovascular health, future clinical studies should include equivalent numbers of participants who are equol producers and nonproducers.
- Clinical studies are needed to determine whether soy and soy isoflavones have plasma lipid–independent benefits on cardiovascular health, particularly in perimenopausal and recently postmenopausal women.

### Mechanisms active on the cardiovascular system

The action of soy, isoflavones, and soy isoflavone metabolites are hypothesized to impact the cardiovascular system by three major mechanisms: 1) directly through ER-mediated effects; 2) through ER-independent effects directly on cardiovascular risk factors and putative atherogenic risk factors; and 3) indirectly through the displacement of animal protein intake.

Structurally similar to 17β-estradiol, soy isoflavones are estrogen agonist/antagonist–like compounds that functionally exert estrogenic and anti-estrogenic effects.[182,183] In animal models, these compounds require the presence of the ER to be anti-atherogenic.[184] Isoflavones are unique among estrogen-like molecules with preferential binding affinity for ER-β rather than ER-α,[182,183] suggesting that isoflavones could exhibit tissue-selective effects given the different tissue distribution of ER-α and ER-β[182] with particular selectivity for vascular tissue, which is ER-β rich.[184,185] However, binding affinities for the ER do not explain the functional complexity of isoflavones. For example, although genistein and equol have a similar 20-fold greater affinity for ER-β than ER-α, the transcriptional expression is greatest for equol relative to all other isoflavones.[186]

The contribution of the protein portion of soy versus the isoflavone portion to the cardiovascular effects reported remains unclear.[187-189] The general approach has been to feed monkeys isolated soy protein that contains its isoflavones (soy [+]) and to compare results when monkeys are fed isolated soy protein that has been alcohol-washed to remove its isoflavones (soy [–]). Monkeys fed soy (+) were found generally to have higher high-density lipoprotein cholesterol (HDL-C) concentrations (~12%) than those fed soy (–). The low-density lipoprotein–C (LDL-C) plus very low LDL-C concentrations of monkeys fed soy (+) were about 16% lower than those fed soy.

These studies suggest that the isoflavones are the primary component of soy responsible for its lipid-lowering effect; however, most studies show that administration of isolated isoflavones is less effective than giving the equivalent amounts as a part of the intact protein.[190,191]

Reduction in blood pressure may be another mechanism by which soy isoflavones improve cardiovascular health. A recent meta-analysis indicates that a daily ingestion of 25 to 375 mg soy isoflavones (aglycone equivalents) for 2 to 24 weeks significantly reduces systolic blood pressure by 1.92 mm Hg compared with placebo in adults with normal blood pressure and prehypertension; there was no effect on diastolic blood pressure.[192]

Beyond standard cardiovascular risk factors, soy isoflavones appear to possess activity in altering putative atherogenic risk factors. Isoflavones, and especially the daidzein metabolic product equol, possess antioxidant activity and are able to protect lipoprotein particles from peroxidation.[193,194]

Comprehensive preclinical studies have been conducted to explore whether and to what extent soy isoflavones may inhibit atherogenesis through anti-inflammatory mechanisms. Long-term (3 y) treatment of surgically postmenopausal monkeys with soy isoflavones was found to result in an anti-inflammatory effect by specifically altering circulating concentrations of sVCAM-1, an important mediator of the initiation and progression of atherosclerosis.[195,196] More importantly, treatment of monkeys with soy isoflavones has been shown to reduce mRNA for inflammation associate genes in atherosclerotic arteries, specifically MCP-1, ICAM-1, and IL-6.[197]

Another potential mechanism by which soy protein may be cardioprotective is by physical displacement of dietary intake of animal protein. Reduction in animal protein intake results in a reduction in dietary cholesterol and saturated fat intake.[198] Reduction in dietary cholesterol and saturated fat positively affects lipid levels, reduces weight, and improves insulin sensitivity.[199]

### Clinical outcomes

Coronary heart disease (CHD) is the major cause of morbidity and mortality among peri- and postmenopausal women. For that reason, there has been intense research on the potential benefit of soy protein and soy isoflavones on plasma lipids and lipoproteins as a means of reducing the risk for CHD.

