2016 NAMS/Pfizer—Wulf H. Utian Endowed Lecture

The history and basic science development of soy isoflavones

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Abstract

This review summarizes the 2016 NAMS/Pfizer—Wulf H. Utian Endowed Lecture that focused on the history and basic science of soy isoflavones. Described is a personal perspective of the background and history that led to the current interest in soy and isoflavones with a specific focus on the role that soy isoflavones play in the health of postmenopausal women. This overview covers the metabolism and physiological behavior of isoflavones, their biological properties that are of potential relevance to aging, issues related to the safety of soy isoflavones, and the role of the important intestinally derived metabolite S-(−)-equol.


It was an honor to have been chosen to present the 2016 NAMS/Pfizer—Wulf H. Utian Endowed Lecture in a continuing series of translational science lectures to honor the pioneering work of Wulf H. Utian in establishing the NAMS network and for his outstanding clinical and scientific contributions to menopausal medicine. This lecture follows on from many previous presentations by legends and leaders in the field of menopause, and it is a privilege to have been selected to present this annual lecture and to overview the history of soy isoflavones. Over the past 30 years, there have been >15,000 publications related to soy isoflavones, but this article will highlight from a personal perspective, the history that has led to current interest in soy and isoflavones, with an emphasis on the key issues and landmark findings pertaining to postmenopausal women’s health.

Despite what has been written and stated in social media and in the lay-journals, the health benefits of diets that include the regular consumption of soy protein are irrefutable.1–3 While soy foods are not commonly consumed in Western populations they are a regular source of high-quality protein for people living in Asian countries and for vegetarians, with average soy food intakes estimated to be in the range of 6 to 8 g/d.4–5 Soy foods are devoid of cholesterol, low in saturated fat, high in unsaturated fatty acids, and oligosaccharides and have no lactose, all features that logically are considered healthful.1,3 Early attempts to introduce soy protein-based foods to Western countries more than 50 years ago proved challenging. It was not until 1999 when the US Food and Drug Administration (FDA) approved a health claim for soy protein and cardiovascular health, based upon the consensus of evidence from >50 clinical studies, that the inclusion of soy protein in a diet low in saturated fat and cholesterol would yield modest reductions in total and low-density lipoprotein (LDL)-cholesterol.6–8 That there occurred renewed interest from food companies in promoting soy foods to the general public. This renaissance was in part driven by our discoveries in 1980 of the presence of high levels of “estrogen-like” compounds in the urine and blood of adults consuming soy foods1–8 and 15 years later in infants fed soy infant formulas.9 Our findings led to the hypothesis, published in 1984, that the regular consumption of foods containing these biologically active isoflavones would be beneficial in the prevention and treatment of many hormone-dependent diseases.7 The rationale for this was based on an attempt to explain low rates of hormone-dependent diseases in Asian countries. This hypothesis gained traction after it was found that the addition of soy protein, to a rodent diet, led to a dose-dependent reduction in the number of mammary tumors induced by potent carcinogens.10 Removal of isoflavones from the soy protein nullified the chemopreventive effect in this breast cancer model10 and later the isoflavone genistein was shown to be chemoprotective.11–14 These findings were a key turning point for many investigations into the biological properties of isoflavones and...
the execution of dietary intervention studies with soy foods. However, the perception that isoflavones, or "phytoestrogens," as they were then classified,15 were "estrogens" because of their similarity in chemical structure to estradiol (Fig. 1) has also caused much controversy, particularly relating to the safety of soy foods, and especially for women with or at risk of breast cancer,16-18 and this is discussed later.

