



WHI and WHIMS follow-up and human studies of soy isoflavones on cognition

Liqin Zhao[†] and Roberta Diaz Brinton

Recent follow-up analyses of the previous findings from the Women's Health Initiative and the Women's Health Initiative Memory Study confirmed some health benefits of estrogen-containing hormone therapy (HT) in women within 10 years from the onset of menopause. However, the potential risks associated with long-term administration of HT, such as breast cancer and stroke, remain a concern for therapy recipients, underlying the need for an alternative treatment that is functionally equivalent but with a greater safety profile. Owing to their structural and functional resemblance to mammalian estrogens and lack of evident adverse effects, research interest in plant-derived phytoestrogens has increased in the past decade. While multiple health-promoting benefits of phytoestrogens have been proposed from basic science, the clinical data remain inconclusive. This review provides a comparative analysis of human studies on the effects of soy-based isoflavones on cognition. Of the eight studies published in 2000–2007, seven were conducted in postmenopausal women, four of which revealed a positive impact of isoflavones on cognitive function. Multiple factors could have contributed to the discrepant outcomes across studies, such as variation in the composition of phytoestrogen interventions and the heterogeneous characteristics of the study population. Thus, a well-designed clinical study based on a standardized stable formulation in a well-characterized study population is required in order to reach a clinical consensus. A formulation composed of select estrogen receptor β -selective phytoestrogens with a rationally designed composition would avoid the potential antagonism present in a mixture and thus enhance therapeutic efficacy. In addition, inclusion of equol in a study formulation offers a potential synergistic effect from equol in both equol-producing and nonproducing individuals, as well as added benefits for men. With respect to the design of study population, a clinically consistent effect could potentially be achieved by stratifying populations based on genotype, age, hormonal history and even diets. Development of an effective phytoestrogen formulation would benefit both women and men to prevent or treat hormone-dependent conditions and, most of all, to improve neurological health and reduce the risk of Alzheimer's disease.

Expert Rev. Neurotherapeutics 7(11), 1549–1564 (2007)

Women's Health Initiative Memory Study & follow-up messages

Estrogen-containing hormone therapy (HT) has long been suggested to promote cognitive health and reduce the risk of developing Alzheimer's disease (AD) in postmenopausal women [1,2]. Until recent years, such an inter-relation has been challenged by results from the Women's Health Initiative (WHI) Memory Study (WHIMS).

The aim of WHIMS, an ancillary study to the WHI hormone program, was to determine the effects of HT on global cognitive function and the risk of developing dementia in postmenopausal women [3]. WHIMS was conducted at 39 WHI clinical centers throughout the USA, involving a total of 7480 women between the ages of 65 and 79 who were free of dementia when the study started in 1995. A total of 2948 women who had undergone a

CONTENTS

Women's Health Initiative Memory Study & follow-up messages

Phytoestrogens: classification, distribution, metabolism & health effects

Human intervention studies of soy isoflavones on cognition

Standardization of human studies on phytoestrogens

Expert commentary & five-year view

Financial & competing interests disclosure

Key issues

References

Affiliations

[†]Author for correspondence
Department of Pharmacology & Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Pharmaceutical Sciences Center, Room 504, 1985 Zonal Avenue, Los Angeles, CA 90089, USA
Tel.: +1 323 442 1436
Fax: +1 323 224 7473
liqinz@usc.edu

KEYWORDS:

Alzheimer's disease, cognition, dementia, hormone therapy, human (clinical) study, phytoestrogen, soy isoflavone, standardization, Women's Health Initiative, Women's Health Initiative Memory Study

hysterectomy were included in the estrogen-alone study (E-Alone; one pill per day of conjugated equine estrogen [CEE], 0.625 mg; brand name Premarin™ by Wyeth). A total of 4532 women who had not had a hysterectomy were included in the combination estrogen plus progestin study (E + P; one pill per day of CEE, 0.625 mg, plus medroxyprogesterone acetate [MPA], 2.5 mg; brand name PremPro™ by Wyeth).

Results from a mean of 4.2 years of follow-up of the E + P arm of the WHIMS revealed that this combination HT not only did not benefit global cognitive function measured with the Modified Mini-Mental State Examination [4], but also doubled the risk for probable dementia in postmenopausal women aged 65 years or older [5]. Results from a mean of 5.4 years of follow-up of the E-Alone arm of the WHIMS showed a similar risk for global cognition and probable dementia in women at the same age range, although these adverse effects were not clinically significant [6,7]. Although these results clearly indicate that estrogen-containing HT does not promote cognition or reduce the risk of developing dementia in women aged 65 years or older, the observed actual number of cases of dementia was small. Overall, 61 women were diagnosed with probable dementia in the E + P study and the ratio between women taking the therapy and women taking the placebo pill was 40:21 [5]. In the E-Alone study, 47 women were diagnosed with probable dementia and the ratio between women taking the therapy and women taking the placebo pill was 28:19 [7]. Translated to a population of 10,000 older women taking the E-Alone therapy, there would be an additional 12 cases of dementia per year; for 10,000 women taking the E + P therapy, there would be an additional 23 cases of dementia per year [5,7]. Owing to an increased incidence of breast cancer, and the increased risks for heart attacks, stroke and blood clots that outweighed the benefits of prevention of hip fractures and colorectal cancer in women on the E + P therapy, all the E + P components of the WHI, including the WHIMS, were halted in July 2002 [8]. In February 2004, owing to the findings of similar risks for stroke and blood clots and no reduction of the risks of coronary heart disease (CHD), all the E-Alone therapy components of the WHI were halted, prior to the planned end date in 2005 [9].

Since then, rigorous debate has ensued in the research community. Multiple factors have been proposed to explain the disparity between the negative outcomes from the WHIMS and positive results from a large number of observational studies [10–13]. First, the combination E + P therapy of the WHIMS included a synthetic progestin, MPA, which may have induced greater adverse impact of E + P than E-Alone therapy on the risk of dementia [14]. Second, the WHIMS was conducted in women aged 65 years or older. It has been hypothesized that the time of initiating HT and duration of HT exposure could be significant regulators of the health impact of HT, particularly on cognition, in postmenopausal women [15]. Specifically, the advanced age at which HT was started in the WHI and WHIMS trials could have contributed to the overall outweighed risks compared with the benefits of HT for women's health [201]. In support of this hypothesis, a prospective study

conducted in Cache County, Utah, USA, revealed that prior HT use was associated with a threefold lower risk of developing AD in women who began HT at the time of menopause and continued the therapy for 10 years [16]. Age-related effects of HT use on cognition were further indicated by results from the recent Research into Memory, Brain Function and Estrogen Replacement (REMEMBER) pilot study, where it was found that early initiation of HT during menopause was beneficial for some cognitive domains, while initiation of HT in late menopause appeared to be detrimental [17]. A summary of a number of observational studies that consistently reported positive results of a prevention paradigm for HT treatment indicates a 20–50% reduction of the risk for developing AD in women who began HT at the time of menopause [16,18–22]. However, some experts argue that observational studies may be systematically biased, since hormone users tend to be healthier and better educated than nonusers [10].

Recent data from a reassessment of findings from the WHIMS further supported such a theory. A follow-up study that was part of the WHIMS, in which 7153 women aged 65–79 years provided information on their past HT exposure, revealed that women who reported using any form of HT before they were 65 years old were nearly 50% less likely to develop AD or other types of dementia than women who did not use such a therapy by that age. However, women who started the E-Alone therapy after the age of 65 years had an approximately 50% increased risk of developing dementia. The risk was nearly double among women using the combined E + P therapy [202]. The impact of 'age factor' on the study outcomes is also illustrated in a recent release of a secondary analysis of the data from both arms of the WHI to explore a trend of the effect of HT on CHD and stroke in relation to the distance from the menopause [23]. Three subgroups of women based on age (50–59, 60–69 and 70–79 years) or years since the onset of menopause (less than 10, 10–19 and 20 years or more) were examined separately. The primary results from the WHI trials showed that there was no benefit of HT for prevention of CHD when data from both younger and older women were collapsed together [8]. The new study further confirmed the previous results. However, the extent of the impact varies by age or years since menopause. That is, women who initiated HT closer to menopause tended to have a reduced CHD risk compared with an increase in CHD risk among women who started the therapy at a time more distant from menopause, although this trend did not meet criteria for statistical significance (Hazard ratio [HR]: 0.93 for 50–59 years, 0.98 for 60–69 years and 1.26 for 70–79 years, $p = 0.16$; or HR: 0.76 for less than 10 years since menopause, 1.10 for 10–19 years and 1.28 for 20 or more years, $p = 0.16$) [23]. A similar trend was observed for total mortality where the effect of HT was more favorable in younger than older women (HR: 0.70 for 50–59 years, 1.05 for 60–69 years and 1.14 for 70–79 years; $p = 0.06$) [23]. However, elevated risk of stroke associated with HT (HR: 1.32) did not appear to be affected by age or time since menopause [23]. In addition, this new study confirmed the overall risk for breast

cancer due to HT. There appeared to be an increased risk of breast cancer even in women who started the E + P treatment within 10 years of menopause [23]. Furthermore, in a most recent report of another follow-up study, the WHI Coronary Artery Calcium Study, involving 1064 women aged 50–59 years at enrollment in the E-Alone arm of the WHI, the computed tomography imaging conducted at 28 of the 40 WHI clinical centers revealed that women receiving the E-Alone therapy for a mean of 7.4 years followed by a mean of 1.3 years of lag time prior to the imaging were 30–40% less likely to have measurable levels of calcified plaque in the coronary arteries, a marker for atherosclerotic plaque burden and predictor for future risk of cardiovascular events, than women receiving the placebo treatment [24]. Women who had an at least 80% adherence to the treatment had a 60% lower risk of severe calcium plaque [24]. These results are consistent with Rossouw's report, suggesting that HT may be protective against heart diseases in younger but not older women [23,24].

Although the later stage outcomes of women who received early HT are still unclear, it is anticipated that these new findings will redress the negative impact of earlier reports from the WHI and WHIMS on the public and offer some reassurance to younger women and their physicians of certain health-promoting benefits that HT can provide if the treatment is initiated soon after the onset of menopause before the endogenous estrogen is significantly diminished as a result of menopause. However, caution should be taken since estrogen has complex effects and potential health risks could become apparent upon long-term administration, as highlighted by a recent disclosure of the correlation between the age-adjusted incidence rate of breast cancer and use of HT in postmenopausal women [25]. Before the question of whether any possible short-term benefit from HT to postmenopausal women in their 50s would extend into older ages if they were to continue using the therapy is answered, HT is recommended to be used on a short-term basis to alleviate menopausal symptoms in recently menopausal women, but should not be used on a long-term basis for the

prevention of diseases such as cardiovascular diseases and cognitive decline that are associated with estrogen deficiency due to menopause. Meanwhile, the problematic aspects associated with HT have spurred research interest in an attempt to find an estrogen alternative therapy that can mimic the beneficial effects of estrogen on women's health while not eliciting the deleterious effects as observed in WHI trials. In the last several years, research has focused on two types of estrogen alternatives: phytoestrogens and synthetic selective estrogen receptor (ER) modulators (SERMs). This article reviews phytoestrogens and their potential effects on human health with a focus on cognition.

