Equol, Soy, and Menopause

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A Brief Historical Overview of the Past Two Decades of Soy and Isoflavone Research1,2

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Abstract

During the past 20 years, a remarkable amount of research into the health effects of soy consumption has been conducted, which in large part can be attributed to the presence of isoflavones in the soybean. Isoflavones first came to the attention of the scientific community in the 1940s because of fertility problems observed in sheep grazing on a type of isoflavone-rich clover. In the 1950s, as a result of their estrogenic effects in rodents, isoflavones were studied as possible growth promoters for use by the animal feed industry, although shortly thereafter, it was shown that isoflavones could also function as antiestrogens. Despite this early work, it was not until the 1990s, largely because of research sponsored by the U.S. National Cancer Institute, that the role of soyfoods in disease prevention began to receive widespread attention. Subsequently, isoflavones and soyfoods were being studied for their ability to alleviate hot flashes and inhibit bone loss in postmenopausal women. In 1995, soy protein attracted worldwide attention for its ability to lower cholesterol. At this same time, isoflavones began to be widely discussed as potential alternatives to conventional hormone therapy. In 2002, it was hypothesized that individuals possessing the intestinal bacteria capable of converting the soybean isoflavone daidzein into the isoflavan equol were more likely to benefit from soy intake. More recently, in vitro and animal research has raised questions about the safety of isoflavone exposure for certain subsets of the population, although the human data are largely inconsistent with these concerns. J. Nutr. 140: 1350S–1354S, 2010.

Asian populations have consumed foods made from soybeans for centuries, whereas in the West, certain subpopulations, namely Seventh-day Adventists and vegetarians, have used soyfoods for ~100 years, although the quintessential soyfood tofu was first introduced on a large scale to the general U.S. population beginning only in the early 1970s. Health-conscious and ecologically minded consumers were particularly attracted to soy at this time because it was perceived as being a source of high-quality protein low in saturated fat that was more efficiently produced than animal sources of protein.

A dramatic increase in soyfood consumption during the last decade of the 20th century occurred because of the belief among many consumers that soyfoods might offer health benefits independent of their nutrient content. This increased interest is best viewed in the context of the general recognition underway at this time that plants contain large numbers of potentially beneficial nonnutritive biologically active components commonly referred to as phytochemicals. This knowledge led to the concept of functional foods [initially referred to as designer foods by the National Cancer Institute (NCI)3] and to soy being one of the first foods widely acknowledged to fall into this category. Like all foods, the soybean contains a number of biologically active components, many of which are being actively investigated including, e.g., saponins and lunasin, but unquestionably it is the isoflavones that are responsible for so much of the scientific interest in this legume.

Isoflavones, which have been known to exist in plants for >100 years, have a relatively limited distribution in nature such that among commonly consumed foods by humans, they are found in physiologically relevant amounts only in soybeans and foods derived from this legume (1), although a variety of plants such as red clover (2) are also rich sources. Consequently,

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3 Abbreviations used: ER, estrogen receptor; NCI, National Cancer Institute; ODS, Office of Dietary Supplements.
isoflavone intake among older adults in Japan and Chinese cities such as Shanghai is ~40 mg/d (3), whereas in Europe and the United States it is likely no more than 3 mg/d (4–10). Although soy protein has also been the subject of considerable investigation, especially in regard to its hypocholesterolemic effects (11), recent scientific interest in soy largely parallels the interest in isoflavones. Of the ~2000 soy-related papers currently published annually, more than one-half are related to isoflavones.

Isoflavones, like many phytochemicals of interest to nutritionists, are phytoalexins, substances formed by the host tissue in response to physiological stimuli, infectious agents, or their products, which accumulate to levels that inhibit the growth of microorganisms (12). Isoflavones possess properties (i.e. anti-fungal, antimicrobial, and antioxidant) that enhance the survival of the soybean (12). For this reason, soybean isoflavone concentrations increase greatly in times of stress, such as when moisture is limited, and are influenced by the environmental conditions under which the soybean is grown (13,14).

In many respects, the biological effects of isoflavones first came to the attention of the scientific community in the 1940s because of breeding problems experienced by female sheep in Western Australia grazing on a type of clover rich in isoflavones (15–17). Three decades later, Setchell et al. (18) established that isoflavone-rich soy, which was part of the standard diet of cheetahs in North American zoos, was a factor in the decline of fertility in these animals. It is easy therefore to understand why nutritionists, if they thought of isoflavones at all at this time, viewed them largely as antinutrients. Interestingly, in the 1950s, isoflavones were being studied by the animal feed industry as possible growth promoters because of their reported estrogenic effects in rodents (19–22). By the 1960s, the determination of the relative binding affinities of isoflavones for estrogen receptor (ER) alpha helped firmly establish these soybean constituents as phytoestrogens (23,24).

For the most part, there was little interest in isoflavones within the nutrition community throughout the 1980s. One notable exception is the now-classic work by the pioneering isoflavone researcher, Kenneth D. R. Setchell, who showed that in response to soy consumption, isoflavone excretion increased dramatically and that only a minority (~25% of Westerners) of participants possessed the intestinal bacteria capable of converting the soybean isoflavone daidzein into the isoflavan equol (25,26). Sixteen years later, Setchell et al. (27) proposed that equol was an especially beneficial compound and that those individuals who possessed equol-producing intestinal bacteria were more likely to benefit from soyfood consumption than those who did not. This hypothesis is currently a very active area of research.

The view toward isoflavones as only being phytoestrogens required modification as a result of Akiyama et al. (28) serendipitously discovering in 1987 that genistein, the primary soybean isoflavone, was an inhibitor of protein tyrosine kinase, an enzyme frequently overexpressed in cancer cells (29). Since then, genistein has been extensively studied for its ability to affect a diverse array of intracellular signaling cascades (30,31) that control cell growth (30). As a result of the protein tyrosine kinase finding, it became clear that isoflavones were complex molecules that could no longer be viewed simply as phytoestrogens and that soyfoods might account for the low incidence of breast cancer in Japan, a notion supported by animal (32) and epidemiologic (33) research published in the early 1990s. As already noted, although largely overlooked, the ability of isoflavonoids to function as antiestrogens and thus possibly reduce risk of hormone-sensitive cancers as a result was already part of the scientific literature in the 1960s (23–26).

In 1990, there was sufficient preliminary evidence for the NCI to sponsor a workshop on the role of soy in reducing cancer risk (34). The findings from this meeting led the NCI to initiate a multi-million dollar research program evaluating the anticancer effects of soyfoods. This declaration of interest, which was largely based on the proposed chemopreventive effects of isoflavones, greatly increased interest in both soy and isoflavones in a wide range of areas. One of these areas was the alleviation of menopausal symptoms.

In 1992, Adlercreutz et al. (35) were the first to suggest that soyfoods, because they contain isoflavones, might at least partially account for why Asians and Japanese women in particular were less likely to report experiencing menopause-related hot flashes. The first trial to examine this hypothesis was published in 1995 (36); since then, >30 trials evaluating the efficacy of isoflavone-containing products have been conducted (see references for reviews) (37,38).

Upon reflection, it is now apparent that in the relatively short recent history of isoflavone and soy research, 1995 was a seminal year. In that year, Anderson et al. (11) published a meta-analysis that attracted widespread attention to the hypocholesterolemic effects of soy protein, although Italian investigators had demonstrated dramatic reductions in cholesterol in hypercholesterolemic participants in response to soy protein as early as the late 1970s (39,40). In many respects, the meta-analysis indirectly led to the approval by the U.S. FDA of a health claim for soy protein and coronary heart disease 4 years later (41). Interestingly, Anderson et al. (11) suggested that isoflavones might account for 60–70% of the cholesterol-lowering effects of soy protein; as a result of this suggestion, considerable investigation of the hypocholesterolemic properties of isoflavones was undertaken (42). Although there is only weak support for the cholesterol-lowering effects of isoflavones, and the cholesterol-lowering potency of soy protein is less than initially thought (43,44), the effects of isoflavones on a variety of coronary heart disease risk factors have been extensively evaluated (45). For example, in 2001, Walker et al. (46) were the first to show that the isoflavone genistein markedly increased nitric oxide-dependent dilation in forearm vasculature.

In the same year in which Anderson et al. (11) published their meta-analysis, a Wake Forest University research group, extremely active in the soy field, helped popularize the notion that isoflavones possessed mixed ER agonist/antagonist properties and were a possible alternative to conventional hormone therapy (47). Soon thereafter, investigators began referring to isoflavones as natural selective ER modulators (48), a classification that gained support from the identification of the second ER, ERβ (49), and the finding that isoflavones preferentially bind to ERβ compared with ERα (50,51). Later work demonstrated that isoflavones also preferentially transactivate ERβ [for a review, see Reiter et al. (52)].

A final landmark development in 1995 was the publication of animal research by Lamartiniere et al. (53–55) showing that isoflavone exposure early in life reduces breast cancer risk during adulthood. In addition to the animal studies, this hypothesis has considerable epidemiologic support (56–59) and is consistent with an emerging school of thought that emphasizes the important role early life events have in the etiology of breast cancer (60–62).

Not surprisingly, given the recognized role of estrogen, there has been considerable interest in the potential skeletal effects of isoflavones, although in 1996, the investigators responsible for
publishing the first animal study to demonstrate skeletal benefits suggested it was the ability of genistein to inhibit tyrosine protein kinase activity that was responsible for this effect (63). That year, the first rodent study was published showing isoflavone-rich soy protein improved bone mineral density (64) and 2 years later the first clinical study showing this was the case in postmenopausal women appeared in the literature (65). Since then, >30 trials have examined the effects of isoflavone-containing products on bone mineral density in postmenopausal women (see references for reviews of the literature) (66,67). The ability to conduct clinical trials, especially those longer in duration, was greatly aided by the development of isoflavone supplements, which first became available in 1996.

Along with research of the possible skeletal benefits of isoflavones for postmenopausal women, there has been interest in understanding the impact of soy on cognitive function. The first clinical trial in this area that reported a benefit was published in 2001 (68). One year before this publication an prospective epidemiologic study, whose primary endpoint was heart disease in men, found an association between tofu intake and the development of cognitive impairment in older age (69). However, other epidemiologic data do not concur with this observation (70). For a review of the clinical trials, the reader is referred to the reference (71).

Not surprisingly, the federal government has funded much of the isoflavone research in the United States, but their involvement in this field is not limited to funding. For example, in 1999, the USDA in conjunction with Iowa State University created an online database of the isoflavone content of foods (72). Also in 1999, The Office of Dietary Supplements (ODS), Office of Research on Women’s Health, and National Institute on Aging sponsored a workshop to evaluate the effects of phytoestrogens on diseases affecting older men and women (73). In 2005, the ODS sponsored a comprehensive review of the soy-related clinical literature, which was conducted by the Agency for Healthcare Research and Quality at Tufts University (74). And in July of 2009, the ODS convened a workshop aimed at providing guidance for future clinical research involving soy (75).

It would be remiss not to mention that although there continues to be considerable enthusiasm for the potential health benefits of soyfoods and isoflavones, concerns about the safety of isoflavones, based largely on their estrogen-like properties, have occurred in parallel. In fact, isoflavones are not just classified as phytoestrogens and mixed estrogen agonists/antagonists but also as endocrine disruptors (76–78). Evaluations of isoflavone safety have been undertaken by governmental and quasi-governmental agencies in several European countries as well as in Japan and Israel and at the time of this writing, the European Food Safety Authority is currently conducting an evaluation. Most notable among the concerns, which in all cases are based almost exclusively on in vitro research (the human research, including both clinical and epidemiologic data, are supportive of safety) is that isoflavone-containing products pose a risk to estrogen-sensitive breast cancer patients and women at high-risk of developing this disease (79) and that isoflavone exposure via the consumption of soy infant formula may harm the long-term development of infants (80). The latter issue was reviewed by the National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction in 2006 (81,82), and 2009 (83). At the most recent meeting the expert panel concluded that there was minimal concern about the safety of soy infant formula (83). For a discussion of the breast cancer (79,84) and infant formula issues (80,85,86), the reader is referred to the references.

Finally, there is recognition of the need to more precisely identify those factors contributing to the inconsistent clinical data such as interindividual differences in isoflavone metabolism, the health status (at-risk compared with normal risk, healthy compared with unhealthy) and metabolic profile (i.e. receptor polymorphisms) of study participants and especially, differences in the chemical composition of intervention products. Establishing those variables that play such a role may go a long way toward achieving a more precise understanding of the health effects of soyfood and isoflavone consumption.

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Literature Cited


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Equol: History, Chemistry, and Formation1,2

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Abstract

Equol, first isolated from equine urine in 1932 and identified 50 years later in human urine as a metabolite of the soy isoflavones, daidzin and daidzein, is produced by intestinal bacteria in some, but not all, adults. This observation led to the term equol-producers to define those adults that could make equol in response to consuming soy isoflavones and the hypothesis that the health benefits of soy-based diets may be greater in equol-producers than in equol nonproducers. By virtue of a chiral center, equol occurs as a diastereoisomer and intestinal bacteria are enantiospecific in synthesizing exclusively the (S)-equol enantiomer, an enantiomer that has selective affinity for the estrogen receptor β. Both enantiomers are of interest from a clinical and pharmacological perspective and are currently being developed as nutraceutical and pharmacological agents. The wide range of biological activities these enantiomers possess warrants their investigation for the treatment of a number of hormone-related conditions involving estrogen-dependent and androgen-related conditions. The following review describes the history, chemistry, and factors governing the intestinal bacterial formation of equol. J. Nutr. 140: 1355S–1362S, 2010.

Introduction

It is now 57 years since the first report appeared describing a new phenolic compound in an estrogenic fraction of pregnant mare urine (1). It was suggested that the compound be given the name equol, after the equine source of the material. Efforts to obtain large-scale quantities of the compound led to the recognition that it was also present in appreciable amounts in the urine of stallions and nonpregnant mares and the conclusion that it was not associated with the presence of high estrogen states. During the autumn months, the amounts of equol declined and by winter it was impossible to isolate it from urine. The authors concluded that, “so far as can be determined, no dietary factor was the cause of this (seasonal) variation…” (2). It later became apparent that this was not the case when in SW Australia reports emerged of a catastrophic “failure to breed” associated with uterine abnormalities and endometriosis in sheep grazing on Trifolium subterraneum clover (3). Reductions in sperm counts and motility were also documented in ewes (4). This clover disease, as it was so-called, was found to be the result of extremely high circulating concentrations of equol, formed by ruminal bacteria from the ingestion of large amounts of the methoxylated isoflavone, formononetin, abundant in several indigent species of clover (5–7). Equol was even found as a component in urinary calculi of sheep and cattle (8). Equol has since been reported to be present in the urine and/or plasma of many other animal species, including cows (9), hens (10–14), monkeys (15,16), chimpanzees (17,18), dogs (19), mice (19), rats (20–22), and pigs (16,23), but there are marked differences in the extent of metabolism of isoflavones into equol by these species. Rodents, e.g., very efficiently convert daidzin/daidzein to equol (24), whereas pigs and humans have been reported to do this less efficiently (16,24). In the decades leading to 1970, a great deal of work was performed defining the metabolism of isoflavones and biological actions of equol (6,25–28). While its estrogenic effects were well documented based upon field observations and classical bioassays, it was not until after the discovery of the first estrogen receptor (ER)5 in the mid-1960s (29) that the relative affinity of equol for the ER could be

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Abbreviations used: BBM, brush border membrane; ER, estrogen receptor; ISP, isolated soy proteins; RMB, relative molar binding.
quantified. When the relative molar binding affinities of a number of phytoestrogens for sheep uterine ER were compared, equol was found to have much higher affinity than its precursor daidzein in competing with radioactive estradiol for binding to the cytosolic receptor (27), supporting the theory that it may be advantageous to be able to convert daidzein to equol (24).