SOYBEAN ISOFLAVONE COMPOSITION, METABOLISM, AND BIOLOGICAL PROPERTIES

All soybeans and most soy foods contain isoflavones in relatively high concentrations.19-24 The predominant forms of isoflavones in soybeans are genistein and daidzein and to a lesser extent glycitein, and these are conjugated to various sugars to form malonylglucosides, acetylglucosides, and glycosides, resulting in a complex and differing composition among foods (Fig. 1). The conjugated forms of isoflavones do not cross the enterocyte and are not bioavailable or bioactive.25 Hydrolysis by intestinal glucosidases is essential to release the bioactive aglycons from the sugars to afford absorption and biological activity.25,26 The isoflavone composition of soy foods is highly variable among different types of soy foods, especially with regard to the levels and relative proportions of these different isoflavone forms.19-24 Unless the soybean or soy food has undergone some form of fermentation process, there will be relatively small proportions of the biologically active unconjugated (aglycon) forms consumed.19,27 Soybeans, and most Western-style soy foods that are generally formulated from purified soy proteins (isolated soy protein, often referred to as ISP), contain the conjugated forms of isoflavones.19-24 Processing has a significant effect on isoflavone content and composition of the soy protein or food, whereby isoflavones can be lost and/or changed in highly processed soy food products.21,23,28 In general, foods made from purified soy proteins can be expected to contain approximately 1.0 to 1.5 mg isoflavones/g of protein, whereas higher isoflavone intakes can be achieved through the consumption of whole soy foods (3.0-3.5 mg/g). In contrast to Western soy food products, many Asian foods have higher proportions of the more bioavailable and biologically active aglycons.19,27,29 This compositional difference, in my opinion, may explain the disparity in the clinical effects observed between dietary intervention studies of soy foods and extracted isoflavones performed in Asian versus Western populations.30

The typical dietary intake of soy protein by adults living in Asian countries is modest, but nevertheless the total intake of isoflavones consumed in this region of the world is typically in the range 25 to 50 mg/d.5 This level of isoflavone intake can be readily accomplished from eating many soy foods available in the USA; foods such as edamame, soymilk, tofu, roasted soy nuts, and texture vegetable protein are such examples. Soy oil has only traces of isoflavones due to the polar nature of the isoflavone glycosides, making them unable to partition into the oil phase. In general, with the exception of
SOY ISOFLAVONES AND MENOPAUSE

vegetarians, soy food consumption and hence isoflavone intake by most Western adults is negligible (<3 mg/d)\textsuperscript{31-34} and too low to expect clinical or biological effects from these bioactive compounds. For example, data from the Framingham Study have shown that the median isoflavone intake by postmenopausal women living in the United States was only 0.154 mg/d (0.99-0.235 mg/d),\textsuperscript{33} consistent with the relatively low intake of fiber, fruits, and vegetables, which are sources of many phytoprotectants. A comprehensive database of the isoflavone levels of many soy foods is available online.\textsuperscript{35} Irrespective of the type or source, when adults consume one serving of a soy food per day, such as a glass of soymilk, a tablespoon of soybeans, or serving of textured vegetable protein, urinary and plasma isoflavone concentrations will exceed by many orders of magnitude the levels of estradiol\textsuperscript{7,15} (Fig. 2). Even greater plasma concentrations were reported for infants raised on soy infant formulas.\textsuperscript{9,36,37}

The pharmacokinetics of isoflavones in humans have been exhaustively defined.\textsuperscript{9,38-43} Isoflavone aglycons on first-pass absorption are efficiently conjugated in the intestine and liver\textsuperscript{25} to predominantly circulate in plasma and be excreted in urine as glucuronides\textsuperscript{8,44-46} and to a lesser extent sulfates.\textsuperscript{47} This phase 2 metabolism is identical to that observed for endogenous steroids, including estrogens. Studies of the behavior of orally administered pure isoflavone aglycons and glycosides and their stable-labeled analogs, isoflavone supplements, and isoflavones in numerous different types of soy foods have been reported on, with the consensus of findings showing that daidzein and genistein exhibit first-order kinetics in being absorbed from the intestine relatively rapidly, reaching maximum plasma concentrations at between 2 and 8 hours postintake, and disappearing from the circulation with a terminal elimination half-life (t\textsubscript{1/2}) of between 6 and 8 hours (Fig. 2). Based upon the pharmacokinetics, the optimal intake/dosing of soy isoflavones to achieve maximum clinical efficacy should ideally be twice to three times daily. Attempts to formulate a slow-release isoflavone supplement with the goal of once a day dosing have been reported.\textsuperscript{35} There are some notable differences in the pharmacokinetics of isoflavones, with aglycons showing a faster rate of absorption and attaining higher plasma concentrations than glycosides,\textsuperscript{38,41,42,49} not surprisingly given the latter’s requirement of intestinal hydrolisis for bioavailability.\textsuperscript{25} For these reasons, we proposed that there would be advantages in consuming fermented soy foods or isoflavone aglycons to optimize efficacy of biological effects and that this could be another factor in explaining the discrepancies in the findings from clinical studies of soy foods performed in Asian versus Western countries.\textsuperscript{30} The “apparent bioavailability” of daidzein and genistein is relatively low, being 30% to 40% and 7% to 15%, respectively,\textsuperscript{50} which can be explained by their extensive intestinal metabolism by the microbiome. Isoflavones undergo a series of reactions catalyzed by bacterial enzymes, and these include reduction, deoxygenation, hydroxylation, and in the case of genistein, C-ring cleavage.\textsuperscript{50} This phase 1 metabolism yields a complex number of metabolites\textsuperscript{30,52} (Fig. 1). S-(-)-equol and O-desmethylangolensin are the endproducts of the metabolism of daidzein,\textsuperscript{8,53} and while the latter metabolite appears of little interest in that is has no apparent biological activity, S-(-)-equol, on the contrary, is an important metabolite from a clinical perspective.\textsuperscript{30,50,54} Indeed, it was our discovery of S-(-)-equol (originally designated as unknown compound 386/192)\textsuperscript{35} in human and animal urine\textsuperscript{56} and the serendipitous finding that it was not a new estrogen, as was first thought, but rather an intestinal bacterial metabolite of isoflavones contained in soy foods that led to a renaissance in the interest in soy foods.\textsuperscript{7,8} Evidence for its bacterial origin was gained from early studies showing the failure to produce S-(-)-equol by germ-free animals fed soy\textsuperscript{57,58} and later by newborn infants who lack a developed microbiome,\textsuperscript{7,9,59} and finally by incubation of a soy protein, textured vegetable protein, with cultured human fecal flora.\textsuperscript{7} Administration of antibiotics can wipe out the production of S-(-)-equol in adults.\textsuperscript{61-63} Despite demonstrating the important role of the microbiome in the metabolism of isoflavones more than 30 years ago, only in the past decade

