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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.

Key Words: Equol – Genistein – Isoflavones – Menopause – Phytoestrogens – Soy.

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] 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 foods[7,8] 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 model,[10] 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

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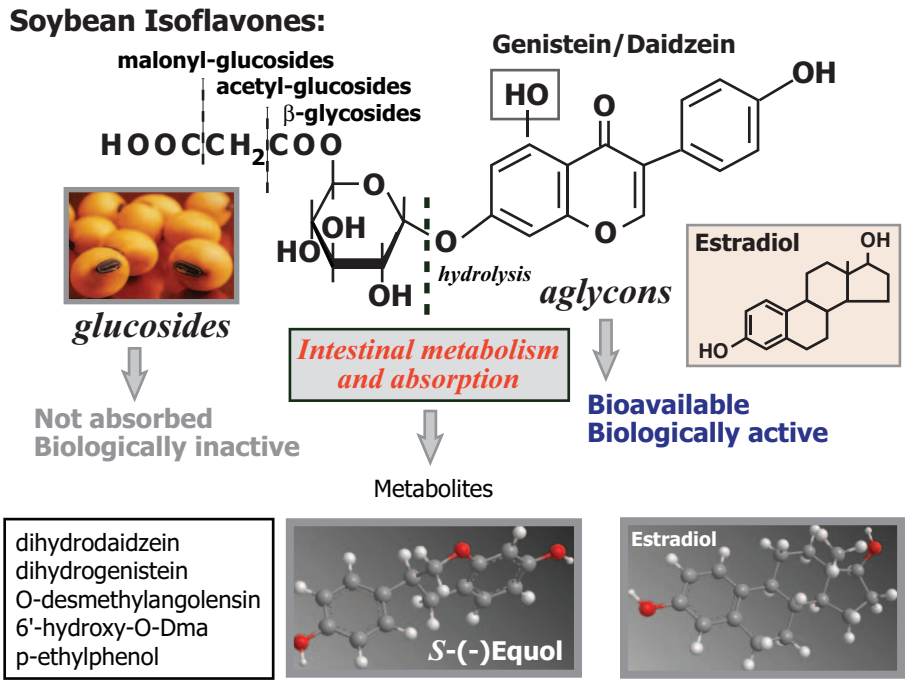

FIG. 1. The complex chemical composition of isoflavones found in soybeans and their metabolism after ingestion. The chemical structures of these nonsteroidal estrogens and the key metabolite *S*-(−)equol are compared to the estrogen estradiol shown in two-dimensional and three-dimensional structures. The biologically active aglycons, genistein and daidzein, are present in only minor amounts in soybeans and most soy foods, and require activation by intestinal metabolism.

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

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vegetarians, soy food consumption and hence isoflavone intake by most Western adults is negligible (<3 mg/d)[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),[31] 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.[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[7,15] (Fig. 2). Even greater plasma concentrations were reported for infants raised on soy infant formulas.[9,36,37]

The pharmacokinetics of isoflavones in humans have been exhaustively defined.[9,38-43] Isoflavone aglycons on first-pass absorption are efficiently conjugated in the intestine and liver[25] to predominantly circulate in plasma and be excreted in urine as glucuronides[8,44-46] and to a lesser extent sulfates.[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 ($t_{1/2}$) 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.[48] 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,[38,41,42,49] not surprisingly given the latter's requirement of intestinal hydrolysis for bioavailability.[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.[30] The ''apparent bioavailability'' of daidzein and genistein is relatively low, being 30% to 40% and 7% to 15%, respectively,[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.[50] This phase 1 metabolism yields a complex number of metabolites[51,52] (Fig. 1). *S*-(−)equol and *O*-desmethylangolensin are the endproducts of the metabolism of daidzein,[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.[30,50,54] Indeed, it was our discovery of *S*-(−)equol (originally designated as unknown compound 386/192)[55] in human and animal urine[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.[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[57,58] and later by newborn infants who lack a developed microbiome,[9,59,60] and finally by incubation of a soy protein, textured vegetable protein, with cultured human fecal flora.[7] Administration of antibiotics can wipe out the production of *S*-(−)equol in adults.[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

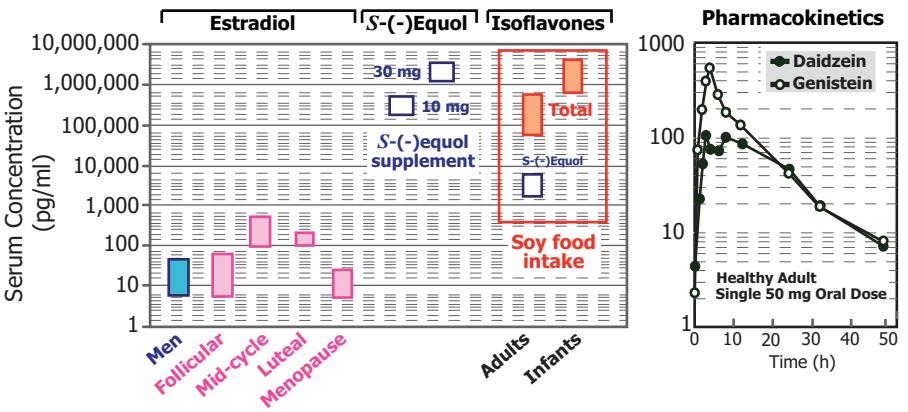

FIG. 2. The typical pharmacokinetic behavior of the isoflavones, daidzein and genistein, in healthy humans (right panel) and the steady-state serum concentrations when consuming soy foods are compared with serum estradiol concentrations in men, premenopausal and postmenopausal women, and children.

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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.[54,72] 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 ERβ, and can be synthesized chemically[89,90] or made naturally by fermentation of soy germ with an equol-producing bacteria such as *Lactococcus garvieae*.[91] It is now under commercial development as a pharmaceutical[92] and available commercially as a ''natural *S*-equol'' supplement.[93,94] Safety and toxicity studies[95,96] have been reported and its pharmacokinetics extensively studied.[46,50,66,94] 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.[95] 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 raloxifen[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,[94] 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-producers'' (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 μg/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*

Target site for action	The ideal estrogen	Estrogens	SERMs (raloxifene)	Soy isoflavones *S*-(−)equol
Lipoproteins	+	+	+	+
Arteries	+	+/−	+	+
Bone	+	+	+	Neutral
Brain	+	+	No effect	(+?)
Breast	Protective	Negative	Protective	Protective
Endometrium	Protective	Negative	Protective	Neutral
Skin	+	+	?	+
Conformational binding to ER	SERM	Estrogen	SERM	SERM

The symbol + designates positive effects, − designates negative effects, and ? designates possible positive effects.
ER, estrogen receptor; SERM, selective estrogen receptor modulator.

