REVIEW

Pharmaceutical Prospects of Phytoestrogens

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Abstract. Interest in the physiologic and pharmacologic role of bioactive compounds present in plants has increased dramatically over the last decade. Of particular interest in relation to human health are the classes of compounds known as the phytoestrogens, which embody several groups of non-steroidal estrogens, including isoflavones and lignans that are widely distributed within nature. The impact of dietary phytoestrogens on normal biologic processes was first recognized in sheep. Observations of sheep grazing on fields rich in clover and cheetahs fed high soy diets in zoos suggested that flavonoids and related phytochemicals can affect mammalian health. Endogenous estrogens have an important role not only in the hypothalamic-pituitary-gonadal axis, but also in various non-gonadal systems, such as cardiovascular systems, bone, and central nervous systems, and lipid metabolism. There have been several clinical studies of hormone replacement therapy (HRT) in post-menopausal women to examine whether HRT has beneficial effects on the cardiovascular system, bone fractures, lipid metabolism, and Alzheimer's disease. In addition, estrogen contributes to the development of some estrogen-dependent cancers, such as breast cancer and prostate cancer and the number of patients with these cancers is increasing in developed countries. Although recent mega-studies showed negative results for classical HRT in the prevention of some of these diseases, the molecules that interact with estrogen receptors are candidate drugs for various diseases, including hormone-dependent cancers. This review focuses on the molecular properties and pharmaceutical potential of phytoestrogens.

Key words: Phytoestrogen, Estrogen receptor

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NUCLEAR receptors are members of a large family of transcription factors responsible for sensing changes in the environment and translating these changes directly into the modulation of transcriptional activity and, thus, phenotypic alterations. Ligands that modulate nuclear receptor activity are generally small lipophilic molecules that passively enter a cell and bind to their cognate receptor. Such ligands include steroid hormones such as estrogen, progesterone, testosterone, and aldosterone, as well as other non-peptide hormones, such as thyroid hormone, vitamin D, cholesterol metabolites, and bile acids. The large number of diseases associated with the inappropriate production or response to these hormones indicates the importance of nuclear receptors as a therapeutic target for pharma-

ceuticals. Recently, the plant sterol guggulsterone, which has been used in Ayurvedic medicine since at least 600 BC to treat a wide variety of ailments, was reported to be a farnesoid receptor antagonist [1, 2]. Guggulsterone is now a candidate drug to treat various life-style mediated diseases such as obesity or hyperlipidemia. St. John's wort, an ancient herbal remedy that has gained widespread popularity, is a potent pregnane receptor ligand [3-5]. Pregnane receptor ligands regulate the expression of CYP3A4, which has an important role in drug metabolism [6, 7]. Thus, many unidentified or identified natural products present in nature might have unknown pharmacologic effects on nuclear receptors. The estrogen receptor (ER) is perhaps the most well-defined nuclear receptor system from the point of view of biologic responses and clinical implications. The presence of phytoestrogens, phytochemicals with substantial estrogenic activity, is well known.

Epidemiologic data indicate that Asian people have

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lower rates of osteoporotic fractures, cardiovascular diseases, postmenopausal symptoms, and certain cancers than Western populations [8]. These health advantages are notably reduced when Asians adopt a Western lifestyle and eating habits [8]. These observations have led researchers to look at the Asian diet for possible answers. Soy is part of the traditional diet in the Asian populations and is rich in phytoestrogens such as genistein and daidzein. An increasing number of researchers are investigating the relation between soy intake and the above-mentioned diseases [8–10]. The number of papers concerning phytoestrogens has drastically increased over the last few years (Fig. 1). The interest in phytoestrogens has extended to their pharmaceutical potential. Indeed, estrogens are already used for various diseases such as postmenopausal syndrome and osteoporosis. In addition, estrogens are thought to be beneficial for hyperlipidemia, and for the prevention of cardiovascular disease, and Alzheimer's disease (AD). In addition, estrogen antagonists are used for treatment for breast cancer. Recently, selective estrogen receptor modulators (SERMs) have become available for treatment for osteoporosis, and there are currently large scale clinical studies being performed to examine the beneficial effects on cardiovascular disease and breast cancer.