FIG. 2. The typical pharmacokinetic behavior of the isoflavones, daidzein and genistein, in healthy humans (right panel) and the steady-state serum concentrations when consuming soy foods are compared with serum estradiol concentrations in men, premenopausal and postmenopausal women, and children.
have specific bacterial species been associated with the formation of \(S(-)\)equol,\(^{30}\) and interestingly, these are not the major bacterial species that colonize the human intestine.\(^{30}\) There are striking differences in the metabolism of isoflavones among animal species,\(^{30,64,65}\) and especially between humans and rodents, something that needs to be considered when extrapolating data from animal studies. Rodents exclusively metabolize daidzein to \(S(-)\)equol, and we described these animals as ‘‘equol-producing machines,’’\(^{54,66}\) whereas not all humans are capable of making \(S(-)\)equol when fed soy foods.\(^{7}\) Furthermore, the frequency of \(S(-)\)equol producers among adults consuming soy foods differs, with typically only 25% to 30% of Western adults, compared with 50% to 60% of Asian adults, capable of making \(S(-)\)equol after consuming soy foods.\(^{67-71}\) The importance of this distinction is that the ability to produce \(S(-)\)equol may define the clinical effectiveness of a soy food diet.\(^{34,72}\) This so-called ‘‘equol hypothesis’’\(^{54}\) gained traction from many, but not all, clinical studies of soy foods showing that ‘‘equol-producers’’ appear to show greater benefits than ‘‘non-equol producers,’’\(^{73-76}\) possibly because of \(S(-)\)equol’s greater biological potency compared with its precursor daidzein. Whether this is because of direct effects of \(S(-)\)equol, or because of a distinct phenotype associated with producing \(S(-)\)equol,\(^{77,78}\) is unclear. There is no good explanation to why certain individuals produce \(S(-)\)equol and others do not, and many studies have investigated the possible roles of the diet.\(^{59,60,68,78-82}\) An absence of the equol-producing microflora is most likely,\(^{30}\) or it is possible that the bacteria may be present, but inactive. Manipulation of the diet by the use of pre and probiotics has failed to stimulate \(S(-)\)equol production.\(^{83,86}\)

