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REVIEW

The Physiological Actions of Isoflavone Phytoestrogens

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Summary

Isoflavones are a subgroup of phytoestrogens, natural plant substances with structure similar to 17-β-estradiol and capable of binding to estrogen receptors (ERs). Isoflavones possess higher affinity to ERβ than to ERα and may have a potency to activate both genomic and non-genomic estrogen signaling pathways. In addition, isoflavones interact with the metabolism of steroid hormones. Therefore, the actions of isoflavones are rather complex and may be related to large number of factors, which are not satisfactorily identified yet. Recently, isoflavones have come into focus of interest due to several reports about their positive effect on human health, in particular prevention of hormone-dependent cancers, cardiovascular diseases, osteoporosis, adverse menopausal manifestations and age-related cognitive decline. Isoflavones may bring new insights into the mechanisms of physiological regulations and increase the possibilities of medical interventions.

Key words

Isoflavones • Estradiol • Estrogen receptor • Sex hormones • Steroid hormones • Hormonal regulation • Phytopharmaceuticals

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Characteristics of phytoestrogens

Phytoestrogens represent a heterogeneous group of herbal substances, the structure of which is similar to that of 17-β-estradiol. They are called estrogen-like molecules or non-steroidal estrogens. In spite of the structural similarity with estradiol, phytoestrogens are diphenolic yet non-steroidal compounds. Currently the group of phytoestrogens includes more than 100 molecules, divided according to their chemical structure into 1) isoflavones (genistein, daidzein, biochanin A, formonetin) (Fig. 1), 2) lignans (matairesinol, secoisolariciresinol-diglucoside), 3) coumestans (coumestrol, 4-methoxycoumestrol), and 4) stilbens (resveratrol). Some of these substances (e.g. resveratrol) act as natural antioxidants and findings concerning their effect on the organism, especially the cardiovascular system, have been repeatedly reported in *Physiological Research* (Rezzanni *et al.* 2008, Carusio *et al.* 2008, Číž *et al.* 2008, Puzserová *et al.* 2006, Jendeková *et al.* 2006, Babál *et al.* 2006, Duarte *et al.* 2004, Zenebe *et al.* 2003, for review see Pecháňová *et al.* 2006). The present article is focused particularly on isoflavones and it summarizes recent knowledge on their biological impact. This group of substances has recently come into focus of interest due to increasing information on their positive effects in prevention of some forms of hormone-dependent cancer, cardiovascular diseases, osteoporosis, adverse menopausal manifestations and cognitive decline.

Occurrence and metabolism of isoflavones

In nature isoflavones occur in more than 300 kinds of plants, mostly in the roots and seeds (Klejdus *et al.* 2005). They are found in red clover, germs of alfalfa, linseed, as well as in extracts of red wine. They can be

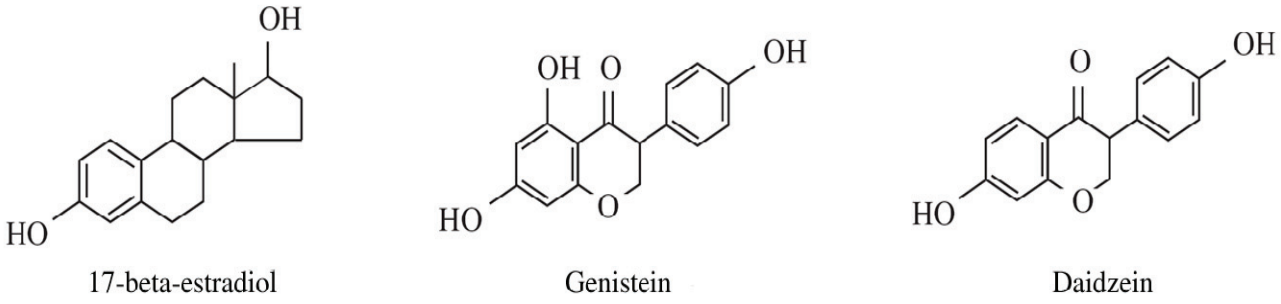

Fig. 1. Molecular structure of 17-β-estradiol and isoflavones genistein and daidzein. (Source: http://pubchem.ncbi.nlm.nih.gov)

produced by some kinds of bacteria and fungi. Soybeans are one of the richest sources of isoflavones in normal food. Dry soybeans contain 1.2-4.2 mg/g isoflavones (Wang and Murphy 1994). Their exact concentration depends on many factors, including the soil in which they are grown, climate, stage of their maturity or level of processing. Generally, higher degree of processing is associated with lower content of isoflavones. The second generation of soy products (e.g. tofu) contains only 6-20 % of the total isoflavone amount found in unprocessed soybeans (Duncan *et al.* 2003).