Biochemistry of phytoestrogens

Phytoestrogens are a group of biologically active plant substances with a chemical structure that is similar to that of estradiol, an endogenous estrogen. This structural similarity accounts for the ability of these compounds to bind to ERs and exert various estrogenic or antiestrogenic effects. There are three main classes of phytoestrogens: isoflavones, coumestans, and lignans, which occur in either plants or their seeds. Resorcylic acid lactones exhibit estrogenic activity and are produced by molds that commonly contaminate cereal crops and hence are better termed mycoestrogens. A single plant often contains more than one class of phytoestrogen. For example, the soy bean is rich in isoflavones, whereas the soy sprout is a potent source of coumestrol, the major coumestan [11]. The major isoflavones, genistein and daidzein (rich in soy), commonly exist as inactive glucosides [12]. They are also derived from the precursors, biochanin A and formononetin, which are converted to genistein and daidzein, respectively, after breakdown by intestinal glucosidases [13]. Daidzein is further partially metabolized to equol and O desmethylangiolensin (O-DMA). The classical phytoestrogens, such as genistein and daidzein have a higher affinity to ER beta than alpha [14]. ER beta is strongly expressed in ovary, uterus, brain, bladder, testis, prostate, and lung [14]. Expression of ER beta appears to occur at different sites in



Fig. 1. The number of papers about phytoestrogens.

the brain than ER alpha [14]. Moreover, ER beta is also expressed in both bone and the cardiovascular system [15]. Although the physiologic significance of ER beta is not completely known, agonistic actions on ER beta and weak (or little) activation of ER alpha is beneficial for osteoporosis, cardiovascular protection, lipid metabolism, and breast cancer. In addition, estrogen can act via non-nuclear receptors that interact with ERs via second messenger systems. There is in vitro evidence that E2 modulates the functions of neural and vascular cells via non-genomic actions. The molecular mechanisms of the non-genomic effects are still under investigation. A transcriptome study using a DNA micro-array system demonstrated that gene expression profiles after estrogen administration are different from those after SERM administration [16]. Therefore, both the genomic effects through classical estrogen receptors and non-genomic effects must be considered.

Recently, several phytochemicals, such as 8-isopenenylnaringenin, resveratrol (found in grapes and wine) [17], 8-prenylnaringenin (in hops), glyceollins (in soy) [18], ginsenoide Rg1 (from Panax notoginseng) [19], and lindleyin (from Rhei rhizoma) [20], are novel Some of these novel classes of phytoestrogens. phytoestrogens exhibit unique estrogenic properties. Resveratrol differentially affects the transcriptional activity of ER alpha and ER beta in a sequencedependent manner [21]. Glyceollins exhibit unique antagonistic effects on ER in both ER-transfected ERnegative HEK293 and ER-positive MCF-7 cells [22]. Ginsenoide does not interact directly with ER. Thus, newly identified phytoestrogens exhibit unique estrogenic activities. Therefore, it is conceivable to speculate that some of these phytoestrogens might act as SERMs and be candidate drugs for various human diseases.

In general, recent advances in our understanding of the mechanisms through which nuclear receptors regulate cellular function make these receptors exciting prospects for drug discovery. Insights into the significant functional changes caused by subtle changes in the ligand, as demonstrated by studies of tamoxifen versus raloxifene, make the identification of tissueselective compounds that maintain a desired efficacy while avoiding harmful effects an attainable goal.