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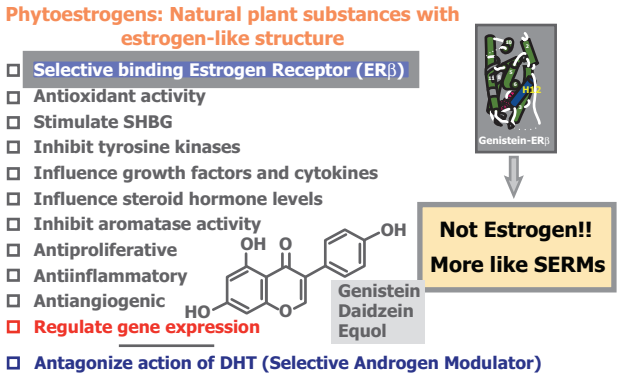

FIG. 3. Some of the important biological properties of soy isoflavones and *S*-(−)equol of potential relevance to human health. The conformational binding of genistein to the estrogen receptor highlights their similarity to that of SERMs such as raloxifene. X-ray crystallographic analysis redrawn from Pike et al.[115] DHT, dihydrotestosterone, ER, estrogen receptor; SHBG, sex hormone binding globulin; SERM, selective estrogen receptor modulator.

note, *S*-(−)equol is not found in plants and therefore is not by definition a phytoestrogen,[30,50] rather a nonsteroidal estrogen. Unlike estradiol that binds with equal affinity to both ERα and ERβ, genistein and *S*-(−)equol both show preferential affinity for ERβ and bind poorly to ERα.[66,110-114] Thus, in this regard, isoflavones and *S*-(−)equol more closely align with the SERMs, tamoxifen and raloxifene,[102] and can be expected to have beneficial tissue-selective effects. X-ray crystallographic studies confirm the binding of genistein to the estrogen receptor to be conformationally similar to that of raloxifene (a SERM developed to have the beneficial effects of estrogen on bone, but without the negative effects on breast and endometrium) and not estradiol.[115] Isoflavones, and particularly *S*-(−)equol, should perhaps be considered as ''good estrogens.''

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.[116-119] Isoflavones also have anti-inflammatory effects.[120,121] 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[122] and in clinical studies where soy isoflavones have been consumed either as a supplement,[123] or in the form of a soy food.[124] Interestingly, changes in gene expression have been shown to be quite different between ''equol-producers'' and ''non-equol producers,''[123] pointing to the expectation of differing interindividual clinical responsiveness to soy foods and isoflavones.[54] 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 hypocholesterolemic effect of soy protein is well-established and was reviewed recently by Messina.[3] A plethora of studies have shown that soy protein lowers LDL-cholesterol, albeit modestly, and increases high-density lipoprotein (HDL)-cholesterol.[125,126] In Japanese adults, serum cholesterol levels were shown to be inversely proportional to soy food intake,[4] whereas in Chinese women higher soy food intake was associated with improved lipid profiles and lower blood pressure.[127,128] 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,[6] 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).[129,130] The removal of isoflavones reduced the effectiveness of the cholesterol-lowering effect of soy protein in a study of hypercholesterolemic adults.[131] How effective soy protein is at lowering serum cholesterol has been debated for some time, but 10 separate meta-analyses, summarized recently,[3] 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

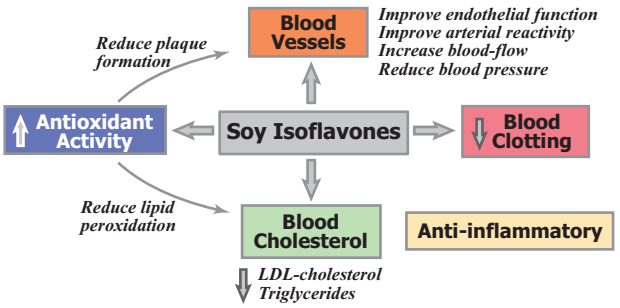

FIG. 4. Potential mechanisms of action for soy isoflavones in reducing risk for cardiovascular disease, the major cause of death in postmenopausal women. LDL, lipoprotein.

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oxidized lipids by macrophages leading to atherosclerosis.[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.[133,134] By contrast, soy isoflavones either lower serum CRP levels, or are neutral in effect,[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.[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.[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.[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,''[146] supportive of the ''equol-producer hypothesis.''[54]

There have been many studies on endothelial function and the vasodilatory effects of soy isoflavones.[148,149] Honore et al[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[148,149]; and reductions in arterial stiffness by soy isoflavones have been demonstrated in adults,[146,147,151,152] including postmenopausal women.[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.[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.[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.[147,159,160] Although the overall reductions in blood pressure by soy foods (isoflavones) are small,[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.[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,[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.[165-168] The potential of soy isoflavones for managing menopausal vasomotor symptoms[169] was first examined 30 years ago,[170] after it was noted that the proportion of Japanese women reporting hot flushes during menopause was significantly lower than that for Western women.[171] The suggestion was that this could be explained by their consumption of soy foods.[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 *S*-(−)equol, bind with much lower affinity to ERα than estradiol (these nonsteroidal estrogens selectively bind ERβ), 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 *S*-(−)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.[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 flashes.[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.[176] As pointed out by Messina,[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

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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.[178] 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.[179] Several additional studies of postmenopausal women given isoflavone supplements or soy foods examined differences between equol-producers and nonproducers with variable outcomes.[180-182] Since it is difficult to convert a non-equol producer to a producer,[60,183] there is now interest in *S*-(−)equol as a nonhormonal agent for menopause,[87] and in two clinical trials of postmenopausal women in Japan, a natural *S*-(−)equol supplement was found to be effective in improving menopausal symptoms,[93,184] 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[185] 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,[186] 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,[87] ''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.[7] 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.[187] 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.[10] These findings caught the attention of the National Institutes of Health to then fund a program to investigate further the potential of isoflavones for cancer protection. Work from Lamartiniere et al[12,13] 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.[188,189] 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.[100,190,191] 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.[192-195] 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,[196,197] its glycoside, genistin,[198] and by daidzein[101]; however, interestingly *S*-(−)equol had no effect on the growth of these estrogen-sensitive cells,[101] in agreement with its failure to stimulate the growth of tumors in the dimethylbenz[a]anthracene chemically-induced breast cancer model.[100] *S*-(−)equol thus appears to be safe where breast cancer is concerned. The significant differences in the metabolism of isoflavones between rodents and humans,[64] 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.[3,199,200] 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[201,202] and three from China,[203-205] 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,[206-208] soy food/isoflavone consumption appears to enhance the effectiveness of treatment with tamoxifen[209] and with the aromatase inhibitor, anastrozole.[204] 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.[210,211] 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,[212] the risk from isoflavone exposure appears extremely small and rare cases of adverse events are always possible. The greatest concerns for postmenopausal

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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 isoflavones[218] and *S*-(−)equol[219-222] 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 in 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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REFERENCES

1. Messina MJ. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am J Clin Nutr* 1999;70 (3 Suppl):439S-450S.
2. Anderson JW, Smith BM, Washnock CS. Cardiovascular and renal benefits of dry bean and soybean intake. *Am J Clin Nutr* 1999;70 (3 Suppl):464S-474S.
3. Messina M. Soy and health update: evaluation of the clinical and epidemiologic literature. *Nutrients* 2016;8:754.
4. Nagata C, Takatsuka N, Kurisu Y, Shimizu H. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. *J Nutr* 1998;128:209-213.
5. Messina M, Nagata C, Wu AH. Estimated asian adult soy protein and isoflavone intakes. *Nutr Cancer* 2006;55:1-12.
6. Food labeling, health claims, soy protein, and coronary heart disease. Food and Drug Administration, HHS. Final rule. *Fed Regist* 1999;64: 57700-57733.
7. Setchell KDR, Borriello SP, Hulme P, Kirk DN, Axelson M. Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *Am J Clin Nutr* 1984;40:569-578.
8. Axelson M, Sjovall J, Gustafsson BE, Setchell KDR. Soya: a dietary source of the non-steroidal oestrogen equol in man and animals. *J Endocrinol* 1984;102:49-56.