In plants, isoflavones are found as glucoconjugates, which are biologically inactive (Brown and Setchell 2001, Wiseman *et al.* 2002). They are hydrolyzed to active forms – aglycones – by the action of intestinal bacteria (Tsuchihaschi *et al.* 2008). In humans, daidzein and genistein are considered the most important biologically active forms of isoflavones. These substances arise both by hydrolysis of biologically inactive forms of glucoconjugates, as mentioned above, and by metabolism from biochanin A and formononetin. Aglycone forms of isoflavones are transported from the intestine to the blood or they are further metabolized directly in the intestine. Degradation of isoflavones occurs in the liver, where they are conjugated with glucuronic acid and to a lesser degree with sulfates. They are excreted from the body in urine or bile. The major portion of daidzein and genistein is eliminated from the body within 24 hours (Axelson *et al.* 1984, Lapčík *et al.* 1997, Setchell *et al.* 2001).

In blood serum, the highest levels of isoflavones are reached within 2-8 hours after consumption. After administration of 125 g of boiled soy, Lapčík *et al.* (1997) found the highest total level of daidzein to be about 500 nmol/l. Adlercreutz *et al.* (1993) examined isoflavone concentrations in relation to regional differences in food. In serum samples of Japanese men, the average concentrations amounted to 107 nmol/l of daidzein and 276 nmol/l of genistein (maximum value: 900 nmol/l for daidzein, 2 400 nmol/l for genistein). These values were on average 20-fold (daidzein) and 40-fold (genistein) higher than in samples of men from Finland. Extensive epidemiological research (WHO-CARDIAC study) has shown that mortalities of coronary artery disease and cancers are lower in populations with high soybean and fish consumption (for review see Yamori 2006). High intake of dietary phytoestrogens may thus contribute to healthy longevity.

Mechanism of action of isoflavones

Interaction with estrogen receptors

On the basis of their structural similarity with 17-β-estradiol, isoflavones are able to bind to estrogen receptors (ERs) (Barnes *et al.* 2000). In the organism, ERs are found in the two forms, ERα and ERβ, with different expression in tissues (Pietras and Szego 1977, Kuiper *et al.* 1997, 1998). Current data indicate that ERα plays a major role in mediating estrogen action in the uterus, hypothalamus/pituitary, skeleton, and other classical estrogen target tissues. ERβ seems to play role in the ovary, cardiovascular system, and brain as well as in several animal models of inflammation (Harris 2007). ERs are members of the steroid/thyroid superfamily of intracellular receptors, located primarily in the membrane of the nucleus. Interaction of isoflavones with ERs leads to the activation of so-called estrogen response elements located on the inner side of the nuclear membrane. In this way genomic mechanism, particularly transcription processes, are affected (Belcher and Zsarnovszky 2001).

The affinity of genistein to ERβ is about 20-30 times higher than to ERα and is comparable to the affinity of 17-β-estradiol (Kuiper *et al.* 1998, Morito *et al.* 2001). The affinity of other isoflavones is approximately 100-500 times lower than that of 17-β-estradiol. Isoflavones act as agonists of ERs, but their

activity is lower than that of 17-β-estradiol. At sufficiently high levels (over about 100 nmol/l for genistein), the effect of isoflavones may approach the effect of endogenous 17-β-estradiol at its physiological level (Kuiper *et al.* 1998). The effect of isoflavones also depends on the level of endogenous estradiol, since isoflavones and estradiol are competing for their binding on ERs. In a state of high levels of endogenous estrogens (e.g. women in the follicular phase of the menstrual cycle), isoflavones may obstruct full estrogen activity by occupying a part of the ERs. On the other hand, in a state with low levels of endogenous estrogens (men, women in menopause, after ovariectomy, etc.), the estrogen activity of isoflavones may become manifest (Kuiper *et al.* 1998, Lephart *et al.* 2002, 2005). In this context, isoflavones are being increasingly used as an alternative or complement of hormonal replacement therapy in postmenopausal women, especially in cases of long-term administration (Davis *et al.* 1998).