Phytoestrogens: classification, distribution, metabolism & health effects

Phytoestrogens are a class of naturally occurring polyphenolic molecules that structurally resemble mammalian estrogens but are distributed in plants [26]. Owing to the structural similarities, phytoestrogens can bind to mammalian ERs; some of them have a binding preference to ER β , but overall they have weaker binding affinities when compared with the female endogenous estrogen 17 β -estradiol. In mammalian systems, through their interactions with ERs, phytoestrogens can moderately interfere with the endogenous estrogen-responsive signaling and result in either estrogenic or antiestrogenic bioactivities, depending on the status of the endogenous estrogens and the distribution of two ER subtypes, ER α and ER β [27,28].

Three major subclasses of phytoestrogens have been identified and chemically defined as isoflavones, lignans and coumestans (TABLE 1) [26]. Isoflavones are predominantly enriched in red clover and legumes such as soybeans; lignans are largely distributed in oilseeds such as flaxseeds; and coumestans are widely distributed in plant sprouts including red clover sprouts and alfalfa sprouts. Mature red clover leaf is the richest known source of total isoflavones, mainly biochanin A and formononetin, along with lesser amounts of genistein and daidzein. Biochanin A and formononetin are *O*-methylated precursor

Table 1. Three main classes of naturally occurring phytoestrogens, isoflavones, lignans and coumestans*.

| Classification | Main source | Main constituent(s) | Concentration (mg/100 g dry weight) [‡] |
|----------------|--------------------|---------------------------------|--|
| Isoflavones | Soybeans | Genistein | 26.8–84.1 |
| | | Daidzein (\rightarrow equol) | 10.5–56.0 |
| | | Glycitein | 6.72–20.40 |
| | Red clover | Biochanin A | 417 |
| | | Formononetin | 647 |
| Lignans | Flax seeds | Matairesinol | 1.087 |
| | | Secoisolariciresinol | 369.9 |
| Coumestans | Red clover sprouts | Coumestrol | 28.1 |

*Equol is not present in nature but can be produced exclusively from daidzein metabolism in 30% of human adults.

[‡]Data from [29–31,203].

molecules of genistein and daidzein, respectively, and are enzymatically converted to genistein and daidzein once ingested in mammals [29]. Soy is the richest source of genistein, rich in daidzein and the known exclusive source of glycitein. Besides red clover and soy, kudzu root is another major botanical source for isoflavones, serving as the richest source of daidzein among all plants [30]. Flaxseed provides the richest dietary source of lignans, with a large amount of secoisolariciresinol and lesser amount of matairesinol [203]. Once ingested, these plant lignans are enzymatically converted to mammalian lignans, secoisolariciresinol to enterodiol, and matairesinol to enterolactone. Coumestrol is a major coumestan, with a structure quite different from isoflavones while highly estrogenic owing to the presence of two phenol groups at both ends which allows it to bind strongly to ERs as does genistein. Only a small trace amount of coumestrol exists in human diets [203].

Among all known phytoestrogens, soy-based isoflavones, owing to their enrichment in human diets (especially in Asian diets), are the most extensively studied. Substantial research had attributed the estrogenic activity of soy products mainly to genistein and daidzein, until the third main constitutive component, glycitein, was characterized [31]. Glycitein accounts for 5–10% of the total isoflavones in soy foods and may be up to 40% in some soy supplements made from soy germ [31]. These soy-derived isoflavones commonly exist as inactive but water-soluble glucosides (genistin, daidzin and glycitin), which, inside the mammalian digestive system, are converted to estrogenically active genistein, daidzein and glycitein by intestinal glucosidases, prior to absorption. Daidzein can be further metabolized to equol and *O*-desmethylangiolesin [32]. Because the bioavailability of estrogenically active soy isoflavones is largely dependent upon the metabolic conversion in the gastrointestinal tract, which can be significantly different between individuals, the estrogenic responses derived from the intake of even an equal amount of the same soy product could be varied in different individuals. One typical case known to date is the production of equol, a structural mimic of daidzein. Unlike genistein and daidzein, equol is not of plant origin, yet can be exclusively produced through the metabolism of daidzein that is catalyzed by intestinal microbial flora following the intake of pure daidzein or soy products [32]. Interestingly, wide variations in the ability to produce equol from daidzein metabolism exist both between animals and humans, and across human populations. It has been found that almost all rodents and monkeys can produce equol in large quantities; however, only approximately 20–35% of human adults have such equol-producing ability [33]. Since it was first identified in human urine [34], equol has been widely recognized for its highly potent estrogenic activity in some ER-distributed tissues, largely through strong binding to both ERs (with an approximately tenfold binding preference to ER β) that are roughly equal to genistein. By contrast, its precursor isoflavone, daidzein, only exhibits weak ER-binding affinity and estrogenic activity [33]. Therefore, it is conceivable that the variation in response to soy products could be significant in two distinct subpopulations, equol-producers and equol nonproducers.

It can be speculated that the clinical efficacy of a soy product in humans could be significantly enhanced by the presence of equol in equol producers as compared with equol nonproducers.

Historically, research interests in the health effects of soy isoflavones were spurred, in large part, by a number of early comparative observational studies, for example, studies conducted in Asian and Western populations, where large differences in the daily dietary intake of total isoflavones and other types of phytoestrogens exist. The daily consumption of total phytoestrogens, mainly soy isoflavones, in Asian populations such as Japanese and Chinese, is estimated to range from 20 to 80 mg, whereas the daily dietary intake of phytoestrogens in Caucasians in the USA has been estimated to be less than 1 mg, of which lignans account for 80% of the total phytoestrogen content, followed by isoflavones (20%) and a trace amount of coumestans ($p < 0.1\%$) [35]. Although it has been argued that high consumption of soy foods is only one of many potentially protective lifestyle factors that distinguish Asian and Western women, it has been associated, to a large extent, with the low incidence rates of a number of hormone-dependent conditions in Asian populations. For example, it was found that only 25% of Japanese and 18% of Chinese postmenopausal women suffer from hot flashes as compared with 85% of North American and 70% of European women [36]. In addition, historically, breast cancer rates in the USA have been four- to seven-times higher than those in Asia [37,38]. An interesting study conducted in Asian-Americans revealed a strong link between a low risk for breast cancers and the intake of tofu in these women [39]. Specifically, a lower breast cancer rate was observed in those Asian-American women who were born in Asia and then immigrated to the USA yet still consumed substantial soy foods, as compared with those women born in the USA who had more Americanized dietary structure with lower intake of soy isoflavones. Moreover, the same study revealed that the reduction of risk was only seen in perimenopausal women, not postmenopausal women [39]. As an example of how the demography and culture might change the dietary structure, in a study in 274 Japanese-American women aged 65 years or older living in King County, Washington State, USA, the mean dietary intake of soy isoflavones (10.2 mg/day) was about a quarter to a half that of women living in Japan, but higher than that consumed by Caucasian women [40].

The high content of soy isoflavones in Asian diets may have also contributed to the low prevalence of AD in Japan and China compared with that in the USA and Europe. FIGURE 1 summarizes the prevalence rates for all-cause dementias and subtypes AD and vascular dementia (VaD), reported in 22 studies across continents, 13 studies conducted in Asia (nine in Japan [41–47] and four in China [48–51]), four studies in North America (three in the USA [52–54] and one in Canada [55]) and five studies in Europe (two in Great Britain [56,57], one in France [58], one in Italy [59] and one in Sweden [60]). Despite a few exceptional cases, it is apparent that the overall prevalence rates for all-cause dementias in Japan and China are similar to the rate in North America, although they are lower than the rate in Europe. However, the prevalence ratios of AD to VaD in Japan and China are

