Critical Review

The Clinical Importance of the Metabolite Equol—A Clue to the Effectiveness of Soy and Its Isoflavones

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ABSTRACT Equol [7-hydroxy-3-(4′-hydroxyphenyl)-chroman] is a nonsteroidal estrogen of the isoflavone class. It is exclusively a product of intestinal bacterial metabolism of dietary isoflavones and it possesses estrogenic activity, having affinity for both estrogen receptors, ERα and ERβ. Equol is superior to all other isoflavones in its antioxidant activity. It is the end product of the biotransformation of the phytoestrogen daidzein, one of the two main isoflavones found in abundance in soybeans and most soy foods. Once formed, it is relatively stable; however, equol is not produced in all healthy adults in response to dietary challenge with soy or daidzein. Several recent dietary intervention studies examining the health effects of soy isoflavones allude to the potential importance of equol by establishing that maximal clinical responses to soy protein diets are observed in people who are good "equol-producers." It is now apparent that there are two distinct subpopulations of people and that "bacterio-typing" individuals for their ability to make equol may hold the clue to the effectiveness of soy protein diets in the treatment or prevention of hormone-dependent conditions. In reviewing the history of equol, its biological properties, factors influencing its formation and clinical data, we propose a new paradigm. The clinical effectiveness of soy protein in cardiovascular, bone and menopausal health may be a function of the ability to biotransform soy isoflavones to the more potent estrogenic isoflavone, equol. The failure to distinguish those subjects who are "equol-producers" from "nonequol producers" in previous clinical studies could plausibly explain the variance in reported data on the health benefits of soy. J. Nutr. 132: 3577–3584, 2002.

KEY WORDS: • *equol* • *isoflavone* • *bacterial metabolism* • *phytoestrogens*

The present renaissance in soy foods is driven largely by documented research on the potential health benefits of soy isoflavones (1–4) and the recent Food and Drug Administration approval allowing manufacturers of soy foods to make a heart health claim for soy foods containing the mandatory 6.25 g of soy protein per serving (5). In reality, this renaissance is largely the consequence of the discovery that soybeans and most soy protein products are the richest source of isoflavones, an important class of bioactive phytoestrogens and, once absorbed, they exceed estradiol levels by several orders of magnitude (6). Their prominence relative to the many other important constituents of soy was evident at the recent 4th International Symposium on The Role of Soy in Preventing and Treating Chronic Diseases (7) in which 75% of the presentations concerned isoflavones, and not protein, a trend seen in previous symposia in this series. In 1984 we first proposed that these nonsteroidal estrogens play a role in the prevention and treatment of hormone-dependent disease (6) after high levels of the metabolite 7-hydroxy-3-(4′-hydroxyphenyl)-chroman (equol)[3] were found in the urine of adults consuming soy foods (8,9). Emerging data from several clinical studies now indicate that this isoflavone metabolite may hold the clue to the mechanism of action and effectiveness of soy in studies of hormone-dependent diseases. We now put forward a case for "bacterio-typing" study subjects for their "equol-producing" status.

Historical perspective

In 1932, Marrian and Haslewood (10) first isolated and elucidated the chemical structure of equol, a contaminant of the estrus-producing hormone hydroxyestrin, found in high levels in pregnant mare's urine. Using differential solvent extraction and recrystallization this "contaminant" was found to have a melting point lower than estrone, and was assigned the chemical composition $C_{15}H_{14}O_3$. This "unknown" was also present in the urine of nonpregnant mares and stallions, leading to the conclusion that its excretion was "quite unspecific for pregnancy" and that there was "no reason to associate its presence in urine with the presence of large amounts of oestrogenic substances" (11). It was named equol for its equine origins. Interestingly, equol could be isolated in large quantities from horse urine during the summer months, but levels

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[3] Abbreviations used: BMC, bone mineral content; BMD, bone mineral density; Cl/*F*, clearance normalized to the bioavailable fraction (*F*); equol, 7-hydroxy-3-(4′-hydroxyphenyl)-chroman; ER, estrogen receptor.