Based on animal research and human epidemiologic evidence, many experts believed that consumption of soy protein and soy isoflavones might improve plasma lipid profiles and thereby reduce the risk of CHD. In 1999, the Food and Drug Administration approved the health claim that 25 g soy protein/day, as part of a diet low in saturated fat and cholesterol,

may reduce the risk of heart disease. Shortly thereafter, the American Heart Association (AHA) recommended dietary soy protein and isoflavones for decreasing the risk of CHD.[200] As more data accumulated, however, the AHA reversed its recommendation and concluded that soy and soy isoflavones had such small effects on plasma lipid profiles that they probably did not reduce CHD risk.[201]

Much of the early interest in the potential cardiovascular benefits of soy resulted from a meta-analysis of 38 studies, which concluded that consumption of 31 to 47 g soy protein/day could reduce plasma concentrations of both total cholesterol and LDL-C).[202] That meta-analysis was followed by a number of reports on the effect of soy protein on the plasma lipid profiles of postmenopausal women, some claiming benefits while others found no effects. There have been two well-controlled studies that seem to have placed the subject into proper perspective. One study concluded that the regular intake of high levels of soy protein (>50 g/d) had only a modest effect on blood cholesterol levels and was seen only in subjects with elevated LDL-C levels (>4.14 mmol/L), although soy protein was potentially helpful when used to replace animal products in the diet.[203] In the second study, 25.6 g soy protein containing 99 mg isoflavones administered to postmenopausal women daily for 12 months resulted in no significant effect on plasma concentrations of total cholesterol, LDL-C, HDL-C, triglycerides, or lipoprotein(a).[204]

These latter findings in women were consistent with a review on the effect of soy protein and soy isoflavones on plasma lipid profiles, largely in postmenopausal women.[190] A total of 17 studies involved soy protein and nine studies focused on soy isoflavone extracts. The authors concluded that both soy protein and soy isoflavone extracts resulted in very small reductions in total plasma cholesterol concentrations—primarily LDL-C values—and no changes in HDL-C levels, and that these decreases were likely too small to be clinically beneficial.

The conclusions above are consistent with the AHA advisory on soy and cardiovascular health, a review of 22 RCTs that compared isolated soy protein isoflavones with milk and other proteins.[201] The AHA advisory concluded that soy reduced plasma concentrations of LDL-C by approximately 3% on average, with no significant effects on HDL-C, triglycerides, lipoprotein(a), or blood pressure. The panel also considered 19 studies involving purified soy isoflavone extract, and found no consistent effects on LDL-C or other plasma lipid risk factors. The overall conclusion was that any cardiovascular benefit from soy protein or isoflavone supplements would be "minimal at best."

### Conclusions

Despite the reviews above, healthcare professionals should not rush to judge whether soy has cardiovascular benefits based solely on the effects on standard lipids. The clinical picture is still evolving. Plasma lipid concentrations are but one surrogate marker for atherosclerosis progression and the development of CHD. In one report, soy supplementation changed lipoprotein subclasses of postmenopausal women.[205] They reported a significant decrease in LDL-C particle number, a stronger indicator of CHD progression than LDL-C plasma concentration. Additionally, although isoflavone treatment of postmenopausal women does not usually improve plasma lipid concentrations, the isoflavones do result in significant improvement in arterial compliance and arterial stiffness, measurements closely associated with the degree of atherosclerosis.[206]

Whether isoflavone-containing soy protein treatment reduces the progression of atherosclerosis in postmenopausal women to an extent greater than its minimal effect on plasma lipids will soon be determined by the results of a prospective RCT—the NIH-supported WISH trial—with a primary endpoint focusing on change in carotid intima-media thickness.