There was little interest in equol for several decades until the chance discovery in 1980 of high concentrations of an unknown estrogen-like compound in rat urine (30) accompanying the mammalian lignans, enterolactone and enterodiol (31–33). At the time, it was referred to as compound 386/192, a notation for the molecular ion and base peak in the mass spectrum of its trimethylsilyl ether derivative. In common with most endogenous steroid hormones, including estrogens, it was conjugated predominantly to glucuronic acid and a lesser extent to sulfuric acid (34). Its presence in such high concentrations in rat urine fortuitously afforded a means of isolating sufficient quantities for structural elucidation studies by infrared spectroscopy, NMR, and GC-MS (35) and the subsequent confirmation that it was identical in chemical structure to the equol first isolated from pregnant mares urine in 1932 by Marrian et al. (1,2). This confirmation was made possible because one of us (K.D.R.S.) was gifted from the curator of the UK Medical Research Council’s Steroid Reference Collection (the late Professor D.N. Kirk) the original 4.0 mg sample of equol isolated from pregnant mares urine by Marrian et al in 1932. Equol was also found to occur as a minor constituent in the urine of many adults. The link between equol and soy came about after a series of studies in which different plant-based foods were fed to rats maintained on a purified diet. The introduction of soy protein led to a huge increase in the urinary excretion of equol and following this observation, the soy isoflavone daidzin was isolated and shown to be a precursor to equol (35). It was also found that the introduction of soy protein to the diet led to an increased excretion of equol in some but not all adults (36), whereas in vitro incubation of cultured fecal flora from equol-producing individuals with either daidzein or soy protein resulted in the formation of equol (36). The finding of high concentrations of equol in the urine of adults consuming soy foods prompted the hypothesis that this nonsteroidal estrogen may be beneficial in the prevention and treatment of many hormone-dependent conditions (36).

Progress in research studies of equol was hampered by the lack of sufficient amounts of the compound for biological and clinical testing and by the divergent interest and focus on genistein, the other soy-derived isoflavone that was shown to be a potent inhibitor of tyrosine protein kinases (37) and a genistein, the other soy-derived isoflavone that was shown to be a potent inhibitor of tyrosine protein kinases (37) and a

![FIGURE 1](https://example.com/figure1.png)

**FIGURE 1** Cumulative number of publications on equol by year since its first identification in human urine.

nonsteroidal estrogens. It has a molecular composition of \( \text{C}_{15}\text{H}_{14}\text{O}_3 \) and a molecular weight of 242.27 Daltons. The heterocyclic structure contains 2 reactive hydroxyls and 1 relatively inert and unreactive oxygen in the central furan ring. Physicochemically, it is nonpolar and relatively insoluble in solution, something that should be considered when conducting in vitro experiments, particularly at high concentrations. It is also extremely acid-labile and can readily be destroyed (>60%) in the general work-up of samples, particularly if acidic hydrolytic steps are used (39). Despite having 2 phenolic rings, it exhibits poor UV absorption characteristics, meaning that HPLC with UV detection is unsuitable for its measurement in most biological fluids. As a result of a chiral carbon at position C-3 of the molecule, equol exists in 2 enantiomeric forms, \( R \)-equol and \( S \)-equol, and the latter is the natural diastereoisomer produced by intestinal bacteria in the intestine of humans and rats (40). This makes it distinct from its precursor isoflavone, daidzein, and the 2 other major isoflavones of soy, genistein and glycitein (41). Equol can be readily synthesized from daidzein by catalytic hydrogenation, but this yields the (±) equol form (42) and it is the form that has been commercially available and mostly utilized in studies of its biological potency and properties. Indeed, unless otherwise stated, it can be assumed that all previously reported experiments used (±)equol and not the individual enantiomers. It cannot always be assumed that the racemate will behave in an identical manner to that of the individual enantiomers and this was recently shown to be the case for its pharmacokinetics (43). It is probable that biological effects may be underestimated when testing with the racemate and this may particularly hold true for binding affinities to receptors. The racemic mixture can be readily separated by chiral chromatography and the earliest studies used this approach to isolate sufficient amounts of each enantiomer to determine the estrogen binding affinities (40,44) and the pharmacokinetics (43). More recently, methods for the selective synthesis of \( S \)-equol (45,46) and \( R \)-equol (45) have been described. Methods for the synthesis of [\(^{13}\)C]labeled isoflavone analogs (47–50) have been described that can represent suitable starting points for the preparation of stable-labeled [\(^{13}\)C]equol for use as tracers in metabolic studies or for internal standards in stable-isotope dilution mass spectrometric assays (43). The synthesis of \( S \)-equol and \( R \)-equol by chiral chemistry (45) now affords the large-scale production of enantiomeric pure compounds for use in clinical and animal studies. Finally, the

**Chemistry**

Equol [7-hydroxy-3-(4′-hydroxyphenyl)-chroman], an isoflavone, belongs to the general class of compounds referred to as

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production of S(-)-equol from daidzein-rich soy germ by a specific equol-producing bacterium, Lactococcus garvieae (51), offers an alternative biological means to producing specifically S(-)-equol (52). With these breakthroughs, it is now possible to study in some detail the effects of equol in animals and humans and such studies will likely shed further light on the relevance of this metabolite to the clinical outcomes.

Role of intestinal bacteria in the formation of S(-)-Equol

The early evidence in support an intestinal bacterial origin for equol in humans and animals was as follows: 1) germ-free animals fed a soy diet do not excrete equol in urine (30); 2) S(-)-equol is not excreted in the urine or found in the plasma of either newborn infants that lack a developed micro flora (53) or in infants up to the age of 4-mo fed exclusively soy infant formula from early life (54,55); 3) incubation of soy, or daidzein with human fecal flora from adults that produce equol, leads to the formation of S(-)-equol (36,40); 4) some antibiotics will knockout the production of equol (18,56,57).

Even though it was known for decades that intestinal bacteria were responsible for the production of S(-)-equol, it is only in recent years that specific bacteria capable of converting daidzin/daidzein to S(-)-equol have been isolated and identified (Table 1). Interestingly, whereas it was reported that 1.59% of injected [14C]genistein was recovered as [14C]equol in domestic fowl (77), metabolic and pharmacokinetic studies in humans administered [13C]daidzein and [13C]genistein tracers show conclusively that in humans, S(-)-equol is formed from daidzein and not genistein (78). Although the major degradative pathway for genistein leads to p-ethylphenol, a phenol first found in goat urine (79) and 4-hydroxyphenyl-2-propionic acid in rats (80), recently it was shown that an anaerobic bacterium from mouse intestine could produce 5-hydroxy-equol from genistein by analogous reactions to those that yield equol from daidzein (66,74). All of these conversions are time dependent and slow, and in humans it takes 12–36 h for the appearance of [13C]equol in plasma after oral administration of [13C]daidzein (78), which is consistent with a colonic origin for its formation.

Biotransformations that take place after oral administration of soy isoflavones are summarized in Figure 2. The production of S(-)-equol from daidzin requires 3 key steps. Daidzin first undergoes hydrolysis to split the glucoside moiety and effect release of the bioavailable aglycon, daidzein. This step is crucial to all soy isoflavones, because the conjugated forms (glucosides) do not cross the enterocyte and are consequently not bioavailable (81). Hydrolysis is very efficient and begins in the proximal intestine by the action of brush border membrane β-glucosidases (82). Bacterial β-glucosidases are also capable of performing this hydrolysis and many of the common bacteria that reside in the intestinal tract do this (62,63,83). The efficiency of this initial hydrolytic step by brush border membrane glucosidases is also exemplified by the very high plasma concentrations of daidzein

### Table 1

List of intestinal bacteria that cultured in vitro have been found to biotransform isoflavones to S(-)-equol or related intermediates

| Reference | Bacterium strain | Source | Reaction
|-----------|------------------|--------|-----------
| Maruo et al., 2008 (58) | Adlercreutzia equolifaciens | Human | Daidzein → Equol |
| Minamida et al., 2006 (59) | Asaccharobacter cellulans AHS1763 | Rat | Daidzein → Equol |
| Minamida et al., 2008 (60) | Asaccharobacter cellulans gen nov sp nov strain do03 | Rat | Daidzein → Equol |
| Ueno and Uchiyama, 2002 (61) | Bacteroides ovatus | Human | Daidzein → Equol |
| Tsangalis et al., 2002 (62) | Bifidobacterium sp | Human | Daidzein → Equol |
| Tsangalis et al., 2002 (62) | Bifidobacterium animalis | Human | Daidzein → Equol |
| Raimondi et al., 2009 (63) | Bifidobacterium sp (22 strains) | Human | Daidzein → Equol |
| Hur et al., 2000 (64) | Clostridium sp HGH6 | Bovine | Daidzein → Dihydrodaidzein |
| Tamura et al., 2007 (65) | Clostridium-like bacterium | Human | Daidzein → Dihydrodaidzein |
| Mathies et al., 2008 (66) | Coriobacteriaceae sp MT189 | Mouse | Daidzein → Equol |
| Mathies et al., 2008 (66) | Coriobacteriaceae sp MT189 | Mouse | Genistein → 5-Hydroxy-equol |
| Wang et al., 2005 (67) | Eggerthella sp Julong 732 | Human | Dihydrodaidzein → Equol |
| Kim et al., 2009 (68) | Eggerthella sp Julong 732 | Human | Dihydrodaidzein → Equol |
| Yokoyama and Suzuki, 2008 (69) | Eggerthella sp YY7918 | Human | Daidzein → Equol |
| Decroos et al., 2005 (70) | Enterococcus faecium | Human | Daidzein → Equol |
| Tamura et al., 2007 (65) | Escherichia coli (HGH21 and HGH6) | Human | Daidzein → Daidzein |
| Yu et al., 2008 (71) | Eubacterium sp D1 and D2 | Pig | Daidzein → Equol |
| Decroos et al., 2005 (70) | Finegoldia magna | Human | Daidzein → Equol |
| Decroos et al., 2005 (70) | Lactobacillus mucasae | Human | Daidzein → Equol |
| Wang et al., 2006 (72) | Lactobacillus sp Nsu-016 | Human | Daidzein → Equol |
| Ishimi et al., 2008 (73) | Lactobacillus garvieae (Lg HE1) | Human | Daidzein → Equol |
| Ueno and Uchiyama, 2002 (61) | Ruminococcus productus | Human | Daidzein → Equol |
| Mathies et al., 2009 (74) | Slackia sp HE8 | Human | Genistein → 5-Hydroxy-equol |
| Mathies et al., 2009 (74) | Slackia sp HE9 | Human | Daidzein → Equol |
| Jin et al., 2009 (75) | Slackia equolifaciens (Strain DZ2) | Human | Freelike aglycon (PUE) |
| Jin et al., 2008 (76) | Streptococcus intermedius | Human | Daidzein → Equol |
| Ueno and Uchiyama, 2002 (61) | Veillonella sp | Human | Daidzein → Equol |
| Decroos et al., 2005 (70) | Veillonella sp | Human | Daidzein → Equol |
| Decroos et al., 2005 (70) | Mixture of Lactobacillus mucosae EP12, Enterococcus faecium EP11, Finegoldia magna EP13, and Veillonella sp | Human | Daidzein → Equol |

1 In most cases the definitive structure of the equol product was not determined, but it can be assumed that in all cases the product is the S(-)-equol enantiomer.
and genistein in infants fed soy infant formula (54,55) that have immature and undeveloped gut microflora (53). Daidzein is reduced to $S$-equol through the intermediate dihydrodaidzein then converted by deoxygenation to yield $S$-equol. Daidzein is also metabolized to O-desmethylangolensin as a result of cleavage across the heterocyclic ring (84), but this metabolite appears to be of little interest in that it has no known biological activity.

Bacteria are enantiomeric in metabolizing daidzein to exclusively $S$-equol and not $R$-(-)equol (40,67,72). It is not completely clear whether the conversion of daidzein to $S$-equol is performed by a single bacterium or whether there are distinctly different bacteria that execute these reactions, or both. The large variability in the levels of dihydrodaidzein and $S$-equol in human urine (85) would indicate that there is more than a single bacterium responsible for producing $S$-equol. Furthermore, the finding that certain antibiotics selectively inhibit the formation of equol but not dihydrodaidzein when human feces from equol-producers are incubated with daidzein also supports this contention (56). The list of intestinal bacteria that can produce equol in culture is ever increasing (Table 1); a number of strains have been isolated that perform only conversion of daidzin/daidzein to dihydrodaidzein whereas others appear to be able to completely convert daidzein to $S$-equol (40,67,72). It is not known but is important to understand if the hypothesis that the ability to produce equol when consuming soy foods (equol hypothesis) is advantageous in terms of enhancing health benefits can be clearly demonstrated (24,106). Thus far, the data are inconclusive, but this is partly because, to our knowledge, none of the clinical studies have preselected participants on the basis of equol-producer status but rather have retrospectively subanalyzed data from equol-producers, and most are underpowered. Significant differences in gene expression between equol-producers and equol nonproducers have been demonstrated in postmenopausal women exposed to an isoflavone supplement, with the most notably significant alterations in expression of a number of estrogen-responsive genes (107).

It would appear that equol-producer status is a relatively stable phenomenon, as evidenced from repeat testing over prolonged periods of time in adults (24,89,95,108). There have been many studies looking at associations between equol-producer status and dietary components, including fat and carbohydrate composition (93,101), PUFA (109), dairy intakes (110), lactose (111), green tea consumption (112), seaweed (113), and soy food intake (103,105,110), but no clear conclusion can be made. Prolonged soy food consumption appears not to be a factor driving equol formation (114). One possible explanation for the differences in the frequency of equol-producers among populations, we speculate, may be related to the type of soy foods consumed (115). There are marked differences in the isoflavone composition of Western and Asian soy foods (Fig. 3) (41). Asians consume a high proportion of isoflavone aglycons, because fermented soy foods account for...
about one-third of the total intake of soy foods (116,117). Based upon the typical 20–50 mg/d intake of total isoflavones by Asians, we estimate that 10–30 mg are ingested in the form of aglycons, which in most adults are absorbed faster than glycosides (43,102,118–121) and may be more easily converted to equol than glycosides. In a recent cholesterol-lowering dietary intervention study of a soy germ-enriched pasta containing predominantly isoflavones in the aglycon form, it was found that 69% of the patients were equol-producers (115), which is considerably higher than the 20–30% expected frequency for Westerners (95). Most dietary intervention studies of soy have used foods or products made from isolated soy proteins as the source of isoflavones (122) or soy isoflavone supplements that contain almost exclusively isoflavone glucosides (119). Isolated soy proteins are not commonly consumed in the Asian diet and this may explain the lower frequency of equol-producers in Western adults. Furthermore, if equol production does enhance the efficacy of soy foods (24,106), then this could explain the variable results obtained from recent studies of soy foods. Future intervention study of a soy germ-enriched pasta containing predominantly isoflavones in the aglycon form (123,126), then this could explain the lower frequency of equol-producers in these populations. Therefore, to define an equol-producer by the equol:daidzein ratio also circumvents the need for accurately timed 24-h urine collections.

**FIGURE 3** Differences in the type of soy foods consumed by Western and Asian populations may account for differences in the frequency of equol-producers.