Phytoestrogens and bone

Several animal studies have provided convincing data on the significant improvement of bone mass or other end points after soy protein or isolated isoflavoneenriched soy extract supplementation [19, 23-25]. Several observational epidemiologic studies have also examined the link between dietary intake of phytoestrogens and bone mass in humans and reported that soy protein and soy phytoestrogen intake are beneficial for maintaining or modestly improving bone mass in postmenopausal women [26-28]. Only a few randomized trials of relatively short duration and small sample size have been conducted in Caucasian populations [19, 29-33]. Some studies revealed that isoflavonerich soy protein had a modest effect in retarding bone loss in perimenopausal [29] and postmenopausal [31, 32] women, but such effects were not observed in other studies [33]. Studies have also reported inconsistent effects of phytoestrogens (or soy protein) on bone markers in postmenopausal women [19]. Arjmandi et al. reported that in postmenopausal women, the soy protein group had significantly reduced urinary deoxypyridinoline excretion (a specific biomarker of bone resorption), and calcium excretion did not change compared to a milk-based protein group [34]. There was also an enhancing effect of soy isoflavones on insulin-like growth factor 1 (IGF-I) synthesis, and the IGF-I concentration is positively related to bone mass in women. Chen et al. reported that in the Chinese population, soy isoflavones have a mild but significant effect on the maintenance of hip bone mineral concentration in postmenopausal women with low initial bone mass [35]. In postmenopausal monkeys, however, soy phytoestrogens are poor substitutes for mammalian estrogens in protecting against bone loss resulting from estrogen deficiency [36]. Thus, the hypothesis of a beneficial effect of soy on bone mass is still speculative, and little is known about the effects of soy isoflavones in Asian populations where soy intake is part of the habitual diet. The optimal dosage and the components responsible for the favorable effects of soy or isoflavone-rich soy protein isolates on bone are still unclear.

As Setchell and Lydeking-Olsen report in their review, diets rich in phytoestrogens have bone-sparing effects over the long term, although the magnitude of the effect and the exact mechanism(s) of action are presently elusive or speculative [37]. A prospective study of the impact of phytoestrogens on fracture rate would provide definitive answers regarding the efficacy of phytoestrogens and their value as a possible alternative to pharmacologic treatment.

Phytoestrogens and cancer protection

Breast cancer is still a major cause of death for women in Western countries. Breast cancer is thought to have multifactorial causation ranging from gene profile to diet and lifestyle and mutations; in particular tumor suppressor genes such as BRCA1, BRCA2, and p53 are likely to be of particular importance. The role of endogenous estrogens in breast cancer risk is widely recognized. Different forms of estrogen metabolism result in the formation of mitogenic endogenous estrogens or the metabolic activation of estrogen, which can result in carcinogenic free-radical mediated DNA damage. To date, 13 studies have assessed the direct relation between the individual dietary intake of soy products and the risk of breast cancer [38-50]. Overall, the results do not show significant protective effects against breast cancer. Four of 11 studies indicate protection, and none of them reported statistically significant breast cancer reductions. There are numerous in vitro studies showing that genistein, and to lesser extent daidzein, inhibits the growth of a wide range of both hormone-dependent and hormone-independent cancer cells. Phytoestrogens might act as antioxidants and/or inhibit blood vessel growth, which is essential for tumor expansion [51, 52]. As mentioned above, however, the overall clinical or epidemiologic information on phytoestrogen consumption and breast cancer risk is still scarce.

The development of endometrial cancer is largely related to prolonged exposure to unopposed estrogens. Therefore, intake of phytoestrogens might influence the incidence of endometrial cancer. In contrast to breast cancer, however, the reports about the effect of phytoestrogens on endometrial cancer are limited. In Hawaii's multiethnic population, a greater consumption of tofu alone or in combination with other soy products is associated with a 50% reduction in the endometrial cancer risk. The risk reduction was strongest among women who had never given birth and had never used estrogen replacement therapy [53]. Horn-Ross *et al.* reported in non-Asian women in the San Francisco Bay area that isoflavone and lignan

consumptions was inversely related to the risk of endometrial cancer. They concluded that some phytoestrogenic compounds, at the level consumed in the typical American-style diet, are associated with a reduced risk of endometrial cancer [54]. Both of these two positive studies for phytoestrogens on endometrial cancer were case-controlled observation studies, and a randomized case-control prospective study must be performed to further address the question.