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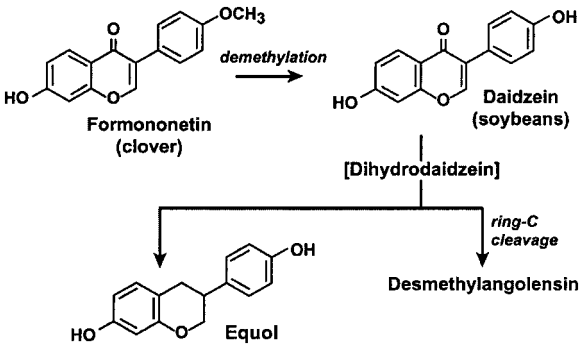

FIGURE 1 Pathway for equol formation after hydrolysis of the glycoside conjugates daidzein from soy, and the methoxylated isoflavone formononetin found in clover.

declined in the autumn, and it proved almost impossible to isolate from urine collected during the winter. This attests to what we now know is seasonal variation in its precursor isoflavones in plants. It was concluded incorrectly that, "So far as can be determined, no dietary factor was the cause of this [seasonal] variation." Through a series of superb analytical studies, the chemical formula of what we now know as equol was deduced (10–12). Little information on equol subsequently appeared in the scientific literature following its characterization until it became associated with devastating reproductive problems and infertility in sheep grazing in South Western Australia. Known as "Clover Disease," it was caused by "estrogenic" isoflavones in species of *Trifolium* clover (13,14).

Metabolism of isoflavones and formation of equol in animals

The metabolism of isoflavones has been well characterized in sheep (15,16) and many other species, including domestic fowl (17), laying hens (18), goats (19) and cows (20). In sheep, formononetin and biochanin A are biotransformed by ruminal bacteria to the demethylated intermediates, daidzein and genistein, and then to the estrogenic isoflavone equol (**Fig. 1**) and the inactive metabolite *p*-ethylphenol, respectively. Plasma equol concentrations were as high as 20 μmol/L in sheep, and this accounted for the pathophysiologic effects on reproduction (21,22), although this was not the case for cattle (16). It has been suggested that humans consuming soy foods (23,24) could be susceptible to similar reproductive problems. However, actual intakes of isoflavones that caused Clover disease were 20–100 g/d, far in excess of 15–50 mg/d [0.5–1.0 mg/(kg body · d)] typically ingested by humans consuming soy foods (25–27). Adults would have to consume daily, >1000 L of soymilk, 8600 soy burgers or 360 kg of tofu to achieve similar levels of intake. It is worth noting that laboratory rodents are normally exposed to doses of daidzein and genistein of 60–80 mg/(kg body · d) from commercial animal feed (28–30), yet these high exposures do not negatively affect breeding. However, isoflavones can induce subtle biological responses at the cellular, molecular and gene-expression levels (30,31). Hence, researchers should be aware of the background diet used in rodent studies. It would be helpful if suppliers of commercial rodent diets were to certify the phytoestrogen content because experimental end points can be influenced by inadvertent exposure to isoflavones.

Equol formation in humans—the crucial role of intestinal microflora

Equol is not normally present in the urine of most healthy adults in more than traces unless soy is consumed. Its identification in human urine followed the fortuitous discovery that the rat, with its large cecum and abundance of microflora, was an "equol-producing machine." Sufficient quantities of equol were isolated from urine and its structure was fully elucidated by mass spectrometry and nuclear magnetic resonance spectroscopy (9). Its formation is exclusively dependent on intestinal microflora. Germ-free animals do not excrete equol (32) and the lack of equol in the plasma of infants fed infant formula (33,34) further highlights the need for an active microflora for its formation (**Fig. 2**). Incubation of textured vegetable protein with cultured human fecal bacteria under anaerobic conditions first confirmed the biotransformation of the soy isoflavone, daidzein, into equol (6). Equol is not a phytoestrogen because it is not a natural constituent of plants yet it is inaccurately classified that way by many investigators. Rather, it is a nonsteroidal estrogen of the isoflavone class that is exclusively a metabolic product of intestinal bacterial metabolism. Equol has subsequently been identified in the urine of pregnant macaque monkeys (35), chimpanzees (36), dogs (37) and mice (30).