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**Literature Cited**

1360S Supplement


Equol: Pharmacokinetics and Biological Actions

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Abstract

Equol [7-hydroxy-3-(4′-hydroxyphenyl)-chroman], an isoflavon produced by intestinal bacteria in response to soy isoflavone intake in some but not all humans, exhibits a wide range of biological properties. It exists as the diastereoisomers S-(+)equol and R-(+)-equol. Intestinal bacteria produce exclusively S-(+)-equol, which has selective affinity for estrogen receptor (ER)-β. The evidence is conflicting on whether there is an advantage to producing S-(+)-equol in response to soy isoflavone intake, but the ability to now synthesize these diastereoisomers opens the way for future clinical trials to directly examine their potential in a number of hormone-dependent conditions. In this review, the plasma and urinary pharmacokinetics of S-(+)-equol and R-(+)-equol are reviewed and summarized, and some of the more recent evidence supporting potential biological effects of S-(+)-equol is considered. J. Nutr. 140: 1363S–1368S, 2010.

Introduction

In Part 1 of this overview of equol (1), the history, chemistry, and factors that influence equol production were reviewed. Part 2 separately reviews the pharmacokinetics and the biological properties of equol that have led to the current interest in this unique isoflavone metabolite.

The hormonal effects of equol are well documented from early observations using estrogen bioassays. It was not until after the discovery of the first estrogen receptor (ER)-α (2) and the discovery that a second ER (ERβ) was present in specific tissues (3) that the relative affinity of equol for both receptors could be quantified (4–6). The results from these studies place the natural soy isoflavone metabolite, S-(+)-equol, into a category of a selective ER modulator and consequently prompts many questions as to whether it could confer some specific benefits in hormone-related conditions.

The ability of both S-(+)-equol and its diastereoisomer, R-(+)-equol, to antagonize the in vivo actions of dihydrotestosterone (7) further makes equol a unique molecule with potential for the treatment or prevention of androgen-mediated conditions. For these reasons equol is currently attracting considerable interest as a potential pharmaceutical or nutraceutical agent. The following will review its pharmacology and biological effects.

Pharmacokinetics of equol

To our knowledge, data from the first pharmacokinetic study of equol was described in a single healthy adult female administered 25 mg of (±)-equol given as a single oral bolus dose (8). The plasma (±)-equol concentration appearance/disappearance curve suggested that equol differed in its pharmacokinetic behavior from the soy isoflavones daidzein and genistein. Most notably it had a much higher apparent bioavailability and slower clearance rate (8). This was confirmed in later studies when the plasma pharmacokinetics of S-(+)-equol and R-(+)-equol were compared in 3 healthy adults (6). More recently, using [13C]labeled tracers, the plasma and urinary pharmacokinetics of enantiomeric pure S-(+)-equol and R-(+)-equol were determined in 12 healthy adults (6 males, 6 females) (9). Both enantiomers were rapidly absorbed and reached peak plasma concentrations after 2–3 h when taken with a meal. In an evaluation of the pharmacokinetics of a new S-(+)-equol–containing supplement (SE5-OH) given to 12 healthy postmenopausal women, the average peak plasma concentration was observed after 1–2 h when taken...
without a meal (10). The differences in the absorption rates of $S$-equol between these 2 studies is explained by a meal-effect altering gastric emptying time and slowing the initial absorption rate. Such differences will influence peak plasma concentrations, as was evident from the much higher dose-adjusted $C_{\text{max}}$ values attained with the $S$-equol supplement given without a meal compared with the pure compounds given with a meal (10). Therefore, in practice, the maximal effect of $S$-equol is more likely to occur if it is administered before a meal. Independent of this difference, the pharmacokinetics of enantiomeric pure $S$-equol was similar to that of the $S$-equol supplement produced by the fermentation of soy germ isoflavones with Lactococcus garvieae (10). $S$-equol has an terminal elimination half-life of 7–8 h in healthy adults and, therefore, steady-state levels will be more readily attained by dosing twice daily to minimize peaks and troughs in circulating concentrations. Within the constraints of small sample-sizes, data from all these studies suggested no obvious gender differences in the pharmacokinetics of $S$-equol. Two interesting findings arose from a comparison of the pharmacokinetics of the $^{13}$C-labeled enantiomers. Racemic ($\pm$) $^{13}$C-eqol showed slower absorption, attained lower peak plasma concentrations, and had lower systemic bioavailability compared with $S$-[($\pm$)C]eqol and $R$-[($\pm$)C]eqol. Also, the apparent systemic bioavailability of $R$-[($\pm$)C]eqol was significantly greater than that of $S$-[($\pm$)C]eqol (9).

$S$-equol and $R$-[($\pm$)C]eqol undergo little biotransformation in humans, save phase II metabolism by conjugation to glucuronic acid and to a minor extent sulfuric acid. $S$-eqol circulates in plasma and is excreted in urine as predominantly the 7-glucuronide conjugate (11–13). In this respect, its metabolism is similar to that of the soy isoflavones daidzein and genistein (14–18). Conjugation is extremely efficient in humans and takes place on first-pass absorption within the enterocyte and also the liver. Uridine diphosphate-$S$-glucuronosyltransferase is widely distributed throughout the gastrointestinal tract and it is probable that it is the uridine diphosphate-$S$-glucuronosyltransferase 1A10 isomer that catalyzes glucuronidation, because this one conjugates genistein (15). For eqol, its major route of elimination is by renal excretion into urine. The percent fractional elimination in urine after oral administration is extremely high and in some adults it can be close to 100% (9,10), which is far higher than that of daidzein (30–40%) and genistein (7–15%) (20,21). Recoveries averaged 82% when $S$-[($\pm$)C]eqol was given as a supplement and 61.3 $\pm$ 19.5% for enantiomeric pure $S$-[($\pm$)C]eqol. The bioavailability of $R$-[($\pm$)eqol]eqol was higher than its diastereoisomer $S$-[($\pm$)eqol]eqol based on the plasma pharmacokinetics and urinary recovery of the $^{13}$C tracers (9). Overall, the very high bioavailability of $S$-[($\pm$)eqol]eqol would indicate that relatively modest doses (10–30 mg twice a day) would result in high steady-state plasma concentrations in the range observed for plasma $S$-[($\pm$)eqol]eqol derived from soy foods.

Endogenous estrogens, as with most hormones, circulate predominantly bound to albumin and sex hormone binding globulin (22) and also to $\alpha$-fetoprotein (23). Less than 5% of estradiol is present in the free (unbound form), which is the fraction that is available for receptor occupancy. For eqol it has been reported that 49.7% circulates in the free form, which is significantly higher than daidzein (18.7% free), its precursor (22). Thus, the biological activity of eqol should be enhanced by its reduced binding to serum proteins and greater availability for receptor binding. In a dose-dependent manner, eqol in vitro inhibits the binding of estradiol and testosterone for serum proteins (24).

### Biological properties of eqol

The diastereoisomers of eqol share many similarities yet some significant differences in biological properties. The more planar-looking $S$-[($\pm$)eqol]eqol enantiomer is strikingly similar in conformational structure to estradiol and, not surprisingly, this enantiomer binds to the ER (Fig. 1). When first isolated from equine urine in 1932, long before the discovery of the first ER (2), it was reported to have no estrogenic activity when injected into ovariectomized mice in doses up to 0.086 mg (25); however, its uterotrophic activity was later acknowledged. The earliest in vitro study of the binding of $S$-[($\pm$)eqol]eqol, isolated from sheep urine showed it to have a relative molar binding affinity of 0.4 compared with estradiol, which was about 4 times the affinity of its precursor, daidzein (26). Later, 5 mg of eqol, presumed to be the racemic form because it was chemically synthesized by methods at the time that were not enantioselective, when injected subcutaneously into 3-wk-old female rats increased uterine weight to the same extent as 0.005 mg of estradiol (27) and it was shown to antagonize the binding of estradiol to the ER. Others have reported similar relative binding affinities using a selection of different in vitro systems (28–30). It should, however, be pointed out that in most cases these early studies would have examined binding to ER$\alpha$, because they predated the discovery of ER$\beta$ (3) and because this is the major ER subtype localized to the uterus (31). These early data are also possibly underestimated because of the use of racemic mixtures. Several more recent studies have since reported the binding characteristics of the individual enantiomers toward ER$\alpha$ and ER$\beta$ (4–6,32–34). $S$-[($\pm$)eqol]eqol produced from incubation of soy

![Estradiol, S-(-)equl, and R-(+)equl](image_url)
isoflavones with enteric bacteria when tested in competitive binding assays with human ERα and ERβ, and in a gene expression assay, was found to bind more strongly to ERβ than to ERα (4). The preferential binding of S(-)-equol to ERβ has been confirmed in multiple studies (5,6,32) and indicates the molecule shares the characteristics of a selective ER modulator in this regard. However, S(-)-equol induces transcription either similarly, or more strongly, with ERα than with ERβ (4,5), as does R(+)-equol (5), indicative of both being agonists. So the differential effects of 2 almost identical molecules on the ER subtypes is quite striking and shows how the presence of a chiral center in the molecule confers quite different biological properties.

Given the present interest in S(-)-equol as a possible pharmaceutical or nutraceutical agent for a number of hormone-dependent disorders (9,10,35), the question of whether the ER agonist action could pose some risk for women with breast cancer or for those in high risk groups remains to be addressed (36–38). Recent studies using animal models of breast cancer have examined the role of equol on the growth of mammary tumors (39,40). In one model, S(-)-equol did not stimulate the growth of human ER positive MCF-7 cells transplanted into the athymic mouse (39). This important finding is in striking contrast to the marked stimulatory effect of the soy isoflavone genistein reported earlier by the same investigators in this same model (41), an observation that led to the issue of whether soy foods are safe for women with breast cancer. To date, there are no human data to support this concern, but 2 recent large prospective clinical studies of breast cancer survivors suggest that soy food consumption is associated with more favorable prognosis, in reducing risk of recurrence and improving survival (42,43). In a different animal model, S(-)-equol did not stimulate the growth of mammary tumors induced by the chemical carcinogen dimethylbenz[a]anthracene, but neither did it prove to be chemopreventive (40). R(+)-equol on the other hand was found in this same model to be potently chemopreventive (40). Combining data from these 2 animal models of breast cancer suggests that S(-)-equol should not increase risk for breast cancer and R(+)-equol could be a useful chemopreventive agent. If these animal data can be reliably extrapolated to humans, then the ability to make equol when consuming soy foods could be advantageous in reducing the risk of breast cancer.

While much of the interest in equol has centered on its estrogenic effects, equol enantiomers have a myriad of other biological properties with the potential to be of value in many clinical areas, including cancer, cardiovascular disease, osteoporosis, and menopausal symptoms (8); several of these areas are discussed below.

Uniquely, both S(-)-equol and R(+)-equol bind dihydrotestosterone and inhibit the in vivo stimulatory effect of this potent androgen on prostate growth (7). Neither S(-)-equol nor R(+)-equol bind to the androgen receptor, but its selective androgen-modulating activity, combined with S(-)-equol having selective affinity for ERβ, suggests that S(-)-equol may have potential in a number of androgen-mediated conditions, in particular prostate cancer treatment or prevention. The pharmaceutical industry has more recently turned its attention toward ERβ agonists in the search for the next generation of drugs to treat prostate cancer (44) and in this regard S(-)-equol may be a potential candidate. A small case control of prostate cancer patients from South Korea, Japan, and the US found a low frequency of equol-producers among the patients compared with age-matched controls (45), while in a separate Japanese study, the risk for prostate cancer was reported to show an inverse dose-response relationship with plasma equol concentrations (46).

Equol can be broadly classified as a polyphenol and due to the high number of p-electrons, it has hydrogen/donor properties and will scavenge free radicals. The in vitro antioxidant property of equol, presumed to be racemic equol, is well documented (47–50) and the antioxidant activities of the individual enantiomers should be similar. (+)Equol has the highest antioxidant activity of all the isoflavones that have been tested. To date there are no in vivo human data on the extent to which administering equol may influence lipid peroxidation, an important risk factor for atherosclerosis, but LDL oxidation by cultured monocyte/macrophages was shown to be inhibited by an antioxidant effect mediated through inhibition of superoxide radical production (51). The effect of equol on inhibition of nitric oxide (NO) production by inducible NO synthase gene expression in murine macrophages was reported as being mediated through upstream signaling pathways, specifically by Akt activation and down-regulation of nuclear factor-κB activity (52); inducible NO synthase is implicated in the development of atherosclerosis. These findings are perhaps not unexpected, because genistein is antiinflammatory by an effect on reducing NO production (53).

Several studies show equol to be a vasorelaxant, inducing endothelial and NO-dependent relaxation (54–60), suggesting equol may be helpful in reducing risk of cardiovascular disease. The isoflavone intermediate dihydrodaidzein and the closely related dehydroequol are also vasodilatory (55,61). No study has yet examined the vasodilatory actions of S(-)-equol or R(-)-equol separately and it is too early to know whether either enantiomer may be effective in the clinical arena. Studies of soy isoflavones have yielded mixed results with regard to the effects on endothelial function (62–69), but equol-producer status was not directly examined. In one recent clinical study of hypercholesterolemic patients, brachial artery-mediated vasodilatation was significantly greater in equol-producers compared with equol-nonproducers after 4 wk of dietary intervention with a soy isoflavone-containing food that resulted in a high proportion of equol-producers (70). Similar differential effects between equol-producers and nonproducers were observed in arterial stiffness in a study of postmenopausal women taking tibolone (71) and these translated into lower diastolic blood pressure (72). This was not the case in the former study (69). Because inflammation plays a key role in the onset of cardiovascular disease (73), it is possible, given equol’s documented effect on the NO pathway, that it may act as an antiinflammatory agent. Serum high sensitivity C-reactive protein concentration, a surrogate marker of inflammation and cardiovascular risk, was shown in a recent study to be reduced in equol-producers by a soy isoflavone-containing food (70). In a recent study, equol and genistein, but not daidzein, modulated the inflammatory response in activated macrophages by inhibition of NO and prostaglandin E2 while regulating gene transcription of cytokines and inflammatory markers (74).

In vitro cell culture and animal studies have provided impressive data on the bone-trophic effects of isoflavones (75), but recent clinical studies of soy isoflavone supplementation in postmenopausal women have proved disappointing (76–80). None of these trials have prescreened for equol-producer status and randomized accordingly and all have used isoflavone mixtures of predominantly conjugated rather than aglycon forms. Interestingly, the aglycon genistein given at a dose of 54 mg/d for 3 y to postmenopausal women was reported to have impressive effects on bone, with increases in spine and hip bone mineral density (81). Studies from Japan show more favorable responses in measures of bone loss in those women who are equol-producers (82). Equol in its racemic form has been shown...
to have modest effects in preventing bone loss in animal models of osteoporosis (83–87) but has yet to be used in clinical trials. S-(-)-equol is also being studied for its effects on reducing the incidence and frequency of menopausal symptoms (88), particularly hot flushes. Data from Japan have indicated that the severity of overall menopausal symptoms is significantly lower in women who are equol-producers treated with a soy isoflavone supplement (89).

In conclusion, S-(-)-equol is a unique nonsteroidal estrogen that binds preferentially to ERβ and at the same time antagonizes the in vivo action of the potent androgen dihydrotestosterone. It occurs as 2 distinct diastereoisomers and both have properties that warrant their further investigation for the prevention and/or treatment of a number of estrogen- and androgen-mediated diseases or disorders as was first proposed in 1984 (90). The ability to now synthesize bulk quantities of enantiomeric pure S-(-)-equol and R-(-)-equol should permit future clinical studies to be conducted that will more clearly define the potential benefits of these diastereoisomers. More importantly, such direct studies of the pure compounds will enable a better understanding of the extent to which there are advantages to producing equol from soy foods, as has been proposed. If the equol hypothesis can be substantiated, then for those adults who are unable to produce equol due to a lack of intestinal equol-producing bacteria or some other factors, one option is to administer these enantiomers in the form of a nutraceutical or pharmaceutical. Major clinical studies are likely to emerge in the near future that will permit a better understanding of the potential value of equol in numerous clinical areas, not just those discussed above.