Phytoestrogens and postmenopausal symptoms

In Asia, only 10% to 20% of postmenopausal women experience hot flashes compared with 70% to 80% of women in Western countries [55–58]. A popular hypothesis to explain this difference is that isoflavones found in soy, a staple in the traditional Asian diet, influences the body's response to the changing hormonal levels of menopause [59]. Dietary supplements containing isoflavones from soy are widely marketed for menopausal symptoms and are increasingly being used by women as an alternative to estrogen.

Clinical studies of the effects of phytoestrogens on the effect on postmenopausal syndrome are summarized in Table 1 [30, 60-72]. Most of the studies of isoflavones used soy products. The results are mixed: some studies report a modest benefit compared with placebo and others do not. There was also a wide range in the amount of total isoflavones used in these studies. The reduction in hot flashes reported in the positive studies was smaller than the reduction reported in a meta-analysis of clinical trials using HRT. Recently, Tice et al. reported that the isoflavone from clover extract (Promensil) had little clinically important effect on hot flashes or other symptoms of menopause [72]. They also reported, however, some evidence for biologic effect of Promensil (Promensil reduced hot flashes more rapidly than placebo). Further research in this area is needed.

Phytoestrogens, Alzheimer disease, and cognitive disorders

Estrogen therapy is one of the most compelling potential strategies for the prevention of dementia, primarily AD. Strong biologic evidence supports the beneficial effects of estrogen on the brain, including

Subjects	Diet	Effects	Reference
252 postmenopausal women	Promensil (82 mg of total isoflavone per day), Rimostil (57 mg of total isoflavone per day, or placebo	Some evidence for a biological effect of Promensil, neither supplement had a clinically important effect on hot flushes or other symptoms of menopause.	Tice et al. 2003
30 women with more than 12 months amenorrhea and experiencing more than five flushes per day.	Isoflazone 80 mg or placebo	Frequency of hot flusheds decreased by 16% in first 4 weeks. 44% reduction was seen in isoflavone group during the subsequent double blind phase.	v d Weijer and Barentsen. 2002
123 postmenopausal women	Soy beverage (containing 90 mg of isoflavone) and placebo.	No significant differences between the soy and placebo groups in the number of hot flushes or hot flush scores.	Van Patten et al. 2002
24 postmenopausal women	Dietary beverage containing isoflavones or an isoflavone-free isocaloric placebo.	No statistically significant differences in the incidence of hot flushes between groups.	Knight et al. 2001
69 postmenopausal women	Isoflavone-rich (80.4 mg/day) soy protein, isoflavone-poor (4.4 mg/day) soy protein.	No evidence that isoflavone-rich or isoflavone-poor soy protein provided relief of vasomotor or other menopausal symptoms.	St Germain <i>et al.</i> 2001
94 postmenopausal women	Soy supplements containing 118 mg of isoflavones compared with casein.	3 months of soy supplements containing phytoestrogens did not provide symptomatic relief compared with placebo.	Kotsopoulos et al. 2000
177 postmenopausal women	Soy isoflavone extract (total 50 mg genistein and daidzein per day) or placebo	Soy isoflavone extract was effective in reducing frequency and severity of flushes.	Upmalis et al. 2000
177 women	Soy tablet or placebo.	No suggestion that the soy product was more effective in reducing hot flushes than the placebo.	Quella et al. 2000
39 postmenopausal women	Soy extract or conjugated equin estrogens.	Severity of hot flushes was reduced in the soy group.	Scambia et al. 2000
41 postmenopausal women	One tablet containing 40 mg isoflavone or placebo.	No significant differences between groups in the reduction in hot flushes.	Baber <i>et al.</i> 1999
51 women	20 g of soy protein (34 mg of phytoestrogen).	No significant effect for frequency of menopausal symptoms.	Washburn et al. 1999
104 postmenopausal women	Isolated soy protein 60 g daily compared with casein	Soy was significantly superior to placebo in reducing the mean number of hot flushes per 24 hours after 4, 8, 12 weeks of treatment.	Albertazzi et al. 1998
42 postmenopausal women	Soy and linseed diets (high in phytoestrogens) and wheat diet (low in phytoestrogens).	High phytoestrogen dietary intake reduced hot flush rate.	Dalais <i>et al.</i> 1998
58 postmenopausal women	Soy flour 45 g	Hot flushes significantly decreased in the soy and wheat four group (40% and 25% reduction) with significant rapid response in the soy flour group.	Murkies et al. 1995

