# Soy consumption during menopause

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*Abstract*

In developed countries, the life expectancy of women is currently extending more than 30 years beyond the age of menopause. The menopausal transition is often associated with complaints. The conflicting results on the effectivity of phytoestrogens to alleviate menopausal symptoms. This discrepancy in treatment effect may be due to the large interindividual variation in isoflavone bioavailability in general and equol production in particular. Equol, a microbial metabolite of daidzein, has been hypothesized as a clue to the effectiveness of soy and its isoflavones, but only about 30-50% of the population harbor an intestinal microbial ecosystem supporting the conversion of daidzein into equol.

There is much concern on breast cancer, since this incidence of this disease increases with age. There is indication that soy phytoestrogens may decrease this breast cancer incidence. In order to evaluate the estrogenic potential of these exposure levels, we studied the isoflavone-derived $E_2\alpha$- and $E_2\beta$-equivalents (*i.e.* 17β-estradiol ($E_2$)-equivalents towards ERα and ERβ, respectively) in human breast tissue. Total isoflavones showed a breast adipose/glandular tissue distribution of 40/60 and their derived $E_2\beta$-equivalents exceeded on average 21 ± 4 and 40 ± 10 times the endogenous $E_2$ concentrations in corresponding adipose and glandular biopsies, respectively, whereas the $E_2\alpha/E_2$ ratios were 0.4 ± 0.1 and 0.8 ± 0.2 in adipose and glandular breast tissue, respectively. These calculations suggest that, at least in this case, soy consumption could elicit partial ERβ agonistic effects in human breast tissue. We are currently characterizing the differential activation of estrogen-responsive genes between dietary isoflavones, the chemopreventive selective ER modulators tamoxifen and raloxifene and exogenous estrogens in a controlled dietary intervention trial that integrates data on the exposure to estrogenically active compounds, expression of isoflavone and estrogen target genes, and epigenetic events.

During the menopause, there is a close relation between the drop in serum estrogen and negative metabolic changes such as the increase in bone resorption and negative change in the serum lipid profile. Randomized controlled trials measuring bone turnover markers in menopausal women revealed that soy isoflavone supplements significantly but moderately decrease the bone resorption marker urinary deoxypyridinoline without significant effects on the bone formation markers serum bone alkaline phosphatase and osteocalcin.

*Key words:* Bone resorption, estrogens, isoflavones, lipid profile, menopause, phytoestrogens, soy.

## Introduction

In developed countries, the life expectancy of women is currently extending more than 30 years beyond the age of menopause. This is in fact, in the evolutionary history of the human race, a relatively new situation. To take one example, the mean life expectancy for women in Belgium is now over 83 years, while it was only 46 years in 1880. In many countries women spend more than one third of their life in menopause. Therefore, we seek to provide an optimal quality of life during this long

period. This means that we have to search for solutions to alleviate menopause-related complaints and, on the other hand, look for ways to prevent diseases such as osteoporosis, breast cancer, and cardiovascular diseases.

The menopausal transition is often associated with complaints. The most prominent are hot flashes, sleeping problems, fatigue, and stiffness of muscles and joints. There are large interindividual differences in the severity of these symptoms, which may be very mild in some women but socially disabling in others. Moreover, the prevalence of hot flashes varies markedly throughout the world. It may be as high as 80% in Europe and North America, whereas in Asian countries, the prevalence is much lower (Boulet et al., 1994; Lethaby et al., 2007; Melby, 2005; Rodstrom et al., 2002). The higher intake of soy food, recognized as the major dietary source of phytoestrogens (Bingham et al., 1998), may partially account for this lower prevalence of complaints in Asian populations (Adlercreutz et al., 1992; Anderson et al., 1999). Menopause-related symptoms usually abate over time without any treatment, but persist for more than twenty years in up to 15% of the women (Rodstrom et al., 2002). Although these complaints are very typical, it is often the woman herself who has to link the symptoms to the menopausal transition. In Belgium it takes up to seven months before these complaints are related to hypoestrogenism by the medical practitioner.