The main dietary origins of equol in humans are soy protein and soy foods because these are the most abundant sources of the isoflavones daidzin and daidzein (38,39), its precursors (8). Phytoestrogen supplements made from extracts of red clover, increasingly popular as alternative therapies for menopausal symptoms, indirectly provide a source of daidzein because the methoxylated isoflavone formononetin in red clover is efficiently biotransformed in the human intestinal tract to daidzein (40). Supplements that contain extracts of the Chinese vine, kudzu (*Radix puerariae* or *Pueraria lobata*) also have appreciable amounts of daidzin and its methoxylated analog, puerarin (40,41).

The metabolism of soy isoflavones in humans is well documented (8,42–45). Isoflavones in soy proteins and most soy foods are conjugated to sugars. The β-glycosides are not absorbed and require hydrolysis for bioavailability and subsequent metabolism (46). Hydrolysis is extremely efficient and occurs along the entire length of the intestinal tract by the action of both the brush border membrane and the bacterial β-glucosidases (47), which are active from relatively early in life. The aglycones are released and further metabolism of daidzein and genistein takes place (Fig. 1). Intestinal biotransformations include dehydroxylation, reduction, C-ring cleavage and demethylation; these are bacterial reactions that take place distally and presumably in the colon. Glycitin, the 6-methoxy analog of daidzin is found in high proportions in

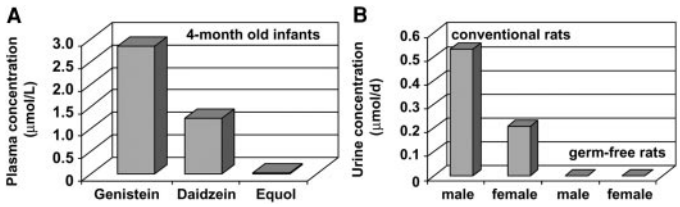

FIGURE 2 Evidence for the bacterial origins of equol: mean plasma equol concentrations in (*A*) 4-mo-old healthy infants fed soy formula exclusively from birth and (*B*) conventional healthy adult rats and germ-free animals. Adapted from data published previously (32,33).

soy germ but is a minor component of most soy foods. This isoflavone has not been studied extensively but has been found to be metabolically stable; its glycoside is readily hydrolyzed to release glycitein but the close proximity of the 6-methoxyl to the 7-hydroxyl sterically hinders its demethylation. Thus, glycitein is not converted to any appreciable extent to daidzein and is therefore not a precursor of equol (40,48).

The formation of equol from daidzein occurs via a pathway that involves the formation of the intermediate dihydrodaidzein (Fig. 1). Studies in healthy adults using [^{13}C]daidzein and [^{13}C]genistein tracers show conclusively that equol is formed from daidzein and not genistein (49). Once formed, equol appears to be metabolically inert, undergoing no further biotransformation, save phase II metabolism. As with daidzein and genistein, the predominant phase II reactions are glucuronidation, and to a minor extent, sulfation (8,50,51). This conjugation appears to take place on first-pass absorption across the enterocyte (52,53) as first indicated from high levels of equol (compound 192/386) in portal venous blood of rats (32). UDP-glucuronyltransferase 1A10 localized to the colon catalyzes the glucuronidation of genistein (51), but the specific isozyme responsible for equol conjugation is presently unknown.