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9. Setchell KDR, Zimmer NL, Cai J, Heubi JE. Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet* 1997;350: 23-27.
10. Barnes S, Grubbs C, Setchell KDR, Carlson J. Soybeans inhibit mammary tumors in models of breast cancer. *Prog Clin Biol Res* 1990;347: 239-253.
11. Barnes S. Effect of genistein on in vitro and in vivo models of cancer. *J Nutr* 1995;125 (3 Suppl):777S-783S.
12. Lamartiniere CA, Moore JB, Brown NM, Thompson R, Hardin MJ, Barnes S. Genistein suppresses mammary cancer in rats. *Carcinogenesis* 1995;16:2833-2840.
13. Lamartiniere CA, Cotroneo MS, Fritz WA, Wang J, Mentor-Marcel R, Elgavish A. Dietary Genistein Protects Against Mammary and Prostate Cancers. 4th International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, San Diego, USA, 2001.
14. Constantinou AI, Mehta RG, Vaughan A. Inhibition of N-methyl-N-nitrosourea-induced mammary tumors in rats by the soybean isoflavones. *Anticancer Res* 1996;16 (6A):3293-3298.
15. Setchell KDR. Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin Nutr* 1998;68 (6 Suppl):1333S-1346S.
16. Setchell KDR. Soy isoflavones: benefits and risks from nature's selective estrogen receptor modulators (SERMs). *J Am Coll Nutr* 2001;20 (5 Suppl):354S-362S[discussion 81S–83S].
17. Messina MJ, Persky V, Setchell KDR, Barnes S. Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr Cancer* 1994;21: 113-131.
18. Messina MJ, Wood CE. Soy isoflavones, estrogen therapy, and breast cancer risk: analysis and commentary. *Nutr J* 2008;7:17.
19. Coward L, Barnes NC, Setchell KDR, Barnes S. Genistein, daidzein, and their b-glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. *J Agric Food Chem* 1993;41: 1961-1967.
20. Murphy P, Tongtong S, Buseman G, et al. Isoflavones in retail and institutional soy foods. *J Agric Food Chem* 1999;47:2697-2704.
21. Murphy PA. Phytoestrogen content of processed soybean products. *Food Technol* 1982;43:60-64.
22. Franke A, Hankin J, Yu M, Maskarinec G, Siew HL, Custer L. Isoflavone levels in soy foods consumed by multiethnic populations in Singapore and Hawaii. *J Agric Food Chem* 1999;47:977-986.
23. Setchell KDR, Cole SJ. Variations in isoflavone levels in soy foods and soy protein isolates and issues related to isoflavone databases and food labeling. *J Agric Food Chem* 2003;51:4146-4155.
24. Wang HJ, Murphy PA. Isoflavone content in commercial soybean foods. *J Agric Food Chem* 1994;42:1666-1673.
25. Setchell KDR, Brown NB, Zimmer-Nechemias L, et al. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am J Clin Nutr* 2002;76:447-453.
26. Day AJ, DuPont MS, Ridley S, et al. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *FEBS Lett* 1998;436:71-75.
27. Nakajima N, Nozaki N, Ishihara K, Ishikawa A, Tsuji H. Analysis of isoflavone content in tempeh, a fermented soybean, and preparation of a new isoflavone-enriched tempeh. *J Biosci Bioeng* 2005;100:685-687.
28. Pandjaitan N, Hettiarachchy N, Ju Z, Crandall P, Sneller C, Dombek D. Evaluation of genistin and genistein contents in soybean varieties and soy protein concentrate prepared with 3 basic methods. *J Food Sci* 2000;65:399-402.
29. Chan SG, Murphy PA, Ho SC, et al. Isoflavonoid content of Hong Kong soy foods. *J Agric Food Chem* 2009;57:5386-5390.
30. Setchell KDR, Clerici C. Equol: history, chemistry, and formation. *J Nutr* 2010;140:1355S-1362S.
31. de Kleijn MJ, van der Schouw YT, Wilson PW, et al. Intake of dietary phytoestrogens is low in postmenopausal women in the United States: the Framingham study (1-4). *J Nutr* 2001;131:1826-1832.
32. van Erp-Baart MA, Brants HA, Kiely M, et al. Isoflavone intake in four different European countries: the VENUS approach. *Br J Nutr* 2003;89 (Suppl 1):S25-S30.
33. van der Schouw YT, Kreijkamp-Kaspers S, Peeters PH, Keinan-Boker L, Rimm EB, Grobbee DE. Prospective study on usual dietary phytoestrogen intake and cardiovascular disease risk in Western women. *Circulation* 2005;111:465-471.
34. Boker LK, Van der Schouw YT, De Kleijn MJ, Jacques PF, Grobbee DE, Peeters PH. Intake of dietary phytoestrogens by Dutch women. *J Nutr* 2002;132:1319-1328.
35. Bhagwat S, Haytowitz D, Holden J. USDA Database for the Isoflavone Content of Selected Foods, Release 2.0. U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory Home Page: 2008. Available at: http://www.ars.usda.gov/nutrientdata/isoflav.
36. Setchell KDR, Zimmer-Nechemias L, Cai J, Heubi JE. Isoflavone content of infant formulas and the metabolic fate of these phytoestrogens in early life. *Am J Clin Nutr* 1998;68 (6 Suppl):1453S-1461S.
37. Franke AA, Halm BM, Ashburn LA. Isoflavones in children and adults consuming soy. *Arch Biochem Biophys* 2008;476:161-170.
38. Setchell KDR, Brown NM, Desai P, et al. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *J Nutr* 2001;131 (4 Suppl):1362S-1375S.
39. Setchell KDR, Brown NM, Desai PB, et al. Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. *J Nutr* 2003;133: 1027-1035.
40. Setchell KDR, Faughnan MS, Avades T, et al. Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women. *Am J Clin Nutr* 2003;77:411-419.
41. Izumi T, Piskula M, Osawa S, et al. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J Nutr* 2000;130:1695-1699.
42. Chang Y, Choue R. Plasma pharmacokinetics and urinary excretion of isoflavones after ingestion of soy products with different aglycone/glucoside ratios in South Korean women. *Nutr Res Pract* 2013;7: 393-399.
43. Zubik L, Meydani M. Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. *Am J Clin Nutr* 2003;77:1459-1465.
44. Doerge DR, Chang HC, Churchwell MI, Holder CL. Analysis of soy isoflavone conjugation in vitro and in human blood using liquid chromatography-mass spectrometry. *Drug Metab Dispos* 2000;28:298-307.
45. Shelnutt SR, Cimino CO, Wiggins PA, Badger TM. Urinary pharmacokinetics of the glucuronide and sulfate conjugates of genistein and daidzein. *Cancer Epidemiol Biomarkers Prev* 2000;9:413-419.
46. Jackson RL, Greiwe JS, Desai PB, Schwen RJ. Single-dose and steady-state pharmacokinetic studies of S-equol, a potent nonhormonal, estrogen receptor beta-agonist being developed for the treatment of menopausal symptoms. *Menopause* 2011;18:185-193.
47. Ronis MJ, Little JM, Barone GW, Chen G, Radominska-Pandya A, Badger TM. Sulfation of the isoflavones genistein and daidzein in human and rat liver and gastrointestinal tract. *J Med Food* 2006;9: 348-355.
48. Setchell KDR, Brzezinski A, Brown NM, et al. Pharmacokinetics of a slow-release formulation of soybean isoflavones in healthy postmenopausal women. *J Agric Food Chem* 2005;53:1938-1944.
49. Nagino T, Kano M, Masuoka N, et al. Intake of a fermented soymilk beverage containing moderate levels of isoflavone aglycones enhances bioavailability of isoflavones in healthy premenopausal Japanese women: a double-blind, placebo-controlled, single-dose, crossover trial. *Biosci Microbiota Food Health* 2016;35:9-17.
50. Setchell KDR, Clerici C. Equol: pharmacokinetics and biological actions. *J Nutr* 2010;140:1363S-1368S.
51. Kelly GE, Nelson C, Waring MA, Joannou GE, Reeder AY. Metabolites of dietary (soya) isoflavones in human urine. *Clin Chim Acta* 1993;223: 9-22.
52. Schwen RJ, Nguyen L, Jackson RL. Elucidation of the metabolic pathway of S-equol in rat, monkey and man. *Food Chem Toxicol* 2012;50:2074-2083.
53. Bannwart C, Adlercreutz H, Fotsis T, Wahala K, Hase T, Brunow G. Identification of O-desmethylangolensin, a metabolite of daidzein and of matairesinol, one likely plant presursor of the animal lignan enterolactone in human urine. *Finn Chem Lett* 1984;4-5:120-125.
54. Setchell KDR, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. *J Nutr* 2002;132:3577-3584.
55. Axelson M, Setchell KDR. Conjugation of lignans in human urine. *FEBS Lett* 1980;122:49-53.
56. Axelson M, Kirk DN, Farrant RD, Cooley G, Lawson AM, Setchell KDR. The identification of the weak oestrogen equol [7-hydroxy-3-(4'-hydroxyphenyl)chroman] in human urine. *Biochem J* 1982;201: 353-357.