**How to deal with menopause-related complaints**

Estrogen supplementation, with or without a progestogen, is the most effective treatment of moderate-to-severe menopause-related vasomotor symptoms and their potential consequences (NAMS, 2010a). However, the Women's Health Initiative (WHI) trial results (Anderson et al., 2004), together with those of the Million Women Study (Banks et al., 2003), provoked a substantial and sustained, worldwide decrease in hormone therapy (Barbaglia et al., 2009). The main reasons for this are an increased risk of breast cancer and the lack of cardiovascular protection. Although the WHI was a randomized trial, it took nearly ten years to understand why it did not answer all our questions. Data from the WHI estrogen-alone trial published in 2011, including the postintervention health outcomes (median conjugated equine estrogen use: 5.9 years; mean follow-up: 10.7 years), suggest greater safety and possible benefit among women in their 50s and potential harm among older women in terms of coronary heart disease, total myocardial infarction, colorectal cancer, total mortality, and the global index of chronic diseases (LaCroix et al., 2011). The WHI

trials are consistent with observational studies indicating that hormone therapy may reduce total mortality by 30% when initiated soon after menopause while starting this treatment after the age of sixty is no longer useful (NAMS, 2010a). These results are in line with other publications (Allison, Manson, 2011; Grodstein et al., 2000; Grodstein et al., 2006; Salpeter et al., 2004; Stram et al., 2011). Yet, the current recommendations from many organizations that hormone therapy should be limited to the treatment of moderate-to-severe menopause-related symptoms, with the lowest effective dose used for the shortest duration necessary, remain appropriate (NAMS, 2010a). Lower doses of unopposed or combined estrogens are better tolerated and may have a more favorable benefit-risk ratio than the standard dose. However, lower doses have not been tested in long-term trials to support this (NAMS, 2010a). Therefore, using lower doses of estrogen, using natural estrogen and progestogens, and starting early after the onset of menopause are definitely steps in the right direction.

This WHI history clearly shows how careful we have to be when we read studies, even though they are randomized controlled trials. Still, many women nowadays are reluctant to hormone therapy and seek alternatives for the relief of menopause-associated symptoms. This is in line with NAMS' recommendation of lifestyle-related strategies and non-prescription remedies, such as phytoestrogens, as the preferred first-line approach to alleviate mild symptoms (NAMS, 2004). Literature on the use of phytoestrogens during menopause is, to say the least, conflicting. Although more than 50 hot flash trials have been conducted, a plethora of well-designed studies using similar interventions and outcome measures in matching study populations does not exist to evaluate the efficacy of isoflavone-containing products to reduce vasomotor symptoms. Despite the inconsistent study results and marked placebo effect, there is some indication of a benefit of isoflavones on hot flash frequency and/or severity, especially when a minimum of 15 mg/d genistein aglycone equivalents is provided (Howes et al., 2006; Jacobs et al., 2009; Lethaby et al., 2007; Messina, 2010; Williamson-Hughes et al., 2006). Furthermore, the discrepancy in treatment effect may be due to the large interindividual variation in isoflavone bioavailability in general and equol production in particular. Equol, a microbial metabolite of daidzein, has been hypothesized as a clue to the effectiveness of soy and its isoflavones (Setchell et al., 2002), but only about 30-50% of the population harbor an intestinal microbial ecosystem supporting the conversion of daidzein into equol (Atkinson et al., 2005). Indeed, Jou et al. (2008) reported that a 6mo-intervention with

Table 1. — Overview of equol-producing cultures.

| Culture | Precursor | Origin | References |
|---|---|---|---|
| *Bacteroides ovatus, Ruminococcus productus* and *Streptococcus intermedius* | Daidzein | Human feces | (Ueno, Uchiyama, 2001) |
| EPC4: *Lactobacillus mucosae* EP1, *Enterococcus faecium* EP2, *Finegoldia magna* EP3, and *Veillonella sp.* EP | Daidzin | Human feces | (Decroos et al., 2005) |
| *Eggerthella* SNU-Julong 732 | DHD | Human feces | (Wang et al., 2005) |
| *Asaccharobacter celatus* AHU1763 (strain do03) | Daidzein | Rat cecum | (Minamida et al., 2006; Minamida et al., 2008; Thawornkuno et al., 2009; Uchiyama et al., 2007) |
| *Lactococcus garvieae* G20-92 | Daidzin | Human feces | (Uchiyama et al., 2007) |
| *Eggerthella* SNU-Julong 732 and *Lactobacillus* sp. Niu-O16 | Daidzein | Human feces | (Wang et al., 2007) |
| Strains PUE and DZE | Puerarin | Human feces | (Jin et al., 2008) |
| *Adlercreutzia equolifaciens* | Daidzein | Human feces | (Maruo et al., 2008) |
| Strain Mt1B8 | Daidzein | Mouse intestine | (Matthies et al., 2008) |
| *Slackia isoflavoniconvertens* | Daidzein | Human feces | (Matthies et al., 2009) |
| *Eggerthella* sp. YY7918 | Daidzein | Human feces | (Yokoyama, Suzuki, 2008) |
| Strains D1 and D2 | Daidzein | Pig feces | (Yu et al., 2008) |