Much interest has developed as to whether women who have the capacity to convert the isoflavone daidzein to equol derive cardiovascular benefits from soy supplementation while those lacking such capacity derive little or no benefits. One study reported rather large reductions in total and LDL-C concentrations in equol-producing women whereas equol-nonproducing women had no improvement in their lipid profiles.[207] It has been suggested that equol production could be induced with probiotics, the most commonly used being *Lactobacilli* and *Bifidobacteria*.[208] In addition, supplements markedly enriched with equol produced by fermentation of daidzein are now on the market.

Whether equol-producing capacity is a determinant of soy's cardiovascular benefits is uncertain at this time. One report found that pasta enriched with soy germ isoflavones improved markers of cardiovascular risk in equol producers,[209] while two other reports found no effect of equol-producing capacity on the cardiovascular benefits of isoflavone supplementation[210] or of soy food consumption.[211] Further, equol-producing capacity has been shown to be associated with favorable vascular function among women being treated with tibolone even when not being given soy supplements and thus not producing equol.[212]

## COGNITIVE EFFECTS

### Key recommendation

- More soy studies are needed to provide clear guidelines on the use of soy for cognitive benefits.

### Mechanisms active on the brain

Both ER-α and ER-β are abundantly expressed in brain and exhibit a pattern of distribution consistent with their roles in reproductive and cognitive function.[213-218] As expected, ER-α occurs in brain regions involved in regulation of reproduction but both occur, particularly ER-β, in brain regions involved in cognition.[213] The expression and localization of ERs are highly dynamic and can vary depending upon brain region, cell type, hormonal status, and neurological condition.

Soy extracts are highly complex and typically consist of an unknown number, type, and ratio of molecules. A portion of

these molecules may act as weak estrogenic agonists while others may act as antagonists of estrogen action.[219-221] This mixed pharmacological profile is consistent with the clinical outcomes but is not consistent with the many preclinical study outcomes that show efficacy of soy-derived molecules that preferentially target ER-β. For example, a formulation of three defined ER-β-preferring isoflavone agonists selected on their ER-β-binding properties induced efficacy in hippocampal cultures across a wide spectrum of estrogenic responses predictive of neuronal defense, survival, and plasticity.[222] Conversely, studies in the nonhuman primate brain indicated that equol was largely ineffective and in certain instances induced a trend toward decline.[223] These findings are consistent with earlier in vitro and in vivo findings indicating that isoflavones administered individually (eg, genistein or equol) might be ineffective alternatives to 17β-estradiol.[224]

Preclinical animal models typically use young ovariectomized animals and therefore do not address whether efficacy of soy depends on age or endocrine state. This issue is critical to understand the effects of soy isoflavones on the intended population of middle-aged women who are transitioning through or have undergone natural menopause to become reproductively senescent. The importance of this age and endocrine difference between the preclinical models and the human condition is highlighted by the data, which suggests but does not definitively demonstrate that soy intervention in young women can be beneficial whereas soy intervention postmenopause is not.[220]

## Clinical outcomes

To understand the impact of soy isoflavones on cognitive function, it is helpful to distinguish trials involving women younger than age 65 from trials involving women over age 65. This distinction addresses the "critical window hypothesis," a current influential hypothesis positing that estrogen can exert beneficial cognitive effects when initiated early in the menopause transition or postmenopausal period but exerts neutral or harmful effects when initiated later in life. Table 4 shows an overview of RCTs discussed below.

### *Studies in younger postmenopausal women*

- In one 3-month trial of 60 mg soy isoflavones/day or placebo in 33 postmenopausal women ages 50 to 65 compared to placebo, soy led to greater improvements in executive function, verbal memory, and figural memory but did not influence anxiety, depression, or menopausal symptoms.[225]
- The same research group conducted a larger trial (n = 50) of that same soy intervention and dose for 6 weeks, but found effects only on mental flexibility and planning.[226] The conclusion from the two trials was that soy had more benefit on executive function than memory.
- In a crossover study also using 60 mg isoflavones/day, women ages 46 to 65 (n = 78) were randomized for soy or placebo for 6 months, followed by a 1-month washout period, followed by the treatment not previously received.[227] Soy led to improvements in working memory