Note added in proof: Work by Setchell et al (9) has shown that the unconjugated fraction of [2-13C]S-(-)-equol accounted for only 0.10 ± 0.05% of the total [2-13C]S-(-)-equol in plasma following oral administration of 20 mg of the [13C]equol tracer to 12 healthy adults.

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Emerging Research on Equol and Cancer

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Abstract

Mechanisms of action of equol described using in vitro studies suggest possible effects of this compound in relation to cancer risk. However, experimental data are lacking with regard to the effects of S-(−)-equol (a gut bacterial product of daidzein), racemic equol, or even daidzein on tumorigenesis in vivo. Rodent studies, using racemic equol or daidzein in equol-producing animals, suggest that equol exposure does not stimulate mammary tumor growth, but there is little evidence that it is protective either. Racemic equol has been shown to inhibit skin carcinogenesis in hairless mice. Epidemiologic studies of associations between urinary or plasma isoflavone concentrations and breast cancer risk in women have reported no association nor increased risk associated with higher equol measures in low-soy-consuming populations but have reported a trend toward decreased cancer risk with increased equol in Asian populations. These population-based differences have been reported for prostate cancer too. Several studies in Asian men report lower equol concentrations or a lower prevalence of equol-producers among men with prostate cancer compared with controls, whereas studies in European populations report no association. Studies using intermediate biomarkers of cancer risk and susceptibility in humans also have examined the effects the equol-producer phenotype in relation to soy intake with varying results. Overall, the role of equol in relation to cancer remains unclear. With the availability of R- and S-equol, animal studies of carcinogenesis and human intervention studies addressing effects of the equol enantiomers on intermediate biomarkers may help to ascertain the role of equol in cancer risk.

Introduction

Equol is a chiral molecule that can exist as 2 distinct optically active isomers, R- and S-equol. The enantiomer S-(−)-equol, is the product of gut bacterial metabolism of the soy isoflavone daidzein. Several actions of equol, including its estrogenic and antioxidant properties and its proliferative and antiproliferative effects, suggest that exposure to the compound may have implications for cancer risk [reviewed in (1,2)]. However, results of in vitro studies can be influenced by whether R- or S-equol or the racemic mixture are used. For example, in binding assays, S-equol had a high and preferential binding affinity for estrogen receptor (ER)β, whereas R-equol bound more weakly with a preference for ERα (3). Further, compared with the racemic mixture, S-equol had no antigenotoxic or antioxidant effects in breast cancer cell lines (2). The objective here was to summarize the available animal studies of equol and tumorigenesis, to update our 2005 review of the epidemiologic literature of equol exposure and cancer risk (1), and to discuss the complexities of conducting research in this area.

Animal studies

Experimental data are lacking with regard to effects of S-(−)-equol, racemic equol, or even daidzein on tumorigenesis. Rodent studies, using racemic equol or daidzein in equol-producing animals, suggest that R- and S-equol combined do not stimulate mammary tumor growth, but there is little evidence that these compounds provide a protective effect either. Lamartiniere et al. (4) reported that in rats, dimethylbenz(a)anthracene-induced mammary tumorigenesis was not affected by feeding daidzein-containing diets. Further, Ju et al. (5) showed that dietary racemic equol administered at 3 doses [250, 500, and 1000 ppm (1.03, 2.06, and 4.12 mmol/kg diet, respectively)] to ovariectomized athymic mice did not stimulate growth of implanted estrogen-dependent human breast tumor

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4 Abbreviations used: EPIC, European Prospective Investigation into Cancer and Nutrition; ER, estrogen receptor; OR, odds ratio.
(MCF-7) cells, increase tumor cell proliferation, or induce estrogen-responsive pS2 expression, despite stimulating growth of MCF-7 cells in vitro. Findings such as these point to the challenges of translating in vitro results to effects in vivo and speak to the need for more in vivo studies that allow for the integration of pharmacokinetic and other factors that may affect the biologic response.

Topical application of racemic equol has been shown to reduce the proportion of tumors progressing from benign papillomas to malignant squamous cell carcinoma and reduce the average diameter of lesions in hairless mice treated with solar-simulated UV radiation and/or dimethylbenz(a)anthracene (6). Further, in mice treated with solar-simulated UV radiation, racemic equol topically applied prior to UV treatment reduced DNA damage as measured by cyclobutane pyrimidine dimers, whereas equol applied after UV treatment did not increase the rate of dimer removal (7). Whether there are differences in effects of the specific equol enantiomers on tumorigenesis remains to be established.

**Epidemiologic studies of equol and cancer**

The association between equol production and cancer risk in humans has not been extensively characterized. Because of the lack of commercially available dietary equol supplements, human exposure to equol historically has been exposure to S-(-)-equol as a result of gut bacterial conversion of daidzein to equol. In 2005 in a review of the literature, Atkinson et al. (1) identified 8 studies of equol and cancer. The studies in Asian men tended to report lower equol concentrations or a lower prevalence of equol producers among men with prostate cancer compared with controls (8–10). The studies of breast cancer yielded inconsistent results, with reports of nonsignificant lower equol excretion in breast cancer cases than controls in Asian and Asian-American populations (11,12), a significant trend toward lower equol excretion in breast cancer across increasing quartiles of equol excretion in an Australian study (13), and higher urine and serum equol associated with breast cancer in the UK (14). In a case-control study of women with histologically confirmed cervical squamous intraepithelial lesions (cases) and normal cytology (controls), plasma equol concentrations were positively associated with cervical squamous intraepithelial lesions risk for the highest relative to the lowest quartile level (15). Some of the limitations of these studies included small sample sizes, insufficient statistical power, and lack of controlled evaluation of equol-producer status.

Since 2005, additional, larger studies have examined the relationship between equol measures and risk of prostate, colon, and breast cancer (Table 1). They continue to report mixed results. A recent study in Japanese men reported reduced risk of prostate cancer across tertiles of plasma equol and genistein (16). This association was limited to men with localized disease. In a study of the Multiethnic Cohort, a cohort including men in 5 ethnic/multi-racial groups (i.e., African Americans, Native Hawaiians, Japanese Americans, Latinos, and Whites), Park et al. (17) reported a nonsignificant association between prostate cancer risk and tertiles of urinary equol. Odds ratios (OR) (95% CI) for the second and 3rd tertiles compared with the lowest tertile were 0.89 (0.58–1.37) and 1.32 (0.84–2.08), respectively (P-trend = 0.08). There was no significant interaction of urinary equol by race/ethnicity or any difference by tumor characteristics.

In European populations, 2 large studies conducted in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts reported no association between equol and risk of prostate cancer and colon cancer (18,19). Two studies of breast cancer, also in EPIC cohorts, similarly reported no association between equol measures and overall breast cancer risk (20,21); however, among ER-positive cases in the Norfolk cohort, urinary equol was associated with a slightly higher risk [OR (95% CI) = 1.07 (1.01–1.112); P = 0.013] in the 95 cases compared with the 329 controls.

Observational studies of equol and disease outcomes, such as the ones described above, present particular challenges. They require sufficient habitual exposure to daidzein to allow for bacterial production of equol. In Asian cohorts, the primary source of daidzein in observational studies is soyfoods and, among individuals excreting measurable amounts of equol, equol excretion is soy protein dose-dependent (22). Therefore, in a population with a range of soy intakes, it is often difficult to tease out whether equol itself is associated with disease risk, whether equol is serving as an additional measure of daidzein or genistein exposure or of soy food intake in general, or whether...
equol is a marker of harboring a particular gut bacterial community (1). To address some of these issues, statistical approaches are needed that adjust for overall soy or isoflavone exposure before testing for equol effects. Studies of equol exposure are more problematic in Western Europe and the US where soy intake is very low. Even among individuals in the highest quintiles of exposure in these populations, equol in blood and urine is low and likely to be below clinically relevant levels (23). Equol exposure in some Western populations also may be due to dietary intake of equol from animal and dairy sources, rather than from daidzein from soy foods (24).

Equol phenotype and intermediate biomarkers in human studies

Studies using intermediate biomarkers of cancer risk and cancer susceptibility in humans have also examined the effect of equol-producer phenotype. Similar to the studies of cancer outcomes in humans, these studies also reflect exposure to S-(-)-equol. In an observational study of postmenopausal women phenotyped for equol production, Fuhrman et al. (25) reported a significant association between equol-producer phenotype and soy intake in association with mammographic density (a biomarker of breast cancer risk) despite no independent associations of phenotype or soy intake individually. In contrast, an intervention study testing the effects of soy protein on mammographic density showed no effect of equol-producer phenotype (26). An isoflavone supplement intervention in men with a personal or family history of colorectal adenoma showed that circulating insulin-like growth factor-I decreased in equol producers but not nonproducers (27). Further, the serum insulin-like growth factor-I change was inversely associated with serum equol concentration. In another study of postmenopausal women, a stronger effect of isoflavone supplementation (900 mg/d for 84 d) on estrogen-responsive genes in peripheral lymphocytes was observed among equol producers compared with nonproducers (28). These studies suggest that there may be a differential response to isoflavones dependent on equol-producer phenotype; however, results are not consistent across studies.

Summary

The role of equol in relation to cancer remains unclear. To date, animal studies using either daidzein or racemic equol are few and there are no studies of S-(-)-equol specifically. The number of epidemiologic studies of equol exposure and cancer risk in humans is also limited and the studies are difficult to interpret. These studies have had to rely on measurement of circulating or urinary equol concentrations in populations routinely consuming soy. The ideal test would be a randomized, placebo-controlled intervention trial of supplemental equol with cancer endpoints. However, given the lack of preclinical data and the lack of consistent effects of equol-producer phenotype in soy-isoﬂavone intervention studies, such an undertaking is unwarranted. With the recent availability of sufficient amounts of R- and S-equol, animal studies of carcinogenesis and human intervention studies that address directly the effects of the equol enantiomers on intermediate biomarkers of cancer risk may help to further ascertain the effects of the equol-producer phenotype and equol itself.

Acknowledgment

The sole author had responsibility for all parts of the manuscript.

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Dietary Equol and Bone Metabolism in Postmenopausal Japanese Women and Osteoporotic Mice\textsuperscript{1,2}

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Abstract

Equol binds to the estrogen receptor with greater affinity than its precursor, daidzein, an isoflavone found in soybeans. Inter-individual differences in the ability to produce equol may lead to differential effects of isoflavone intervention on human health. Here, we review previously published work from our laboratory on equol producer status and bone health in humans and in a mouse model of osteoporosis. We performed a 1-y, double-blind, randomized trial to compare the effects of isoflavone (75 mg of isoflavone conjugates/d; equivalent to 47 mg/d of the aglycone form) with those of placebo on bone mineral density (BMD), fat mass, and serum isoflavone concentrations in 54 early postmenopausal Japanese women classified by their equol-producer phenotype. Isoflavone intervention increased the serum equol concentration in equol producers but not in nonproducers (P < 0.04). The annualized changes in BMD in the total hip and intertrochanteric regions in the isoflavone-treated equol producers (−0.46 and −0.04%, respectively) were less than in the nonproducers (−2.28 and −2.61%, respectively). The annualized change in fat mass was lower in the equol producers compared with the nonproducers in the isoflavone group. The annualized changes in BMD and fat mass did not differ between the equol producers and nonproducers in the placebo group. Equol also inhibited bone loss and fat accumulation in estrogen-deficient osteoporotic mice. Our data suggest that prevention of bone loss and fat accumulation in early postmenopausal women by isoflavones may depend on an individual’s equol-producing capacity. J. Nutr. 140: 1373S–1376S, 2010.

Effects of the intestinal metabolites of daidzein, equol, on bone metabolism

Epidemiological studies indicate that women who have high soy intake have less risk for osteoporosis than those consuming a typical Western diet (1). Additionally, several recently published studies report that isoflavone supplementation decreases risk for osteoporosis (2–4). In general, these studies administered isoflavones (40–200 mg/d) for 1–2 y and measured bone mineral density (BMD).\textsuperscript{3} The results from human studies are inconsistent (5). A recent meta-analysis of 10 randomized, controlled trials indicated that isoflavone intervention significantly attenuated bone loss in postmenopausal women (6,7). However, another meta-analysis concluded that isoflavones do not affect bone loss (8). One potential reason for these inconsistencies is individual differences in isoflavone metabolism. Recent studies suggest that the clinical effectiveness of isoflavones on bone metabolism might be due to individual differences in the ability to produce the daidzein metabolite, equol, in the intestine (9).

Equol is an isoflavone and a nonsteroidal estrogen (9). It binds to both estrogen receptors and induces transcription more strongly than other isoflavones, especially estrogen receptor-\(\alpha\) (10). Moreover, equol is a chiral molecule, which exists as enantiomers R (\(+\))-equol and S (\(\text{−}\))-equol. In humans, the intestinal bacterial metabolism of daidzein to equol results in S-equol production only (11). Humans may be the only species that exhibits inter-individual variability in equol production. Several animals, including rats (12), mice (13), and chimpanzees (14), excrete equol. O-desmethylangolensin (O-DMA), another daidzein metabolite, is found in ~80–90% of the human population. O-DMA is less potent than equol (15). O-DMA binds to both estrogen receptors and induces transcription more weakly than equol (16).

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\textsuperscript{3} Abbreviations used: BMD, bone mineral density; Lc., Lactococcus; O-DMA, O-desmethylangolensin; OVX, ovariec timized.
Adapted from (37) with permission by Wolters Kluwer/Lippincott, Williams & Wilkins.

Inflammatory cytokines, such as tumor necrosis factor-stimulation of osteoclast formation, a process enhanced by several mechanisms by which estrogen deficiency causes bone loss is via estrogen-deficient animals. It is now recognized that one of the without estrogenic activity in the reproductive organs of reproductive organs (33). Similarly, equol may inhibit bone loss due to ovariectomy. We have reported that administration of equol (0.5 mg/d subcutaneously) inhibited bone loss of the whole body and femur in ovariectomized mice without producing O-DMA (34). Interestingly, the strain, Lc. 20–92, can also cleave glycosidic bonds of daidzein. Here we review our previously published work on equol producer status and bone health in humans and a mouse model of osteoporosis.

Effects of equol on bone metabolism in animals
Estrogen deficiency is associated with increased bone turnover and acceleration of bone loss, which leads to an increased susceptibility to bone fracture. A number of studies have reported that the soybean isoflavones, genistein and daidzein, dosed dependently inhibit bone loss in both female and male osteoporotic animal models without causing notable effects on reproductive organs (33). Similarly, equol may inhibit bone loss due to ovariectomy. We have reported that administration of equol (0.5 mg/d subcutaneously) inhibited bone loss of the whole body and femur in ovariectomized mice without uterine hypertrophy (34). Although 17β-estradiol administration (0.03 μg/d subcutaneously) prevented ovariectomized-induced bone loss from all regions, uterine hypertrophy occurred in mice (34). These results suggest that similar to selective estrogen receptor modulators, isoflavones, including equol, inhibit bone loss without estrogenic activity in the reproductive organs of estrogen-deficient animals. It is now recognized that one of the mechanisms by which estrogen deficiency causes bone loss is via stimulation of osteoclast formation, a process enhanced by several inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β. Furthermore, Nakamura et al. (35) recently reported apoptosis and Fas ligand expression upregulation in trabecular osteoclasts from wild type but not estrogen receptor knockout (EαδOc/dOc) mice after estrogen treatment. These results support a model in which estrogen regulates the life span of mature osteoclasts via the induction of the Fas/Fas ligand system and help explain the osteoprotective function of estrogen and selective estrogen receptor modulators, including isoflavones.