Recently, knowledge has been increasing on the existence and function of membrane ERs. It has been estimated that these represent about 2-3 % of all ERs (Levin 2001). Since membrane ERs are not directly involved in the regulation of transcription, the term "non-genomic" action with rapid effect has been coined (McEwen 2002, Patisaul 2005, Watson *et al.* 2007). Activation of membrane ERs initiates a cascade of intracellular mechanisms, which may include control of the activity of G-proteins, adenylate cyclase, phospholipase or protein kinase (Simoncini *et al.* 2003). Activation of these cascades results in rapid effects on cell metabolism, including changes in membrane permeability, ion concentration, production of nitric oxide (NO), etc. This mechanism is assumed to play an important role in tissues that are not considered to be classical targets of estradiol action. In the cardiovascular system, this mechanism is associated with rapid vasodilatation of blood vessels due to increased activity of endothelial NO-synthase (Liu *et al.* 2004, Pechanova and Simko 2007, Vera *et al.* 2007). In the CNS, the non-genomic way of action may affect excitability of neurons due to changes in cell membrane permeability (McEwen and Alves 1999). It has been speculated that these processes may be related to the effects of estradiol and isoflavone phytoestrogens on cognitive functions.

Interaction with the metabolism of steroid hormones

Isoflavones interact with sex steroids in multiple ways. Influence on the metabolism of sex hormones may be quite complex and may depend on several factors including species, sex, age, hormonal status, etc. Moreover, the dose and duration of isoflavones administration may not be linearly related to the treatment effect, which could add to the significant variability of research findings. Isoflavones were found to inhibit the activity of both 5α-reductase, which catalyzes the conversion of testosterone to 5α-dihydrotestosterone, and aromatase P450, which mediates the conversion of testosterone to estradiol (Adlercreutz *et al.* 1993, Evans *et al.* 1995, Kao *et al.* 1998, Brooks and Thompson 2005). On the other hand, it has been reported that aromatase activity is inhibited by low concentration of isoflavones only, whereas high isoflavone levels increase the activity of this enzyme (Almstrup *et al.* 2002). Isoflavones bind to the sex hormone binding globulin (SHBG), and stimulate its synthesis (Adlercreutz *et al.* 1987, Berrino *et al.* 2001). Alterations in SHBG concentration may yield changes in the concentration of circulating steroid hormones.

In rats fed with high amounts of isoflavones, reduced plasma testosterone was found (Weber *et al.* 2001), but no changes were reported in several other studies (Lund *et al.* 2001, Lephart *et al.* 2003, Wang *et al.* 2004). Berrino *et al.* (2001) reported that consumption of soy-rich diet reduced free testosterone in men. No effect on free testosterone, but an increase in total serum testosterone was found by others (Celec *et al.* 2007, Low *et al.* 2007). This has been explained by increased SHBG synthesis with consequently enhanced uptake of free testosterone, which, in turn, stimulates testosterone production. Therefore, at increased concentration of total testosterone in serum, the level of free testosterone may not change (Celec *et al.* 2005). In women, soy-rich diet decreased (Berrino *et al.* 2001), or had no effect on serum testosterone levels (Celec *et al.* 2005).

In normal rats, no alterations of estradiol level were reported after isoflavones administration (Weber *et al.* 2001, Lund *et al.* 2001, Lephart *et al.* 2003). However, increased estradiol serum levels were found in ovariectomized rats (Kawakita *et al.* 2009). In women before the menopause, either no effect (Celec *et al.* 2005) or a decrease in serum estradiol were reported (Nagata *et al.* 1998, Kurzer 2002). No effect (Petrakis *et al.* 1996), a decrease (Berrino *et al.* 2001, Low *et al.* 2005), but even an increase (Adlercreutz and Mazur 1997) were reported in postmenopausal women. In men, alterations in estradiol serum concentrations were not detected (Celec

et al. 2005, Ostatníková *et al.* 2007).

In addition to the interactions of isoflavones with the metabolism of sex steroids the effects on thyroid gland hormones were reported. Hampl *et al.* (2008) found that a short-term diet with a high amount of soybeans led to a transient increase in thyrotropin concentration. This effect was observed only in men and not in women, whereby the triiodothyronine and thyroxine serum concentrations were not changed.