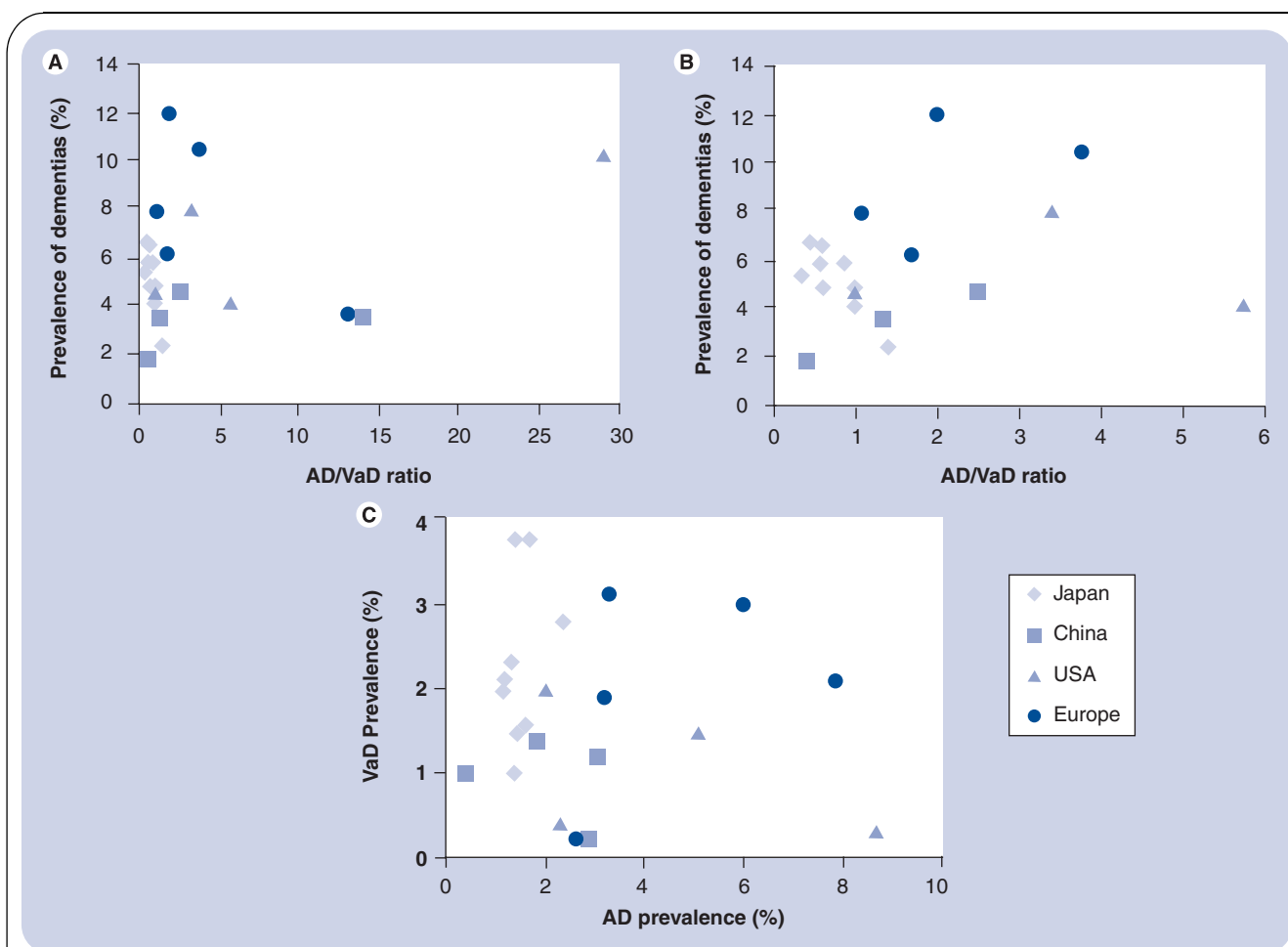


Figure 1. Summary of prevalence rates for all-cause dementias, and subtypes Alzheimer disease and vascular dementia, in Japan, China, North America and Europe. It indicates that: excluding the three exceptional cases (A), the overall prevalence rates for all-cause dementias in Japan (5.1%) and China (3.3%) are similar to the rate in North America (5.5%), and approximately half of the rate in Europe (9.1%) (B is an x-axis justified version of A). The figure also demonstrates that the prevalence ratios of AD to VaD in Japan (0.8%) and China (1.4%) are lower than the ratios in North America (3.4%) and Europe (2.1%) (A,B), as a result of lower prevalence rates for AD and similar rates for VaD in Japan (1.5% for AD vs 2.3% for VaD) and China (1.8% for AD versus 1.2% for VaD) compared with North America (3.1% for AD vs 1.3% for VaD) and Europe (5.1% for AD vs 2.5% for VaD) (C). These data suggest that AD is more prevalent in Western than Asian countries (~2.5-fold), while the prevalence of VaD is similar. Moreover, AD is more prevalent than VaD in Western countries (~2.2-fold); in comparison, the prevalence of AD and VaD is similar in Asian countries.
AD: Alzheimer's disease; VaD: Vascular dementia.

lower than the ratios in both North America and Europe, due to lower prevalence rates for AD and similar rates for VaD in Japan and China compared with North America and Europe. These data suggest that AD is more prevalent in Western than Asian populations (~2.5-fold), while the prevalence of VaD is similar. Moreover, AD is more prevalent than VaD in Western populations (~2.2-fold). In comparison, the prevalence of AD and VaD is similar in Asian populations, although the AD/VaD ratio has been increasing owing to greater longevity and a higher percentage of elderly, which leads to increased risk of AD [61]. In addition to the age factor, the AD prevalence rates in women have been consistently found to be higher than those in men across both Asian and Western nations [46,51–53].

Additional evidence for a potential role of dietary intake of soy isoflavones in preventing the onset of AD in Asian populations can be found in two large-scale studies conducted in Asian-Americans

that had adapted towards Western style diets [40]. The Honolulu-Asia Aging Study (HAAS) in a cohort of 3734 Japanese-American men (71–93 years) living in Hawaii, found the overall prevalence rates for all-cause dementias (9.3%) and AD (5.4%) [62] were similar to the rates reported in Europe (FIGURE 1; 9.1 and 5.1%, respectively) [56–60], plus a higher prevalence rate for VaD (4.2%) [62] than that in Europe (FIGURE 1; 2.5%) [56–60]. Similarly, the Kame Project, in a cohort of 3045 Japanese Americans (65 years or older) living in King County, Washington State, USA, found the overall prevalence rates for all-cause dementias (6.8%), AD (3.9%) and VaD (1.6%) [63] were similar to the rates reported in North America (FIGURE 1; 5.5, 3.1 and 1.3%, respectively) [52–55]. In agreement with these studies conducted in a single-cohort population, a retrospective analysis on data collected at AD Research Centers of California, compared the frequency of dementia etiologies between 1992

and 2002 in four ethnic groups [64]. Cases for all the neurodegenerative causes of dementia were identified for 452 Asian and Pacific Islander (API), 472 black, 675 Latino and 2926 white patients. No statistical differences were found among study groups, but there was a trend of less AD in API [64]. Taken together, these comparisons suggest that the prevalence of AD in older Japanese immigrants is similar to that among Caucasians in the USA and Europe, and higher than that among the matching population living in Japan. This trend correlates well with the daily intake of soy isoflavones among these three groups of populations. The dietary change associated with migration from Japan to the USA may have contributed to the increased risk for AD. However, this dietary factor appears to have less or no effect on the pathogenesis of VaD. Despite population-based epidemiological research which suggests a positive correlation between soy isoflavone intake and a reduction of AD prevalence, an adverse relationship was reported as part of HAAS, where higher midlife tofu consumption was associated with cognitive impairment and brain atrophy in late life [65].

In the last 5 years, research interest in soy isoflavones and other phytoestrogens have greatly expanded, largely owing to the revelations of health risks associated with estrogen-containing HT, in particular those seen in the WHI and WHIMS trials. In view of the promising results from *in vitro* culture and animal studies, it has been proposed that phytoestrogens could play a positive role in those areas when estrogen has demonstrated its effectiveness, particularly in postmenopausal women, such as alleviation of the climacteric symptoms [36], preservation of the bone mineral density [66,67], reduction of the risks of cardiovascular diseases [68] and improvement of cognitive function (reviewed later). However, at this time, the mixed results across studies remain problematic.

In addition to the impact of soy isoflavones on a woman's health, recent research revealed a reduction of risk of prostate cancer associated with soy products in men. A case-control study to compare the percentage of equol producers between prostate cancer patients and cancer-free control subjects resident in Japan, Korea and the USA, revealed that the incidence of prostate cancer was significantly higher in equol nonproducers, suggesting that the ability to produce equol or equol itself may be closely associated with reduced risk of prostate cancer [69]. The underlying mechanism in support of clinical findings indicated that equol can effectively block the action of the potent male hormone dihydrotestosterone (DHT), which normally stimulates prostate growth [70]. Unlike many conventional DHT blockers that inhibit a certain enzyme that converts testosterone to DHT, equol does not prevent DHT from being made but prevents it from functioning. It directly binds to DHT which prevents DHT from binding to the androgen receptor and thereby preventing the prostate from growing [70]. Since DHT is also relevant to male pattern baldness, acne and excess body hair, it has been proposed that equol may offer some help in treating these androgen-mediated conditions.

In addition to the research and discussions on the health-promoting effects of phytoestrogens, another important area of debate is the relationship between soy intake and breast cancer,

especially in women at high risk [71]. Concern has arisen from a number of *in vitro* and animal studies revealing that treatment with soy isoflavones increased the growth of breast cancer cells [72,73]. However, there is no clinical data in support of such a link. And, in fact, as reviewed earlier, epidemiological studies suggested that high dietary intake of soy isoflavones may be associated with reduced risk of breast cancer in Japanese and Chinese women. It is argued that the differences between non-human and human, for example, metabolism, and differences in experimental design and methods, would induce different outcomes. Thus, it is inadequate to extrapolate the conclusions from *in vitro* and animal data to humans [74]. At this time, before further clinical data are available for a definite conclusion, it is recommended that "the impact of isoflavones on breast tissue needs to be evaluated at the cellular level in women at high risk for breast cancer" [75].

Human intervention studies of soy isoflavones on cognition

The effects of phytoestrogens on the nervous system and cognitive function have received much less attention than their effects in the peripheral systems, although increasing evidence suggests that as mammalian estrogen does, phytoestrogens may play a role in the brain [76,77]. Both ER subtypes, ER α and ER β , are enriched throughout the brains of both rodents and humans, providing the structural basis required for estrogen actions in the brain [78–80]. It is demonstrated that activation of either ER α or ER β is able to activate the downstream signaling cascades leading to estrogen promotion of neuroprotection against various neurodegenerative insults [81,82]. ER β appears to play a more central role in the regulation of brain development and mediation of estrogen promotion of neuronal plasticity and memory function [83]. Results of both *in vitro* cellular and *in vivo* animal studies indicate that phytoestrogens at a physiologically relevant concentration are effective at promoting neuroprotection, neurogenesis and memory [84–88]; however, the neuroendocrine actions of high-dose phytoestrogens are largely antiestrogenic [76,89] or cytotoxic [90,91]. Clinical research on the impact of phytoestrogens on brain function has been sparse. Few available observational studies showed that a short-term consumption of soybeans had a small positive effect on the cognitive function in women aged 25–40 years [92,93]. Eight randomized and placebo-controlled intervention studies of soy isoflavones on cognition, which were reported in 2001–2007, are identified (TABLE 2) and reviewed below in an attempt to identify a trend for the correlation, particularly in postmenopausal women.