Table 1. Clinical studies of the effect of soy (or isoflavones) on postmenopausal syndrome

neurotrophic effects, reduction in beta-amyloid accumulation, enhanced neurotransmitter release and activity, and protection against oxidative damage [73, 74]. ERs, including ER alpha and ER beta, are located throughout the brain, especially in areas involved in learning and memory, such as the hippocampus and amygdala [75]. Enzymes necessary for sex steroid biosynthesis have been identified in these same regions, suggesting that estrogen has an important role in the brain. In addition, both prospective and case-control studies found that women who took estrogen had up to a 50% lower risk of developing AD [76–79].

One of the largest clinical studies that demonstrated the effectiveness of HRT for preventing AD was reported by Zandi et al. [78]. This study was a part of the Cache County Study. They pointed out that prior HRT use is associated with a reduced risk of AD, but there is no apparent benefit from current HRT use, unless such use has exceeded 10 years. On the other hand, Shumaker et al. reported in the Women's Health Initiative (WHI) study, that estrogen plus progestin therapy did not have any beneficial effects on dementia or cognitive impairment [80]. The effect of unopposed estrogen on the prevention of developing dementia and AD will be evaluated with the ongoing estrogen arm of Women's Health Initiative Memory Study (WHIMS). The addition of progestin might have a differential effect on the risk of developing dementia, because progestins, especially medroxyprogesterone, reportedly modify the beneficial effects of estrogen [81, 82]. The molecular mechanisms underlying the effects of estrogen on learning and memory are not understood. A clinical study of the effectiveness of SERMs on AD is now underway. Phytoestrogens are a candidate novel drug for AD. Although there are a few papers about the beneficial effects of phytoestrogens on memory or the central nervous system [83, 84], the effects of phytoestrogens on the central nervous system in humans are poorly understood. Thus, reports about the effects of phytoestrogen (and endogenous estrogen) on the central nervous system, especially on learning and memory, are controversial. We still need further study.