The potential of \(S(-)\)equol for treating menopause has been recently reviewed,\(^{87}\) and was highlighted in a position statement by NAMS.\(^{88}\) \(S(-)\)equol shows selective affinity for ER\(\beta\), and can be synthesized chemically\(^{89,90}\) or made naturally by fermentation of soy germ with an equol-producing bacteria such as \textit{Lactococcus garvieae}.\(^{91}\) It is now under commercial development as a pharmaceutical\(^{91}\) and available commercially as a ‘‘natural \(S(-)\)equol’’ supplement.\(^{53,54}\) Safety and toxicity studies\(^{95,96}\) have been reported and its pharmacokinetics extensively studied.\(^{46,50,66,64}\) Although equol was infamous for having caused devastating reproductive abnormalities in sheep grazing on red clover\(^{97,98}\) and was associated with infertility in captive cheetah fed soy-containing diets,\(^{99}\) in humans it appears to have a relatively safe profile with no apparent negative effect on the uterus at high oral doses.\(^{75}\) Furthermore, unlike estrogen, \(S(-)\)equol did not stimulate the growth of mammary tumors in 2 different animal models of human breast cancer,\(^{100,101}\) findings that would be consistent with the known breast protective effects of selective estrogen receptor modulators (SERMs) such as tamoxifen and raloxifene\(^{102}\) (Table 1). Furthermore, NAMS recently concluded that soy isoflavones do not increase the risk of breast and endometrial cancers.\(^{103}\) Compared with the soy isoflavones, genistein and daidzein, \(S(-)\)equol has a more favorable pharmacokinetic profile.\(^{94}\) It is almost completely bioavailable,\(^{84}\) because it is not metabolized further, save conjugation to form mainly the 7-O-glucuronide,\(^{52,104}\) and oral administration of as little as 10 mg/d of \(S(-)\)equol yields plasma concentrations that are consistent with those found in adults consuming modest amounts of soy foods and who are ‘‘equol-producing’’ (Fig. 2).

The biological activities and properties of soy isoflavones are expansive and have been reviewed elsewhere,\(^{15,50,105-107}\) with much of the data coming from in vitro and cell culture studies and from in vivo animal models. Figure 3 summarizes a selection of the many reported properties of soy isoflavones and the metabolite \(S(-)\)equol, and this review will consider those of potential relevance to human disease prevention and treatment in menopause. Importantly, soy isoflavones and \(S(-)\)equol are not ‘‘estrogens’’ as is often perceived. This strong association with estrogen has created concerns, particularly for postmenopausal women, especially following the negative perceptions of estrogen; ‘‘life is estrogen-dependent’’ as is clearly evident with menopause.\(^{108}\) Furthermore, it is generally not appreciated that over a 30-year period ranging from 50 to 80 years of age the average daily synthesis rate of estrogen by men is almost four times greater than that of postmenopausal women (estrogen 55 vs 15 \(\mu\)g/d; estrogen is not just a female hormone).

The chemical and conformational structure of isoflavones and \(S(-)\)equol are remarkably similar to estradiol, and it is for this reason that they were classified as phytoestrogens.\(^{15,109}\) Of

### TABLE 1. Comparison of what the clinical features should be for an ‘‘ideal estrogen’’ at different target sites with that of the features of estrogens, the selective estrogen receptor modulator (SERM) raloxifene and soy isoflavones, and the intestinal metabolite \(S(-)\)equol

<table>
<thead>
<tr>
<th>Target site for action</th>
<th>The ideal estrogen</th>
<th>Estrogens</th>
<th>SERMs (raloxifene)</th>
<th>Soy isoflavones (S(-))equol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoproteins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arteries</td>
<td>+</td>
<td>(+/-)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bone</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Neutral</td>
</tr>
<tr>
<td>Brain</td>
<td>+</td>
<td>+</td>
<td>No effect</td>
<td>(+?)</td>
</tr>
<tr>
<td>Breast</td>
<td>Protective</td>
<td>Negative</td>
<td>Protective</td>
<td>Protective</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Protective</td>
<td>Negative</td>
<td>Protective</td>
<td>Neutral</td>
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<tr>
<td>Skin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>SERM</td>
</tr>
<tr>
<td>Conformational binding to ER</td>
<td>SERM</td>
<td>Estrogen</td>
<td>SERM</td>
<td></td>
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</tbody>
</table>

The symbol + designates positive effects, – designates negative effects, and ? designates possible positive effects. ER, estrogen receptor; SERM, selective estrogen receptor modulator.
The lack of equol and bind poorly to ER). equol is likely to be found in plants and therefore is not by itself a phytoestrogen.

Overall, for all biological effects, the conversion of daidzein to S(-)-equol of potential relevance to human health. The conformational binding of genistein to the estrogen receptor highlights their similarity to that of SERMs such as raloxifene. X-ray crystallographic analysis redrawn from Pike et al. DHT, dihydrotestosterone; ER, estrogen receptor; SHBG, sex hormone binding globulin; SERM, selective estrogen receptor modulator.