We report here for the first time, the bioavailability and metabolism of equol in one healthy adult. The plasma appearance and disappearance profile in that subject is shown in **Figure 3**. Equol, when given as a single-bolus oral dose (25 mg), was rapidly absorbed, attaining a maximal plasma concentration after 4–6 h and thereafter disappearing with a terminal elimination half-life of 8.8 h. It showed similarity in its pharmacokinetics to other isoflavones, although the slower plasma clearance was striking (Cl/*F* = 6.85 L/h) compared with its precursor, daidzein (Cl/*F* = 17.5 L/h). This slow clearance contributes to the maintenance of high plasma concentrations in rats; plasma concentrations of equol are normally far in excess of daidzein or genistein in this species (30).

Factors influencing equol production in humans

About 30–50% of the adult population do not excrete equol in urine when challenged daily with soy foods (6,54–57) and the reasons are unclear. Furthermore, even when the pure compounds are administered, thereby removing any influence of the food matrix, many people do not convert daidzein to equol (40,49). This phenomenon has led to the terminology of being an "equol-producer" or "nonequol producer" to describe these two distinct populations; cut-off values have been derived empirically, permitting assignment of individuals to each of these categories. In our experience, people who have plasma equol concentrations of <40 nmol/L (10 μg/L) can be classified as "nonequol producers"; concentrations >83 nmol/L (20 μg/L) define "equol producers." This distinction can also be derived from urine, with an equol producer defined as someone excreting >1000 nmol/L (56,57). Although the excretion of equol is highly variable among individuals, there is a large demarcation between those that can and cannot produce equol, consistent with a precursor-product relationship in the enzyme kinetics. There is consequently an inverse relationship between urinary daidzein and equol levels, and thus far no significant gender differences have been defined (56).

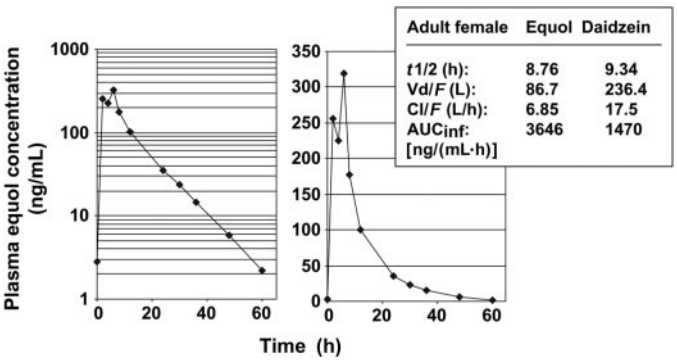

FIGURE 3 The plasma appearance/disappearance curve for equol expressed as log/linear (*left panel*) and linear/linear (*right panel*) plots depicting its pharmacokinetics in a healthy adult female after oral administration of 25 mg of the pure (±)equol. Computed pharmacokinetics for equol are compared with our previously published data (40) on the pharmacokinetics of daidzein in healthy women. Abbreviations: $t_{1/2}$, half-life; V_d/F, volume of distribution normalized to the bioavailable fraction (*F*); Cl/*F*, clearance normalized to the bioavailable fraction, *F*; AUC, area under the curve.

In studies by Cassidy (58,59) using an in vitro model of human colonic fermentation, it was observed that the conversion of daidzein to equol by cultured human fecal flora could be manipulated. Specifically, a high nonstarch polysaccharide milieu, which stimulates bacterial fermentation, led to rapid and complete conversion of daidzein to equol, yet under conditions mimicking low carbohydrate intake, equol was not formed. This suggests that other components of the diet likely play an important role in intestinal biotransformation of daidzein to equol. A study of 24 healthy adults by Rowland et al. (57) found that the good equol producers consumed less fat as a percentage of energy compared with poor equol producers (26 ± 2.3 vs. 35 ± 1.6%), and more carbohydrates (55 ± 2.9 vs. 47 ± 1.7%). Lampe et al. (56) similarly showed that women, and not men, who were equol excretors consumed a significantly higher percentage of energy as carbohydrate compared with nonequol excretors, and they also consumed greater amounts of plant protein and dietary fiber. It was suggested that, among women, dietary fiber or other components of a high fiber diet promote the growth and/or the activity of bacterial populations responsible for equol production in the colon. However, a later study found no effect on urinary equol excretion if dietary fiber intake was doubled by the supplementation of 16 g/d of wheat bran (60). Whether this is sufficient fiber to significantly alter intestinal dynamics is uncertain. Interestingly, wheat bran does not alter urinary enterolactone and enterodiol excretion (60,61), and these two lignans are formed in the colon by the same type of metabolic reactions as those involved in equol formation (62).