135 mg/d isoflavones (65.4 mg/d daidzein aglycone equivalents, 17.1 mg/d genistein aglycone equivalents) improved menopausal symptoms only in so-called equol producers. Despite the worldwide quest for dietary applications enhancing equol production, strategies to convert non-producers to equol produces with either pre- or probiotics have, thus far, proven elusive (Bonorden et al., 2004; Lampe et al., 2001; Nettleton et al., 2004; Nettleton et al., 2005; Steer et al., 2003). Several specific equol-producing cultures have been isolated and characterized (Table 1), but, to the best of our knowledge, none of these have been applied in vivo as a probiotic (Decroos et al., 2006). However, some have been used in another approach, which is the administration of equol as either a pharmaceutical or neutraceutical agent or as a food additive (Ishiwata et al., 2009; Yee et al., 2008). In a first randomized, placebo-controlled intervention trial, equol supplementation (3 × 10 mg/d during 12 weeks) significantly alleviated menopause-associated mood-related symptoms compared to placebo in peri- and post-menopausal Japanese non-producers (Ishiwata et al., 2009). Hence, in order to identify subpopulations with a specific exposure profile and possibly response to treatments with phytoestrogens or mixtures thereof, personalized screenings are recommended (Bolca et al., 2009). Whereas the feasibility of large (sub)population screenings or individual screenings of consumers or patients depends on the development, validation, and implementation of rapid, easy, and more elegant, urine-based screening assays such as immunoassays (Bennetau-Pelissero et al., 2000; Bennetau-Pelissero et al., 2003; Brouwens et al., 2003; Creeke et al., 1998; Hampl et al., 1998; Lapcik et al., 2004; Lapcik et al., 1998; Lapcik et al., 2003; Makela et al., 2000; Schaefer et al., 2005; Shinkaruk et al., 2008; Vitkova et al., 2004; Wang et al., 1994; Wyns et al., 2011), in experimental settings and clinical trials, fecal incubations enable the phenotyping and possibly inclusion or stratification of study participants without the need for one or more dietary interventions (Bolca et al., 2007a; Bolca et al., 2007b; Bolca et al., 2009).

**Isoflavones and breast cancer**

There is a lot of controversy, confusion, and concern about the 'soy-breast cancer' relation (Messina, Wu, 2009; Stubert, Gerber, 2009). Although soy products contain several bioactive phytochemicals, most cancer research has focused on isoflavones and genistein in particular. The substantially lower breast cancer prevalence in populations with a high soy consumption (one-third reduction in both pre- and postmenopausal breast cancer risk), as well as the increased risk observed upon migration and westernization of Asian people, initiated soy-breast cancer research 20-30 years ago. Both significant and null results have been obtained regarding the (protective) impact of soy isoflavones on hormone-related breast

cancer risk factors such as plasma steroid and sex-hormone-binding globulin levels, urinary 2-hydroxy-estrone/16α-hydroxy-estrone ratios, and menstrual cycle length (Messina, Wu, 2009; Stubert, Gerber, 2009). Yet, the findings of Helferich's group, reporting a growth stimulatory effect of genistein on mammary tumors in a heavily criticized xenograft model (Allred et al., 2004; Allred et al., 2001b; Allred et al., 2001a; Hsieh et al., 1998; Ju et al., 2001; Messina, Wu, 2009), has raised concern the safety of dietary phytoestrogens, especially for patients with existing estrogen-sensitive tumors and women at high risk of developing breast cancer. Conversely, results from clinical studies, in which breast biopsies were analyzed or breast tissue density measured as a marker of breast cancer risk (Atkinson et al., 2004; Marini et al., 2008; Maskarinec et al., 2004; Maskarinec et al., 2009; Maskarinec et al., 2003), are reassuring and contrast with the proliferative effects of combined estrogen-progestogen therapy (Chlebowski et al., 2009; Chlebowski et al., 2010).