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57. Axelson M, Setchell KDR. The excretion of lignans in rats: evidence for an intestinal bacterial source for this new group of compounds. *FEBS Lett* 1981;123:337-342.
58. Axelson M, Sjovall J, Gustafsson BE, Setchell KDR. Origin of lignans in mammals and identification of a precursor from plants. *Nature* 1982; 298:659-660.
59. Brown NM, Galandi SL, Summer SS, et al. *S*-(−)equol production is developmentally regulated and related to early diet composition. *Nutr Res* 2014;34:401-409.
60. Setchell KDR, Brown NM, Summer S, et al. Dietary factors influence production of the soy isoflavone metabolite S-(−)equol in healthy adults. *J Nutr* 2013;143:1950-1958.
61. Blair RM, Appt SE, Clarkson TB. Treatment with antibiotics reduces plasma equol concentration in cynomolgus monkeys (*Macaca fascicularis*). *J Nutr* 2003;133:2262-2267.
62. Atkinson C, Berman S, Humbert O, Lampe JW. In vitro incubation of human feces with daidzein and antibiotics suggests interindividual differences in the bacteria responsible for equol production. *J Nutr* 2004;134:596-599.
63. Halm BM, Franke AA, Ashburn LA, Hebshi SM, Wilkens LR. Oral antibiotics decrease urinary isoflavonoid excretion in children after soy consumption. *Nutr Cancer* 2008;60:14-22.
64. Setchell KDR, Brown NM, Zhao X, et al. Soy isoflavone phase II metabolism differs between rodents and humans: implications for the effect on breast cancer risk. *Am J Clin Nutr* 2011;94:1284-1294.
65. Gu L, House SE, Prior RL, et al. Metabolic phenotype of isoflavones differ among female rats, pigs, monkeys, and women. *J Nutr* 2006;136: 1215-1221.
66. Setchell KDR, Clerici C, Lephart ED, et al. S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am J Clin Nutr* 2005;81:1072-1079.
67. Setchell KDR, Cole SJ. Method of defining equol-producer status and its frequency among vegetarians. *J Nutr* 2006;136:2188-2193.
68. Rowland IR, Wiseman H, Sanders TA, Adlercreutz H, Bowey EA. Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut microflora. *Nutr Cancer* 2000;36:27-32.
69. Lampe JW, Karr SC, Hutchins AM, Slavin JL. Urinary equol excretion with a soy challenge: influence of habitual diet. *Proc Soc Exp Biol Med* 1998;217:335-339.
70. Song KB, Atkinson C, Frankenfeld CL, et al. Prevalence of daidzein-metabolizing phenotypes differs between Caucasian and Korean American women and girls. *J Nutr* 2006;136:1347-1351.
71. Akaza H, Miyanaga N, Takahima N, et al. Comparisons of percent equol producers between prostate cancer patients and controls: case-controlled studies of isoflavones in Japanese, Korean and American residents. *Jpn J Clin Oncol* 2004;34:86-89.
72. Lampe JW. Is equol the key to the efficacy of soy foods? *Am J Clin Nutr* 2009;89:1664S-1667S.
73. Ahuja V, Miura K, Vishnu A, et al. Significant inverse association of equol-producer status with coronary artery calcification but not dietary isoflavones in healthy Japanese men. *Br J Nutr* 2017;117: 260-266.
74. Birru RL, Ahuja V, Vishnu A, et al. The impact of equol-producing status in modifying the effect of soya isoflavones on risk factors for CHD: a systematic review of randomised controlled trials. *J Nutr Sci* 2016;5:e30.
75. Yoshikata R, Myint KZ, Ohta H. Relationship between equol producer status and metabolic parameters in 743 Japanese women: equol producer status is associated with antiatherosclerotic conditions in women around menopause and early postmenopause. *Menopause* 2017;24: 216-224.
76. Igase M, Igase K, Tabara Y, Ohyagi Y, Kohara K. Cross-sectional study of equol producer status and cognitive impairment in older adults. *Geriatr Gerontol Int* 2017; doi: 10.1111/ggi.13029 [Epub ahead of print].
77. Frankenfeld CL, Atkinson C, Thomas WK, et al. High concordance of daidzein-metabolizing phenotypes in individuals measured 1 to 3 years apart. *Br J Nutr* 2005;94:873-876.
78. Frankenfeld CL, Atkinson C, Thomas WK, et al. Familial correlations, segregation analysis, and nongenetic correlates of soy isoflavone-metabolizing phenotypes. *Exp Biol Med (Maywood)* 2004;229:902-913.
79. Nagata C, Ueno T, Uchiyama S, et al. Dietary and lifestyle correlates of urinary excretion status of equol in Japanese women. *Nutr Cancer* 2008;60:49-54.