Whether there are specific components of the diet that influence bacterial conversion of daidzein to equol remains to be definitively established because the data are equivocal at present. From our many studies of repeated administration of isoflavones or soy foods to the same adults, a consistent observation has been that those who are "equol producers" seem to remain "equol producers" over time (49). Lampe et al. (60) also noted that being an equol converter was a relatively stable phenomenon. This then begs the important question, i.e., can we take someone who does not make equol and convert them to an equol-producer? Certainly, it is possible to do the reverse; excessive use of antibiotics, which wipe out intestinal flora, is likely to do this, as it does in blocking the formation of the lignans, enterolactone and enterodiol (62). It is conceivable that the use of prebiotics or probiotics could induce equol production but this remains to be established. Uehara et al. (63) showed that dietary fructopolysaccharides alter the bioavailability of daidzein and genistein in rats but there was no

mention in that study of what it did for equol; this is surprising given that equol is the major isoflavone in rat plasma (30).

Identifying the bacterial species responsible for converting daidzein to equol is a major challenge given the large number of bacteria that reside in the colon and small intestine. Recently, Ueno et al. (64) identified equol producers from healthy Japanese adults after consumption of 70 g tofu and culturing of their fecal flora. Three strains of bacteria that reportedly converted pure daidzein to equol in vitro were the gram-positive strains of *Streptococcus intermedius* spp., and *Ruminococcus productus* spp. and gram-negative *Bacteroides ovatus* spp.

Biological properties of equol

There is a paucity of data on the biological activity of equol. Unlike the isoflavones from soy, daidzein and genistein, or those in clover, formononetin and biochanin A, equol is unique in having a chiral center due to the lack of a double bond in the hetrocyclic ring. Therefore, two distinct optically active isomers occur (**Fig. 4**). When both are modeled, the *R*- and *S*- isomers differ conformationally and this will undoubtedly influence ligand binding in the cavity of the dimerized estrogen receptor (ER) complex. (-)Equol was originally reported to have no estrogenic activity in the ovariectomized mouse (10); later, however, other investigators using bioassays found racemic equol to be a weak estrogen, whereas its precursors daidzein or formononetin were inactive (21). Metabolism has to be considered in assessing estrogenic potency because formonentin and daidzein act as proestrogens, i.e., they are activated to greater potency by the action of bacterial flora in converting these to equol. We have compared equol with genistein for its effect on the uterus of immature rats and find it a potent stimulator of growth (unpublished data). Uterine weights of immature rats killed after subcutaneous injection of (±)equol show that equol is more than twice as estrogenic as genistein in this model when allowing for the fact that half of the injected dose is an inactive enantiomer. Many different in vitro assay systems have been employed to measure the estrogenicity of isoflavones. Independent of the assay system used, data for the relative molar binding affinities of equol compared with its precursor daidzein, and with estradiol are consistent and in general agreement with the original values of 0.4, 0.1 and 1.0, respectively, published by Shutt and Cox (21) for their binding to sheep uterine cytosol. These early studies however, predated the recognition of distinct ER subtypes and the discovery of ERβ (65); therefore, these relative binding affinities almost certainly reflect affinities toward ERα because this is the predominant estrogen receptor in the uterus (66).