Phytoestrogens and the cardiovascular system

It is well known that menopause is an important risk factor for cardiovascular disease because estrogen has a protective role against atherosclerosis. In vitro, genistein inhibits the proliferation of many vascular cells and inhibits angiogenesis, which is an atherosclerotically important process [85]. Neovascularization is associated with advanced lesions and genistein inhibits this function in cultured endothelial cells [52, 86]. ER alpha and beta are both expressed in vascular endothelial and smooth muscle cells, and myocardial cells [87]. Interestingly, in the rat carotid artery, ER alpha is expressed at low levels, whereas ER beta expression is more than 40-fold higher [15]. Zhu et al. reported that in mice deficient in ER beta have abnormal vascular function and hypertension [88]. These results suggest that ER beta-specific ligands might be cardiovascular-protective. Based on these in vitro and animal model studies, HRT was performed for many years to prevent cardiovascular disease. In 2000, the American Heart Association updated their guideline for "soy protein and cardiovascular disease" [89]. In this guideline, it is suggested that daily consumption of more than 25 g of soy protein with its associated phytochemicals intact can improve lipid profiles in hypercholesterolemic humans. The mechanisms by which soy modulates blood cholesterol and lipoprotein levels require further research. Soy protein without isoflavones appears to be less effective [90-92]. Consuming isoflavones without soy protein does not lower cholesterol, but might provide other cardiovascular benefits. The effects of using soy extracts of isoflavones as dietary supplements are largely unknown and cannot be recommended. Apparently there is a synergy among the components of intact soy protein, which provides the maximum hypocholesterolemic benefit. A variety of clinical trials demonstrated that consuming 25 to 50 g/d of soy protein is both safe and effective in reducing LDL cholesterol by approximately 4% to 8% [93]. The beneficial effects of soy are proportionally greater in people with hypercholesterolemia. Lichtenstein has noted that the judicious substitution of soy for animal protein can result in lower saturated fat and cholesterol intake, thereby indirectly resulting in a more favorable blood cholesterol level and potentially reducing the risk of coronary heart de Kleijn et al. reported in their disease [94]. Framingham Offspring Study that a high intake of phytoestrogens in postmenopausal women is associated with a favorable metabolic cardiovascular risk profile [95]. Recently, Nikander et al. studied the effects of phytoestrogens on serum levels of C-reactive protein and E-selectin levels, and plasma nitric oxide levels, vascular surrogate markers for 3 months [96]. They found the phytoestrogen diet did not affect any other marker. The subject of the study, however, were postmenopausal women who had been treated for breast cancer. Eleven of 64 women had received chemotherapy and three subjects had used tamoxifen. Therefore, the subjects of this study seem to be different from general postmenopausal subjects. On the other hand, van der Schouw et al. demonstrated that a higher than usual dietary intake of phytoestrogens is associated with low aortic stiffness in postmenopausal women [97]. They concluded that phytoestrogens have a protective effect on the risk of atherosclerosis

and arterial degeneration through an effect on arterial walls, especially among older women. Squadrito et al., in a randomized double-blind, controlled study, also showed that 1 year of genistein therapy improves endothelium function in postmenopausal women [98]. An in vitro study demonstrated that the phytoestrogen equol increases nitric oxide availability by inhibiting superoxide production. Teede *et al.* showed that a soy protein isolate improved pulse wave velocity, whereas flow-mediated vasodilatation declined in a randomized double-blind study [99]. Adipose-specific plasma protein, adiponectin, has anti-atherogenic and antiinsulin-resistance properties. Nagasawa et al. reported that a calorie-restricted diet raises adiponectin mRNA expression and its plasma level in mice, but there were no significant differences between the soy protein and casein protein group [100]. In 2002, a WHI study aborted the mega-study because HRT did not have beneficial effects on protecting cardiovascular events in menopausal women [101]. In 2003, Zhang et al. reported direct evidence that soy food consumption might reduce the risk of coronary heart disease in women [102]. They found in this large prospective cohort study that soy food consumption was significantly and inversely associated with the risk of coronary heart disease among Chinese women. The beneficial effects of estrogens or phytoestrogens on cardiovascular diseases need to be confirmed.

Phytoestrogens and glucose metabolism and obesity

Many studies in animals as well as in humans suggest that soy has beneficial effects on diabetes mellitus, and several studies in obese humans and animals suggest that soy as a source of dietary protein has significant antiobesity effects (Table 2) [103-117]. Mahalko et al. fed different sources of fiber to type 2 diabetic subjects for 2 to 4 weeks and observed beneficial effects of soy hulls on glucose tolerance, lipid indexes, and glycated hemoglobin [110]. Tsai et al. observed that in obese subjects with type 2 diabetes, soy polysaccharides significantly reduced increases in postprandial serum glucose and triacylglycerol concentrations [108]. These effects appear to be due to smaller increases in glucagons and pancreatic polypeptides and larger increases in somatostatin concentrations. There was no significant effect on serum insulin