Among the eight human intervention studies, five studies revealed a positive effect of soy isoflavone administration on select cognitive activities. The first study, conducted in London, UK, examined the effects of supervised intake of high versus low soy diets on attention, memory and frontal lobe function in young healthy adults (average age 25 years) of both sexes [94]. Results from this 10-week intervention study showed that intake of the high-soy diet (100 mg total isoflavones per day) significantly improved both verbal and nonverbal short-term memory, long-term memory and mental flexibilities in

Table 2. Human intervention studies of soy isoflavones on cognition (2000–2007).

| Design | Location | Population | Intervention | Duration | Outcomes | Ref. |
|----------|---------------|---|--|----------|--|------|
| RP | London, UK | 27 healthy young adults (15 males and 12 females); mean age: 25 years | High soy diet (100 mg total isoflavones/day) vs low soy diet (0.5 mg total isoflavones/day); two diets were matched for protein, carbohydrates and fat composition | 10 weeks | In both males and females: ↑ DMTS-CANTAB (short-term nonverbal memory; $p < 0.05$) ↑ logical memory and recall Weschler revised (short-term verbal memory; $p < 0.05$) ↑ picture recall (long-term episodic memory; $p < 0.05$) → category fluency (verbal fluency and semantic memory) → PASAT (sustained attention) ↑ IDEB-CANTAB (mental flexibility; $p < 0.05$) ↑ mood In females only: ↑ letter fluency (frontal function; $p < 0.01$) ↑ SoC-CANTAB (planning ability; $p < 0.05$) | [94] |
| RDBP, Pa | London, UK | 50 healthy PMW; 51–66 years (mean age 58.0 years); >1 year of menopause (mean: 9.1 years, 66% >5 years); no HT in the previous 12 months | Soy supplement (60 mg total isoflavones/day) | 6 weeks | ↑ DMTS-CANTAB (short-term nonverbal memory; $p < 0.03$) → logical memory and recall Weschler revised (short and long-term verbal memory) → picture recall (long-term episodic memory) → category fluency (verbal fluency and semantic memory) → PASAT (sustained attention) ↑ IDEB-CANTAB (mental flexibility: simple rule reversals; $p < 0.05$; compound rule reversals; $p < 0.03$) ↑ SoC-CANTAB (planning ability; $p < 0.05$) | [95] |
| RDBP, Pa | London, UK | 33 healthy PMW; 50–65 years (mean age: 57.8 years); >1 year of menopause (mean 8.1 years); no HT in the previous 12 months | Soy supplement (60 mg total isoflavones/day) | 12 weeks | → DMTS-CANTAB (short-term nonverbal memory) → logical memory and recall Weschler revised (short-term verbal memory) ↑ picture recall (long-term episodic memory; $p < 0.03$) → category fluency (verbal fluency and semantic memory) ↑ PASAT (sustained attention; $p < 0.03$) ↑ IDEB-CANTAB (mental flexibility: simple rule reversals; $p < 0.05$; compound rule reversals; $p < 0.003$) ↑ SoC-CANTAB (planning ability; $p < 0.05$) | [96] |
| RDBP | San Diego, US | 53 healthy PMW; 55–74 years (mean age: 60.7 years); >2 years of menopause (mean age: 10.9 years); no HT currently (43% past users for 1–6 years, 0–13 years since last use) | Soy supplement (110 mg total isoflavones/day) | 6 months | → logical memory and recall Weschler (short and long-term verbal memory) ↑ category fluency (verbal fluency and semantic memory; $p = 0.03$) → Trails A: Halstead-Reitan (visuomotor tracking and attention) ↑ Trails B: Halstead-Reitan (visuomotor tracking and attention; $p = 0.08$ in all women; $p = 0.007$ in women at ages 50–59 years) | [97] |

→ no change in performance; ↑ improvement in performance. CANTAB: Cambridge Neuropsychological Test Automated Battery; CO: Crossover design; DMTS: Delayed Matching To Sample; HT: Hormone therapy; IDEB: Attentional Set Shifting; MMSE: Mini-Mental State Examination; Pa: Parallel design; PASAT: Paced Auditory Serial Addition Test; PMW: Postmenopausal women; RDBP: Randomized double-blind placebo-controlled; RP: Randomized placebo-controlled; SoC: Stockings of Cambridge.

Table 2. Human intervention studies of soy isoflavones on cognition (2000–2007) (cont.).

| Design | Location | Population | Intervention | Duration | Outcomes | Ref. |
|----------|----------------------|---|--|-----------|---|-------|
| RDBP, CO | Milan & Rome, Italy | 76 healthy PMW; mean age: 49.5 years; >1 year of menopause (mean 5.7 years); no HT use in the previous 8 weeks | Soy supplement (60 mg total isoflavones/day; 40–45% genistein; 40–45% daidzein; 10–20% glycitein) | 6 months | ↑ six of eight measures in Digit Symbol Test (psychomotor performance; pairs recalled correctly; $p < 0.05$), Digit Span Test (immediate auditory attention and mental flexibility; backward recall of digits; $p = 0.05$) and Visual Scanning Test (distractibility and visual inattentiveness) ↑ eight of nine self-rating mood scales, five significant, $p < 0.05–0.001$; eight of eight visual analogue scales, seven significant, $p < 0.05–0.001$ ↑ preference to isoflavone treatment (49 participants) vs nine to placebo vs 18 no preference | [98] |
| RDBP | Utrecht, Netherlands | 175 healthy PMW; 60–75 years (mean age: 66.6 years); >1 year of menopause (mean 18.0 years); no HT in the previous 6 months | Soy protein supplement (99 mg total isoflavones/day; 52 mg genistein, 41 mg daidzein and 6 mg glycitein) | 12 months | → 16 measures in tests of memory: Rey Auditory Verbal Learning Test, Digit Span Test, Doors Test; complex attention: Trail-making Test, Digit Symbol Substitution Test; verbal skills: Verbal Fluency Test, Boston Naming Task; global cognition and dementia: MMSE | [99] |
| RDBP, Pa | Hong Kong, China | 176 (168 complete) healthy PMW; 55–76 years (mean age: 63.5 years); >5 years of menopause (mean 13.8 years); no HT in the previous 6 months | Soy supplement (80 mg total isoflavones/day) | 6 months | → 15 measures in tests of learning and memory: Hong Kong List Learning Test, Rey-Osterrieth Complex Figure Test and Wechsler Memory Scale-Revised; executive function: Trail-making Test, Verbal Fluency Test; attention and concentration: Digit Span Test; motor control: Finger Tapping Test; language: Boston Naming Test; visual perception: Rey-Osterrieth Copy Trial; global cognition and dementia: MMSE | [100] |
| RDBP | Pullman, US | 79 healthy PMW; 48–65 years (mean age: 56.1 years); 1–35 years of menopause (mean 7.7 years); no HT in the previous 6 months | Soy milk (72 mg total isoflavones/day; 37 mg genistein, 31 mg daidzein and 4 mg glycitein) or soy supplement (70 mg total isoflavones/day; 33 mg genistein, 30 mg daidzein and 7 mg glycitein) | 16 weeks | → 16 measures in tests of memory: Digit Ordering Test, Color Matching Test, Benton Visual Retention Test, Visual Pattern Recognition; selective attention: Stroop Test | [101] |

→ no change in performance; ↑ improvement in performance. CANTAB: Cambridge Neuropsychological Test Automated Battery; CO: Crossover design; DMITS: Delayed Matching To Sample; HT: Hormone therapy; IDEE: Attentional Set Shifting; MMSE: Mini-Mental State Examination; Pa: Parallel design; PASAT: Paced Auditory Serial Addition Test; PMW: Postmenopausal women; RDBP: Randomized double-blind placebo-controlled; RP: Randomized placebo-controlled; SoC: Stockings of Cambridge.

both sexes. In comparison, a significantly improved performance in two other tests of frontal lobe function, letter fluency and planning, associated with the high-soy diet, were observed only in females. There was no effect of diet on tests of attention and in a category generation task related to semantic memory [94]. Despite the small sample size and other limitations, this study clearly indicates that soy isoflavones have the potential to benefit cognition in both women and men.

Following the first study, the same investigators conducted another two studies using the same cognitive test battery but in postmenopausal women aged 50–66 years [95,96]. These two studies revealed a similar beneficial effect of soy isoflavones on some of the measures of cognitive function in older women. In summary, women receiving 60 mg total isoflavones per day for 6 or 12 weeks had a better overall performance on tests of episodic memory, although there were variations with regard to the specific tasks. Furthermore, in comparison with a less robust effect in memory tests, it was consistently found in both studies that soy isoflavone administration significantly improved performance on both tests of frontal lobe function, mental flexibility and planning ability [95,96]. In addition, the 12-week study found an additional benefit of soy isoflavones on sustained attention [96], which, however, was not observed in the 6-week study [95], suggesting that longer duration of the treatment may offer greater health benefits.

Concurrently, a separate study, the Soy and Postmenopausal Health In Aging Study (SOPHIA), was launched in the USA [97]. In comparison with the studies conducted by File *et al.* [95,96], in this study, Kritz-Silverstein and her group examined a higher dose of soy isoflavone administration (110 mg total isoflavones per day) for a longer duration (6 months) in an average older population of postmenopausal women (55–74 years) [97]. Kritz-Silverstein reported that owing to a learning effect over time, women in both the isoflavone treatment group and the placebo group showed an improvement in performance on all five cognitive tests with a focus on attention and verbal memory. However, women in the active treatment group tended to show greater improvement for four of the five tests than women in the placebo group, with a significant difference in category fluency (23% improvement in the treatment group vs 3% improvement in the placebo group), a test of verbal memory. Results were similar after adjustment for age and education. Furthermore, the investigators conducted a stratified analysis of both groups on change in test scores in younger (aged 50–59 years) versus older (aged 60–74 years) women after adjustment for education, and they found a significant improvement in trails B, a test of visuo-motor tracking and attention, in the active treatment group compared with the placebo group among the younger women. No such an improvement was observed among the older women [97]. These results suggest that soy isoflavones may have a favorable effect on cognitive function, particularly verbal memory, in postmenopausal women. Furthermore, soy isoflavones may have greater impact in perimenopausal to early menopausal women on select cognitive functions relative to the impact in older postmenopausal women.

In support of those early studies, a recent study conducted in Milan and Rome, Italy, revealed that daily administration of 60 mg total isoflavones in postmenopausal women with a mean age of 49.5 years and a mean of 5.7 years of menopause, for 6 months, was associated with a small-to-moderate improvement in performance in three standard cognitive tests [98]. More impressively, women receiving the soy supplement treatment had significantly better mood than women on placebo treatment, as reflected by the three self-rating mood scales and the eight visual analogue scales, where five of nine and seven of eight scores, respectively, were statistically significant between two treatment groups. Furthermore, 48 participants reported a preference to isoflavone treatment, as compared with eight to placebo and 18 reporting no preference [98]. These results are particularly significant in that they provide strong support for a possible role of soy isoflavones in alleviating or reversing the psychological disturbances often associated with menopause and improving the overall quality of life in postmenopausal women.

In contrast to the above studies, three studies failed to demonstrate a favorable effect of soy isoflavone intake on cognitive function, including the study with the longest duration [99] among all eight studies published to date. In this study conducted in Utrecht, Netherlands, Kreijkamp-Kaspers *et al.* investigated the effects of a 12-month intervention of a soy protein supplement, containing 99 mg total isoflavones per day (52 mg genistein, 41 mg daidzein and 6 mg glycitein), on cognitive function, along with measures of bone mineral density and plasma lipids, in postmenopausal women aged 60–75 years [99]. These women were more advanced in age (mean age 66.0 years) and had been postmenopausal for a mean of 18 years. In addition, approximately 21 and 19% of women were on active cholesterol-lowering and antihypertensive medication, respectively [99]. No significant differences in performance were found in all measures that are sensitive to cognitive aging, including memory, verbal skills, complex attention functions and risk of dementia, between the soy protein supplement group and the placebo group [99]. Similarly, no significant changes were found in measures of bone mineral density and lipid profiles between two groups [99].