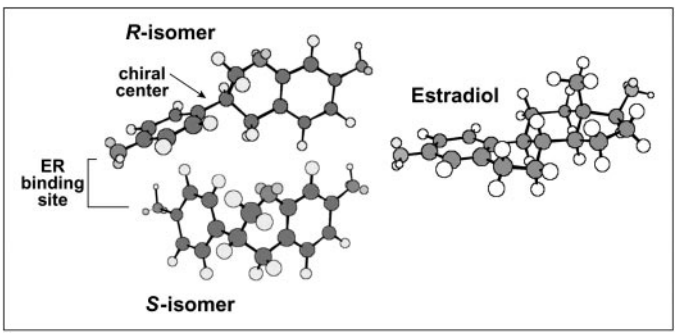

FIGURE 4 Molecular modeling of the chemical structures of the optical isomers of equol compared with estradiol.

Although Kuipers et al. (67) did not test equol, genistein was found to have a high binding affinity for ERβ, whereas daidzein and formononetin bound poorly by comparison, although still with preference for ERβ. Phytoestrogens are unique among many estrogen-like substances for their preferential binding to ERβ protein, and this may explain some of the reported effects of soy isoflavones in tissues expressing this receptor subtype, such as the bone, brain and vascular endothelium. Recently, the binding affinity of equol for human ERα and ERβ was found to be similar to that of genistein, but equol induced transcription more strongly than any other isoflavone, especially with ERα (68). Interestingly, daidzein showed poor affinity and transcriptional activity in these in vitro systems. This suggests that it could be advantageous to convert daidzein to equol to enhance its estrogenic potency in vivo.

Nagel et al. (69) showed that 49.7% of equol circulates in the free or unbound form. This is considerably greater than the proportion of free daidzein (18.7%) or estradiol (4.6%); because it is the unbound fraction that is available for receptor occupancy, this may effectively contribute to enhancing the overall potency of equol. Equol binds to sex hormone binding globulin and competitively inhibits estradiol and testosterone binding in a dose-dependent manner (70). It also binds to α-fetoprotein (71), although the clinical importance of this is uncertain.

Equol possesses other properties of relevance to cellular function. As a polyphenol, it shares with flavonoids the ability to be a hydrogen/electron donor and therefore can scavenge free radicals. Equol has the greatest antioxidant activity of all the isoflavones tested when measured in vitro in the ferric reducing ability of plasma, Trolox equivalent antioxidant capacity and copper(II)- or ferric(III)-induced liposomal peroxidation assays (72–74). Although isoflavones are considered weak antioxidants when tested in vitro, their in vivo effect may be sufficient to account for the reduced ex vivo lipid peroxidation that has been observed in all but one study (75) when adults consume soy protein diets (76–79). Given the superior antioxidant activity of equol compared with other isoflavones, a case can be made for being an "equol-producer" because this may provide greater inhibition of lipid peroxidation and therefore greater reduction in risk for cardiovascular disease.

Measurement of equol in biological samples

The measurement of equol has been largely neglected in most studies because soy research became highly focused on genistein after it was found to be a potent inhibitor of tyrosine kinases and several growth factors (80,81). Other reasons may have been the lack of commercially available supplies of equol as a pure compound for testing and the fact that the universal approach to measuring isoflavones, i.e., HPLC with UV detection, cannot detect equol with any degree of reliability or sensitivity due to equol's poor UV absorption characteristics. Although immunoassays for daidzein and genistein have been developed (82–85), these antibodies show negligible cross-reactivity with equol and therefore fail to detect this important metabolite. Cross-reactivity of equol in some immunoassays measuring estradiol has been noted as a methodological problem (86). Presently, stable-isotope dilution mass spectrometry with selected ion monitoring remains the only reliable sensitive and specific method for measuring equol in plasma and urine (9,40,49,50).