The role of equol status in the effects of isoflavones on bone health in humans
Equol production depends on an individual’s intestinal flora. Research has shown that ~30–50% of individuals in the population studied are capable of producing equol from daidzein (36). Lydeking-Olsen et al. (36) conducted a 2-y, randomized, placebo-controlled trial to investigate the effectiveness of isoflavone-supplemented soymilk (76 mg/d aglycone isoflavones content) for prevention of bone loss in postmenopausal women aged 41–75 y (mean age 58 y). The lumbar spine BMD for women who consumed soymilk with low levels of isoflavones (n = 22) decreased (4.2%; P = 0.01) by the end of the study; however, the lumbar spine BMD of women who consumed soymilk with isoflavones (n = 23) did not change compared with baseline. Equol producers (n = 10), defined by a cutoff level of 10 ng/mL (40 mmol/L) plasma equol, had a 2.4% increase in lumbar spine BMD compared with the 0.6% increase for equol nonproducers (n = 12) after the 2-y intervention with isoflavone-enriched soymilk.

We assessed the effects of equol-producing activity on BMD in postmenopausal Japanese women (37). Study participants were 68 healthy women aged 45–60 y who had undergone natural menopause within the previous 5 y, where menopause was defined as at least 12 mo beyond the last menstrual cycle. Fifty-four women (29/34 in the placebo, 25/34 in the isoflavone group) completed the 1-y intervention and their data were used for equol analysis. Study participants in the isoflavone group received a daily dose of 75 mg of isoflavone conjugates (38.3 mg daidzin, 0.2 mg malonyl-daidzin, 2.1 mg acetyldaidzin, 0.6 mg daidzein, 8.6 mg genistin, 0.6 mg acetylgenistin, 0.2 mg genistin, and 24.4 mg glycitin with glycitein) in capsule form (Fujiflavone P40, Fujicco; 47 mg aglycon form) with dextrin and those in the placebo group received capsules containing only placebo treatment. Values are means ± SD, n = 10–15. *Different from EQ producers, P < 0.05. Adapted from (37) with permission by Wolters Kluwer/Lippincott, Williams & Wilkins.
dextrin. Fifty-six percent of study participants were equol producers, as assessed by equol production in fecal suspension incubated with daidzein under anaerobic conditions. In the placebo group, there were 15 and 14 equol producers and nonproducers, respectively; and in the isoflavone group, there were 15 and 10 equol producers and nonproducers, respectively. Serum daidzein concentrations, as determined by reverse-phase HPLC, increased to 3-fold the baseline value in equol producers and nonproducers who consumed isoflavone for 1 y (P = 0.002). However, there was no difference in the serum daidzein concentration between equol producers and nonproducers. On the other hand, serum equol increased only in equol producers in the isoflavone group after 1 y (P = 0.04). Serum equol did not change in equol producers in the placebo group. Because equol is a metabolite of daidzein, it has a slower plasma clearance rate than daidzein (10). Therefore, whereas the serum levels of daidzein did not significantly differ between the equol producers and nonproducers, the equol levels were significantly higher in the equol producers. Equol producers tended to have lower serum concentrations than nonproducers (P = 0.15), but the longer half-life of equol in the bloodstream could explain why there were no significant differences in daidzein levels. After 1 y, serum genistein did not differ between equol producers and nonproducers in the isoflavone group.

In the isoflavone intervention group, the percent change in bone loss in the total hip (–0.46%) and the hip intertrochanteric region (–0.04%) of equol producers was lower (P < 0.05) than that of equol nonproducers (–2.28 and –2.61%, respectively) (Student’s t test; Fig. 1) (38).

A 2-factor analysis of covariance revealed no significant main effect on the percent change in BMD in any region after 1 y. The percent change in bone loss in the total hip and the hip intertrochanteric region did not differ between equol producers and nonproducers in the placebo group. Equol producers had a significantly lower annualized change in fat mass than nonproducers in the isoflavone group, but this was not the case for the placebo group. Based on these results, the effects of isoflavone on bone and fat mass might depend on equol-producing activity in postmenopausal Japanese women (37). Isolavone supplementation (47 mg/d aglycone equivalent) with a normal diet for 1 y did not affect serum levels of estrogen, follicle-stimulating hormone, luteinizing hormone, progesterone, triiodothyronine, thyroxine, and thyroid stimulating hormone in postmenopausal Japanese women (39).

Isolation of equol-producing bacteria from human feces

Uchiyama et al. (32) detected 3 equol-producing bacterial strains from human feces (32,39). They selected the Lc. 20–92 strain as the most appropriate bacteria for food usage, because it is homologous to Lc. Garvieae, which is widely used in Italian cheese. Using real time-PCR and the particular primer for Lc. garvieae, they found that Lc. 20–92 exists in the feces of postmenopausal Japanese women (32). Lc. garvieae was found in 35.3% of the postmenopausal Japanese women (39). Interestingly, women with Lc. garvieae were not always equol producers, suggesting that other bacteria may play a role in human equol production. Several other factors such as hydrogen gas and short chain fatty acids, which can affect the environmental conditions in the colon, may also be necessary for equol production.

Conclusions

Several factors such as race, age, diet, timing of exposure, and individual variations including genetic differences and variation in intestinal microflora influence the effects of isoflavones on bone health. The preventive effects of daidzein on bone loss in postmenopausal women might depend on an individual’s equol-producing capacity. In research from our laboratory, supplementation of the normal diet for 1 y with an additional 47 mg/d of isoflavone aglycon equivalent did not have adverse effects in postmenopausal women. Further studies are required to address the numerous questions on the potential benefits, mechanisms of action, and safety of isoflavones for postmenopausal women.

Acknowledgment

The sole author had responsibility for all parts of the manuscript.

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Equol, via Dietary Sources or Intestinal Production, May Ameliorate Estrogen Deficiency-Induced Bone Loss¹-³

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Abstract

Equol, a product of intestinal metabolism of daidzein, is chemically similar to estrogen (without the lipophilic moiety) and has higher estrogen receptor-β binding affinity than its parent precursor. In 2004, a long-term, randomized controlled trial that characterized postmenopausal women by their equol-producing status showed stronger advantages to lumbar spine bone mineral density (BMD) in equol- compared with nonequol-producers. Subsequent studies have related equol status of participants to change in bone turnover markers or BMD in response to soy isoflavone interventions. To our knowledge, we are the first to prescreen women for equol-producing status prior to initiating an intervention. In menopausal Western women, equol status did not affect the modest, but significant, reduction in bone resorption achieved with a soy isoflavone intervention. J. Nutr. 140: 1377S–1379S, 2010.

Introduction

Dietary soy isoflavone supplements have been studied for their effect on ameliorating bone loss associated with menopause. However, studies of dietary equol as a potential dietary supplement are limited. The reported studies of oral and i.v. equol administration on bone in animal models used high doses to inhibit bone loss, which caused adverse effects on reproductive tissues. We conducted a dose-ranging study (0, 50, 100, and 200 mg equol/kg diet) of dietary racemic equol (50% R-equol, 50% S-equol) in 6-mo-old ovariectomized (OVX) Sprague Dawley rats. Doses were selected to achieve similar serum concentrations in postmenopausal women consuming medium and high amounts of isoflavones. We found that tissue (heart, intestine, kidney, and liver) equol concentrations reflected the diet, but only the highest dose studied (200 mg/kg) increased the femoral calcium content. Uterine, but not mammary gland, epithelial tissues were stimulated at this dose. These findings suggested limited benefit with the potential for adverse effects of equol as a dietary supplement.

Equol may be a bone antiresorption agent

Endogenous estrogen helps with maintaining bone mass and its deficiency with transition to menopause has been associated with rapid bone loss (1,2). When circulating levels decrease, bone resorption rates exceed bone formation rates, leading to accelerated bone loss for 3–5 y until bone formation rates catch up (3). Estrogen therapy was a mainstay of osteoporosis prevention in postmenopausal women until adverse cardiovascular and breast cancer health events were reported in women randomized to a conjugated estradiol treatment (premarin) in the Women’s Health Initiative trial (4). Replacements for estrogen have been highly sought that have the antiresorption properties of estrogen therapy without the adverse effects on reproductive tissues.

Soy isoflavones have been studied the most for their potential to replace estrogen therapy. These studies have had mixed results (5–15). Equol has received much attention in the last 5 y. Equol, the end product of intestinal metabolism of daidzein, is chemically similar to estrogen (without the lipophilic moiety) and has 80 times more estrogen receptor-β binding affinity than its parent precursor (16). Estrogen receptor-β is dominant in bone and is presumed to be a primary mode of action of estrogen.
and phytoestrogens such as isoflavones (16). Other modes of action, including suppressing osteoclastogenesis, have also been proposed (14,15). Osteoclast formation was inhibited by equol in a dose-dependent manner (10–1000 nmol/L) in a coculture system of mouse bone marrow and primary osteoclast cells (17).

All rodents, but not all humans, have gut microflora that convert daidzein to equol. Many approximate that only 30–50% of the population possesses the gut microflora needed for bacteria intestinal conversion of daidzein to equol (18,19). Here, we explore equol as a dietary supplement on bone properties as well as the effect of equol-producing status on bone antiresorptive responsiveness to soy.

**Dietary equol, calcium metabolism, and bone properties in estrogen deplete rodent models**

Studies of dietary equol as a potential dietary supplement are limited. Early studies administered equol by i.v. injection or osmotic pump, because it was only recently made affordable by synthesis in adequate quantities for feeding studies. The reported studies of oral and i.v. equol administration on bone in animal models used high doses, which inhibited bone loss (20,21) but caused adverse effects on reproductive tissues (21). Plasma equol levels ranged from 1550 nmol/L (20) to 8433 nmol/L (21). Daily subcutaneous injections of 0.5 mg equol/d in mice (20) as well as oral dosing of 10 µg equol/g body weight-d (21) in rats maintained bone mineral density (BMD) of OVX animals to similar levels of SHAM rats. Consumption of 1000 mg/kg dietary equol led to increased uterine epithelial proliferation and plasma equol levels of 6–8 µmol/L (22) in mice. Dietary equol at 400 mg/kg diet attenuated OVX-induced trabecular bone loss at the lumbar spine but had a mild uterotropic activity, decreased weight gain at these concentrations, and circulating equol concentrations of 2460 nmol/L (23). Equol supplementation also improved biomechanical and histomorphometric measures of femurs in OVX rats (24) as well as aided in healing of osteoporotic fractures in OVX rats (25).

We conducted a dose-ranging study (0, 50, 100, and 200 mg equol/kg diet) of dietary racemic equol (50% R-equol, 50% S-equol) in 6-mo-old OVX Sprague Dawley rats. Doses were selected to achieve serum concentrations similar to those of postmenopausal women consuming medium (56 mg/d, 3–39 µmol/L) or high (90 mg/d, 5–49 µmol/L) amounts of isoflavones (26). We found that heart, intestine, kidney, and liver tissue equol concentrations reflected the diet, but only the highest dose studied (200 mg/kg) increased femoral calcium content (27). Calcium absorption and retention tended (P = 0.07) to be higher at equol concentrations >100 mg/kg. Mammary gland epithelial tissues were not stimulated, but uterine epithelial tissues were stimulated at this dose. These findings suggest limited benefit with the potential for adverse effects of equol as a dietary supplement.

**New method for examining effect of soy phytoestrogens on bone**

We have developed a novel method using the rare isotope, $^{41}$Ca, to prelabel the skeleton and then monitor bone resorption by measuring urinary $^{41}$Ca excretion by accelerator MS (28). The advantages of this method are its extreme sensitivity and specificity compared with traditional methods. We have used this method to study the effect of dose-ranging intervention of soy isoflavones in soy protein isolates (28) as well as to compare several botanical supplements with traditional therapies of osteoporosis (29). Response to the botanical supplements reflected their genistein content. The supplement with the highest genistein content (158 mg/d) was soy derived and suppressed bone resorption about one-third as well as either estradiol (1 mg/d) and medroxyprogesterone (2.5 mg/d) or a bisphosphate (Actonel, 5 mg/d). We are using this method to determine the response to soy feeding in equol-producers compared with nonproducers and also to determine the effective dose in a dose-response study of purified genistein. This method has promise for determining drug-botanical interactions.

**Effect of equol-producing status on response to soy**

Recent evidence suggests that the effect of soy isoflavone treatment on health is dependent on the ability to convert daidzein to equol. Soy isoflavone supplementation has a stronger effect on the expression of estrogen responsive genes (genes with estrogen response elements in promoter regions) in equol producers compared with nonequol producers (30). A long-term, randomized controlled trial that characterized postmenopausal women by their equol-producing status showed stronger advantages to lumbar spine BMD after a 2-y intervention in equol (2.4% increase) compared with nonequol producers (0.6% increase) (8). Several subsequent studies have related equol status of subjects to change in bone turnover markers or BMD in response to an intervention containing soy isoflavones (19,31). Equol-producing ability determined by fecal equol levels in 68 of 122 postmenopausal Japanese women was associated with reduced total hip BMD after 24 wk of isoflavone treatment (31). Both urinary equol and O-desmethylangolensin levels indicating the presence of gut metabolizing microflora following 3 d of soy food consumption were related to BMD in 92 postmenopausal women (18). O-desmethylangolensin, but not equol, producers had greater total, leg, and head BMD compared with nonproducers (P < 0.05).

**Equol metabolism in young adults**

The form of equol provided for supplementation also plays a key role in metabolism and could alter potential health benefits of equol. Currently, there is only one pharmacokinetic study to our knowledge that examined the effect of S-equol, R-equol, and racemic equol on circulating equol levels in adults (32). A single oral dose of 20 mg of S-$^{13}$C-equol, R-$^{13}$C-equol, or racemic $^{13}$C-equol was administered to healthy young men (n = 6) and women (n = 6) and plasma was collected for 48 h. Compared with other phytoestrogens (genistein and daidzein), equol was more bioavailable and rapidly absorbed. Differences in bioavailability and absorption were associated with the form of equol consumed. R-$^{13}$C-equol had higher bioavailability and fractional absorption than S-$^{13}$C-equol. However, both enantiomers had higher absorption, plasma concentrations, and bioavailability than the racemic mixture.

**Future directions**

Although evidence suggests that equol may attenuate estrogen deficiency-related bone loss, there are various factors that could influence the health benefit potential of equol, such as dose, form of equol, and the ability for intestinal production of equol. Other lifestyle traits could also affect the health protective actions of equol. For instance, our dose-response equol study revealed that equol had a detrimental effect on tibia calcium content that could be contributed to the estrogen-like effects during mechanical loading (27). Future research should determine the bone health effect of equol supplementation in physically active postmenopausal women. Overall, a deeper understanding of equol and interaction with lifestyle factors is needed to thoroughly assess the bone health benefit of equol.
Acknowledgments
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Menopausal Hot Flashes: A Review of Physiology and Biosociocultural Perspective on Methods of Assessment

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Abstract

Hot flashes continue to be a troublesome problem for menopausal women the world over. After >50 y of research, we still do not understand the etiology and mechanism of hot flashes, nor do we know how estrogen, the major pharmaceutical treatment, works to reduce hot flashes. We are gaining insight into sociocultural complexities that may affect how and whether women report hot flashes. And we are becoming more sophisticated in our research tools (be it questionnaires, physiological monitors, or brain imaging techniques). New aspects of hot flash research, including neuroimaging and the study of genetic polymorphisms, when combined with increasingly nuanced ways of asking questions of culturally distinct populations, provide challenges but rich complexity from which a better understanding will emerge. J. Nutr. 140: 1380S–1385S, 2010.