Isoflavones and bone tissue

Low serum levels of 17-β-estradiol are associated with lower calcium availability and activation of bone resorption-accelerating cytokines (IL-1, IL-6, IL-11 and TNF), leading to the dominance of bone resorption over bone synthesis and subsequent bone decalcification (Slemenda *et al.* 1987). Therefore, osteoporosis is a considerable complication in postmenopausal women. Even short-term administration of estrogens enhances bone density. Nevertheless, in light of relatively frequent vascular complications and increased risk of estrogen-dependent cancers, they are not recommended as long-term hormonal replacement therapy (Colditz *et al.* 1995, Jick *et al.* 1996). Long-term administration of isoflavones was found to affect positively bone metabolism (Arjmandi *et al.* 1996, Blair *et al.* 1996). Six-month genistein administration to postmenopausal women led to a significant increase in bone density and concurrent reduction in the concentration of biochemical markers of bone resorption (Turhan *et al.* 2008). After twelve-month genistein administration, the increase in bone density was comparable to the effects of estrogen hormonal replacement therapy (Potter *et al.* 1998, Morabito *et al.* 2002). Importantly, the administration of isoflavones seems not to be associated with the above mentioned health risks of estrogen therapy (Cornwell *et al.* 2004).

Polkowski and Mazurek (2000) suggested that the positive effect of isoflavones on bone metabolism may be mediated by at least two mechanisms. The first is the impact on osteoclasts *via* activation of apoptosis. The second is the inhibition of tyrosine-kinase activity via modulation of membrane ERs with consecutive changes in the activity of alkaline phosphatase. In accordance with this hypothesis, Blair *et al.* (1996) reported that cell osteoclast cultures washed by a genistein concentrate exhibited decreased tyrosine-kinase activity with subsequently reduced bone remodeling.

Isoflavones and the cardiovascular system

Cardiovascular diseases represent the main cause of death in western countries. In Asia, the incidence of cardiovascular diseases is about eight times lower than in western countries (Beaglehole 1990). Besides hereditary factors, the disproportion in the incidence of cardiovascular diseases is assumed to be caused by nutritional factors. Soy is an important component of food in eastern countries so that a long-term intake of isoflavones by this population is high. This suggests that isoflavones may exert a protective effect on the cardiovascular system (Adlercreutz 1990, Anderson *et al.* 1999, Yamori 2006). There is growing evidence that isoflavones affect the cardiovascular system *via* several mechanisms. These include regulation of vasoactivity and alteration of lipid metabolism.

Current data indicate that soy isoflavones affect vascular tone through a combination of mechanisms including endothelial-dependent and endothelial-independent vasodilator systems and inhibition of constrictor mechanisms. These processes involve both classical genomic as well as non-genomic mechanisms of action. Activation of nuclear ERs by isoflavones was found to increase expression of endothelial NO-synthase (eNOS), reduce oxidative stress and increase NO bioavailability (Mahn *et al.* 2005). Through a rapid non-genomic way of action (after activation of membrane ERs) isoflavones modulate several signaling pathways, including cAMP/protein kinase A, phosphatidylinositol-3-kinase (PI3-kinase)/protein kinase B (Akt), and extracellular signal-regulated kinase 1 and 2 (ERK 1/2), resulting in increased eNOS activity and NO production (Liu *et al.* 2004, Joy *et al.* 2006, Fu and Simoncini 2007, for review see Mann *et al.* 2007). PI3-kinase/Akt pathway mediates induction of heme oxygenase 1 (HO-1) and other antioxidant defense genes in oxidative stress (Siow *et al.* 2007). Shin *et al.* (2007) reported increased NO plasma concentration by genistein, which was accompanied with elevated expression of cyclo-oxygenase-2 (COX-2) and prostaglandin E_2 production. Expression of eNOS and inducible NOS (iNOS) was unchanged. This indicates that genistein increased NOS activity directly and/or it increased NO bioavailability by decreasing oxidative stress. Hermenegildo *et al.* (2005) showed that isoflavones increased cyclooxygenase-2 (COX-2) expression and prostacyclin production in endothelial cells *via* ERs-specific mechanisms. It has been reported that isoflavones decrease vascular

contraction through inhibition of RhoA/Rho-kinase signaling (Seok *et al.* 2008). Furthermore, isoflavones inhibit tromboxane A_2 receptors in smooth muscle cells and platelets and may thus decrease thrombogenicity (Guerrero *et al.* 2005). Isoflavones were also found to inhibit growth and migration of vascular smooth muscle cells, which may protect against the development of atherosclerosis (Kanazawa *et al.* 1993, Anthony *et al.* 1998, Pan *et al.* 2001).