In agreement with Kreijkamp-Kaspers's findings, a similar study conducted in Hong Kong, China, found no significant effect of daily intake of a total of 80 mg soy isoflavones in Chinese postmenopausal women aged at 55–76 years (mean age 63.5 years) and with at least 5 years (mean 13.8 years) of menopause on 13 standardized neuropsychological tests of memory, executive function, attention, motor control, language, visual perception and a global function assessment, as compared with the placebo group [100]. This is the first and only study conducted in a population for which soy is a staple food throughout life. The mean usual dietary isoflavone intake in this study population was approximately 20 mg/day. The investigators postulated that the insignificant finding of a positive effect of soy supplement in a Chinese population of postmenopausal women may be related to their lifetime habitual intake of soy foods that could have had some overall protective effects on

cognitive function, even among the placebo group, and thus could have lessened the between-group differences of the trial results [100].

In another recent study conducted in the USA, Fournier and colleagues examined the respective effects of soy milk and a soy isoflavone supplement intervention over a 16-week period on several cognitive tasks that measured behavioral responses (accuracy and response time) in 79 healthy postmenopausal women [101]. In comparison with the study population included in the other two studies that failed to show a effect [99,100], women recruited in this study were relatively younger at a mean age of 56.1 years (range 48–65 years), but had a similar wide range of years since the onset of menopause (1–35 years, mean 8 years). It was found neither soy milk containing 72 mg total isoflavones per day nor soy isoflavone supplement containing 70 mg total isoflavones per day yielded any appreciable improvement on performance in all tests of selective attention or working (verbal and visual-spatial), visual-spatial short-term and long-term memory [101].

In summary, eight intervention studies addressing the relationship between soy isoflavones and cognition in humans are identified. Of them, one study conducted in young adults of both sexes showed a beneficial effect of a high soy diet on select cognitive activities. Among seven studies conducted in postmenopausal women, four demonstrated a beneficial effect of soy supplementation primarily on verbal memory and frontal lobe function. Three studies failed to demonstrate such an effect, including a study conducted on Chinese postmenopausal women with a high exposure to soy foods throughout life [100]. To conclude, although a subset of studies report promising findings, a definite conclusion cannot be drawn yet owing to the inconsistencies across studies. Thus, it is important to address the potential factors that may have contributed to the mixed outcomes and take these factors into the design of future studies.

Standardization of human studies on phytoestrogens

As exemplified in TABLE 2, the data remains sparse and inconclusive with respect to the effects of phytoestrogens on human health. The overall results derived from basic science research in cell cultures and animals are encouraging, however, there is either a lack of clinical data in support of preclinical findings, or the results derived from clinical studies have been inconsistent. Multiple factors could have contributed to the disparities between the *in vitro/in vivo* preclinical and clinical data, or data from various clinical studies. One main impact factor that could have contributed to the discrepant clinical outcomes is a lack of standardization across studies, including, but not limited to, the composition of the study interventions, the profile of the characteristics of the study populations and the outcomes measured. Elaborated below is the potential impact from the variations in these key elements on clinical results. It is anticipated these analyses would emphasize the importance of standardization of these elements in order to reach a clinical consensus in search for a definite answer for phytoestrogen use in relation to human health.

Phytoestrogen composition

Significant research has related the health effects of soy-based foods and products to two most extensively studied isoflavones, genistein and daidzein. Increasing evidence indicates that besides genistein, daidzein and recently discovered glycitein, there are a number of additional structurally similar and hormonally active phytoestrogenic molecules present in soy foods and other products, and the types and amounts of these molecules can be significantly different from source to source. First, the complex biosynthetic pathway leading to the production of various phytoestrogenic molecules in soy plants could be readily altered in response to external stimuli, such as environmental factors and growth conditions, adding more complexities in the phytoestrogen profile. For instance, soy plants grown under stressed conditions were found to involve increased biosynthesis of isoflavonoid phytoalexin compounds, glyceollins, which can represent up to 56% of the total isoflavone composition [102]. Increased accumulation of glyceollins can be also present in soy foods prepared from stress-treated soy such as freezing and fermentation [102]. Second, the contents and present forms (aglycons vs glucosides and solid vs liquid) of three main constitutive phytoestrogens, genistein, daidzein and glycitein, naturally present in different soy foods, could be significantly different leading to marked variations in bioavailability. As revealed in a comparative analysis by Cassidy *et al.*, consumption of tempeh (~50% as aglycons) resulted in higher serum peak levels of both genistein and daidzein compared with textured vegetable protein (predominantly isoflavone glucosides, <15% as aglycons). However, soy milk (<15% as aglycons) was absorbed faster and peak levels of isoflavones were attained earlier than with either tempeh or textured vegetable protein [103]. Additional variability in phytoestrogen composition in different soy supplements could be introduced from the preparation protocols. An extensive analysis of 33 commercially available phytoestrogen supplements or extracts revealed that there were not only considerable differences in actual isoflavone content from what was claimed by the manufacturers, but an abundance of peaks of unknown origin and chemical structure was found in many of the supplements [104]. Although the bioactivity of these unknown molecules remains undefined, their potential impact on the overall estrogenic activity and effects of a soy product on human health must not be neglected. Based on the facts and clinical data summarized in TABLE 2, one could reasonably conceive that the discrepant outcomes across studies could be, in large part, originated from the heterogeneity in the types and relative amounts of both known and unknown but intrinsically hormonally active molecules present in the soy products studied.

A complex formulation comprising a mixture of various phytoestrogenic molecules could induce either a synergistic or antagonistic effect depending on the composition and interactions among these molecules. One possible antagonism could be derived from the antagonistic interactions among components that have preference to bind to and activate either ER α or ER β . Research in primary neuron cultures revealed that coadministration of an ER α -selective agonist and an ER β -selective agonist

was less effective than treatment with either single agonist in various neuroprotective measurements, suggesting that although both ER α and ER β contribute to estrogen promotion of neuronal survival, simultaneous activation of both ER α and ER β in the same context may diminish the efficacy [81]. Thus, it can be extrapolated that such an antagonism may occur in a complex formulation composed of various known and unknown constituents, such as a soy-derived supplements or extracts, which may offset the overall effect and cause clinical insignificance.

Another possible antagonism present in a complex formulation could be induced by molecules that are predominantly antiestrogenic. For instance, glyceollins that are rich in soy plants grown under stressed conditions and processed such as fermented soy foods, exerted potent antiestrogenic effects in a number of hormone-responsive systems, whereas genistein and daidzein were found to be estrogenic at similar concentrations [102]. In addition, most phytoestrogens possess a biphasic estrogenic and antiestrogenic activity depending on the dose, target tissue and/or estrogen status in a mammalian system. For instance, studies have shown that some phytoestrogens tend to exert an estrogenic effect at relatively low doses, and develop antiestrogenic [105] or cytotoxic [90,91] effects at higher doses. This dose and tissue-related biphasic effect has significant implications for the design of a clinical study with a specific clinical end point. For example, in a study addressing the relationship between phytoestrogen use and cancer prevention, an overall antiestrogenic activity would be advantageous, whereas in a study that investigates the protective effects of phytoestrogens on the cardiovascular system, bone mass and cognition, an estrogenic activity would be desired. Moreover, the dose of phytoestrogens studied could significantly impact the efficacy and safety. On one hand, a low dose could be insufficient to induce a clinically significant effect. As revealed in the observational Study of Women's Health Across the Nation (SWAN) that included a cohort of midlife Chinese and Japanese women aged 42–52 years, low dietary genistein intake, 6.8 and 3.5 mg/day, respectively, could be a factor for the lack of association between genistein intake and measures of cognitive performance in either ethnic groups [106]. On the other hand, a high dose during long-term treatment could raise the red flag for safety reasons. This can be illustrated by results from two clinical studies that assessed the effects of phytoestrogens on histological characteristics of endometrium, a target tissue with high risk for neoplasia associated with the use of steroidal estrogen-containing HT. One study revealed that a 5-year exposure to 150 mg soy isoflavones significantly increased the occurrence of endometrial hyperplasia, although the absolute number was relatively small, six cases of 154 participants on isoflavone treatment (3.8%) versus 0% in placebo group [107]. However, at a low dose that was sufficient to alleviate menopausal hot flashes to a large degree (56.4% reduction in the mean number of hot flashes) [108] and to reduce cardiovascular risk markers [109], no adverse effects in the endometrium were observed in women exposed to a daily intake of 54 mg pure genistein after 2 years [109].

In summary, the phytoestrogen composition may serve as a major determinant of the overall effects of a soy-based product on human health. An optimal composition and dose should be determined based on the target clinical outcomes investigated. Standardization of such a composition would eliminate the variation related to the phytoestrogens studied and make the data from various studies comparative.

Study population

The characteristics of the study population is another potential factor that may affect study outcomes. Recent re-analyses of the findings from the WHI and WHIMS trials evidently indicate that the estrogen-containing HT may benefit younger women who start HT treatment within 10 years of the onset of menopause, while it appears to have an insignificant or even detrimental effect in older women who start the treatment 10 or more years later after the onset of menopause. Owing to the structural and functional resemblances between mammalian estrogens and phytoestrogens, it can be extrapolated that, as in the WHI and WHIMS trials, age and hormonal history of women may also regulate the health effects of phytoestrogens. In support of this hypothesis, Tempfer *et al* reviewed that in selected studies involving women with early natural postmenopause and mild-to-moderate vasomotor symptoms, a strong link between the phytoestrogen treatment and reduced hot flashes was observed [110]. Health benefits associated with early interventions with soy-based products are to some extent in concordance with the habitual consumption of a substantial amount of soy foods in Asian populations through life.