Introduction

Hot flashes are one of the most common symptoms experienced by women around the world during the transition to and through menopause. Whereas prevalence rates tend to be higher in Western countries than, e.g., in Asian countries, rates vary widely and are likely influenced by a range of factors (1). In the U.S., hot flashes are one of the main reasons women at menopause seek medical help or look for dietary supplements and over-the-counter remedies for relief (2,3). In 2002, after publication of the results from the large NIH-funded Women’s Health Initiative study, which indicated that estrogen was harmful on a number of indices, many women stopped taking this hormone therapy (4) and for >30%, bothersome, often severe hot flashes returned (5). These women re-experienced hot flashes they thought they had left far behind. Yet, while women have complained of these “spells” of heat sensation from time immemorial and despite this considerable public health problem, at the start of the 21st century, we still do not understand the etiology or physiology, nor do we know how the most widely used pharmaceutical treatment to date, estrogen, works to relieve hot flashes.

This article aims to provide a brief overview of some aspects of the biology of hot flashes and methods used in research, given insights we now have into biosociocultural factors that influence how we observe and the phenomenon being observed. Emerging areas of research are also discussed.

Hot flashes: the phenomenon

The terms hot flash and hot flush are used interchangeably and are typically synonymous, referring to a sudden sensation of heat and sweating, most notably on the upper body. Hot flashes occur primarily and most intensively in peri- and postmenopausal women. They also may occur when estrogen drops suddenly and rapidly, such as after removal of the ovaries of premenopausal women, with chemically induced menopause, and also in breast cancer patients treated with selective estrogen receptor modifiers such as tamoxifen. Men can experience hot flashes, particularly when testosterone levels fall rapidly, such as in men with prostate cancer treated medically or surgically. In both women and men, these are situations where there is an abrupt drop in sex steroid hormones, resulting in hot flashes.

Hot flashes can occur day or night; when they occur at night, they are called night sweats. Each particular episode lasts between 3 and 10 min and can recur with varying frequency (6). Some women experience hot flashes hourly or daily, while for others they may occur only occasionally; a small percent of women report not having any hot flashes. Whether this is a...
matter of a threshold of perception or there are genetic, environmental, or lifestyle factors that preclude hot flashes is a subject of increasing interest.

Although for most women, hot flashes begin and have their peak occurrence during the peri- and early postmenopausal periods, typically when a woman is in her late 40s and early 50s, at times they may begin when menstrual cycles are still regular (6). The majority of women have hot flashes for a year or two, but ~15% may have them nonstop for 10, 20, or 30 y (6). And while every woman’s estrogen level decreases during the transition to and through menopause, it remains an enigma as to why some women’s bodies seem to adjust and hot flashes taper off, while others continue having hot flashes for many years. In addition, for some women whose hot flashes were treated successfully by estrogen, the hot flashes resume after cessation of estrogen treatment.

**Physiological changes during a hot flash**

Thermography provides a visual snapshot of the skin surface manifestation of the pattern of thermoregulatory changes during a hot flash and provides insights into underlying physiological changes (Supplemental Fig. 1). One can see the skin areas that warm during the hot flash (fingers, neck, face) and cool as the hot flash subsides.

Figure 1 graphically illustrates some of the primary physiological changes that occur during a hot flash (7). “Sensation” is a subjective rating of the sense of hot flash intensity on a scale of 0–10 (10 being the most intense). At the onset of a hot flash, there is a sudden increase in sweating. Heart rate increases to normal. During a hot flash, many of the easily observed physiological changes involve the thermoregulatory and vascular systems. This remains an area of incomplete research.

![Figure 1](image)

**Figure 1** Physiological changes during a hot flash. Characteristic physiological changes during a hot flash: sensation (SENS), heart rate, internal body temperature ($T_{eso}$ = esophageal), skin temperatures ($T_{for}$ = forehead, $T_{fin}$ = finger), and ambient temperature ($T_{a}$ = ambient). Reproduced from Kronenberg and Downey (7) © 2008 NRC Canada or its licensors. Reproduced with permission.

Hot flashes occur worldwide, and starting in the 1970s, research documenting such occurrence increased substantially. A wide

**Mechanisms of hot flashes**

Estrogen has been studied and used to treat hot flashes for >60 y, but the mechanism by which it works is still in question. This is due to relatively little examination of basic hot flash physiology, which has not received the research attention that has been accorded clinical trials of therapeutic agents. Thus, the body of knowledge on the endocrinology, neurophysiology, thermoregulatory physiology, and other aspects of hot flash biology is limited.

One long-standing assumption has been that hot flashes involve transient dysregulation of the thermoregulatory system, triggering homeostatic heat loss mechanisms to return the system to normal. During a hot flash, many of the easily observed physiological changes involve the thermoregulatory and vascular systems. This remains an area of incomplete research.

Clearly, estrogen plays some role as a mediator of hot flashes. Hot flashes occur as estrogen levels decline and they are alleviated for the most part by treatment with estrogen (9). Estrogen priming is likely important. Young women with low estrogen levels do not have hot flashes, but if given estrogen and then withdrawn from it, they will have hot flashes. And premenopausal women whose ovaries are removed most often experience hot flashes almost immediately. However, low estrogen levels alone do not explain the presence of hot flashes. Investigators have searched for correlations between estrogen levels and the occurrence of hot flashes in women with and without hot flashes as well as with individual hot flashes with conflicting results. Some population studies have demonstrated inverse relationships between estrogen levels and hot flashes (10,11), as have more recent studies (12). Others, including Freedman et al. (13), have not corroborated these results when small groups of symptomatic and asymptomatic women were compared.

There has been examination of the levels of circulating hormones, including sex steroids and gonadotropins, among others. Substances such as luteinizing hormone (14,15), B-endorphin, adrenocorticotropic hormone (16), and others show pulsatile activity that is temporally correlated with hot flash occurrence, but not causally related (7). Freedman et al. (17) have proposed, based on several studies, that elevated brain norepinephrine plays a role in the etiology of hot flashes. Examining the complex interactions of the multiple systems that are involved has not been substantively undertaken. Thus, at this time, there is no definitive determination of what it is that triggers individual hot flashes or explains why some women do or do not experience them.

**Sociocultural issues in measuring hot flashes**

Hot flashes occur worldwide, and starting in the 1970s, research documenting such occurrence increased substantially.
distribution of the prevalence of hot flashes around the globe continues to be examined, with reports, particularly in Asian countries, of prevalence less than that in the US and other Western countries (1,18). Interest in understanding these differences has raised questions about whether these differences are due to genetic, cultural, environmental, or lifestyle factors such as diet and exercise.

Research in Japan has provided particular insight. Japanese women have a high dietary intake of soy (relative to Western countries) and it was hypothesized that this might explain why they have fewer hot flashes than women in the US, Canada, and Europe. Basic science research has established that isoflavones (compounds in soy and other plants) have estrogen-like activity (19). Interest in the relationship between the soy consumption of different populations and hot flash prevalence led to epidemiological studies comparing level of dietary soy intake and frequency of hot flashes in countries such as Japan, where an inverse association between soy intake and hot flashes has been demonstrated (20,21). Clinical studies of soy foods and soy isoflavones to treat hot flashes proliferated, with mixed results, although there was a tendency toward a beneficial effect (22).

However, it is now known that there are confounding factors that make this a more complex issue and might contribute to explaining the differing results among the clinical trials. The isoflavone daidzein is biotransformed by intestinal microflora to the active metabolite, equol. The presence of the particular bacterial species responsible for this conversion differs among people in different countries and thus some people are “equol producers” and some are not (19,23).

Further complexity may relate to a critical period for soy consumption relative to its beneficial health effects. This has come to light while trying to understand the observation of lower breast cancer rates in populations with high soy food consumption. Exposure during adulthood did not support these observations. Data are accumulating to support a protective effect of soy when it is consumed during early life (childhood/adolescence) with regard to breast cancer later in life (24). Soy foods are a staple in Asian cuisine and are consumed throughout life, suggesting their role in breast cancer prevention. Consumption of soy or genistein affects gene expression and activates estrogen receptor α, thereby affecting BRCA1 (25,26). Similarly, it is possible that consuming soy during puberty may affect whether one experiences hot flashes during menopause; initiating soy consumption or isoflavone supplementation at age 50 y may not have the same outcome of reducing hot flashes.

The Japanese experience
In the 1980s, Lock et al. (27) studied menopause among Japanese women and asked women about symptoms, including those typically experienced by American and Canadian women; they also asked the Japanese women (and their physicians) how they would describe this time of life and the symptoms they experienced. The respondents used a combination of 3 terms (sudden feeling of heat; feeling hot or flushed; rush of blood to the head) to encompass what Western women and physicians mean by the single term hot flash. The Japanese women reported fewer hot flashes than their Western counterparts. The researchers concluded that fewer Japanese women were experiencing hot flashes and night sweats.
Twenty years later, Melby (28) conducted a follow-up study in Japan, this time focusing on the more linguistically nuanced terminologies as she came to understand how Japanese women spoke about their sensations of heat and sweating during the menopausal years. Melby included Lock’s symptom list in her questionnaire but asked about hot flash terms separately, and found there was an increase in symptom reporting in the time since Lock’s study. There was an increase in those reporting each of the symptoms comprising the nuanced components of hot flashes (Table 1) (28). Some of the explanation for increased symptom reporting may involve the globalization of the Western approach to and discussion of menopause. Zeserson (29) commented on the media’s impact on how women discuss and view their symptoms. In the case of hot flashes, Zeserson (29) noted that Japanese women began using the Americanized expression “botto furasshu” and became conversant with the Western terminology of “hot flash,” having heard this in the media. As described earlier, there are a number of measurable physiological changes that constitute a hot flash.

Some women’s heart rate increases, some women sweat, while others flush and feel a sensation of heat. In Western countries, women describe whether they flush or sweat during a hot flash; they name this constellation of changes as a “hot flash” or “hot flush.” Japanese women describe an individual component of hot flash sensation if they experience it. In Japan, this time focusing on the more linguistically nuanced terminologies as she came to understand how Japanese women spoke about their sensations of heat and sweating during the menopausal years. Melby included Lock’s symptom list in her questionnaire but asked about hot flash terms separately, and found there was an increase in symptom reporting in the time since Lock’s study. There was an increase in those reporting each of the symptoms comprising the nuanced components of hot flashes (Table 1) (28). Some of the explanation for increased symptom reporting may involve the globalization of the Western approach to and discussion of menopause. Zeserson (29) commented on the media’s impact on how women discuss and view their symptoms. In the case of hot flashes, Zeserson (29) noted that Japanese women began using the Americanized expression “botto furasshu” and became conversant with the Western terminology of “hot flash,” having heard this in the media. As described earlier, there are a number of measurable physiological changes that constitute a hot flash.

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TABLE 1 Symptoms recalled in previous 2-wk period in Japanese women: comparison across 20 y1

<table>
<thead>
<tr>
<th>Romanized Japanese symptom</th>
<th>English symptom</th>
<th>Melby, n = 140</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katakori</td>
<td>Shoulder stiffness</td>
<td>51.7 %</td>
<td>62.1 %</td>
</tr>
<tr>
<td>Zutsuu</td>
<td>Headache</td>
<td>27.7 %</td>
<td>39.3 %</td>
</tr>
<tr>
<td>Youtsuu</td>
<td>Lumbago</td>
<td>22.4 %</td>
<td>35.0 %</td>
</tr>
<tr>
<td>Benpi</td>
<td>Constipation</td>
<td>21.1 %</td>
<td>25.0 %</td>
</tr>
<tr>
<td>Hieshou</td>
<td>Chilliness</td>
<td>16.3 %</td>
<td>29.3 %</td>
</tr>
<tr>
<td>Iraia</td>
<td>Irritability</td>
<td>11.9 %</td>
<td>28.6 %</td>
</tr>
<tr>
<td>Fumin</td>
<td>Insomnia</td>
<td>11.4 %</td>
<td>11.4 %</td>
</tr>
<tr>
<td>Kansetsutsuu</td>
<td>Aches and pains in the joints</td>
<td>10.9 %</td>
<td>15.0 %</td>
</tr>
<tr>
<td>Kaze o yoku hiku</td>
<td>Frequent colds</td>
<td>10.4 %</td>
<td>15.0 %</td>
</tr>
<tr>
<td>Kyuu na nekkon nobose, hoteri</td>
<td>Any hot flash</td>
<td>9.5 %</td>
<td>22.1 %</td>
</tr>
<tr>
<td>Ki ga meiru</td>
<td>Depression</td>
<td>7.9 %</td>
<td>22.9 %</td>
</tr>
<tr>
<td>Memai</td>
<td>Dizziness</td>
<td>7.2 %</td>
<td>10.7 %</td>
</tr>
<tr>
<td>Kyuu na hakkun</td>
<td>Sudden perspiration</td>
<td>4.2 %</td>
<td>8.6 %</td>
</tr>
<tr>
<td>Ne ase</td>
<td>Night sweats</td>
<td>3.2 %</td>
<td>6.4 %</td>
</tr>
</tbody>
</table>

1 Adapted from Melby 2005 (28) and Lock et al. (27). Reprinted by permission of Elsevier.

Interestingly, Melby (31) administered to both men and women in Japan the same symptom questionnaire. Both groups reported shoulder stiffness (the most prevalent symptom reported by women) and men reported more night sweats than did the women (Table 2) (31). How do we determine which symptoms are influenced by cultural factors and expectations and which are a function of hormonal or other physiological change?

Do women of Japanese ancestry really have fewer hot flashes than western women or do they just not report them?

Theories have been offered to explain the differences in hot flash prevalence around the world, including genetics, physiology, expectation, lifestyle (soy foods, exercise), or other environmental factors. Brown et al. (32) looked elsewhere to examine whether part of the explanation for fewer hot flashes among Japanese-American women might involve a lack of reporting rather than a lack of occurrence of hot flashes. Their study, conducted in Hawaii, compared objectively measured hot
flashes (using the measure of skin conductance) with self-report in women of Japanese descent and European descent living in Hilo, Hawaii. They administered a postal questionnaire asking about symptoms experienced in the past 2 wk and followed that with 24-h objective monitoring with diary reporting in a subset of women. The postal survey indicated that Japanese women had fewer symptoms such as backache, hot flashes, night sweats, depression, and trouble sleeping (Fig. 3). However, the ambulatory monitor revealed no difference between the groups in the percentage of women exhibiting objective hot flashes (Fig. 4).

There was a distinct difference between what was reported in the postal questionnaires and the ambulatory monitoring. Japanese-American women were significantly less likely to report hot flashes in the previous 2 wk, yet, they had the same frequency of objectively recorded hot flashes as the European-Americans. The authors concluded that fewer reported hot flashes in women of Japanese ancestry might be a consequence of reporting bias likely due to cultural perceptions of what is acceptable to discuss and report. The study of Brown et al. (33) highlights the value of objectively measuring hot flashes. There can be cultural differences in how and whether one reports hot flashes; diary use often underreports hot flashes such as those that occur during sleep or during a busy period when a hot flash is not noticed or goes unreported.

Clinical research has been hampered by the lack of a hot flash monitor that is accurate and easy-to-use in ambulatory subjects. Most of the portable monitors available for measuring skin conductance (or other physiological signals) are cumbersome to wear and have wires that do not facilitate ease of use. A new, miniature, wireless monitor, developed by Bahr Management in collaboration with researchers at the University of California, San Francisco, promises increased accuracy and ease of use (34,35).