80. Bolca S, Possemiers S, Herregat A, et al. Microbial and dietary factors are associated with the equol producer phenotype in healthy postmenopausal women. *J Nutr* 2007;137:2242-2246.
81. Gardana C, Canzi E, Simonetti P. The role of diet in the metabolism of daidzein by human faecal microbiota sampled from Italian volunteers. *J Nutr Biochem* 2009;20:940-947.
82. Védrine N, Mathey J, Morand C, et al. One-month exposure to soy isoflavones did not induce the ability to produce equol in postmenopausal women. *Eur J Clin Nutr* 2006;60:1039-1045.
83. Lampe JW, Skor HE, Li S, Wahala K, Howald WN, Chen C. Wheat bran and soy protein feeding do not alter urinary excretion of the isoflavan equol in premenopausal women. *J Nutr* 2001;131:740-744.
84. Nettleton JA, Greany KA, Thomas W, Wangen KE, Adlercreutz H, Kurzer MS. Short-term soy and probiotic supplementation does not markedly affect concentrations of reproductive hormones in postmenopausal women with and without histories of breast cancer. *J Altern Complement Med* 2005;11:1067-1074.
85. Greany KA, Nettleton JA, Wangen KE, Thomas W, Kurzer MS. Probiotic consumption does not enhance the cholesterol-lowering effect of soy in postmenopausal women. *J Nutr* 2004;134:3277-3283.
86. Bonorden MJ, Greany KA, Wangen KE, et al. Consumption of *Lactobacillus acidophilus* and *Bifidobacterium longum* do not alter urinary equol excretion and plasma reproductive hormones in premenopausal women. *Eur J Clin Nutr* 2004;58:1635-1642.
87. Utian WH, Jones M, Setchell KDR. S-equol: a potential nonhormonal agent for menopause-related symptom relief. *J Womens Health (Larchmt)* 2015;24:200-208.
88. North American Menopause Society. Nonhormonal management of menopause-associated vasomotor symptoms: 2015 position statement of The North American Menopause Society. *Menopause* 2015;22: 1155-1172.
89. Setchell KDR, Sirokin V, Girindus America Inc, Children's Hospital Medical Center, assignees. Method for the enantioselective hydrogenation of Chromenes. US Patent US 7528267. 2009.
90. Heemstra JM, Kerrigan SA, Doerge DR, Helferich WG, Boulanger WA. Total synthesis of (S)-equol. *Org Lett* 2006;8:5441-5443.
91. Yee S, Burdock GA, Kurata Y, et al. Acute and subchronic toxicity and genotoxicity of SE5-OH, an equol-rich product produced by *Lactococcus garvieae*. *Food Chem Toxicol* 2008;46:2713-2720.
92. Jackson RL, Greiwe JS, Schwen RJ. Emerging evidence of the health benefits of S-equol, an estrogen receptor beta agonist. *Nutr Rev* 2011;69: 432-448.
93. Ishiwata N, Melby MK, Mizuno S, Watanabe S. New equol supplement for relieving menopausal symptoms: randomized, placebo-controlled trial of Japanese women. *Menopause* 2009;16:141-148.
94. Setchell KDR, Zhao X, Shoaf SE, Ragland K. The pharmacokinetics of S-(-)equol administered as SE5-OH tablets to healthy postmenopausal women. *J Nutr* 2009;139:2037-2043.
95. Schwen RJ, Nguyen L, Plomley JB, Jackson RL. Toxicokinetics and lack of uterotropic effect of orally administered S-equol. *Food Chem Toxicol* 2012;50:1741-1748.
96. Schwen R, Jackson R, Proudlock R. Genotoxicity assessment of S-equol in bacterial mutation, chromosomal aberration, and rodent bone marrow micronucleus tests. *Food Chem Toxicol* 2010;48:3481-3485.
97. Shutt D. The effects of plant estrogens on animal reproduction. *Endeavour* 1976;35:110-113.
98. Shutt D, Braden A. The signficance of equol in relation to the oestrogenic responses in sheep ingesting clover with a high formononetin content. *Aust J Agric Res* 1968;19:545-553.
99. Setchell KDR, Gosselin SJ, Welsh MB, et al. Dietary estrogens: a probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology* 1987;93:225-233.
100. Brown NM, Belles CA, Lindley SL, et al. The chemopreventive action of equol enantiomers in a chemically-induced animal model of breast cancer. *Carcinogenesis* 2010;31:401-409.
101. Ju YH, Fultz J, Allred KF, Doerge DR, Helferich WG. Effects of dietary daidzein and its metabolite, equol, at physiological concentrations on the growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in ovariectomized athymic mice. *Carcinogenesis* 2006;27: 856-863.