The impact of the characteristics of the study population could also be derived from the interindividual differences in equol production in humans. As reviewed earlier, equol binds to both ER α and ER β , although with a preference for ER β , at affinities comparable to genistein. In comparison, daidzein, the precursor isoflavone of equol, mainly binds to ER β at a ten- to 100-fold lower affinity than equol [111]. Furthermore, it was found that equol was most potent in inducing the transcriptional expression of β -galactosidase in yeast cells, especially in those transfected with ER α , among all test isoflavones [111]. In comparison, daidzein only induced a very weak transcription [111]. The substantial differences in the binding and estrogenic activity between daidzein and equol could dramatically influence the overall responses to soy consumption in humans. Therefore, the failure to factor these differences into human studies could be another major cause of the disparity in outcomes across studies. The more potent estrogenic activity associated with equol implicates that individuals with equol-producing ability could be more sensitive to phytoestrogen treatment [33]. In support of such a theory, a recent 1-year randomized trial revealed that an intake of 75 mg isoflavone conjugates per day in Japanese postmenopausal women offered a more favorable effect in preserving the bone mineral density in the 'equol-producers', with an annualized change of -0.04%, compared with the nonproducers, with a change of -0.46%. Overall, women in the isoflavone group exhibited a smaller change than the control group (-2.28

and -2.6%, respectively) [112]. A similar result was observed in an independent study conducted in an American postmenopausal study [113]. These studies confirm that the presence of equol in the circulating system can enhance the overall efficacy of isoflavones, at least on the bone system.

In addition to the intrinsic metabolic environment, equol production can be affected by other external factors. One factor is the form in which daidzein exists in a soy product. In a soy food or product that is made from soy plants grown under a normal condition, daidzein along with other isoflavones is present mainly in the inactive sugar-conjugated glycoside forms. The glycosides of genistein and daidzein are referred to as genistin and daidzin, respectively. However, in a stress-processed soy food or product made from soy germ, daidzein along with other isoflavones exist mainly in the unconjugated hormonally active forms, aglycons. A study by Setchell *et al.* revealed that equol appeared in the plasma of half of the women who ingested daidzin, the glucoside conjugate of daidzein, while it was not found in the plasma of the women who consumed daidzein [104]. The other known factor is the dietary matrix, which may impact the composition of the intestinal microflora and thus the amount of the equol produced in 'equol-producers'. Two human studies showed that a high equol production was associated with a high fiber diet [114,115]. In addition, since rodents have much greater capacity to produce equol, it may explain the difference between results derived from animal models that largely showed significant benefits of phytoestrogens, for example, on brain function, and data from human studies which demonstrated a heterogeneous picture.

On the other hand, owing to the potent estrogenic activity of equol through activation of ER α , the predominant ER subtype mediating estrogen functions in female reproductive tissues, the enhanced responsiveness to soy isoflavones in 'equol-producers' could lead to increased risk of estrogenic stimulation in the breast and endometrium upon a long-term use. In a study reported by Unfer *et al.*, six (3.8%) of 154 participants receiving soy tablets containing a total of 150 mg isoflavones daily for 5 years were diagnosed with endometrial hyperplasia, which was statistically significant compared with the placebo group (0%) [107]. It would be insightful to investigate whether the increased occurrence of endometrial hyperplasia is linked to the equol-producing status of individuals.

In summary, the heterogeneity in the characteristic profile of a study population could obscure the positive response from a favorable subpopulation and bring the overall effects of a soy product down below a clinically detectable range. A stratified investigation justified by defined characteristics including age, hormone status, equol-producing capacity and even habitual diet, would likely provide insights into a 'window of opportunity' for the use of phytoestrogens to maximally reap their health benefits.

Expert commentary & five-year view

Despite the fact that recent follow-up analyses of the previously collected data and findings from WHI and WHIMS have confirmed some health benefits of estrogen-containing HT in younger postmenopausal women, the potential risks, such as breast cancer

and stroke, remain. Phytoestrogens structurally resemble mammalian estrogens and thus may provide similar functional outcomes. Clinical data on the impact of phytoestrogens on human health is promising, while not convincing.

Identification of factors that may have caused discrepant outcomes across studies will be crucial for future design of human research. Standardization of the study interventions and populations may be essential in reaching a clinical consensus. Phytoestrogens bind to ERs at modest affinities, which may account for their weak-to-moderate estrogenic activity and low benefit to human health. Such small effects could be significantly impacted by external factors such as the composition of the study interventions and the heterogeneity of the characteristics of the human subjects. Thus, a high bar for the standardization of the key elements will be required in future human studies in search for a definite answer.

ER β has been suggested as a novel therapeutic target for the development of the an novel estrogen alternative therapy [83]. Selective targeting of ER β has three major advantages:

- To reduce antagonistic interactions that may occur in a complex preparation
- To minimize adverse effects associated with the activation of ER α in reproductive tissues
- To take advantage of additional beneficial health effects exclusively mediated by ER β [83,116]

One can envision that:

- A formulation composed of rationally selected ER β -selective phytoestrogens would offer a greater effect than a complex formulation mixed with both ER α and ER β -selective components;
- A composition composed of phytoestrogens with a rationally defined content that can induce a synergistic rather than antagonistic effect would likely generate enhanced and comparative results across studies that seek an estrogenic effect;
- A composition with inclusion of equol would impair the potential influence of the interindividual differences in the capacity to produce equol on the study outcomes, and thus elevate the estrogenic response across the study subjects;
- Inclusion of equol would be beneficial in men to help prevent male hormone-dependent disorders, such as prostate cancer.

In conclusion, estrogen-containing HT and alternative therapies will continue to be one of the most important focuses for research because they address serious issues that affect the health and quality of life of millions of women. Phytoestrogens, in particular soy-derived isoflavones, have potential therapeutic promise in both women and men. Criticism of such promise may lead to the loss of the opportunity to take advantage of the potential health benefits of these products, which have been widely consumed in Asian populations for centuries. Standardization of future human studies may be the only path that leads to a clinical consensus.

Financial & competing interests disclosure

The authors gratefully acknowledge the grant support from the Alzheimer's Association to L Zhao (NIRG-05-13838) and the Kenneth T and Eileen L Norris Foundation to RD Brinton. The authors have no other relevant affiliations or financial involvement

with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Recent follow-up analyses of the data and findings previously collected from the Women's Health Initiative and the Women's Health Initiative Memory Study confirmed some health benefits of the currently available estrogen-containing hormone therapy (HT) in younger women within 10 years from the onset of menopause.
- Specifically, in the CNS, estrogen-containing HT is associated with an approximate 50% reduction of risk for Alzheimer's disease (AD) or other dementias when started before the age of 65 years; by contrast, initiation of HT after 65 years of age increases the risk of developing dementias by 50% for the estrogen (conjugated equine estrogen [CEE])-alone therapy and the risk can be doubled for the combined estrogen (CEE) plus progestin (medroxyprogesterone acetate) therapy.
- Despite the plentiful health-promoting benefits in postmenopausal women, potential risks associated with the long-term administration of estrogen-containing HT, such as breast cancer and stroke, remain a serious concern for therapy recipients, underlying the medical need for an alternative treatment that is functionally effective but safe.
- Plant-derived estrogenic compounds, known as phytoestrogens, structurally resemble mammalian estrogens and exert weak-to-moderate estrogenic/anti-estrogenic activity in a concentration- and tissue-dependent manner. Owing to the lack of evident adverse effects as seen with estrogens, research interest in developing phytoestrogens into a potential estrogen alternative therapy has greatly expanded in the past decade.
- Eight human studies, seven conducted in postmenopausal women, were published in 2000–2007 that sought to determine the impact of phytoestrogens, in particular soy-based isoflavones, on cognitive function. However, data from these clinical studies are mixed and thus inconclusive.
- Multiple factors could have contributed to the discrepant outcomes across studies, such as variation in the composition of phytoestrogens present in the study interventions and the heterogeneous profile of the characteristics of the study population. Thus, well-designed clinical studies based on a standardized formulation in a well-characterized study population are required in order to reach a clinical consensus.
- From the study intervention perspective, a formulation composed of rationally selected estrogen receptor- β -selective phytoestrogens would avoid the potential antagonism present in a mixture and thus enhance therapeutic efficacy. Moreover, inclusion of equol in a study formulation offers a potential synergistic effect from equol in both equol-producing and nonproducing individuals, as well as added benefits for men.
- From the study population perspective, a clinically consistent effect could be achieved by stratifying populations based on genotype, age, hormonal history and even diets.
- In summary, given the current status of research, development of an effective phytoestrogen formulation would have therapeutic potential to benefit both women and men to prevent or treat hormone-dependent conditions, and most of all, to reduce age- and hormone deficiency-related risk of cognitive decline and AD.

References

- 1 Brinton RD. A women's health issue: Alzheimer's disease and strategies for maintaining cognitive health. *Int. J. Fertil. Womens Med.* 44, 174–185 (1999).
- 2 McEwen B. Estrogen actions throughout the brain. *Recent Prog. Horm. Res.* 57, 357–384 (2002).
- 3 Shumaker S. Hormone therapy in dementia prevention: the Women's Health Initiative Memory Study. *Neurobiol. Aging* 17(Suppl. 4), S9 (1996).
- 4 Rapp SR, Espeland MA, Shumaker SA *et al.* Effect of estrogen plus progestin on global cognitive function in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA* 289, 2663–2672 (2003).
- 5 Shumaker SA, Legault C, Rapp SR *et al.* Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA* 289, 2651–2662 (2003).
- 6 Espeland MA, Rapp SR, Shumaker SA *et al.* Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. *JAMA* 291, 2959–2968 (2004).
- 7 Shumaker SA, Legault C, Kuller LH *et al.* Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. *JAMA* 291, 2947–2958 (2004).