Emerging areas of interest

Brain imaging techniques such as functional MRI are being used to examine brain function during hot flashes. Initial studies of brain activation during hot flashes have found that the insula and anterior cingulated cortex are activated during hot flashes (36). Better understanding of the neural control of hot flashes will provide further insight into mechanisms.

Another area of growing interest is the relationship between hot flashes and polymorphisms of genes involved in estrogen function, such as sex steroid metabolizing enzymes and estrogen receptors. Given that estrogen plays some role in the hot flash phenomenon, investigators are examining variation in genes coding for enzymes involved in estrogen synthesis and hormone interconversion for a possible role in the variance in observed circulating hormone levels (37). Genetic polymorphisms are also being studied in an attempt to explain observations of race/ethnic differences in hot flash prevalences (38), such as seen in the Study of Women Across the Nation in the US. Two studies indicate that there are certain race/ethnicity associations between polymorphisms for sex metabolizing hormones (39,40). This line of research is in its infancy but may provide new insights into the often conflicting and variable results of studies examining factors that might predict who most is at risk for hot flashes.

The study of equol for the treatment of hot flashes holds promise and clinical and basic science studies are underway in the US, Japan, and elsewhere. To better study soy, equol, or any herbal or pharmaceutical agent for the treatment of hot flashes, researchers need better measurement techniques, including better questionnaires that ask questions in a culturally sensitive context. More research on the basic underlying physiology and brain function during hot flashes is needed to better understand mechanisms that can lead to the design of treatments targeting these mechanisms.

Summary

Hot flashes remain a particularly bothersome problem for a majority of menopausal women in the US and elsewhere and a primary reason for which women in the menopausal years seek treatment. Yet, after >40 y of studying hot flash physiology, we still do not understand the etiology, nor do we understand how the most effective treatment to date, estrogen, works to reduce hot flashes. There has been substantial examination of and insight into cross-cultural differences, the nuances of linguistic expressions,
and dietary and other factors that are providing means of stratifying results to better understand the variability in responses. Newer research is focusing on brain function and genetic polymorphisms, both of which add new levels of complexity to what is obviously a very rich and compelling area for investigation. After so many years with insufficient research attention, the time and measurement tools are now ready for a more sophisticated examination of hot flashes. Any additional understanding of mechanism would provide valuable guidance for those wishing to study and develop new treatments for this age-old problem.

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Equol Improves Menopausal Symptoms in Japanese Women1,2

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Abstract

It has been well documented that the frequency of vasomotor menopausal symptoms, such as hot flashes and night sweats, of Japanese menopausal women is less than that of Western women. High intake of soy isoflavones in the traditional Japanese diet has been postulated as the possible explanation of the difference. Epidemiological studies have reported that the content of equol, which is a biologically active metabolite of the isoflavone, daidzein, is lower in the women who complain of severe vasomotor symptoms. To investigate the involvement of equol in the manifestation of menopausal symptoms, especially vasomotor symptoms, and the possible therapeutic role of a supplement containing equol (natural S-equol developed by Otsuka Pharmaceutical) on the menopausal symptoms of Japanese women, 3 randomized clinical trials were conducted. The studies indicated that a daily dose of 10 mg of natural S-equol improved menopausal symptoms. In the confirmation study, menopausal women who were equol nonproducers who consumed 10 mg/d of natural S-equol for 12 wk had significantly reduced severity and frequency of hot flashes as well as a significant reduction in the severity of neck or shoulder stiffness. The equol-ingesting group also showed trends of improvement in sweating and irritability and a significant improvement in the somatic category symptoms. Thus, it is concluded that the supplement containing natural S-equol, a novel soybean-derived functional component, has a promising role as an alternative remedy in the management of menopausal symptoms. J. Nutr. 140: 1386S–1389S, 2010.

Introduction

Menopause health care is one of the hot issues in aging societies. The majority of health problems seen during menopause are a consequence of the health conditions in previous stages of life. In addition, the state of health during menopause shows ethnic and individual variations and fluctuates during the transition from pre- to postmenopause in the same individual. In particular, it has been widely recognized that the type, severity, and pattern of menopausal symptoms exhibit typical ethnic variation. Vasomotor symptoms such as hot flashes and night sweating are the typical and common complains of women in Western societies; on the contrary, Japanese women in general experience milder vasomotor symptoms during menopause than women in Western countries. They mainly complain of shoulder stiffness and general fatigue, which are classified as somatic symptoms (1–3).

As one of the factors relating to such differences, dietary habits of consuming soy-rich products, including phytoestrogen and soy isoflavones, in the traditional Japanese diet have been postulated (4,5). In Japan it has been reported that isoflavone intake is ~31.7 mg/d (4). In the practice of menopausal health care, dietary soy isoflavones, which potentially have properties of phytoestrogens, have been empirically proposed as an alternative in medical therapy, but their efficacies have not been fully elucidated.

The principles of health care are the prevention of disorders and diseases and the promotion of health conditions to provide a better quality of life on an individual basis. To conduct menopausal medical care appropriately, it is necessary to provide evidence-based alternative medicines as much as possible. Thus, the current situation of menopause medical care strongly indicates that the exclusive investigation into the possibility of soy products as an alternative should be conducted. Based on results from a basic investigation, clinical trials are needed to prove the efficacy of soy isoflavones.

Equol and menopausal symptoms in Japanese women

Soy isoflavone contains 3 compounds: daidzein, genistein, and glycine. An active daidzein metabolite, equol, is one of the
principal isoflavones found in soybeans and most soy foods (6). It was reported that approximately one-half of the Japanese population and 70–80% of the U.S. population cannot produce equol after ingesting soy foods or consuming isoflavones directly (7–9). One-half of middle-aged Japanese women (n = 95) excreted detectable equol levels in 24-h urine, but ~70–80% of the younger generation of women (n = 68) and men (n = 75) showed undetectable levels [10 μg/L (41.3 nmol/L) of equol was the detectable limit by HPLC in the current study] (unpublished data). The inability of some humans to produce equol has been attributed to individual differences in gut microflora (10). A study on urinary isoflavone excretion of Japanese women (n = 106, aged 29–78 y) reported that 24-h urinary equol excretion ranged from 0.4 to 123.6 μmol (11).

Uchiyama et al. (12) investigated the relationship between urinary excretion of isoflavones and menopausal symptoms in peri- and postmenopausal Japanese women. Surveys were mailed to 116 dietitians (aged 40–60 y) living in Fukuoka prefecture located in Kyushu Island in southwest Japan, and 108 participated in the study (93.1% response rate). The 24-h urine samples were collected for the measurement of isoflavonoids by HPLC using methods reported elsewhere (13) and the simplified menopausal index (SMI) (14) was used to assess 10 menopausal symptoms. Urinary excreted amounts of total isoflavonoids, genistein, daidzein, and equol of all participants were 38.9 ± 29.2, 19.6 ± 15.1, 10.0 ± 8.9, and 9.3 ± 14.1 μmol/24 h (mean ± SD), respectively. Genistein and daidzein were detected in all samples, but equol was only detected in the urine of 51.6% of participants.

From the total participants, 46 postmenopausal women (whose last menstrual period had occurred >6 mo prior) were selected for the analysis of menopausal symptoms using SMI (14). The participants were divided into 2 groups according to their SMI score: a high score group (SMI score >15, daily life was disturbed, n = 23) and a low score group (SMI score <15, daily activities were not disturbed, n = 23). Urinary excretion of genistein and daidzein for women in the high and low score groups did not differ; however, the amount of equol excreted in the urine was lower in the high score group than in the low score group (P < 0.05) (Fig. 1). These results indicate that women who can metabolize daidzein into equol by their own intestinal bacteria report milder menopausal symptoms.

**Development of natural S-equol supplement**

To access the efficacy of equol as an alternative treatment for menopausal symptoms, a natural S-equol supplement was developed. Equol exists as an enantiomer, S-isomer and R-isomer, but only the S-isomer is biologically produced in vivo. A lactic acid bacterium, *Lactococcus garvieae* (*Lactococcus* 20–92 strain), with equol-producing capabilities was identified and isolated from human feces (15,16). Recently, a standardized product of natural S-equol supplement was developed by Otsuka Pharmaceutical (17). The supplement was formed from fermentation of a soy germ solution by *Lactococcus* 20–92. The concentration of S-equol in the supplement ranged from 0.5 to 0.8% of natural S-equol and the supplement does not contain the R-equol isomer. The micronutrient content of the supplement was not significantly different from the original material. Fermentation with *Lactococcus* 20–92 revealed that except for isoflavones and some amino acids, the levels of protein, fat, carbohydrate, and other components were not changed by fermentation. The levels of protein, fat, and carbohydrate in the supplement were 38.3, 14.5, and 26.8%, respectively (17). The safety of the supplement was confirmed in genotoxicity, acute and subchronic toxicity, and reproductive and development toxicity tests (17,18).

**Clinical trials on the effects of natural S-equol on menopausal symptoms of Japanese women**

Three randomized clinical studies (pilot study, dose-finding study, and confirmation study) were conducted to investigate the ability of the natural S-equol supplement to relieve menopausal symptoms in Japanese women. In this section, the results of 3 clinical trials are discussed. The protocols were approved by the Institutional Review Board of each site and the studies were carried out according to the guidelines of the Declaration of Helsinki. All women provided written informed consent to participate in the study. The first study has been previously published (13) and the other studies will be submitted for publication separately.

**Pilot study of natural S-equol supplement**

Ishiwata et al. (13) conducted a randomized, double-blind, placebo-controlled trial to investigate the effects of the natural S-equol supplement on menopausal symptoms and mood states in pre-, peri-, and postmenopausal Japanese women. Each package of supplement contained 10, 0.8, and 2.0 mg of equol, daidzein, and genistein, respectively. A total of 134 healthy Japanese female participants (aged 40–59 y) were divided into 3 groups: EQ-1 (n = 44, 10 mg of equol/d), EQ-3 (n = 46, 30 mg of equol/d), and placebo (n = 44). The women were allowed to consume a maximum of 20 mg soy isoflavones from their diet each day. Menopausal symptom score and mood score were self-reported by filling out a menopausal symptom scale and the Profile of Mood States questionnaires. The 24-h urine samples were collected for measurement of isoflavones. Physical examinations and biochemical analyses were conducted at baseline and at the end of the 12-wk period.

It was described in the report (13) that 127 participants (94.8%) completed the trials. Participants who excreted >10 μg/L (41.28 nmol/L) of equol (HPLC detection limit) in 24-h urine samples were defined as equol nonproducers. At baseline, equol producers (n = 46) had fewer total menopausal symptoms (P < 0.05) than nonproducers (n = 81). Somatic symptom scores were reduced in the EQ-3 group compared with the placebo group of postmenopausal equol nonproducers (P < 0.05) (Fig. 2). Postmenopausal nonproducers also reported beneficial effects on...
Confirmation study of natural S-equol supplement

To confirm the physiological effects of the natural S-equol supplement in menopausal Japanese women who were equol nonproducers, an intervention trial (randomized double-blind, placebo-controlled, parallel-group) was conducted. This study consisted of a screening period, ingestion period for 12 wk, and follow-up period for 6 wk. One hundred and sixty postmenopausal women with normal BMI who reported having hot flashes at least once per day were enrolled in the study.

Before enrollment to the trial, all participants were asked to take a placebo for 4 wk (screening period). Participants who showed placebo effects of 50% reduction of SMI after the period were excluded from the final enrollment. During the ingestion period, participants were divided into placebo (n = 60) and equol (n = 66, 10 mg equol/d) groups. The supplement containing equol (5 mg), daidzein (1.2 mg), and genistein (1.4 mg) was orally ingested twice per day (morning and evening) for 12 wk.

The baseline frequencies of hot flashes in the placebo and equol groups were 2.9 ± 2.1 and 3.2 ± 2.4/d (mean ± SEM), respectively. The difference between the 2 groups was not significant. Women who ingested 10 mg/d of natural S-equol for 12 wk had reductions in the frequency and severity of hot flashes compared with those in the placebo group (P = 0.0092 and P = 0.0154, respectively). A greater reduction of the severity of neck or shoulder stiffness was observed for the equol group than that of placebo group, when evaluated using both the questionnaire (P = 0.0149) and the visual analogue scale method (P = 0.0070). After the 6-wk follow-up, the ingested supplement was washed out and rebound responses were observed in hot flashes and neck or shoulder stiffness. No serious adverse events were reported. The results of vital sign and clinical laboratory tests indicated no changes (P > 0.10) after ingestion of 10 mg equol/d for 12 wk.

Discussion and conclusion

Because the mental and physical conditions of women in menopause are complicated, health care in this life stage has to be done holistically. By the systemic evaluation of these conditions, the personal management for each woman should be selected. Lifestyle improvements, including diet habits, exercise, and relaxation, should be conducted before medical treatment.

Among the health problems in menopause, menopausal symptoms, especially vasomotor symptoms like hot flashes and sweating, disturb daily activities and quality of life seriously in the great portion of women in Western societies and also for a certain number of Japanese women. In the majority of such women, the symptoms cannot be controlled by nonmedical management and need to be treated by some medical interventions.

Although the mechanism underlying vasomotor symptom has not been fully clarified, the results of various basic and clinical studies have indicated that dramatic changes in the hormonal environment, especially the sharp decline of estrogen, during menopause play key roles. Hormone replacement therapy (HRT) has been widely adapted as a potent evidence-based medicine for menopausal symptoms. The report of a large-scaled study in the United States, the Women’s Health Initiative published in 2002 (19), indicated increasing risks associated with a kind of HRT regimen after long-term use. It is, however, still convincing that the first choice of medical treatment for severe vasomotor symptom is tailor-made HRT under appropriate diagnostic and follow-up medical care.

At the same time, the development of medical treatments for the women suffering from health problems caused by estrogen
deficiency including severe menopausal symptoms who cannot use HRT has been a great issue in clinical practice. It is also expected in menopausal health care that a medical intervention will improve the quality of life in the majority of menopausal Japanese menopausal women who complain of moderate- to mild-grade menopausal symptoms that are not severe enough to be treated by HRT.

In the clinical trial in which a subjective score is used as the end point of evaluation, how to obtain solid data showing the efficacy of a product is particularly critical. The severity of menopausal symptoms is one of the typical cases, which is easily influenced by nonspecific factors inducing placebo effects.

The series of studies described in this paper were attempts to investigate whether natural S-equol supplementation can be a new alternative. To confirm the efficacy of the compound strictly in clinical trials, the confirmation study was designed to include 3 periods. The screening period aimed to exclude the placebo effects of the intervention. At the same time, equol nonproducers were selected by the results of 3 separate measurements of urinary equol contents. Furthermore, special attention was paid to minimizing the bias caused by evaluation methods of interviewers who assessed changes of symptoms. In the follow-up period, it was predicted that a rebound response should appear if the ingested natural S-equol supplement had actual biological effects suppressing menopausal symptoms. As the result, such rebound patterns were observed in the responses of hot flashes and neck or shoulder stiffness. It is indicative that result, such rebound patterns were observed in the responses of the ingested natural S-equol supplement had actual biological effects suppressing menopausal symptoms. As the result, such rebound patterns were observed in the responses of hot flashes and neck or shoulder stiffness. It is indicative that these procedures for standardizing the enrolled population and arrangements adopted in the study made certain contributions to clarifying the importance of equol’s effects on certain menopausal symptoms.

This study confirmed the positive effects of equol on menopausal symptoms and was the first report, to our knowledge, based on a pausal symptoms. Clarifying the importance of equol’s effects on certain menopausal symptoms is one of the typical cases, which is easily influenced by nonspecific factors inducing placebo effects.

The sole author had responsibility for all parts of the manuscript.