FIG. 3. Some of the important biological properties of soy isoflavones and S(-)-equol of potential relevance to human health. The conformational binding of genistein to the estrogen receptor highlights their similarity to that of SERMs such as raloxifene. X-ray crystallographic analysis redrawn from Pike et al. DHT, dihydrotestosterone; ER, estrogen receptor; SHBG, sex hormone binding globulin; SERM, selective estrogen receptor modulator.

Isoflavones are extremely powerful antioxidants having greater antioxidant activity than vitamin C or vitamin E when compared in several in vitro activity tests, with S(-)-equol having the highest antioxidant activity. Isoflavones also have anti-inflammatory effects. Such properties mean a diet containing soy isoflavones can potentially reduce oxidative stress and inflammation, which play key roles in many diseases.

There is convincing evidence that consumption of soy isoflavones can significantly alter gene expression, especially of estrogen-responsive genes and genes involved in oxidative stress mechanisms. These changes have been noted in animals and in clinical studies where soy isoflavones have been consumed either as a supplement, or in the form of a soy food. Interestingly, changes in gene expression have been shown to be quite different between “equol-producers” and “non-equol producers,” pointing to the expectation of differing interindividual clinical responsiveness to soy foods and isoflavones. Overall, for all biological effects, the conversion of daidzein to S(-)-equol is likely to be beneficial. Table 1 presents a comparison of the general characteristics of the ideal estrogen for postmenopausal women with the features of estrogens, the SERM raloxifene, and soy isoflavones and S(-)-equol.

CLINICAL EFFECTS OF SOY AND ISOFLAVONES RELATED TO MENOPAUSE

Cardiovascular disease

Cardiovascular disease (CVD) remains the major cause of death in postmenopausal women. The hypercholesterolemic effect of soy protein is well-established and was reviewed recently by Messina. A plethora of studies have shown that soy protein lowers LDL-cholesterol, albeit modestly, and increases high-density lipoprotein (HDL)-cholesterol. In Japanese adults, serum cholesterol levels were shown to be inversely proportional to soy food intake, whereas in Chinese women higher soy food intake was associated with improved lipid profiles and lower blood pressure. The lack of cholesterol and favorable fatty acid profile of soy foods will undoubtedly contribute to lower serum cholesterol levels. While the approved claims for soy foods in reducing risk of CVD in the USA and Europe were based upon soy protein, the role of isoflavones was not considered at that time, but subsequent evidence indicates isoflavones probably play an important role in many of the risk factors for CVD (Fig. 4). The removal of isoflavones reduced the effectiveness of the cholesterol-lowering effect of soy protein in a study of hypercholesterolemic adults. How effective soy protein is at lowering serum cholesterol has been debated for some time, but 10 separate meta-analyses, summarized recently, conclude that soy protein reduces serum LDL-cholesterol on average 4% to 6%. Although this is unlikely to be helpful as a treatment strategy for patients with genetic hyperlipidemias requiring medications, for the rest of the adult population, including postmenopausal women, a diet containing soy protein, with its lack of cholesterol, should confer long-term benefits because every 1% reduction in serum cholesterol equates to a 2% to 3% risk reduction for CVD; the real value of soy and isoflavones may be in CVD prevention (Fig. 4).

Serum cholesterol is not the only risk factor for CVD, a disease that is considered to be manifest initially by inflammation of the endothelium, oxidative stress, and deposition of...
oxidized lipids by macrophages leading to atherosclerosis.\textsuperscript{132} High blood pressure then becomes one of the earliest phenotypic signs of atheroma and early heart disease. Serum C-reactive protein (CRP), a surrogate marker of inflammation, is increased in postmenopausal women taking estrogen therapy.\textsuperscript{133,134} By contrast, soy isoflavones either lower serum CRP levels, or are neutral in effect,\textsuperscript{134-141} consistent with their anti-inflammatory activity. A meta-analysis of 14 studies of postmenopausal women concluded that soy isoflavones lower serum CRP, and more so in those women with the highest levels.\textsuperscript{142} Based on their antioxidant properties, isoflavones can potentially reduce oxidative stress, as was evident from reductions in lipid peroxidation in a number of, although not all, clinical studies.\textsuperscript{136,143-145} Lipid peroxidation is difficult to determine due to the instability of oxidized lipids, which may account for the variability in the reported findings. In our studies, a novel soy germ pasta containing predominantly isoflavones in aglycon form, as a result of a matrix effect that hydrolyzed the isoflavone glycosides of the germ during manufacture, increased plasma total antioxidant activity, decreased lipid peroxidation, and decreased plasma CRP, a surrogate marker of inflammation. These changes were observed in two clinical trials.\textsuperscript{146,147} We attributed these effects to be because the food contained mainly the bioactive aglycon forms of isoflavones, rather than the inactive glycosides. And, this is an example where the interaction of isoflavones with the food matrix can be an important factor in outcomes. A subanalysis found greater responses to the diet in those patients who were defined as "equol-producers,"\textsuperscript{148} supportive of the "equol-producer hypothesis."\textsuperscript{149}