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102. Jordan VC, Morrow M. Tamoxifen, raloxifene, and the prevention of breast cancer. *Endocr Rev* 1999;20:253-278.
103. North American Menopause Society. 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). *Menopause* 2011;18:732-753.
104. Gardana C, Simonetti P. Long-term kinetics of daidzein and its main metabolites in human equol-producers after soymilk intake: identification of equol-conjugates by UPLC-orbitrap-MS and influence of the number of transforming bacteria on plasma kinetics. *Int J Food Sci Nutr* 2017;68:496-506.
105. Barnes S, Peterson TG. Biochemical targets of the isoflavone genistein in tumor cell lines. *Proc Soc Exp Biol Med* 1995;208:103-108.
106. Ronis MJ. Effects of soy containing diet and isoflavones on cytochrome P450 enzyme expression and activity. *Drug Metab Rev* 2016;48: 331-341.
107. Shao ZM, Shen ZZ, Fontana JA, Barsky SH, Genistein's. ER-dependent and independent'' actions are mediated through ER pathways in ER-positive breast carcinoma cell lines. *Anticancer Res* 2000;20:2409-2416.
108. Radd S, Setchell KDR. *Eat to Live*. NewLeaf, Hodder Headline, Australia Pty Limited later Gill, and Macmillan Ltd; 2002; 79-90.
109. Farnsworth N, Bingel A, Cordell G, Crane F, Fong H. Potential value of plants as sources of new antifertility agents II. *J Pharm Sci* 1975;64: 717-754.
110. Kuiper GG, Carlsson B, Grandien K, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 1997;138:863-870.
111. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 1998;139:4252-4263.
112. Morito K, Hirose T, Kinjo J, et al. Interaction of phytoestrogens with estrogen receptors alpha and beta. *Biol Pharm Bull* 2001;24:351-356.
113. Muthyala RS, Ju YH, Sheng S, et al. Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through estrogen receptors alpha and beta. *Bioorg Med Chem* 2004;12:1559-1567.
114. Kostelac D, Rechkemmer G, Briviba K. Phytoestrogens modulate binding response of estrogen receptors a and b to the estrogen response element. *J Agric Food Chem* 2003;51:7632-7635.
115. Pike AC, Brzozowski AM, Hubbard RE, et al. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. *EMBO J* 1999;18:4608-4618.
116. Arora A, Nair MG, Strasburg GM. Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. *Arch Biochem Biophysics* 1998;356:133-141.
117. Mitchell J, Gardner P, McPhail D, Morrice P, Collins A, Duthie G. Antioxidant efficacy of phytoestrogens in chemical and biological model systems. *Arch Biochem Biophysics* 1998;360:142-148.
118. Choi EJ. Evaluation of equol function on anti- or prooxidant status in vivo. *J Food Sci* 2009;74:H65-H71.
119. Ruiz-Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP, Rice-Evans CA. Antioxidant activity of phytoestrogenic isoflavones. *Free Radic Res* 1997;26:63-70.
120. Salzman A, Szabo C. Genistein is a potent inhibitor of inducible nitric oxide synthase expression in human epithelial cells (Abstract). Symposium on Phytoestrogen Research Methods. Tucson, AZ, 1997.
121. Salzman AL, Preiser F -C, Setchell K D R, Szabo C. Isoflavone-mediated inhibition of tyrosine kinase: a novel anti-inflammatory approach. *J Med Food* 1999;2:179-181.
122. Diel P, Schulz T, Smolnikar K, Strunck E, Vollmer G, Michna H. Ability of xeno- and phytoestrogens to modulate expression of estrogen-sensitive genes in rat uterus: estrogenicity profiles and uterotropic activity. *J Steroid Biochem Mol Biol* 2000;73:1-10.
123. Niculescu MD, Pop EA, Fischer LM, Zeisel SH. Dietary isoflavones differentially induce gene expression changes in lymphocytes from postmenopausal women who form equol as compared with those who do not. *J Nutr Biochem* 2007;18:380-390.
124. Setchell KDR, Nardi E, Battezzati PM, et al. Novel soy germ pasta enriched in isoflavones ameliorates gastroparesis in type 2 diabetes: a pilot study. *Diabetes Care* 2013;36:3495-3497.
125. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids [see comments]. *N Engl J Med* 1995;333:276-282.
126. Jenkins DJ, Mirrahimi A, Srichaikul K, et al. Soy protein reduces serum cholesterol by both intrinsic and food displacement mechanisms. *J Nutr* 2010;140:2302S-2311S.
127. Ho S, Woo J, Leung S, Sham A, Lam T, Janus E. Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population. *J Nutr* 2000;130:2590-2593.
128. Yang G, Shu XO, Jin F, et al. Longitudinal study of soy food intake and blood pressure among middle-aged and elderly Chinese women. *Am J Clin Nutr* 2005;81:1012-1017.
129. Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. *Am J Clin Nutr* 2005;81: 397-408.
130. Zhuo XG, Melby MK, Watanabe S. Soy isoflavone intake lowers serum LDL cholesterol: a meta-analysis of 8 randomized controlled trials in humans. *J Nutr* 2004;134:2395-2400.
131. Crouse JR 3rd, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. *Arch Intern Med* 1999;159:2070-2076.
132. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med* 1999;340:115-126.
133. Cushman M, Legault C, Barrett-Connor E, et al. Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. *Circulation* 1999;100:717-722.
134. Manning PJ, Sutherland WH, Allum AR, de Jong SA, Jones SD. Effect of hormone replacement therapy on inflammation-sensitive proteins in post-menopausal women with type 2 diabetes. *Diabet Med* 2002;19: 847-852.
135. Jenkins DJA, Kendall CWC, Connelly PW, et al. Effects of high-and-low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. *Metabolism* 2002;51:919-924.
136. Jenkins DJA, Kendall CWC, Garsetti M, et al. Effect of soy protein foods on low-density lipoprotein oxidation and ex vivo sex hormone receptor activity: a controlled crossover trial. *Metabolism* 2000;49: 537-543.
137. D'Anna R, Baviera G, Corrado F, Cancellieri F, Crisafulli A, Squadrito F. The effect of the phytoestrogen genistein and hormone replacement therapy on homocysteine and C-reactive protein level in postmenopausal women. *Acta Obstet Gynecol Scand* 2005;84:474-477.
138. Yildiz MF, Kumru S, Godekmerdan A, Kutlu S. Effects of raloxifene, hormone therapy, and soy isoflavone on serum high-sensitive C-reactive protein in postmenopausal women. *Int J Gynaecol Obstet* 2005;90: 128-133.
139. Hall WL, Vafeiadou K, Hallund J, et al. Soy-isoflavone-enriched foods and inflammatory biomarkers of cardiovascular disease risk in post-menopausal women: interactions with genotype and equol production. *Am J Clin Nutr* 2005;82:1260-1268, quiz 365-366.
140. Hilpert KF, Kris-Etherton PM, West SG. Lipid response to a low-fat diet with or without soy is modified by C-reactive protein status in moderately hypercholesterolemic adults. *J Nutr* 2005;135:1075-1079.
141. Nikander E, Metsa-Heikkila M, Tiitinen A, Ylikorkala O. Evidence of a lack of effect of a phytoestrogen regimen on the levels of C-reactive protein, E-selectin, and nitrate in postmenopausal women. *J Clin Endocrinol Metab* 2003;88:5180-5185.
142. Dong JY, Wang P, He K, Qin LQ. Effect of soy isoflavones on circulating C-reactive protein in postmenopausal women: meta-analysis of randomized controlled trials. *Menopause* 2011;18:1256-1262.
143. Tikkanen M, Wahala K, Ojala S, Vihma V, Adlercreutz H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. *Proc Natl Acad Sci USA* 1998;95:3106-3110.
144. Wiseman H, O'Reilly J, Adlercreutz H, et al. Isoflavone phytoestrogens consumed in soy decrease F2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. *Am J Clin Nutr* 2000;72:395-400.
145. Jenkins DJA, Kendall CWC, Jackson C-J, et al. Effects of high-and low-isoflavone soy foods on blood lipids, oxidized LDL, homocystine and blood pressure in hyperlipidemic men and women. *Am J Clin Nutr* 2002;76:365-372.
146. Clerici C, Setchell KDR, Battezzati PM, et al. Pasta naturally enriched with isoflavone aglycons from soy germ reduces serum lipids and improves markers of cardiovascular risk. *J Nutr* 2007;137: 2270-2278.