concentrations. Anderson et al. studied the effect of soy protein in type 2 diabetic subjects with obesity, hypertension, and proteinuria [106]. They observed no beneficial effect on renal function or proteinuria in these subjects when soy protein was half of the daily protein intake. They did, however, observe a reduction in hyperlipidemia and in cholesterol and triacylglycerol concentrations. Hermansen et al. reported that, in type 2 diabetic subjects soy protein with its associated isoflavone and fiber reduced LDL cholesterol, apolipoprotein B-100, and triacylglycerol as compared with a casein diet with cellulose, but had no effect on glucose metabolism, as indicated by the lack of change in hemoglobin A1c [103]. Recently, Jayagopal et al. reported that phytoestrogen supplementation for 12 weeks significantly lowers mean values for fasting insulin, insulin resistance, HbA1c, total cholesterol, cholesterol/HDL cholesterol ratio, and free thyroxin in postmenopausal type 2 diabetic patients [118]. They also reported no significant change in HDL cholesterol, triglycerides, and body weight. In this paper, they concluded that short-term dietary phytoestrogen supplementation reduces insulin resistance and improves glycemic control in postmenopausal women with type 2 diabetes, while also reducing their cardiovascular risk by lowering LDL cholesterol.

Although the anti-diabetic effects of isoflavones are explained by their alpha-glucosidase activity, inhibition of the intestinal brush border uptake of glucose or tyrosine kinase inhibitory properties [119-121], the precise molecular mechanisms by which soy diets or phytoestrogens exert their beneficial effects on diabetes and obesity are unclear. Interesting findings reported by Dang et al. demonstrated that a high concentration of genistein acts as an agonist to peroxisome proliferators activated receptor (PPAR) gamma in an in vitro reporter gene assay system [122]. They demonstrated that at low concentrations genistein acts as estrogen, stimulating osteogenesis and inhibiting adipogenesis, but at high concentrations, genistein acts as a ligand for PPAR gamma, leading to the upregulation of adipogenesis and the downregulation of osteogenesis. This finding is consistent with the phenomenon of a soy diet improving insulin resistance. Heim et al. reported that genistein enhances osteogenesis and represses adipogenic differentiation of human primary bone marrow stromal cells in vitro [123]. The results of these two papers might explain, at least in part, the effects of phytoestrogen (such as genistein) on obesity