There have been many studies on endothelial function and the vasodilatory effects of soy isoflavones.\textsuperscript{148,149} Honore et al\textsuperscript{150} first showed that a soy diet fed to monkeys improved vascular reactivity, and confirmed genistein to have potent vasodilatory effects in women and men probably mediated through a nitric oxide-dependent mechanism. Two separate meta-analyses conclude that soy isoflavones improves endothelial function in postmenopausal women\textsuperscript{148,149}; and reductions in arterial stiffness by soy isoflavones have been demonstrated in adults,\textsuperscript{146,147,151,152} including postmenopausal women.\textsuperscript{153-155} Equol has been shown to increase endothelial nitric oxide synthase gene expression leading to increased production of the vasodilatory molecule, nitric oxide in human aortic endothelial cells.\textsuperscript{156,157} Chronic administration of the aglycon genistein at a relatively high dose of 54 mg/d to healthy postmenopausal women was reported to improve brachial artery flow-mediated dilatation.\textsuperscript{158} Improvements in endothelial function should in the long-term translate to lower blood pressure, and several studies of soy foods have reported reductions in systolic and diastolic blood pressure presumed to be due to isoflavones contained in the food.\textsuperscript{147,159,160} Although the overall reductions in blood pressure by soy foods (isoflavones) are small,\textsuperscript{161-163} these effects are of relevance to menopause and clinically significant in reducing risk for stroke and CVD. Drawing definitive conclusions on the effectiveness of soy isoflavones on CVD risk factors is difficult because of the inconsistency among the study designs, the short duration of the studies, and the often relatively small sample sizes. Furthermore, the forms in which the isoflavones are delivered have varied widely, especially when soy foods have been used in dietary intervention versus supplements. A major question to be answered is whether consumption of isoflavones in the form of the more biologically active aglycons is more efficacious than consuming isoflavone glycosides.\textsuperscript{30} Limited data support this contention, and in Asia, fermented soy foods have proportionally high amounts of isoflavone aglycons.

**Vasomotor symptoms**

After the publication of the Women’s Health Initiative study of negative effects from estrogen replacement therapy,\textsuperscript{164} the evaluation of alternative “natural” therapies for managing the symptomology of menopause accelerated and soy isoflavones figured prominently in a battery of botanical products investigated.\textsuperscript{165-168} The potential of soy isoflavones for managing menopausal vasomotor symptoms\textsuperscript{169} was first examined 30 years ago,\textsuperscript{170} after it was noted that the proportion of Japanese women reporting hot flushes during menopause was significantly lower than that for Western women.\textsuperscript{171} The suggestion was that this could be explained by their consumption of soy foods.\textsuperscript{172} Based upon the estrogenicity of isoflavones, it was believed that these nonsteroidal estrogens could for some women provide relief from hot flushes. It should, however, be stressed that since isoflavones, including genistein; and the metabolite $\delta$-equol, bind with much lower affinity to ERs than estradiol (these nonsteroidal estrogens selectively bind ER$\beta$), isoflavones will never be as potent as estrogen therapy in relieving hot flushes. In an era of personalized medicine, data, however, indicate that soy isoflavones and $\delta$-equol can provide valuable help for some women, particularly those experiencing the highest frequency and severity of hot flushes during menopause. Meta-analyses have been published on soy isoflavones and hot flushes.\textsuperscript{165,173,174} The most recent comprehensive meta-analysis examined the results from 19 clinical trials of soy isoflavones for treating hot flushes, and concluded that soy isoflavone supplements, derived by extraction or chemical synthesis, were significantly more effective than placebo in reducing both the frequency and severity of hot flushes.\textsuperscript{175} Of significance was the finding that those isoflavone supplements with the highest genistein content (>18.8 mg) were the most effective. This observation is in agreement with an early report suggesting that isoflavone supplements low in genistein (eg, this would include those made with soy germ) were not effective on postmenopausal symptoms.\textsuperscript{176} As pointed out by Messina,\textsuperscript{177} the inconsistency in the findings among studies of soy isoflavones effects on vasomotor symptoms, apart from the high placebo effect, is that not all of the studies used the same type, source, or dose of isoflavones, and clearly composition is important. Furthermore, there are insufficient data to know whether "equol-producer” status has any bearing on the effectiveness of isoflavones for managing vasomotor
symptoms. In this regard, a Japanese study of 180 postmenopausal women found using a “simplified menopausal score” questionnaire that equol-producers, defined from their urinary equol excretion, experienced the fewest symptoms of menopause, including hot flushes. The first US study to examine this relationship reported on 375 postmenopausal women of whom 35% were equol-producers and those in the highest quartile of urinary equol excretion reported a lower than average frequency and severity of vasomotor symptoms. Several additional studies of postmenopausal women given isoflavone supplements or soy foods examined differences between equol-producers and nonproducers with variable outcomes. Since it is difficult to convert a non-equol producer to a producer, there is now interest in S(-)equol as a nonhormonal agent for menopause, and in two clinical trials of postmenopausal women in Japan, a natural S(-)equol supplement was found to be effective in improving menopausal symptoms. Although hot flushes were not included in the symptoms monitored. Subsequently, a 12-week study to determine the effects of S(-)equol on hot flushes was conducted by Aso et al that showed the frequency of hot flushes decreased by 58% with S(-)equol compared with 34% in the placebo group, this difference being highly statistically significant. Other than one small trial of S(-)equol in US women showing a cumulative effect of S(-)equol in reducing hot flush frequency, the potential of S(-)equol for menopausal vasomotor symptoms remains to be fully elucidated and questions over the optimal dosage for efficacy remain. However, as pointed out by Utian et al, preliminary evidence warrants clinicians discussing the potential of S(-)equol for the alleviation of vasomotor symptoms with patients’.