Renal factors may also play a role in hypotensive action of isoflavones since isoflavones were reported to increase renal blood flow and sodium excretion (Martin *et al.* 2008). Central mechanisms of blood pressure regulation are a further possible target of isoflavone action. It was found that estrogens, acting through ERβ and the NO system in the hypothalamus, attenuate blood pressure responses to psychological stress (Gingerich and Krukoff 2005). Injections of estradiol into the rostral ventrolateral medulla, a region where sympathetic premotor neurons for the maintenance of basal vasomotor tone are located, lowered systemic arterial pressure, which was mediated *via* ERβ and iNOS-derived NO, but not nNOS- and eNOS-derived NO (Shih 2009). It is conceivable that these mechanisms could be invoked by isoflavones, considering their selective affinity to ERβ.

Although the clinical potency of isoflavones is a matter of debate (Hodgson *et al.* 1999, Sacks *et al.* 2006), a significant hypotensive effect of isoflavones administration was found in several controlled studies. Soy, isoflavones or their metabolites were found to improve endothelial function as well as to decrease blood pressure and arterial stiffness (Washburn 1999, Squadrito *et al.* 2003, Teede *et al.* 2003, Nestel *et al.* 2007). Rivas *et al.* (2002) found significant correlation between urinary genistein and blood pressure reduction in patients with mild-to-moderate hypertension treated with soy milk. Reduction of blood pressure was reported in normotensive and hypertensive women in association with soybean consumption (Yang *et al.* 2005, Welty *et al.* 2007). In rats the data are less conclusive (Mahn *et al.* 2005, Douglas *et al.* 2006). Interestingly, a shorter survival time was observed in spontaneously hypertensive rats treated with isoflavones (Gilani *et al.* 2009).

A diet with high intake of soy was reported to decrease plasma triglyceride levels, total cholesterol, LDL cholesterol and increase HDL cholesterol (Cassidy *et al.* 1995, Potter *et al.* 1998, Washburn *et al.* 1999, Merz-Demlow *et al.* 2000, Wangen *et al.* 2001, Teede *et al.* 2001, Jayagopal *et al.* 2002, Sanders *et al.* 2002, De Kleijn *et al.* 2002). However, it has been suggested that other substances occurring in soybeans, apart from isoflavones, may contribute to these effects (Anderson *et al.* 1995, Geller and Studee 2006). On the other hand, some studies showed no effect of isoflavones on serum cholesterol levels or plasma lipids (Gooderham *et al.* 1996, Samman *et al.* 1999, Simons *et al.* 2000, Dewell *et al.* 2002, Aubertin-Leheudre *et al.* 2008; for review see Sacks *et al.* 2006).

Isoflavones and hormone-dependent cancers

It is known that some types of tumors, such as breast, prostate and colon cancers have a lower incidence in Asian countries compared to the population of western countries (Adlercreutz *et al.* 1992, Adlercreutz and Mazur 1997). Environmental factors appear to contribute largely to the development of these tumors (Ziegler *et al.* 1993). Asian immigrants to western countries who changed their dietary habits (lower intake of soybeans and fiber sources, higher consumption of meat products) suffer more frequently from these forms of cancers (Bingham *et al.* 1998).

Animals fed with high doses of soybeans showed lower incidence of breast and mammary gland cancer (Barnes *et al.* 1990). In postmenopausal women, consumption of isoflavones was found to be associated with reduction of breast cancer incidence (Ingram *et al.* 1997), mammary gland density (Atkinson *et al.* 2004), and proliferation ability of mammary gland cells (Palomares *et al.* 2004). These effects have been associated with the ability of isoflavones to increase serum SHBG concentration, thereby reducing the bioavailability of sexual hormones in hormone-dependent tissues (Kurzer 2002). Moreover, in peripheral tissues, isoflavones inhibit enzymes involved in the processes of cell proliferation (e.g. tyrosine kinase) (Gerosa *et al.* 1993, Blair *et al.* 1996) and reduce estradiol availability through the inhibitory effect on aromatase P450 (Kao *et al.* 1998).

On the other hand, it has been reported that high doses of genistein may activate cell proliferation in estrogen-dependent tumors (Hsieh *et al.* 1998, McMichael-Phillips *et al.* 1998). In contrast, Mertens *et al.* (2004) reported that the administration of isoflavones in the dose of 100 mg/day over a period of 12 months had no effect on cell proliferation of the contralateral

unaffected breast in women who had been treated for breast cancer in the past. Similarly, Fabian *et al.* (2005) reported that isoflavones did not affect the number of atypical cells in the breast tissue. Due to these uncertain and contradictory findings, high isoflavone intake is not recommended in patients with known diagnosis of estrogen-dependent breast cancer (Hulka 1996, Messina *et al.* 2006).