Clinical implications of equol

The hormonal influence of equol in humans was first apparent from studies showing that the daily consumption of 45 mg of isoflavones from 60 g of textured vegetable protein led to a significant increase in menstrual cycle length, not observed if soy was devoid of isoflavones, and follicular phase length correlated significantly with urinary equol excretion (87,88). More recently, data from three clinical studies support our contention in this paper that it is important to define individuals by their "equol-producing" status and that this probably should be done before enrolment in dietary intervention studies. This could be considered as effectively "bacteriotyping" individuals on the basis of whether they possess the bacterial flora necessary to produce equol and is easily achieved by mass spectrometry of plasma or urinary analysis. We speculate that failure in all previous dietary intervention studies to do this could explain the variable responses reported for the actions of soy protein and isoflavones.

Much of the early interest in phytoestrogens focused on their potential as an alternative to hormone replacement therapy for relieving hot flushes associated with menopausal estrogen deficiency (89), and many phytoestrogen supplements have since flooded the market (40). Thus far, 14 clinical trials investigating the effects of phytoestrogen-rich foods, or isoflavone supplements on menopausal symptoms have been reported. The results have been variable and largely disappointing (90–103). Overall, most of the studies had a large placebo effect and the reductions in the severity and frequency of hot flushes in postmenopausal women were at best modest compared with the effectiveness of estrogen therapy. Epidemiologic evidence from a community-based study by Nagata et al. (104) found that the incidence of hot flushes was inversely related both to the amount of soy foods consumed and the daily intake of isoflavones; thus, there is circumstantial evidence for a role for isoflavones. Interestingly, a recent study of 180 Japanese women given a standardized questionnaire to evaluate the severity of menopausal symptoms found that symptoms of hot flushes occurred in only 5% of the women (105). The daily isoflavone intake from soy foods was calculated to be 22 ± 14 mg, much lower than doses used in clinical studies of isoflavones, and 53.5% of the group were found to be equol-producers on the basis of urinary equol excretion. Interestingly, all of the equol-producers recorded the least severe symptoms as assessed by a simplified menopausal index score. These data suggest that equol-producers comprise a distinct subpopulation that may gain the most benefit from soy isoflavones for relief of hot flushes and it may explain anecdotal reports by many women of phytoestrogen's effectiveness in relieving hot flushes. None of the 14 cited studies above on soy isoflavones and hot flushes stratified women according to equol status.

The role that soy isoflavones play in preventing osteoporosis remains to be fully elucidated (106). Many in vitro studies using cell cultures of osteoclasts and osteoblasts (107–111) and in vivo rodent models of ovarian estrogen-deficient osteoporosis (112–115) have yielded convincing evidence that isoflavones reduce bone turnover. The approval in some countries of the synthetic isoflavone, Ipriflavone, for the treatment of osteoporosis gave support for investigations of soy isoflavones, even though a large multicenter 3-y study has subsequently found Ipriflavone to be ineffective and not without side effects (116). Nevertheless, a number of bone and soy studies have thus far been performed with variable outcomes. Short-term studies of 12 wk or less in which surrogate markers of bone turnover such as urinary pyridinoline and deoxypyrodinoline cross-links, plasma/serum osteocalcin, alkaline phosphatase and insulin growth factor-1 were used have indicated reduced bone turnover when soy foods containing isoflavones were included in the diet (117–120). Several studies of 9 mo duration or less have been completed (95,121,122) since the landmark study by Potter et al. (123) showed a bone-sparing effect of a diet containing 90 mg/d of soy isoflavones for 6 mo. All of these studies measured changes in bone mineral density (BMD) at various sites and results were conflicting, with 2 of the 4 showing no effect.