Breast cancer

In 1984, we first proposed that high levels of soy isoflavones would confer protection against many hormone-dependent diseases with our focus at that time on breast cancer. This hypothesis was based upon findings of high circulating concentrations of isoflavones in adults consuming soy foods and epidemiological data of much lower rates of breast cancer in women living in Asia compared with those in Western countries. In collaboration with Stephen Barnes at the University of Alabama in Birmingham and with funding from the American Cancer Society and the American Institute for Cancer Research, we showed for the first time that soy protein with isoflavones, as opposed to soy protein devoid of isoflavones, in a dose-dependent manner, protected against the development of breast cancer in two classical animal models of chemically-induced mammary cancer. These findings caught the attention of the National Institutes of Health to fund a program to investigate further the potential of isoflavones for cancer protection. Work from Lamartiniere et al and others definitively confirmed genistein to be chemoprotective in these models, and furthermore it was found that the earlier in life the exposure to genistein the greater was the chemoprotective effect. It was then suggested that consumption of soy foods early in life by Asians could be an important factor in explaining the low rates of breast cancer in those countries because isoflavones remodel the morphology of the breast in a beneficial manner; genistein and S(-)equol both cause differentiation of cancer-susceptible terminal end buds into less cancer-susceptible lobules. Incidentally, estrogen also has the same effect, which may explain the lower risk for breast cancer in women who have term pregnancies and have children early in adult life. This “early exposure” hypothesis is supported by the findings from several human studies of greater soy food consumption in adolescent years correlating with lower risk for breast cancer later in adult life. The notion that soy isoflavones could be protective against breast cancer was contradicted by later animal studies showing that the growth of human MCF-7 breast cancer cells implanted into athymic mice was stimulated in the presence of genistein, its glycoside, genistin, and by daidzein; however, interestingly S(-)equol had no effect on the growth of these estrogen-sensitive cells, in agreement with its failure to stimulate the growth of tumors in the dimethylbenz[a]anthracene chemically-induced breast cancer model. S(-)equol thus appears to be safe where breast cancer is concerned. The significant differences in the metabolism of isoflavones between rodents and humans, and the marked differences observed in different animal models of breast cancer and among different isoflavones, makes it difficult to evaluate the significance of the animal findings to postmenopausal women and those women at high risk for breast cancer. For this reason, one should primarily rely on the human data, summarized in several excellent reviews by Messina. Data are now highly supportive of soy foods and isoflavones being safe for breast cancer survivors and for reducing breast cancer risk. There have now been five independent 3 to 7-year prospective epidemiological studies, two from the USA and three from China, which included a total of >11,000 breast cancer survivors, and the consensus of these studies was that soy intake was associated with statistically significant reductions in breast cancer recurrence and with decreased mortality. Furthermore, in contrast to findings from animal studies, soy food/isoflavone consumption appears to enhance the effectiveness of treatment with tamoxifen and with the aromatase inhibitor, anastrozole. The American Cancer Society and the American Institute for Cancer Research have endorsed the conclusions that soy food consumption is safe and improves the prognosis for breast cancer survivors. It is time to place greater importance on the human data, since humans are clearly the best and most appropriate animal model for humans, rather than to continue to promote the negativity citing data from animal studies.