**TABLE 4.** *Results from randomized, placebo-controlled trials of soy isoflavones and cognitive functions*

| | | | | Outcome measures | | | |
|---|---|---|---|---|---|---|---|
| | **Author (date)** N (age) | **Design** | **Treatment (total isoflavone/day)** | **Hippocampal-dependent** | **Prefrontal-dependent: executive function** | **Mood** | **Attention** |
| Age < 65 years | **Duffy et al, (2003)**[225] 33 (50-65 y) | Parallel | Oral supplement (60 mg); 12 wk | ↑ Verbal and figural memory | ↑ **Mental flexibility and planning** | Anxiety, depression, sleepiness, menopausal symptoms | |
| | **File et al, (2005)**[226] 50 (51-66 y) | Parallel | Oral supplement (Novasoy, 60 mg); 6 wk | Short-term nonverbal memory<br>Verbal memory | ↑ **Mental flexibility and planning**<br>Category fluency | | Sustained attention |
| | **Casini et al, (2006)**[227] 78 (48-65 y) | Cross-over | Oral tablet (60 mg) 24 wk | | ↑ **Working memory and psychomotor speed** | ↑ **Profile of mood states and Beck Depression Inventory**<br>Speilberg state-trait anxiety | Attention |
| | **Fournier et al, (2007)**[228] 79 (50 y) | Parallel | Soy milk (60-100 mg), cow's milk soy supplement (Novasoy, 70 mg); 16 wk | Figural memory | ↓ **Working memory** | | Attention<br>Selective attention |
| | **Basaria et al, (2009)**[83] 84 (56 y) | Parallel | 20 g soy protein powder (160 mg); 12 wk | | Visuospatial (mental rotation)<br>Fluency<br>Trails B | | Psychomotor speed<br>Fine motor |
| >65 y | **Kreijkamp-Kaspers et al, (2004)**[204] 202 (60-75 y) | Parallel | 25.6 g soy protein (99 mg); 12 months | Verbal memory, naming | Working memory<br>Verbal fluency<br>Trails A and B | | Global cognition<br>Psychomotor speed<br>Working attention |
| Young and Older | **Kritz-Silverstein et al, (2003)**[229] 56 (55-59 y vs 60-74) | Parallel | Oral supplement (110 mg); 6 months | ↑ Young: verbal memory<br>↓ Old: verbal memory | ↑ **Young: mental flexibility (trails A, B)**<br>↑ **Old and young: category fluency**<br>Old: mental flexibility | | |
| | **Ho et al,(2007)**[230] 191 Chinese, (55-64 y vs 65+) | Parallel | Oral supplement (80 mg) and vitamin (400 mg calcium, 5 mg Zinc, 150 mg Mg, and 100 IU vitamin D); 6 months | Old and young: visual and verbal memory, object naming | Old and young: verbal fluency, working memory, executive function | | Global cognition<br>Attention |

and reduced depression and fatigue (executive function and verbal memory were not assessed). As working memory functions are dependent on prefrontal functions, these findings might be seen as supporting the two earlier clinical trials. The majority of participants (n = 49) preferred soy treatment.

- A clinical trial in 78 women (mean age, 50 y) randomized participants to receive 16 weeks of treatment with 1) cow's milk and a placebo supplement, 2) soy milk (72 mg isoflavones/d) and a placebo supplement, or 3) cow's milk and a soy isoflavone supplement (70 mg isoflavones/d).[228] Soy milk led to decreases in working memory, but the authors cautioned that the finding may have been obtained by chance and has yet to be shown as reliable. This caution seems warranted given that the soy supplement did not produce a similar result.
- The cognitive effects of a soy protein powder in a younger postmenopausal group have been investigated.[83] A total of 84 women (mean age, 56) were randomized to receive 20 g of soy protein containing 160 mg total isoflavones/day or placebo (20 g whole milk protein) for 12 weeks. Soy led to an improvement in four quality of life domains (vasomotor, psychosexual, physical, and sexual) but had no effect on visuospatial functions, psychomotor speed, fluency, a simple measure of mental flexibility (Trail-making test B), or fine motor skills.