Acknowledgment
The sole author had responsibility for all parts of the manuscript.

Literature Cited
Cautions and Research Needs Identified at the Equol, Soy, and Menopause Research Leadership Conference\textsuperscript{1–3}

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Abstract

This summary addresses the progress and limitations of existing research on the physiologic properties of the isoflavone daidzein metabolite equol. Previous research demonstrating that physiological equol is its S-enantiomer has led to the preparation of S-\textsuperscript{(-)}equol-enriched products formed by the bacterial fermentation of soy germ. Although this product has interesting properties as described in this workshop, the following important issues must be addressed: 1) the product should be evaluated against a preparation containing an equal amount of pure S-\textsuperscript{(-)}equol to determine whether other components resulting from the fermentation are contributing to the physiological effects; 2) evaluation of the cellular mechanisms of S-\textsuperscript{(-)}equol using cell culture methods should be conducted at concentrations consistent with those encountered physiologically (in the nmol/L range) and in several cell lines representing a target tissue; and 3) in follow-up studies in animal models and in human clinical trials, standardized preparations of S-\textsuperscript{(-)}equol should be made available.

Research opportunities now exist to determine whether equol’s apparent effects on menopausal symptoms (hot flashes, sleep disturbances, bone health) in equal producers can be extended to equal nonproducers. It will be important to ensure that such research is not complicated by cultural differences, differences in lifetime exposure to soy products, experimental techniques, and other variables. Further areas of research that would benefit from the availability of S-\textsuperscript{(-)}equol preparations include its use in skin care (either as an antioxidant or as an estrogen receptor agonist) and in the treatment of brain injury as well as postmenopausal cognitive decline. J. Nutr. 140: 1390S–1394S, 2010.

Introduction

The presentations at the Equol, Soy and Menopause Research Leadership Conference, held on June 16, 2009 in Washington DC, were organized into 3 general areas: soy and isoflavone research and metabolism; the relationship of equol to chronic diseases in humans; and the potential benefits of equol to women experiencing adverse symptoms during menopause. The following is a discussion of the cautions associated with conducting and interpreting research in the area and a summary of the research needs identified at the conference.

Cautions

Physiological equol vs. synthetic equol. There are 2 distinct areas of research on equol: studies on equol manufactured by fermentation using human intestinal bacteria from materials containing its precursor, daidzein, and studies on chemically synthesized equol. Equol can exist as the diastereoisomers S-\textsuperscript{(-)}equol and R-\textsuperscript{(+)}equol; however, S-\textsuperscript{(-)}equol is the only form produced from daidzein metabolism in humans (1). Information most relevant to the health effects of equol in humans will necessarily derive from studies on human intestinal bacteria-produced S-\textsuperscript{(-)}equol and not synthetic equol (2,3), which is a racemic mixture of S-\textsuperscript{(-)}equol and R-\textsuperscript{(+)}equol.
A majority of conference presentations concerned equol-enriched products containing S-(-)-equol that are being used in preclinical and clinical studies. These studies required Institutional Review Board and Institutional Animal Use Committee approval as well as consideration of safety issues. Human studies may involve interactions with the FDA, because a specific manufactured product is involved.

**Possible contribution of other substances to biological effects currently attributed to equol.** S-(-)-equol produced by bacterial fermentation of soy germ with human intestinal bacteria contains other isoflavone metabolites, some of which are possibly nonphysiologic. It remains to be determined whether the biological activities currently attributed to S-(-)-equol in the fermented product are solely due to S-(-)-equol alone. The research need can be addressed by conducting studies on pure S-(-)-equol, which can now be chemically manufactured, and the bacterial products of daidzein metabolism. If the same amount of pure S-(-)-equol and a bacterially produced S-(-)-equol preparation lead to different outcomes, the other components of the product may contribute to the effects currently attributed to S-(-)-equol alone.

**Experimental design.** Although convenient, preclinical studies in animals are not necessarily relevant to the human response to isoflavones because of differences in equol metabolism. In rodents and nonhuman primates (4–6) fed soy, the principal circulating metabolic form of the isoflavones is S-(-)-equol, being 75% of the total isoflavones, and 4-5 times larger than the concentrations of daidzein and genistein. Experiments in rats suggest that a slower urinary clearance of equol compared with daidzein and genistein (7) accounts for its increased circulating amounts. Indeed, because very high circulating levels of equol result from fairly modest exposure to isoflavones in animal models, the relevance of animal studies to human experience is called into question.

In contrast to most other animals, humans do not make large amounts of equol and only ~30% of humans are capable of synthesizing equol (8–13), although in Japan and Korea, a larger group of individuals is able to synthesize equol (14). The recent work of Setchell et al. (15,16) on the pharmacokinetics of orally administered S-(-)-equol and R-(-)+equol in human participants raises interesting questions about equol’s bioavailability. Using 2-13C-labeled equol, an oral dose of 20 mg of S-(-)-equol produced peak blood concentrations of all equol forms (conjugated and unconjugated) that were ~3 times higher than those for daidzein and genistein (after correcting for the different sizes of the doses) (17). It is possible that equol, unlike daidzein and genistein, is a terminal metabolite and is not dissipated into other chemical forms during transit through the intestine and intestinal wall into the blood stream.

**Types of cell cultures and concentrations of isoflavones.** Most studies using cell cultures to study mechanisms of action of isoflavones utilize levels ~2 orders of magnitude above the concentrations found in human exposure. Also, the use of unconjugated isoflavones does not match what is found in human blood (>95% conjugated metabolites). The cell lines typically used in these studies were developed because of their ability to be cultured rather than they are fully representative of the disease of interest. In addition, different types of cancer cells have a different pattern of metabolism for isoflavones (18,19). Attempts to determine the half maximal inhibitory concentration effects of genistein on human breast cancer cell proliferation revealed a correlation with the metabolites but not with genistein (18). Consideration of the cell line used in a preclinical study therefore is crucial to drawing conclusions.

**Standardization of analytical methods.** Analytical standardization represents another research need. Certified reference values do not exist for any soy isoflavone products. The Office of Dietary Supplements has contracted the National Institutes of Standards and Technology to manufacture of food matrix standards for several dietary supplements. A standard reference for soy is currently in development (20). In the past, concerns about the variability in the values being reported for estrogen receptors previously led the National Cancer Institute to develop a pooled standard reference product that was distributed to laboratories performing estrogen receptor analysis. The National Center for Complimentary and Alternative Medicine considers the use of standard products to be an extremely important point in its studies; products used in the center-funded studies have to go through extensive prereview by a Product Quality Working Group. In the absence of a certified standard, there should be a pooled sample that various laboratories can measure to check variability.

**Research needs**

What follows is a list of some of the most pressing research questions in the field.

**Assuming there is a benefit from S-(-)-equol in equol producers, would nonproducers also benefit from equol?** Some studies have reported a beneficial effect of S-(-)-equol. Equol producer status is determined by the presence of the bacteria and the conditions in the intraluminal space, i.e. the correct redox potential. Additional studies are needed to determine whether acute or chronic administration of S-(-)-equol to equol producers and nonproducers results in the same outcomes. Effects of equol on hot flashes, sensitivity to changes in temperature, sleep disturbance, and other symptoms reported as adverse effects by a majority of postmenopausal women are complicated by cultural differences, differences in lifetime exposure to soy products, study techniques, and other variables.

**Improved sensitivity of questionnaire instruments.** Questionnaires were developed for different purposes. Even scales designed to measure global quality of life should pair with symptom profiles appropriate for the topic of interest. There is a need for more sensitive questionnaire instruments to measure changes in the perception of quality of life to improve our objectives in outcome research. Further advancement will come from studying the underlying neurobiology, metabolic pathways, and measurable physiologic responses.

**Is S-(-)-equol a marker for other metabolic events in soy consumers?** The factors that control bacterial equol production require further investigation. Additional information is needed about why there are fewer equol producers in Western populations (~30%) compared with Eastern populations (~50%), and the sensitivity of equol production to redox potential, both microscopically and to the gut in general. Whether S-(-)-equol is a marker for a different bioactive substance should also be investigated. Equol may be a surrogate marker for a minor metabolite, perhaps not even derived from the isoflavonoids. Examples of minor, but incredibly bioactive, compounds are the prostaglandins, thromboxanes, and leukotrienes, all metabolites of long-chain PUFA.

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Are differences in response to equol related to time of exposure? In some Asian cultures, soy is a traditional part of the diet. In the West, the US, and Europe, individuals were not typically exposed to dietary soy in large amounts until the early 1990s. In contrast, Asian women who were in menopause between 2005 and 2009 have soy exposures dating back to the 1950s and 1960s. The differential effects of administration of soy or soy-derived materials to individuals with different exposure histories may be due to strong epigenetic phenomena, as is the case with exposure to soy and breast cancer. Adolescent exposure to soy is considered to be extremely important to determine future adult breast cancer risk (21,22). More recent studies suggest that childhood exposure to isoflavones may influence responses of adults to soy (23,24). Care should be taken to ensure that the right questions are being asked in nutritionally based epidemiological studies.

Because of differences in exposure history, outcomes of studies conducted in the US may not be comparable to outcomes in societies with large soy consumption from birth such as Japan or China. Furthermore, soy consumption patterns in the West have changed and a finding of undetectable blood concentrations of isoflavones today is rare. Controlling for different exposures in different eras is extremely important.

Reduction of variation on studies. Policy decisions are being made about the risk of specific chronic diseases based on studies on consumption of nutritional components. However, most studies for which answers to public health questions are sought are statistically inadequate. An obstacle that needs to be surmounted is having studies that are sufficiently statistically robust that loss of patients from the study or compliance problems do not corrupt the study conclusion. Clinical trials that are designed to provide answers for a whole population may not be affordable because of the large number of participants required. In addition, the frequent failure of a trial to execute the study as planned may yield insufficiently clear answers. However, as mechanisms become identified, it will be important to use this information to more carefully select the group to be studied. This can be achieved by narrowing the study group using the best available science about possible mechanisms. For instance, one could identify single-nucleotide polymorphisms in a receptor and then group individuals according to their responses. Another transforming approach is to use an advanced technology such as accelerator MS to be able to examine bone turnover with the use of calcium-41. The extreme sensitivity and specificity of accelerator MS for this rare isotope and the extended period of observation (5–10 y in the same participant) allows for multiple interventions to be tested in a pairwise manner, thereby substantially lowering the person-to-person variance in the experiment. Such an approach has been used to evaluate the effect of different soy supplements and standard therapies (residronate or estradiol and medroxyprogesterone acetate) on bone turnover in perimenopausal women (25).

Mechanisms of action of isoflavones beyond the estrogen receptor. Isoflavones may also elicit effects through mechanisms that do not involve the estrogen receptor (ER).4 ER receptor activation is very complex and involves coactivators, repressors, and >30 other proteins (26). Isoflavones may affect other protein targets in the pathway. In contrast to peripheral targets (brain, breast, heart, etc.), cells lining the intestinal lumen may be exposed to very high concentrations of isoflavones (an estimated 50 or 60 µmol/L) and thereby other less sensitive mechanisms may come into play. Another region of the body where isoflavone concentrations are high (with the exception of genistein) is prostatic fluid, where isoflavone levels are 20–30 times higher than that in the blood (27–29). And because urine concentrates excreted compounds, the bladder is also exposed to high concentrations of isoflavones.

Other areas of work regarding the mechanisms of action of receptors are being pursued. Exosomes are 50- to 90-nm vesicles secreted by a wide range of mammalian cell types that carry proteins through the blood and thereby target other cells; this is an endocrine-like mechanism. Several polyphenols influence the way in which these exosomes are created, their content, and their fusion to other cells (30). Equol may regulate this process. Other isoflavone-dependent targets are the tumor necrosis factor-α pathway (31–33) and PPARα- and PPARγ-mediated pathways (34–37).

Importance of aglycones. The aglycones are present in fermented soy foods. They are more rapidly absorbed in the upper small intestine and thereby cause higher peak concentrations during the first enterohepatic cycle. Because they are products of fermentation with microorganisms, the aglycones undergo additional hydroxylation (e.g. to 6- or 8-hydroxyiso-flavones) prior to the food being consumed (38). There are questions about whether this rises to increased bioactivity of the fermented soy food.

Role of intestinal microorganisms in human health. The microbiome is the most populous cell type in the human body. There are more cells in the gastrointestinal tract than in the rest of the body. Moreover, recent studies have shown that if microorganisms from the intestines of an obese mouse are transferred to a lean mouse, the lean mouse becomes obese, indicating that the metabolic state can affect either the composition or metabolic capacity of the microbiome in one animal’s body, which can then directly affect another animal’s health (39–41). Additional research is needed to determine whether isoflavones alter the microbiome and hence affect human health. The effect of high alcohol consumption and polyphenols on the microbiome and whether equol production plays a role in the physiological effects of alcohol should be determined.

The skin: another area where equol may have important bioactivity. Does equol have a beneficial effect in skin health? Many topical applications containing polyphenols that protect the skin have been developed. This area has 2 complementary areas, one where the added polyphenols have purported activity in sustaining the physical health and youthfulness of the skin and another where the added polyphenols may actually protect against skin cancer later in life. Both may involve antioxidant and antiinflammatory activities of the polyphenols.

Estrogen-like compounds in sustaining neuronal health and numbers. It is widely accepted that estrogen has benefits in brain health and function, including cognitive function. Given that at least some of the principal benefits of equol may be due to its similarity with estrogen, the effects of equol, especially taken as an additive, in the brain require further study. However, there has been controversy regarding the efficacy of estrogen or estrogen-like compounds in protecting cognitive function or
preventing age- or postmenopause-linked cognitive decline. Nonetheless, cognitive dysfunction induced by ovariectomy clearly was attenuated by added estrogen or estrogen-like compounds, including the soy isoflavones (42). It will be important to determine whether equol alone can have the same benefit as the soy isoflavones in animal models of dementia including Alzheimer’s disease. Estrogen and genistein independently prevented age-related mitochondrial dysfunction (43); in such models, it will be important to determine whether equol mimics or suppresses estrogen or genistein effects. An intriguing brain area where phytoestrogens and/or phytochemicals enriched in antioxidant activity may have activity is in modulating neurogenesis. In brain regions involved in cognition, neurogenesis is restricted to the dentate gyrus of the hippocampus [see (44)]. While there is complexity, recent studies showed that ablating neurogenesis prevented normal learning and memory, suggesting a requirement for neurogenesis in normal hippocampal functions in learning (45). If ablation of neurogenesis inhibits learning and memory, it is reasonable to propose that any compounds that enhance learning and memory have such actions by enhancing or protecting neurogenesis. Previous studies reported that grape seed extract enriched in the oligomeric proanthocyanidins enhanced cognitive function in young adult rats (46). Thus, it was not surprising that grape seed extract modulated neurogenesis in developmentally immature mouse brain (J. Cutts, L. Overstreet-Wadiche, and H. Kim, unpublished observations); however, it is also clear that not all increased neurogenesis was associated with “beneficial” effects in the brain, because increased neurogenesis was correlated with chemically induced seizures (47). Thus, neurogenesis will be a critically important area to explore for potential activity of the soy isoflavones, including equol. There is particular interest in identifying compounds that are safe in the brain whether or not they have the highest activity. The brain and the brain region are thus organs for much further investigation regarding the actions of phytoestrogens, including equol.

Recovery from traumatic brain injury. Screens of compounds from existing drugs with good safety profiles and from dietary supplements in a search for ‘safe’ agents have recently revealed that daidzein and biochanin A are strongly active in supporting recovery from traumatic brain injury (48). Genistein and daidzein are also being studied for their roles in wound recovery.

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Literature Cited