In cell cultures, high doses of genistein and biochanin A inhibit the growth of prostate cancer cell lines (Peterson and Barnes 1993). In rats, reduced prostate volume and weight were found after administration of isoflavones (Lund *et al.* 2001, Fritz *et al.* 2002). It has been reported that higher plasma concentrations of genistein are associated with lower incidence of prostate cancer in men (Travis *et al.* 2009).

Isoflavones and the central nervous system

Similarly to estradiol, isoflavones pass through the blood-brain barrier. In rats, consumption of large amounts of soybeans increased the concentration of isoflavones in basal parts of the hypothalamus, the hippocampus, the cerebellum, and the frontal cortex (Lephart *et al.* 2000, 2002, Lund *et al.* 2001). This corresponds well with regional expression of ERβ, which bind isoflavones (Osterlund *et al.* 1998). In contrast to peripheral tissues, the activity of aromatase P450 in the brain seems to be unaffected by dietary isoflavones (Lephart *et al.* 2000). Surprisingly, decreased activity of 5α-reductase in the hypothalamus and amygdala has been reported at low, but not at high isoflavones intake (Weber *et al.* 1999, Lephart *et al.* 2000).

In a series of studies, Lephart and coworkers reported the effects of isoflavones on sexually dimorphic hypothalamic structures in rats. The sexually dimorphic nucleus of the preoptic area (SDN-POA) is larger in males, whereas the anteroventral periventricular nucleus (AVPN) has larger volume in females. Both nuclei are involved in sexual behavior and the control of gonadotropin release (De Jonge *et al.* 1989, Simerly 1998). Consumption of large amounts of soybeans decreased the volume of SDN-POA in males but not in females. Conversely, AVPN was diminished in females but not in males (Lephart *et al.* 2005). In both sexes, the diet compromised sexual behavior and copulation (Lund 2001, Lephart *et al.* 2001, 2002). It has been hypothesized that the effects of isoflavones on these structures may be related to calbindin expression. Calbindin is a calcium-binding protein with protective effect against apoptosis (Iacopino *et al.* 1992). It has been found that isoflavones decrease the expression of calbindin in the hypothalamus (Lephart *et al.* 2000, Watson *et al.* 1998). Low levels of calbindin may promote apoptosis resulting in volume reduction. Expression of calbindin is high in the SDN-POA of males, whereas it is high in the AVPN of females (Lephart 1996). Due to this region-specific calbindin expression, isoflavones may have different impact in males and females.

Human studies focused mostly on the effect of isoflavones on cognitive functions. In general, beneficial effects have been reported (Karvaj *et al.* 2007). Long-term administration of soy or preparations of isolated isoflavones, used as an alternative hormonal replacement therapy, have been found to improve learning, logical thinking and planning ability in postmenopausal women (Duffy *et al.* 2003, Kritz-Silvertein *et al.* 2003, Kreijkamp-Kaspers *et al.* 2007). Even short-term intensive soy consumption (>170 mg isoflavones/day, during 7 consecutive days) in young healthy adult women, but not in men, significantly improved spatial visualization and mental rotation (Ostatníková *et al.* 2007, Celec *et al.* 2005, 2007). However, this improvement seems to be obvious only in highly demanding tasks (Pilšáková *et al.* 2009). In odds with above cited studies, White *et al.* (2000) reported that a regular consumption of tofu in men compromised cognition and accelerated brain atrophy. Similar finding were reported by Hogevorst *et al.* (2008) for both sexes. It is unclear whether this negative effect is associated with isoflavones, other phytoestrogens or potential toxins related to tofu preparation. On the other hand, epidemiological studies point to lower rates of dementia in Asian population (White *et al.* 1996, Liu *et al.* 2003).

Conclusions

Biological actions of isoflavones are manifold and include several physiological systems. Isoflavones affect multiple signaling pathways through the activation of both intracellular and membrane ERβ, as well as interaction with the metabolism of steroid hormones. Therefore, the impact of isoflavones on physiological processes in the organism seems to be very complex and may be related to large number of factors, which are not satisfactorily identified yet. Despite increasing number of studies, there is still a long way to a firm knowledge on the biological potency of isoflavones and their impact on human health. Nevertheless, isoflavone phytoestrogens

are very promising substances that may provide us with new ideas on the mechanisms of physiological regulations and therapeutic interventions. Encouraging preliminary findings have promoted intensive research in this area. We may thus hope that many of the open questions about the impact of isoflavones will be answered in the near future.

Conflict of Interest

There is no conflict of interest.

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