### *Study in older postmenopausal women*

A large trial randomly assigned 202 postmenopausal women ages 60 to 75 to 25.6 g soy protein containing 99 mg isoflavones (52 mg genistein, 41 mg daidzein, 6 mg glycetein) or total milk protein as a powder on a daily basis for 12 months.[204] There were no significant differences between soy and placebo treatment on any cognitive outcome, including a sensitive test of verbal memory and Trail-making test B.

### *In younger versus older postmenopausal women*

- In one study, early and late postmenopausal women (n = 56; ages 55-74) were randomized to receive an oral supplement containing 110 mg isoflavones/day or placebo for 6 months.[229] Overall, soy improved category fluency, a test that depends on integrity of prefrontal cortex. In younger women (ages 50-59), soy improved verbal memory, category fluency, and attention. Older women (ages 60-74) improved in category fluency but decreased verbal memory. This study concluded that the effects of soy isoflavones may depend on age.
- A larger sample of 191 women (ages 55-76) was randomized to receive 80 mg soy isoflavones per day or placebo for 6 months.[230] Participants also received daily vitamins containing calcium, magnesium, and vitamin D. Measures included verbal and figural memory, working memory, verbal fluency, executive function, attention, simple motor speed, object naming, and global cognitive functioning. Soy had no effect on cognition in either the younger group (<age 65), the older group (>age 65), or the two groups combined. It is unclear whether results from this population would generalize to women who did not have the same history of intake of soy isoflavones from food.

Neither of the two large RCTs (n = ~200) above in women over age 65 found a benefit on cognitive function.[229,230] Smaller clinical trials suggest benefit for planning and complex measures of mental flexibility in early postmenopausal women. These data suggest that soy effects on cognitive function might be most apparent early in the postmenopausal period and most reliably observed on tests whose performance depends on the integrity of the prefrontal lobes.

### Conclusions

The clinical trial literature of the effects of soy and soy isoflavones on cognitive function suggests some benefit for soy on cognitive function in women younger than age 65 with little benefit for women over age 65. These preliminary findings support a "critical window" hypothesis similar to that of HT that posits that younger postmenopausal women treated close to the final menstrual period will derive more cognitive benefit from HT than older women treated many years after the final menstrual period. Larger studies are needed for definitive support, particularly in younger postmenopausal women. The ongoing WISH trial will provide important new insights into the effects of soy on cognition from a sample of about 300 healthy postmenopausal women randomly allocated to 25 g/day of isoflavone-rich soy protein versus milk protein placebo.

## FURTHER RESEARCH ON SOY & SOY ISOFLAVONES IN MIDLIFE WOMEN

During this review of soy and soy isoflavones, the following areas were identified that require further research.

- Applicability of rodent studies to human risk
- "Critical window" hypothesis wherein younger postmenopausal women derive more benefit from isoflavone therapy than older women
- Effects of isoflavones on endogenous estrogen metabolism
- Effects of soy isoflavones, particularly in the form of supplements, on Western populations
- Efficacy of isoflavones in racially and ethnically diverse populations and in younger perimenopausal women
- Equol effects on cardiovascular system
- Greater standardization and documentation of soy interventions in clinical trials
- Human studies with equol nonproducers, soy products with higher genistein content, and higher doses of isoflavones
- Interrelations of diet and supplement use with equol production
- Intervention studies that include adequate sample size, description of isoflavone quantity and type, verification of delivery, and evidence-based outcomes
- Mechanisms for defining or identifying equol producers

- Potential for differing effects of individual isoflavones and isoflavones in combination
- Potential for life-stage-specific risks or benefits
- Potential interactions with prescription and over-the-counter medications
- Role of primary isoflavones in equol producers versus nonproducers
- Understanding of the health reasons for use of soy isoflavones and supplements

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