With regard to safety, this is best judged by the historical consumption of soy by millions of people worldwide and the realization that due largely to genetic polymorphism and diversity nothing can be absolutely safe. Notwithstanding that allergy to soy protein has a prevalence of about 0.5% in the general population, the risk from isoflavone exposure appears extremely small and rare cases of adverse events are always possible. The greatest concerns for postmenopausal
women regarding the safety of soy isoflavones have related to potential adverse effects on the breast, discussed above, and the uterus and thyroid, driven by negative effects observed in animal studies fed isoflavones; these issues have been addressed in previous reviews.3,16,87,199 The European Food Safety Agency (EFSA) recently concluded that for postmenopausal women, soy isoflavones do not adversely affect the breast, uterus, and thyroid,213 and there has finally been the recognition that soy may be beneficial for postmenopausal women, while the metabolite S(−)-equol, despite limited data, holds promise for menopause-related symptom relief.87

**Skin**

Estrogens play a key role in maintenance of skin health and quality, and over a 15 to 18-year period of menopause, most women can typically expect a 2.1% decrease per year in skin collagen content and 1.3% decrease per year in skin thickness in the absence of taking estrogen therapy. Estrogens function in skin to improve collagen content and quality, increase skin thickness, reduce wrinkling, improve vascularization, accelerate wound healing, and reduce sebaceous gland activity.214 Skin thickness is proportional to collagen content and is inversely correlated with risk for osteoporosis.215,216 Estrogen therapy increases skin thickness in postmenopausal women,217 and not surprisingly, attention has more recently focused on the potential of soy isoflavones218 and S(−)-equol219-222 for maintaining skin health during menopause and aging. For skin, isoflavones are protective in animal models and cell cultures against damage from ultraviolet light and photoaging.223-225 In principle, isoflavones should have effects in skin because there is a high expression of ERβ and androgen receptors, and the antioxidant and anti-inflammatory properties are beneficial. Beyond estrogen action, S(−)-equol has been shown to antagonize the action of dihydrotestosterone one of the most potent androgens that is involved in many skin conditions.226 Investigations of effects from both topical and oral routes of administration of isoflavones have shown some promise in terms of wrinkle reduction and collagen stimulation, despite limitations of the studies and the variability in study designs.227-230 In most of the studies, isoflavones were given in mixtures, rather than in pure form. The availability of S(−)-equol as a pure compound or a supplement now permits a direct evaluation of the effects of this isoflavone metabolite and will enable elucidation of potential mechanisms of action in skin. In this regard, topical administration of S(−)-equol was compared against its unnatural enantiomer R(+)equol and the racemate using human monolayer fibroblast cultures. In this cell culture model, it was shown to cause an increase in expression of collagen, elastin, and tissue inhibitor of metalloproteinases (TIMP) genes.222,231 A decreased gene expression of metalloproteinases was also observed, and all of these findings are consistent with beneficial antiaging and antioxidant effects. Currently, there are few data on the effects of oral administration of S(−)-equol on skin. A limited 12-week study of Japanese postmenopausal women given an oral S(−)-equol supplement reported a significant decrease in wrinkle area compared with placebo.219 Overall, isoflavones and S(−)-equol appear to hold promise for preserving skin structure and health during menopause.

**CONCLUSIONS**

It was not possible to review all aspects of soy isoflavones in this lecture, and the reader is guided to other more recent publication sources and references contained therein.3,30,50,87,177,199,200 Moreover, this was an attempt at the time available to present from a personal perspective some of the landmark research studies that have taken place over the past 40 years in this area of soy isoflavones that have seen translation to clinical application emphasizing the relevance to menopausal health. This has been a journey from the early discovery in urine of several unusual unknown estrogen-like compounds, through their identification as isoflavones and S(−)-equol, to identifying their origins in soy foods, illustrating the importance of the diet and the microbiome in metabolism and efficacy, and ultimately leading to a broad evaluation of their potential health effects.

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