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Model	Diet	Amount and duration	Effects	Reference
Humans				
Type 2 diabetic subjects	Soy protein and fiber compared with casein with cellulose	50 g protein/day, 20 g fiber/day, and 150 mg isoflavones/day for 6 wk	Decreased LDL cholesterol, triacylglycerol, and apolipoprotein B-100; no change in HDL cholesterol and hemoglobin A1c	Hermansen, et al. 2001
Mildly obese subjects	Soy protein compared with animal protein	28–29% of energy as protein for 4 days	Decreased 24-h energy expenditure	Mikkelsen, et al. 2000
Type 2 diabetic subjects with obesity and hypertension	Soy protein diet compared with animal-protein diet	1 g protein/kg body wt for 8 wk	Decreased total cholesterol and triacylglycerol	Anderson and Gerner. 1998
Obese subjects	Very-low-energy diet with soy protein compared with low-energy-diet with casein	375–425 kcal/d for 60– 70 days	Decreased body weight but a greater reduction in cholesterol and triacylglycerol	Bosello, et al. 1998
Obese women	Low-energy diets with soy protein or lean meat	Low-energy diets for 16 wk	Similar decrease (9%) in body weight with both diets	Yamashita, et al. 1998
Obese women	Soy-based liquid formula compared with milk-based liquid formula	1000 kcal/day for 4 wk	No significant difference in body weight decrease between the 2 diets	Jenkins, et al. 1989
Obese type 2 diabetic subjects	Soy polysaccharide compared with low fiber	10 g fiber as single meal	Decreased postprandial hyperglycemia and triacylglycerol: no effect on serum insulin	Tsai, <i>et al</i> . 1987
Type 2 diabetic subjects	Soy hull	26–52 g fiber/day for 2– 4 wk	Improved glucose intolelerance and decreased VLDL cholesterol, triacylglycerol, and glycated hemoglobin	Mahalko, <i>et al</i> . 1984
Animals				
Obese yellow mice	Soy-protein isolate and hydrolysate compared with casein-proetin isolate and hydrolysate	35% protein for 4 wk at 60% of energy	Decreased body weight fat and plasma glucose	Aoyama, et al. 2000
Diabetes-prone BioBreeding rats	Soy protein compared with animal and nonanimal protein	20-23.4% protein for 160 days	Decreased frequency and delayed onset of type 1 diabetes	Atkinson, et al. 1998
Wistar fatty rats with type 2 diabetes	Soy protein compared with casein with either saturated or polyunsaturated fat	18% protein and 10% fat (tallow, corn, or fish oil) for 3 wk	Increased insulin receptor messenger RNA concentrations in liver and adipose tissue with soy protein	Iritani, <i>et al</i> . 1997
Alloxan-diabetic mice	Defatted soy flour added to whole-durum meal	7–12% soy flour (9% protein) for 28 days	Decreased hyperglycemia and hyperlipidemia	Taha, et al. 1996
Nonobese diabetic mice	Soy protein with nicotinamide	40–200 days	Inhibited pancreatin insulitis	Reddy, et al. 1995
Streptozotocin-induced diabetic rats	Soy compared with casein	20% protein for 2–3 wk	Decreased eicosapentaenoic acid and increased arachidonic acid	Ikeda, et al. 1993
Genetically obese mice	Soy-protein isolate and hydrolysate compared with casein-protein isolate and hydrolysate	35% protein for 2 wk at 60% of energy	Decreased body weight, plasma glucose, and perirenal fat pad weight	Saito, et al. 1991
Gold thioglucose-induced obese mice	Soy saponin plus casein	15% casein with total saponin (10–100 mg \cdot day ⁻¹ \cdot kg body wt ⁻¹)	Decreased body fat	Kawano-Takahashi, <i>et al.</i> 1986

Table 2. Clinical and animal studies of soy (or isoflavones) on metabolic profiles

and insulin sensitivity in human.

Emerging evidence suggests that diets rich in phytoestrogens can have beneficial effects on many aspects of diabetes and obesity. Additional studies are needed to further elucidate the biologic and physiologic mechanisms by which phytoestrogens improve glucose tolerance and insulin sensitivity.

Conclusion

Whereas female life expectancy is 83 years in industrialized countries, the age of spontaneous menopause has remained stable at approximately 50 years of age. The fact that women now live more than one third of their lives after menopause has encouraged research efforts to prevent estrogen-deficiency related diseases using phytoestrogens. As mentioned in this review and a recently published review [124], the beneficial effects of phytoestrogens are still under investigation. One of the major reasons for the diverse results of phytoestrogens is the different compounds and doses that are given in the animal studies or clinical studies. The diversity of isoflavone contents in the compounds used in animal or clinical studies might complicate the understanding of the precise effects of phytoestrogens. Another important factor that complicates the understanding of the effects of soy isoflavones is the metabolites such as equol, a possibly more potent estrogenic compound than its precursor daidzein. All animals produce equal, whereas only 30% of human are reported to be able to metabolite daidzein to equol [125]. The ability to metabolize phytoestrogens to more potent compounds affects the estrogenicity of soy-related products. Phytoestrogens might be a promising alternative to conventional HRT methods and warrant further examination. Systematic studies from basic research to epidemiologic research are needed to answer these questions.

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