- 8 Rossouw JE, Anderson GL, Prentice RL *et al.* Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the women's health initiative randomized controlled trial. *JAMA* 288(3), 321–333(2002).
- 9 The Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative Randomized Controlled Trial. *JAMA* 291, 1701–1712 (2004).
- 10 Henderson VW. Estrogen-containing hormone therapy and Alzheimer's disease risk: understanding discrepant inferences from observational and experimental research. *Neuroscience* 138, 1031–1039 (2006).
- 11 Yesufu A, Bandelow S, Hogervorst E. Meta-analyses of the effect of hormone treatment on cognitive function in postmenopausal women. *Women's Health* 3, 173–194 (2006).
- 12 Zhao L, O'Neill K, Brinton RD. Selective estrogen receptor modulators (SERMs) for the brain: current status and remaining challenges for developing NeuroSERMs. *Brain Res. Brain Res. Rev.* 49, 472–493 (2005).
- 13 Sherwin BB. The clinical relevance of the relationship between estrogen and cognition in women. *J. Steroid Biochem. Mol. Biol.* 106, 151–156 (2007).
- 14 Brinton RD, Nilsen J. Effects of estrogen plus progestin on risk of dementia. *JAMA* 290, 1706 (2003).
- 15 Brinton RD. Estrogen therapy for prevention of Alzheimer's disease not for rehabilitation following onset of disease: the healthy cell bias of estrogen action. In: *Synaptic Plasticity: Basic Mechanisms to Clinical Applications*. Taylor & Francis Group, NY, USA 131–157 (2004).
- 16 Zandi PP, Carlson MC, Plassman BL *et al.* Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. *JAMA* 288, 2123–2129 (2002).
- 17 MacLennan AH, Henderson VW, Paine BJ *et al.* Hormone therapy, timing of initiation, and cognition in women aged older than 60 years: the REMEMBER pilot study. *Menopause* 13, 28–36 (2006).
- 18 Matthews K, Cauley J, Yaffe K, Zmuda JM. Estrogen replacement therapy and cognitive decline in older community women. *J. Am. Geriatr. Soc.* 47, 518–523 (1999).
- 19 Steffens DC, Norton MC, Plassman BL *et al.* Enhanced cognitive performance with estrogen use in nondemented community-dwelling older women. *J. Am. Geriatr. Soc.* 47, 1171–1175 (1999).
- 20 Jacobs DM, Tang MX, Stern Y *et al.* Cognitive function in nondemented older women who took estrogen after menopause. *Neurology* 50, 368–373 (1998).
- 21 Baldereschi M, Di Carlo A, Lepore V *et al.* Estrogen-replacement therapy and Alzheimer's disease in the Italian Longitudinal Study on Aging. *Neurology* 50, 996–1002 (1998).
- 22 Waring SC, Rocca WA, Petersen RC, O'Brien PC, Tangalos EG, Kokmen E. Postmenopausal estrogen replacement therapy and risk of AD: a population-based study. *Neurology* 52, 965–970 (1999).
- 23 Rossouw JE, Prentice RL, Manson JE *et al.* Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA* 297, 1465–1477 (2007).
- 24 Manson JE, Allison MA, Rossouw JE *et al.* Estrogen therapy and coronary-artery calcification. *N. Engl. J. Med.* 356, 2591–2602 (2007).
- 25 Ravdin PM, Cronin KA, Howlader N *et al.* The decrease in breast-cancer incidence in 2003 in the United States. *N. Engl. J. Med.* 356, 1670–1674 (2007).
- 26 Kurzer MS, Xu X. Dietary phytoestrogens. *Annu. Rev. Nutr.* 17, 353–381 (1997).
- 27 Dixon RA. Phytoestrogens. *Annu. Rev. Plant Biol.* 55, 225–261 (2004).
- 28 Zhao L, Brinton RD. Structure-based virtual screening for plant-based ER β -selective ligands as potential preventative therapy against age-related neurodegenerative diseases. *J. Med. Chem.* 48, 3463–3466 (2005).
- 29 De Rijke E, Zafra-Gomez A, Ariese F, Brinkman UA, Goojic C. Determination of isoflavone glucoside malonates in *Trifolium pratense* L. (red clover) extracts: quantification and stability studies. *J. Chromatogr. A* 932, 55–64 (2001).
- 30 Mazur W, Adlercreutz H. Naturally occurring oestrogens in food. *Pure Appl. Chem.* 70, 1759–1776 (1998).
- 31 Song TT, Hendrich S, Murphy PA. Estrogenic activity of glycitein, a soy isoflavone. *J. Agric. Food Chem.* 47, 1607–1610 (1999).
- 32 Setchell KD, Borriello SP, Hulme P, Kirk DN, Axelson M. Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *Am. J. Clin. Nutr.* 40, 569–578 (1984).
- 33 Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol – a clue to the effectiveness of soy and its isoflavones. *J. Nutr.* 132, 3577–3584 (2002).
- 34 Axelson M, Kirk DN, Farrant RD, Cooley G, Lawson AM, Setchell KD. The identification of the weak oestrogen equol [7-hydroxy-3-(4'-hydroxyphenyl)chroman] in human urine. *Biochem. J.* 201, 353–357 (1982).
- 35 de Kleijn MJ, van der Schouw YT, Wilson PW *et al.* Intake of dietary phytoestrogens is low in postmenopausal women in the United States: the Framingham study(1–4). *J. Nutr.* 131, 1826–1832 (2001).
- 36 Maskarinec S. The effect of phytoestrogens on hot flashes. *Nutrition Bytes* 9(2), 5 (2003).
- 37 Ziegler RG. Phytoestrogens and breast cancer. *Am. J. Clin. Nutr.* 79, 183–184 (2004).
- 38 Henderson BE, Bernstein L. The international variation in breast cancer rates: an epidemiological assessment. *Breast Cancer Res. Treat.* 18, S11–S17 (1991).
- 39 Wu AH, Ziegler RG, Horn-Ross PL *et al.* Tofu and risk of breast cancer in Asian-Americans. *Cancer Epidemiol. Biomarkers Prev.* 5, 901–906 (1996).
- 40 Rice MM, LaCroix AZ, Lampe JW *et al.* Dietary soy isoflavone intake in older Japanese American women. *Public Health Nutr.* 4, 943–952 (2001).
- 41 Kawano H, Ueda K, Fujishima M. Prevalence of dementia in a Japanese community (Hisayama): morphological reappraisal of the type of dementia. *Jpn J. Med.* 29, 261–265 (1990).
- 42 Hasegawa K. The clinical issues of age-related dementia. *Tohoku J. Exp. Med.* 161, 29–38 (1990).
- 43 Shibayama H, Kasahara Y, Kobayashi H *et al.* Prevalence of dementia in a Japanese elderly population. Unusual cases of presenile dementia with Fahr's syndrome. *Acta Psychiatr. Scand.* 74, 144–151 (1986).
- 44 Ichinowatari N, Tatsunuma T, Makiya H. Epidemiological study of old age mental disorders in the two rural areas of Japan. *Jpn J. Psychiatry Neurol.* 41, 629–636 (1987).
- 45 Ueda K, Kawano H, Hasuo Y, Fujishima M. Prevalence and etiology of dementia in a Japanese community. *Stroke* 23, 798–803 (1992).
- 46 Kiyohara Y, Yoshitake T, Kato I *et al.* Changing patterns in the prevalence of dementia in a Japanese community: the Hisayama study. *Gerontology* 40, 29–35 (1994).
- 47 Fukunishi I, Hayabara T, Hosokawa K. Epidemiological surveys of senile dementia in Japan. *Int. J. Soc. Psychiatry* 37, 51–56 (1991).
- 48 Liu HC, Chou P, Lin KN *et al.* Assessing cognitive abilities and dementia in a predominantly illiterate population of older individuals in Kinmen. *Psychol. Med.* 24, 763–770 (1994).

- 49 Li G, Shen YC, Chen CH, Zhao YW, Li SR, Lu M. An epidemiological survey of age-related dementia in an urban area of Beijing. *Acta Psychiatr. Scand.* 79, 557–563 (1989).
- 50 Zhang MY, Katzman R, Salmon D *et al.* The prevalence of dementia and Alzheimer's disease in Shanghai, China: impact of age, gender, and education. *Ann. Neurol.* 27, 428–437 (1990).
- 51 Wang W, Wu S, Cheng X *et al.* Prevalence of Alzheimer's disease and other dementing disorders in an urban community of Beijing, China. *Neuroepidemiology* 19, 194–200 (2000).
- 52 Bachman DL, Wolf PA, Linn R *et al.* Prevalence of dementia and probable senile dementia of the Alzheimer type in the Framingham Study. *Neurology* 42, 115–119 (1992).
- 53 Folstein MF, Bassett SS, Anthony JC, Romanoski AJ, Nestadt GR. Dementia: case ascertainment in a community survey. *J. Gerontol.* 46, 132–138 (1991).
- 54 Evans DA, Funkenstein HH, Albert MS *et al.* Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. *JAMA* 262, 2551–2556 (1989).
- 55 Group CSoHaAW. Canadian Study of Health and Aging: study methods and prevalence of dementia. *Can. Med. Assoc. J.* 150, 899–913 (1994).
- 56 Brayne C, Calloway P. An epidemiological study of dementia in a rural population of elderly women. *Br. J. Psychiatry* 155, 214–219 (1989).
- 57 O'Connor DW, Pollitt PA, Hyde JB *et al.* The prevalence of dementia as measured by the Cambridge Mental Disorders of the Elderly Examination. *Acta Psychiatr. Scand.* 79, 190–198 (1989).
- 58 Dartigues JF, Gagnon M, Michel P *et al.* The Paquid research program on the epidemiology of dementia. Methods and initial results. *Rev. Neurol. (Paris)* 147, 225–230 (1991).
- 59 Rocca WA, Bonaiuto S, Lippi A *et al.* Prevalence of clinically diagnosed Alzheimer's disease and other dementing disorders: a door-to-door survey in Appignano, Macerata Province, Italy. *Neurology* 40, 626–631 (1990).
- 60 Fratiglioni L, Grut M, Forsell Y *et al.* Prevalence of Alzheimer's disease and other dementias in an elderly urban population: relationship with age, sex, and education. *Neurology* 41, 1886–1892 (1991).
- 61 Shigeta M. Epidemiology: rapid increase in Alzheimer's disease prevalence in Japan. *Psychogeriatrics* 4, 117–119 (2004).
- 62 White L, Petrovitch H, Ross GW *et al.* Prevalence of dementia in older Japanese-American men in Hawaii: the Honolulu-Asia Aging Study. *JAMA* 276, 955–960 (1996).
- 63 Graves AB, Larson EB, Edland SD *et al.* Prevalence of dementia and its subtypes in the Japanese American population of King County, Washington state. The Kame Project. *Am. J. Epidemiol.* 144, 760–771 (1996).
- 64 Hou CE, Yaffe K, Perez-Stable EJ, Miller BL. Frequency of dementia etiologies in four ethnic groups. *Dement. Geriatr. Cogn. Disord.* 22, 42–47 (2006).
- 65 White L, Petrovitch H, Ross G *et al.* Brain aging and midlife tofu consumption. *J. Am. Coll. Nutr.* 19, 242–255 (2000).
- 66 Reinwald S, Weaver CM. Soy isoflavones and bone health: a double-edged sword? *J. Nat. Prod.* 69, 450–459 (2006).
- 67 Weaver CM, Cheong JM. Soy isoflavones and bone health: the relationship is still unclear. *J. Nutr.* 135, 1243–1247 (2005).
- 68 Geller SE, Studee L. Soy and red clover for mid-life and aging. *Climacteric* 9, 245–263 (2006).
- 69 Akaza H, Miyayama N, Takashima N *et al.* Comparisons of percent equol producers between prostate cancer patients and controls: case-controlled studies of isoflavones in Japanese, Korean and American residents. *Jpn J. Clin. Oncol.* 34, 86–89 (2004).
- 70 Lund TD, Munson DJ, Haldy ME, Setchell KD, Lephart ED, Handa RJ. Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. *Biol. Reprod.* 70, 1188–1195 (2004).
- 71 Rice S, Whitehead SA. Phytoestrogens and breast cancer – promoters or protectors? *Endocr. Relat. Cancer* 13, 995–1015 (2006).
- 72 Ju YH, Allred KF, Allred CD, Helferich WG. Genistein stimulates growth of human breast cancer cells in a novel, postmenopausal animal model, with low plasma estradiol concentrations. *Carcinogenesis* 27, 1292–1299 (2006).
- 73 Ju YH, Fultz J, Allred KF, Doerge DR, Helferich WG. Effects of dietary daidzein and its metabolite, equol, at physiological concentrations on the growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in ovariectomized athymic mice. *Carcinogenesis* 27, 856–863 (2006).
- 74 Setchell KD. Assessing risks and benefits of genistein and soy. *Environ. Health Perspect.* 114, A332–A333 (2006).
- 75 Messina M, McCaskill-Stevens W, Lampe JW. Addressing the soy and breast cancer relationship: review, commentary, and workshop proceedings. *J. Natl Cancer Inst.* 98, 1275–1284 (2006).
- 76 Patisaul HB. Phytoestrogen action in the adult and developing brain. *J. Neuroendocrinol.* 17, 57–64 (2005).
- 77 Lephart ED, Setchell KD, Lund TD. Phytoestrogens: hormonal action and brain plasticity. *Brain Res. Bull.* 65, 193–198 (2005).
- 78 Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor- α and - β mRNA in the rat central nervous system. *J. Comp. Neurol.* 388, 507–525 (1997).
- 79 Taylor AH, Al-Azzawi F. Immunolocalisation of oestrogen receptor- β in human tissues. *J. Mol. Endocrinol.* 24, 145–155 (2000).
- 80 Perez SE, Chen EY, Mufson EJ. Distribution of estrogen receptor α and β immunoreactive profiles in the postnatal rat brain. *Brain Res. Dev. Brain Res.* 145, 117–139 (2003).
- 81 Zhao L, Wu TW, Brinton RD. Estrogen receptor subtypes α and β contribute to neuroprotection and increased Bcl-2 expression in primary hippocampal neurons. *Brain Res.* 1010, 22–34 (2004).
- 82 Zhao L, Brinton RD. Estrogen receptor α and β differentially regulate intracellular Ca^{2+} dynamics leading to ERK phosphorylation and estrogen neuroprotection in hippocampal neurons. *Brain Res.* 1172, 48–59 (2007).
- 83 Zhao L, Brinton RD. Estrogen receptor β as a therapeutic target for promotion of neurogenesis and prevention of neurodegeneration. *Drug Dev. Res.* 66, 103–117 (2006).
- 84 Perez-Martin M, Salazar V, Castillo C *et al.* Estradiol and soy extract increase the production of new cells in the dentate gyrus of old rats. *Exp. Gerontol.* 40, 450–453 (2005).
- 85 Burguete MC, Torregrosa G, Perez-Asensio FJ *et al.* Dietary phytoestrogens improve stroke outcome after transient focal cerebral ischemia in rats. *Eur. J. Neurosci.* 23, 703–710 (2006).
- 86 Schreihofner DA, Do KD, Schreihofner AM. High-soy diet decreases infarct size after permanent middle cerebral artery occlusion in female rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R103–R108 (2005).
- 87 Zhao L, Chen Q, Brinton RD. Neuroprotective and neurotrophic efficacy of phytoestrogens in cultured hippocampal neurons. *Exp. Biol. Med.* 227, 509–519 (2002).