In a study with premenopausal women, randomly assigned to either a soy (45 mg isoflavones/d during 8-14 d; n = 28) or control (n = 23), Hargreaves et al. (1999) reported a significant increase in breast nipple aspirate pS2 levels, an estrogen-regulated protein, compared to baseline. However, no effects were observed on breast cell proliferation (as measured by tritiated thymidine and Ki67), estrogen receptor (ER) and progesterone receptor (PR) status, Bcl-2 expression, apoptosis, and mitosis. In another (pilot) study without a control, breast nipple aspirate fluid volume significantly increased in premenopausal women (n = 14) during and after discontinuation of a 5mo-treatment with 75 mg isoflavones/d, whereas a minimal increase or no response was found in postmenopausal women (n = 10) (Petrakis et al., 1996). In contrast, Cheng et al. (2007) conducted a 12wk-trial involving healthy postmenopausal women randomly assigned to a placebo (n = 25) or soy-derived supplement (36 mg isoflavones/d, n = 26) and reported no effect on breast cell proliferation or the expression of ERα, ERβ, PRα, PRβ, and βcx. Similarly, the apoptosis/mitosis ratios in breast cancer biopsies (n = 17) upon a 2wk-isoflavone supplementation (200 mg isoflavones/d) were not significantly different from those of untreated controls (n = 26) (Sartippour et al., 2004). Qin et al. (2009) in turn, observed no significant changes in cytology, but methylation and antiestrogenic effects (as measured by serum C3 levels) compared to baseline in premenopausal women (n = 34) consuming 40 mg or 140 mg isoflavones/d through one menstrual cycle. Finally, larger-scale, randomized placebo-controlled trials with pre- and post-menopausal women (n = 30-406) failed to show changes in breast mammographic density upon 1-3y-treatments with 43.5-120 mg isoflavones/d (Atkinson et al., 2004; Marini et al., 2008; 2004; Maskarinec et al., 2009; Maskarinec et al., 2003).

Moreover, the equol production and producer phenotype were studied in relation to breast cancer risk. A high urinary equol excretion was associated with a substantial reduction in breast cancer risk in a case-control study (mean age = 54 years) (Ingram et al., 1997). Additionally, more favorable plasma steroid and sex-hormone-binding globulin profiles, consistent with a lower risk for breast cancer, were observed in equol-producing premenopausal women compared to non-producers (Duncan et al., 2000). Frankenfeld et al. (2004b), however, did not find such hormonal differences in serum of post-menopausal women, but equol producers had a higher urinary 2-hydroxy-estrone/16α-hydroxy-estrone ratio, which has been related to a lower breast cancer risk although the value of this marker is under debate (Stanczyk, Bretsky, 2003). Similar results were obtained in breast cancer survivors (Nettleton et al., 2005) and young to middle-aged women (Atkinson et al., 2003). Finally, a lower mammographic breast density was observed in equol-producing postmenopausal women compared to non-producers (Frankenfeld et al., 2004a), whereas no differences were found in pre-menopausal women (Atkinson et al., 2009).

Based on these findings, an inverse correlation between isoflavone intake and the risk of breast cancer seems more likely than a positive one. Yet, in view of the safety concerns, clinical trials accurately measuring the real individual exposure to isoflavones and metabolites rather than the isoflavone intake, are essential. In this respect, Bolca et al. (2010) measured the levels of isoflavones that actually reach the breast tissue in a bioactive form and found that, upon a 5d-soy-supplementation, human breast adipocytes and mammary gland epithelial cells were exposed to up to 20-25 pmol/g total isoflavone aglycones and 900-1150 pmol/g total isoflavone glucuronides. In order to evaluate the estrogenic potential of these exposure levels, these were converted to isoflavone-derived $E_2\alpha$- and $E_2\beta$-equivalents (i.e. 17β-estradiol ($E_2$)-equivalents towards ERα and ERβ, respectively). Total isoflavones showed a breast adipose/glandular tissue distribution of 40/60 and their derived $E_2\beta$-equivalents exceeded on average $21 \pm 4$ and $40 \pm 10$ times the endogenous $E_2$ concentrations in corresponding adipose and glandular biopsies, respectively, whereas the $E_2\alpha/E_2$ ratios were $0.4 \pm 0.1$ and $0.8 \pm 0.2$ in adipose and glandular breast tissue, respectively. These calculations suggest that, at least in this case, soy consumption could elicit partial ERβ agonist (Pike et al., 1999) effects

in human breast tissue. However, since estrogen-induced cell proliferation and breast carcinogenesis have mainly been linked to ERα signaling, whereas ERβ can antagonize ERα-dependent transcription (Gustafsson, 1999), rather protective effects would be expected. Yet, the clinical implications of these findings require further investigation. Therefore, we are currently characterizing the differential activation of estrogen-responsive genes between dietary isoflavones, the chemopreventive selective ER modulators tamoxifen and raloxifene (Barrett-Connor et al., 2006; Cuzick et al., 2007; Ettinger et al., 1999; Fisher et al., 1998; Powles et al., 2007; Vogel et al., 2006), and exogenous estrogens in a controlled dietary intervention trial that integrates data on the exposure to estrogenically active compounds, expression of isoflavone and estrogen target genes, and epigenetic events.