In the first 2-y study to be completed in which postmenopausal women were randomized to consume two 250 mL glasses of soymilk each day, either with or without isoflavones, bone loss measured by change in lumbar spine BMD was prevented by isoflavone-rich soymilk (124). Lumbar spine BMD and bone mineral content (BMC) decreased 4.0 and 4.3%, respectively ($P < 0.01$) over the 2-y period in the group consuming soymilk with negligible amounts of isoflavones; this is close to the 5–7% loss in bone mass that would be normally expected in the first 2 y of natural menopause. By contrast, those consuming soymilk that contained 50 mg isoflavones showed increases of 1.1 and 2% in lumbar spine BMD and BMC, respectively. Thus soy protein with isoflavones, as opposed to without isoflavones, maintained stable bone mass over a 2-y period. It should be mentioned that this difference was not observed after 1 y. Given the slow rate of bone turnover, it is our opinion that the variability in data from previous bone studies is more likely a consequence of the short duration of dietary intervention with soy foods. However, the most striking observation from the study was that the "equol-producers," comprising 45% of the women, showed significant mean increases of 2.4 and 2.8%, respectively, for BMD and BMC in the lumbar spine, compared with increases of only 0.6 and 0.3% in those women who did not produce equol (**Fig. 5**). These data indicate the importance of taking equol status into consideration in clinical studies investigating the effects of soy on bone turnover because there are clearly distinct differences in the responses.

After first proposing the importance of "bacterio-typing" for equol at The 4th International Soy Symposium on the Role of Soy in Preventing and Treating Chronic Disease in San Diego (7), Howe et al. re-examined data from a completed randomized, placebo-controlled, crossover, lipid-lowering study performed in 23 mildly hypercholesterolemic premenopausal women that had initially found no significant changes in plasma lipids when soy foods were compared against dairy foods (personal communication, P.R.C. Howe, University of Woolagong, NSW, Australia). In that study, 8 of the 23

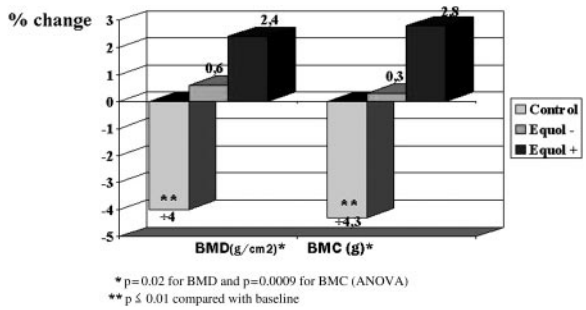

FIGURE 5 Changes in bone mineral density (BMD) and content (BMC) in postmenopausal women over a 2-y period of soymilk consumption stratified according to their equol status. Adapted from Lydeking-Olsen et al. (124).

women were equol producers (35%), and after reevaluating the results and stratifying according to equol status, it was found that consuming 5 servings a day of soy for 5 wk, compared with dairy foods, significantly lowered plasma total cholesterol, LDL cholesterol, LDL/HDL cholesterol ratio, triglycerides and lipoprotein(a) by 8.5, 10.0, 13.5, 21 and 11%, respectively, only in the "equol-producers." Similarly, a subgroup analysis of a 2-y study on lipids in normo- and hypercholesterolemic postmenopausal women also found that soy had the greatest hypocholesterolemic effect in equol-producers. Plasma total cholesterol concentrations significantly decreased 7.2% ($P = 0.04$ relative to baseline) in equol producers compared with only 3.0% (NS) in nonequol producers (125). The failure of soy protein to lower cholesterol in normocholesterolemic subjects (126), with few exceptions (87,127,128), could conceivably be due to heterogeneity in study populations with regard to the metabolism of soy isoflavones.

Conclusions

Overall, recent evidence suggests that it is important to stratify people by "bacterio-typing" according to their ability to produce equol rather than perform data analysis on end points from an entire heterogeneous study population, when in reality it may be two distinct subpopulations. There is good rationale for expecting greater efficacy in equol-producers because equol binds with greater affinity to estrogen receptors than daidzein from which it is derived. Ironically, some 20 y after first identifying equol in human urine and discovering that it was associated with soy food ingestion (6,8,9), we may have come full circle. Could it be that this largely forgotten isoflavone may prove the most important in explaining the mechanism of action of soy isoflavones in disease prevention and treatment? We propose this to be the case and present a new paradigm to explain the clinical effectiveness of these phytoestrogens from soy foods.

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