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147. Clerici C, Nardi E, Battezzati PM, et al. Novel soy germ pasta improves endothelial function, blood pressure, and oxidative stress in patients with type 2 diabetes. *Diabetes Care* 2011;34:1946-1948.
148. Li SH, Liu XX, Bai YY, et al. Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials. *Am J Clin Nutr* 2010; 91:480-486.
149. Beavers DP, Beavers KM, Miller M, Stamey J, Messina MJ. Exposure to isoflavone-containing soy products and endothelial function: a Bayesian meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis* 2012;22:182-191.
150. Honore EK, Williams JK, Anthony MS, Clarkson TB. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. *Fertil Steril* 1997;67:148-154.
151. Pase MP, Grima NA, Sarris J. The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. *Am J Clin Nutr* 2011;93:446-454.
152. Hoshida S, Miki T, Nakagawa T, et al. Different effects of isoflavones on vascular function in premenopausal and postmenopausal smokers and nonsmokers: NYMPH study. *Heart Vessels* 2011;26:590-595.
153. Teede HJ, McGrath BP, DeSilva L, Cehun M, Fassoulakis A, Nestel PJ. Isoflavones reduce arterial stiffness: a placebo-controlled study in men and postmenopausal women. *Arterioscler Thromb Vasc Biol* 2003;23: 1066-1071.
154. Nestel PJ, Yamashita T, Sasahara T, et al. Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. *Arterioscler Thromb Vasc Biol* 1997;17:3392-3398.
155. Tormala R, Appt S, Clarkson TB, et al. Equol production capability is associated with favorable vascular function in postmenopausal women using tibolone; no effect with soy supplementation. *Atherosclerosis* 2008;198:174-178.
156. Mahn K, Borras C, Knock GA, et al. Dietary soy isoflavone induced increases in antioxidant and eNOS gene expression lead to improved endothelial function and reduced blood pressure in vivo. *FASEB J* 2005;19:1755-1757.
157. Joy S, Siow RC, Rowlands DJ, et al. The isoflavone equol mediates rapid vascular relaxation: Ca2+-independent activation of eNOS/Hsp90 involving ERK1/2 and Akt phosphorylation in human endothelial cells. *J Biol Chem* 2006;281:27335-27345.
158. Squadrito F, Altavilla D, Crisafulli A, et al. Effect of genistein on endothelial function in postmenopausal women: a randomized, double-blind, controlled study. *Am J Med* 2003;114:470-476.
159. Rivas M, Garay RP, Escanero JF, Cia P, Alda JO. Soy milk lowers blood pressure in men and women with mild to moderate essential hypertension. *J Nutr* 2002;132:1900-1902.
160. Welty FK, Lee KS, Lew NS, Zhou JR. Effect of soy nuts on blood pressure and lipid levels in hypertensive, prehypertensive, and normotensive postmenopausal women. *Arch Intern Med* 2007;167:1060-1067.
161. Dong JY, Tong X, Wu ZW, Xun PC, He K, Qin LQ. Effect of soya protein on blood pressure: a meta-analysis of randomised controlled trials. *Br J Nutr* 2011;106:317-326.
162. Taku K, Lin N, Cai D, et al. Effects of soy isoflavone extract supplements on blood pressure in adult humans: systematic review and meta-analysis of randomized placebo-controlled trials. *J Hypertens* 2010;28: 1971-1982.
163. Liu XX, Li SH, Chen JZ, et al. Effect of soy isoflavones on blood pressure: a meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis* 2012;22:463-470.
164. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 2002;288:321-333.
165. Nelson HD, Vesco KK, Haney E, et al. Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. *JAMA* 2006;295:2057-2071.
166. Lethaby A, Marjoribanks J, Kronenberg F, Roberts H, Eden J, Brown J. Phytoestrogens for menopausal vasomotor symptoms. *Cochrane Database Syst Rev* (12):2013;CD001395.
167. North American Menopause Society. The role of isoflavones in menopausal health: consensus opinion of The North American Menopause Society. *Menopause* 2000;7:215-229.
168. Kronenberg F, Cushman LF, Wade CM, Kalmuss D, Chao MT. Race/ethnicity and women's use of complementary and alternative medicine in the United States: results of a national survey. *Am J Public Health* 2006;96:1236-1242.
169. Kronenberg F. Menopausal hot flashes: a review of physiology and biosociocultural perspective on methods of assessment. *J Nutr* 2010; 140:1380S-1385S.
170. Murkies AL, Lombard C, Strauss BJ, Wilcox G, Burger HG, Morton MS. Dietary flour supplementation decreases post-menopausal hot flushes: effect of soy and wheat. *Maturitas* 1995;21:189-195.
171. Lock M, Kaufert P, Gilbert P. Cultural construction of the menopausal syndrome: the Japanese case. *Maturitas* 1988;10:317-332.
172. Adlercreutz H, Hamalainen E, Gorbach S, Goldin B. Dietary phyto-oestrogens and the menopause in Japan. *Lancet* 1992;339:1233.
173. Howes LG, Howes JB, Knight DC. Isoflavone therapy for menopausal flushes: a systematic review and meta-analysis. *Maturitas* 2006;55: 203-211.
174. Bolanos R, Del Castillo A, Francia J. Soy isoflavones versus placebo in the treatment of climacteric vasomotor symptoms: systematic review and meta-analysis. *Menopause* 2010;17:660-666.
175. Taku K, Melby MK, Kronenberg F, Kurzer MS, Messina M. Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: systematic review and meta-analysis of randomized controlled trials. *Menopause* 2012;19:776-790.
176. Williamson-Hughes PS, Flickinger BD, Messina MJ, Empie MW. Isoflavone supplements containing predominantly genistein reduce hot flash symptoms: a critical review of published studies. *Menopause* 2006;13:831-839.
177. Messina M. Soybean isoflavones warrant greater consideration as a treatment for the alleviation of menopausal hot flashes. *Womens Health (Lond)* 2014;10:549-553.
178. Uchiyama S, Ueno T, Shirota T. The relationship between soy isoflavones and the menopausal symptoms in Japanese perimenopausal women. *Ann Nutr Metab* 2001;45 (Suppl 1):113(abstract).
179. Newton KM, Reed SD, Uchiyama S, et al. A cross-sectional study of equol producer status and self-reported vasomotor symptoms. *Menopause* 2015;22:489-495.
180. Jou HJ, Wu SC, Chang FW, Ling PY, Chu KS, Wu WH. Effect of intestinal production of equol on menopausal symptoms in women treated with soy isoflavones. *Int J Gynaecol Obstet* 2008;102: 44-49.
181. Crawford SL, Jackson EA, Churchill L, Lampe JW, Leung K, Ockene JK. Impact of dose, frequency of administration, and equol production on efficacy of isoflavones for menopausal hot flashes: a pilot randomized trial. *Menopause* 2013;20:936-945.
182. Liu ZM, Ho SC, Woo J, Chen YM, Wong C. Randomized controlled trial of whole soy and isoflavone daidzein on menopausal symptoms in equol-producing Chinese postmenopausal women. *Menopause* 2014;21: 653-660.
183. Franke AA, Lai JF, Halm BM, et al. Equol production changes over time in postmenopausal women. *J Nutr Biochem* 2012;23:573-579.
184. Aso T. Equol improves menopausal symptoms in Japanese women. *J Nutr* 2010;140:1386S-1389S.
185. Aso T, Uchiyama S, Matsumura Y, et al. A natural S-equol supplement alleviates hot flushes and other menopausal symptoms in equol nonproducing postmenopausal Japanese women. *J Womens Health (Larchmt)* 2012;21:92-100.
186. Jenks BH, Iwashita S, Nakagawa Y, et al. A pilot study on the effects of S-equol compared to soy isoflavones on menopausal hot flash frequency. *J Womens Health (Larchmt)* 2012;21:674-682.
187. Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in. *Int J Cancer* 1999;83:18-29.
188. Lamartiniere CA, Cotroneo MS, Fritz WA, Wang J, Mentor-Marcel R, Elgavish A. Genistein chemoprevention: timing and mechanisms of action in murine mammary and prostate. *J Nutr* 2002;132:552S-558S.
189. Lamartiniere CA, Moore J, Holland M, Barnes S. Neonatal genistein chemoprevents mammary cancer. *Proc Soc Exp Biol Med* 1995;208: 120-123.
190. Lamartiniere CA, Murrill WB, Manzolillo PA, et al. Genistein alters the ontogeny of mammary gland development and protects against chemically-induced mammary cancer in rats. *Proc Soc Exp Biol Med* 1998; 217:358-364.
191. Brown NM, Lamartiniere CA. Xenoestrogens alter mammary gland differentiation and cell proliferation in the rat. *Environ Health Perspect* 1995;103:708-713.