- 88 Lund TD, West TW, Tian LY *et al.* Visual spatial memory is enhanced in female rats (but inhibited in males) by dietary soy phytoestrogens. *BMC Neurosci.* 2, 20 (2001).
- 89 Patisaul HB, Dindo M, Whitten PL, Young LJ. Soy isoflavone supplements antagonize reproductive behavior and estrogen receptor α - and β -dependent gene expression in the brain. *Endocrinology* 142, 2946–2952 (2001).
- 90 Linford NJ, Dorsa DM. 17 β -estradiol and the phytoestrogen genistein attenuate neuronal apoptosis induced by the endoplasmic reticulum calcium-ATPase inhibitor thapsigargin. *Steroids* 67, 1029–1040 (2002).
- 91 Linford NJ, Yang Y, Cook DG, Dorsa DM. Neuronal apoptosis resulting from high doses of the isoflavone genistein: role for calcium and p42/44 mitogen-activated protein kinase. *J. Pharmacol. Exp. Ther.* 299, 67–75 (2001).
- 92 Ostatnikova D, Celec P, Hodosy J, Hampl R, Zdenek P, Kudela M. Short-term soybean intake and its effect on steroid sex hormones and cognitive abilities. *Fertil. Steril.* (2007) (In Press).
- 93 Celec P, Ostatnikova D, Caganova M *et al.* Endocrine and cognitive effects of short-time soybean consumption in women. *Gynecol. Obstet. Invest.* 59, 62–66 (2005).
- 94 File SE, Jarrett N, Fluck E, Duffy R, Casey K, Wiseman H. Eating soya improves human memory. *Psychopharmacology (Berl.)* 157, 430–436 (2001).
- 95 File SE, Hartley DE, Elsbagh S, Duffy R, Wiseman H. Cognitive improvement after 6 weeks of soy supplements in postmenopausal women is limited to frontal lobe function. *Menopause* 12, 193–201 (2005).
- 96 Duffy R, Wiseman H, File SE. Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones. *Pharmacol. Biochem. Behav.* 75, 721–729 (2003).
- 97 Kritz-Silverstein D, Von Muhlen D, Barrett-Connor E, Bressel MA. Isoflavones and cognitive function in older women: the Soy and Postmenopausal Health In Aging (SOPHIA) Study. *Menopause* 10, 196–202 (2003).
- 98 Casini ML, Marelli G, Papaleo E, Ferrari A, D'Ambrosio F, Unfer V. Psychological assessment of the effects of treatment with phytoestrogens on postmenopausal women: a randomized, double-blind, crossover, placebo-controlled study. *Fertil. Steril.* 85, 972–978 (2006).
- 99 Krejckamp-Kaspers S, Kok L, Grobbee DE *et al.* Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. *JAMA* 292, 65–74 (2004).
- 100 Ho SC, Chan AS, Ho YP *et al.* Effects of soy isoflavone supplementation on cognitive function in Chinese postmenopausal women: a double-blind, randomized, controlled trial. *Menopause* 14, 489–499 (2007).
- 101 Fournier LR, Ryan Borchers TA, Robison LM *et al.* The effects of soy milk and isoflavone supplements on cognitive performance in healthy, postmenopausal women. *J. Nutr. Health Aging* 11, 155–164 (2007).
- 102 Burow ME, Boue SM, Collins-Burow BM *et al.* Phytochemical glyceollins, isolated from soy, mediate antihormonal effects through estrogen receptor α and β . *J. Clin. Endocrinol. Metab.* 86, 1750–1758 (2001).
- 103 Cassidy A, Brown JE, Hawdon A *et al.* Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. *J. Nutr.* 136, 45–51 (2006).
- 104 Setchell KD, Brown NM, Desai P *et al.* Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *J. Nutr.* 131, S1362–S1375 (2001).
- 105 Wang TT, Sathyamoorthy N, Phang JM. Molecular effects of genistein on estrogen receptor mediated pathways. *Carcinogenesis* 17, 271–275 (1996).
- 106 Huang MH, Luetters C, Buckwalter GJ *et al.* Dietary genistein intake and cognitive performance in a multiethnic cohort of midlife women. *Menopause* 13, 621–630 (2006).
- 107 Unfer V, Casini ML, Costabile L, Mignosa M, Gerli S, Di Renzo GC. Endometrial effects of long-term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study. *Fertil. Steril.* 82, 145–148 (2004).
- 108 D'Anna R, Cannata ML, Atteritano M *et al.* Effects of the phytoestrogen genistein on hot flashes, endometrium, and vaginal epithelium in postmenopausal women: a 1-year randomized, double-blind, placebo-controlled study. *Menopause* 92(8), 3068–3075 (2007).
- 109 Atteritano M, Marini H, Minutoli L *et al.* Effects of the phytoestrogen genistein on some predictors of cardiovascular risk in osteopenic, postmenopausal women: a 2-year randomized, double-blind, placebo-controlled study. *J. Clin. Endocrinol. Metab.* 92(8), 3068–3075 (2007).
- 110 Tempfer CB, Bentz EK, Leodolter S *et al.* Phytoestrogens in clinical practice: a review of the literature. *Fertil. Steril.* 87(6), 1243–1249 (2007).
- 111 Morito K, Hirose T, Kinjo J *et al.* Interaction of phytoestrogens with estrogen receptors α and β . *Biol. Pharm. Bull.* 24, 351–356 (2001).
- 112 Wu J, Oka J, Ezaki J *et al.* Possible role of equol status in the effects of isoflavone on bone and fat mass in postmenopausal Japanese women: a double-blind, randomized, controlled trial. *Menopause* 14(5), 866–874 (2007).
- 113 Frankenfeld CL, McTiernan A, Thomas WK *et al.* Postmenopausal bone mineral density in relation to soy isoflavone-metabolizing phenotypes. *Maturitas* 53, 315–324 (2006).
- 114 Lampe JW, Karr SC, Hutchins AM, Slavin JL. Urinary equol excretion with a soy challenge: influence of habitual diet. *Proc. Soc. Exp. Biol. Med.* 217, 335–339 (1998).
- 115 Rowland IR, Wiseman H, Sanders TA, Adlercreutz H, Bowey EA. Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut flora. *J. Nutr.* 36, 27–32 (2000).
- 116 McCarty MF. Isoflavones made simple – genistein's agonist activity for the β -type estrogen receptor mediates their health benefits. *Med. Hypotheses* 66, 1093–1114 (2006).

Websites

- 201 Women's Health Initiative Hormone Therapy Study
<http://www.nih.gov/PHTindex.htm>
- 202 Hormone therapy may protect against Alzheimer's (May 2, 2007)
<http://healthday.com/Article.asp?AID=604162>
- 203 Common phytoestrogens and their sources
<http://www.herbalchem.net/Introductory.htm>

Affiliations

- Liqin Zhao, PhD
Department of Pharmacology & Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA
Tel.: +1 323 442 1436
Fax: +1 323 224 7473
liqinz@usc.edu
- Roberta Diaz Brinton, PhD
Department of Pharmacology & Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA
Tel.: +1 323 442 1430
Fax: +1 323 224 7473
rbrinton@usc.edu