**Other health effects**

The consumption of soy in Western countries is progressively increasing due to the more frequent addition of soy flour or soy protein to daily consumed basic foods, such as bakery goods and meat products ('second-generation products'), the success of new generation soy foods (e.g., soy burgers and soy desserts) thanks to the improved food processing able to cope with the 'soy-taste challenge', and the growing consumers' awareness of the impact of a healthy diet together with the advertising on soy's beneficial health effects.

Indeed, the US Food and Drug Administration awarded a health claim for soy protein (25 g/d) and coronary heart disease based on its cholesterol-lowering effects (FDA, 1999). Cardiovascular disease is the leading cause of mortality and morbidity in women and hypoestrogenism has been linked to early adverse vascular changes, resulting in an increased risk after menopause (Woodard et al., 2011). An estimated 25-50% of the cardiovascular protection by estrogens has been attributed to decreases in low-density lipoprotein (LDL) and total cholesterol, an improved clearance of chylomicron-remnants, and attenuation of the postprandial decrease in high-density lipoprotein (HDL) cholesterol, which are mostly mediated through hepatic ERα and LXR (Turgeon et al., 2006; Westerveld, 1998). Recent meta-analyses and reviews have concluded that soy protein lowers LDL cholesterol by 3-5%, which is a modest but meaningful reduction (Messina, 2010). In addition, both rapid and longer-term actions of estrogens on the vasculature contribute to their atheroprotective effects. $E_2$ acutely enhances vasorelaxation through the activation of endothelial NO

synthase by ligand-bound ER, and regulates the expression of endothelial and inducible NO synthase in the cardiovascular system as well (Mendelsohn, 2000). After reviewing randomized placebo-controlled trials, Li et al. concluded that oral isoflavone supplementation significantly improves vascular endothelial function in postmenopausal women with low baseline flow-mediated dilatation (Li et al., 2010). Other, less well-studied factors that may influence cardiovascular health include effects on the circulation, blood pressure, coagulation, and fibrinolysis (Turgeon et al., 2006). The meta-analysis by Taku et al. (2010a) revealed that soy isoflavone supplements significantly decrease systolic but not diastolic blood pressure in normal or prehypertensive adults, without an observed dose-response relationship.

Osteoporosis, characterized by compromised bone strength, increases the risk of fractures. In particular, hip and spine fractures are associated with substantial morbidity and mortality in post-menopausal, especially older women (NAMS, 2010b). As more women will grow older in the near future, osteoporosis becomes a major health threat in our society. In normal bone remodeling, bone resorption is balanced by bone formation. The menopause triggers a rapid phase of bone loss that results from the loss of $E_2$-mediated suppression of bone resorption and an impaired compensatory bone formation associated to estrogen deficiency and aging (as reviewed by Khosla and Riggs (2005)). Additionally, longstanding estrogen deficiency may lead to a chronic negative calcium balance through the loss of estrogens' enhancing effects on the intestinal calcium absorption and renal tubular calcium reabsorption. Unless compensated by dietary calcium intake, this will result in secondary hyperparathyroidism and contribute to the late, slow phase of bone loss. However, data suggesting any benefit of dietary isoflavones in the prevention or treatment of postmenopausal osteoporosis are relatively weak (NAMS, 2010b). Although Marini et al. (2008) found that 2 years of genistein administration (54 mg/d) increases bone mineral density at the lumbar spine and femoral neck in osteopenic women, significant favorable effects on bone mineral density upon soy isoflavone supplementation are unlikely (Liu et al., 2009). Moreover, randomized controlled trials measuring bone turnover markers in menopausal women revealed that soy isoflavone supplements significantly but moderately decrease the bone resorption marker urinary deoxypyridinoline without significant effects on the bone formation markers serum bone alkaline phosphatase and osteocalcin (Taku et al., 2010b). Bisphosphonates, therefore, remain the first-line drugs for treating

postmenopausal women with osteoporosis (NAMS, 2010b).

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