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192. Korde LA, Wu AH, Fears T, et al. Childhood soy intake and breast cancer risk in Asian American women. *Cancer Epidemiol Biomarkers Prev* 2009;18:1050-1059.
193. Wu AH, Wan P, Hankin J, Tseng CC, Yu MC, Pike MC. Adolescent and adult soy intake and risk of breast cancer in Asian-Americans. *Carcinogenesis* 2002;23:1491-1496.
194. Shu XO, Jin F, Dai Q, et al. Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. *Cancer Epidemiol Biomarkers Prev* 2001;10:483-488.
195. Lee SA, Shu XO, Li H, et al. Adolescent and adult soy food intake and breast cancer risk: results from the Shanghai Women's Health Study. *Am J Clin Nutr* 2009;89:1920-1926.
196. Ju YH, Allred CD, Allred KF, Karko KL, Doerge DR, Helferich WG. Physiological concentrations of dietary genistein dose-dependently stimulate growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in athymic nude mice. *J Nutr* 2001;131:2957-2962.
197. Hsieh CY, Santell RC, Haslam SZ, Helferich WG. Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. *Cancer Res* 1998;58: 3833-3838.
198. Allred C, Ju Y, Allred K, Jongsoo C, Helferich W. Dietary genistin stimulates growth of estrogen-dependent breast cancer tumors similar to that observed with genistein. *Carcinogenesis* 2001;22:1667-1673.
199. Messina M. Impact of soy foods on the development of breast cancer and the prognosis of breast cancer patients. *Forsch Komplementmed* 2016;23:75-80.
200. Messina M, Loprinzi C. Soy for breast cancer survivors: a critical review of the literature. *J Nutr* 2001;131:3095S-3108S.
201. Caan BJ, Natarajan L, Parker B, et al. Soy food consumption and breast cancer prognosis. *Cancer Epidemiol Biomarkers Prev* 2011;20:854-858.
202. Guha N, Kwan ML, Quesenberry CP Jr, Weltzien EK, Castillo AL, Caan BJ. Soy isoflavones and risk of cancer recurrence in a cohort of breast cancer survivors: the Life After Cancer Epidemiology study. *Breast Cancer Res Treat* 2009;118:395-405.
203. Shu XO, Zheng Y, Cai H, et al. Soy food intake and breast cancer survival. *JAMA* 2009;302:2437-2443.
204. Kang X, Zhang Q, Wang S, Huang X, Jin S. Effect of soy isoflavones on breast cancer recurrence and death for patients receiving adjuvant endocrine therapy. *CMAJ* 2010;182:1857-1862.
205. Zhang YF, Kang HB, Li BL, Zhang RM. Positive effects of soy isoflavone food on survival of breast cancer patients in China. *Asian Pac J Cancer Prev* 2012;13:479-482.
206. Du M, Yang X, Hartman JA, et al. Low-dose dietary genistein negates the therapeutic effect of tamoxifen in athymic nude mice. *Carcinogenesis* 2012;33:895-901.
207. Ju YH, Doerge DR, Allred KF, Allred CD, Helferich WG. Dietary genistein negates the inhibitory effect of tamoxifen on growth of estrogen-dependent human breast cancer (MCF-7) cells implanted in athymic mice. *Cancer Res* 2002;62:2474-2477.
208. Ju YH, Doerge DR, Woodling KA, Hartman JA, Kwak J, Helferich WG. Dietary genistein negates the inhibitory effect of letrozole on the growth of aromatase-expressing estrogen-dependent human breast cancer cells (MCF-7Ca) in vivo. *Carcinogenesis* 2008;29:2162-2168.
209. Nechuta SJ, Caan BJ, Chen WY, et al. Soy food intake after diagnosis of breast cancer and survival: an in-depth analysis of combined evidence from cohort studies of US and Chinese women. *Am J Clin Nutr* 2012;96:123-132.
210. Rock CL, Doyle C, Demark-Wahnefried W, et al. Nutrition and physical activity guidelines for cancer survivors. *CA Cancer J Clin* 2012;62: 243-274.
211. American Institute for Cancer Research (AICR). Soy is Safe for Breast Cancer Survivors; 2012 Available at: at www.aicr.org/cancer-research-update.
212. Katz Y, Gutierrez-Castrellon P, Gonzalez MG, Rivas R, Lee BW, Alarcon P. A comprehensive review of sensitization and allergy to soy-based products. *Clin Rev Allergy Immunol* 2014;46:272-281.
213. EFSA Panel on Food Additives and Nutrient Sources added to Food. Scientific opinion on the risk assessment for peri-and post-menopausal women taking food supplements containing isolated isoflavones. *EFSA J* 2015;13:4246.
214. Shah MG, Maibach HI. Estrogen and skin. An overview. *Am J Clin Dermatol* 2001;2:143-150.
215. Meema HE, Sheppard RH, Rapoport A. Roentgenographic visualization and measurement of skin thickness and its diagnostic application in acromegaly. *Radiology* 1964;82:411-417.
216. Black MM, Bottoms E, Shuster S. Skin collagen content and thickness in systemic sclerosis. *Br J Dermatol* 1970;83:552-555.
217. Maheux R, Naud F, Rioux M, et al. A randomized, double-blind, placebo-controlled study on the effect of conjugated estrogens on skin thickness. *Am J Obstet Gynecol* 1994;170:642-649.
218. Irrera N, Pizzino G, D'Anna R, et al. Dietary management of skin health: the role of genistein. *Nutrients* 2017;9; doi: 10.3390/nu9060622.
219. Oyama A, Ueno T, Uchiyama S, et al. The effects of natural S-equol supplementation on skin aging in postmenopausal women: a pilot randomized placebo-controlled trial. *Menopause* 2012;19:202-210.
220. Lephart ED. Resveratrol, 4' acetoxy resveratrol, R-equol, racemic equol or S-equol as cosmeceuticals to improve dermal health. *Int J Mol Sci* 2017;18:1193.
221. Magnet U, Urbanek C, Gaisberger D, et al. Topical equol preparation improves structural and molecular skin parameters. *Int J Cosmet Sci* 2017.
222. Gopaul R, Knaggs HE, Lephart ED. Biochemical investigation and gene analysis of equol: a plant and soy-derived isoflavonoid with antiaging and antioxidant properties with potential human skin applications. *Biofactors* 2012;38:44-52.
223. Widyarini S, Husband AJ, Reeve VE. Protective effect of the isoflavonoid equol against hairless mouse skin carcinogenesis induced by UV radiation alone or with a chemical cocarcinogen. *Photochem Photobiol* 2005;81:32-37.
224. Widyarini S. Protective effect of the isoflavone equol against DNA damage induced by ultraviolet radiation to hairless mouse skin. *J Vet Sci* 2006;7:217-223.
225. Widyarini S, Spinks N, Husband AJ, Reeve VE. Isoflavonoid compounds from red clover (*Trifolium pratense*) protect from inflammation and immune suppression induced by UV radiation. *Photochem Photobiol* 2001;74:465-470.
226. Lund TD, Munson DJ, Haldy ME, Setchell KDR, Lephart ED, Handa RJ. Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. *Biol Reprod* 2004;70:1188-1195.
227. Izumi T, Saito M, Obata A, Arii M, Yamaguchi H, Matsuyama A. Oral intake of soy isoflavone aglycone improves the aged skin of adult women. *J Nutr Sci Vitaminol (Tokyo)* 2007;53:57-62.
228. Jenkins G, Wainwright LJ, Holland R, Barrett KE, Casey J. Wrinkle reduction in post-menopausal women consuming a novel oral supplement: a double-blind placebo-controlled randomized study. *Int J Cosmet Sci* 2014;36:22-31.
229. Accorsi-Neto A, Haidar M, Simoes R, Simoes M, Soares-Jr J, Baracat E. Effects of isoflavones on the skin of postmenopausal women: a pilot study. *Clinics (Sao Paulo)* 2009;64:505-510.
230. Skovgaard GR, Jensen AS, Sigler ML. Effect of a novel dietary supplement on skin aging in post-menopausal women. *Eur J Clin Nutr* 2006;60:1201-1206.
231. Lephart ED. Protective effects of equol and their polyphenolic isomers against dermal aging: microarray/protein evidence with clinical implications and unique delivery into human skin. *Pharm Biol* 2013;51: 1393-1400.

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