

The Physiological Actions of Isoflavone Phytoestrogens

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Received October 4, 2009

Accepted February 25, 2010

On-line April 20, 2010

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 ER β than to ER α 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, formononetin) (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

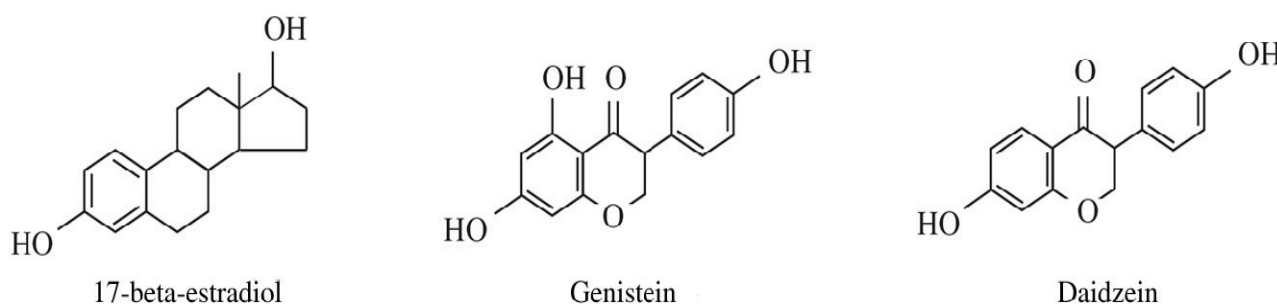


Fig. 1. Molecular structure of 17- β -estradiol and isoflavones genistein and daidzein. (Source: <http://pubchem.ncbi.nlm.nih.gov>)

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, Lapčik *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, Lapčik *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, ER α and ER β , with different expression in tissues (Pietras and Szego 1977, Kuiper *et al.* 1997, 1998). Current data indicate that ER α plays a major role in mediating estrogen action in the uterus, hypothalamus/pituitary, skeleton, and other classical estrogen target tissues. ER β 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 ER β is about 20-30 times higher than to ER α 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 5 α -reductase, which catalyzes the conversion of testosterone to 5 α -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 cyclooxygenase-2 (COX-2) and prostaglandin E₂ 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 thromboxane A₂ 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 ER β 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* ER β 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 ER β .

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 ER β , 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 5 α -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 Hogevoorst *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 ER β , 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.

Acknowledgements

Supported by research grants VEGA 2/0160/08 and APVV-0538-07. I.R. was supported by a postdoctoral fellowship of the Action Austria-Slovakia No. 57s07.

References

- ADLERCREUTZ H: Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. *Scand J Clin Lab Invest Suppl* 201: 3-23, 1990.
- ADLERCREUTZ H, MAZUR W: Phyto-oestrogens and western diseases. *Ann Med* 29: 95-120, 1997.
- ADLERCREUTZ H, HÖCKERSTEDT K, BANNWART C, BLOIGU S, HÄMÄLÄINEN E, FOTSIS T, OLLUS A: Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *J Steroid Biochem* 27: 1135-1144, 1987.
- ADLERCREUTZ H, MOUSAVI Y, CLARK J, HÖCKERSTEDT K, HÄMÄLÄINEN E, WÄHÄLÄ K, MÄKELÄ T, HASE T: Dietary phytoestrogens and cancer: in vitro and in vivo studies. *J Steroid Biochem Mol Biol* 41: 331-337, 1992.
- ADLERCREUTZ H, BANNWART C, WÄHÄLÄ K, MÄKELÄ T, BRUNOW G, HASE T, AROSEMENA PJ, KELLIS JT Jr, VICKERY LE: Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J Steroid Biochem Mol Biol* 44: 147-153, 1993.
- ALMSTRUP K, FERNANDEZ MF, PETERSEN JH, OLEA N, SKAKKEBAEK NE, LEFFERS H: Dual effects of phytoestrogens result in U-shaped dose-response curves. *Environ Health Perspect* 110: 743-748, 2002.
- ANDERSON JJB, ANTHONY MS, CLINE JM, WASHBURN SA, GARNER SC: Health potential of soy isoflavones for menopausal women. *Public Health Nutr* 2: 489-504, 1999.
- ANDERSON JW, JOHNSTONE BM, COOK-NEWELL ME: Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* 333: 276-282, 1995.
- ANTHONY MS, CLARKSON T, WILLIAMS JK: Effects of soy isoflavones on atherosclerosis: potential mechanisms. *Am J Clin Nutr* 68: 1390-1393, 1998.
- ARJMANDI BH, ALEKEL L, HOLLIS BW, AMIN D, STACEWICZ-SAPUNTZAKIS M, GUO P, KUKREJA SC: Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. *J Nutr* 126: 161-167, 1996.
- ATKINSON C, WARREN RM, SALA E, DOWSETT M, DUNNING AM, HEALEY CS, RUNSWICK S, DAY NE, BINGHAM SA: Red-clover-derived isoflavones and mammographic breast density: a double-blind, randomized, placebo-controlled trial. *Breast Cancer Res* 6: 170-179, 2004.
- AUBERTIN-LEHEUDRE M, LORD C, KHALIL A, DIONNE IJ: Isoflavones and clinical cardiovascular risk factors in obese postmenopausal women: a randomized double-blind placebo-controlled trial. *J Womens Health (Larchmt)* 17: 1363-1369, 2008.
- AXELSON M, SJÖVALL J, GUSTAFSSON BE, SETCHELL KD: Soya - a dietary source of the non-steroidal oestrogen equol in humans and animals. *J Endocrinol* 102: 49-56, 1984.
- BABÁL P, KRISTOVÁ V, ČERNÁ A, JANEGA P, PECHÁŇOVÁ O, DANIHEL L, ANDRIANTSITOHAINA R: Red wine polyphenols prevent endothelial damage induced by CCl₄ administration. *Physiol Res* 55: 245-251, 2006.
- BARNES S, GRUBBS C, SETCHELL KD, CARLSON J: Soybeans inhibit mammary tumors in models of breast cancer. *Prog Clin Biol Res* 347: 239-253, 1990.
- BARNES S, KIM H, DARLEY-USMAR V, PATEL R, XU J, BOERSMA B, LUO M: Beyond ER α and ER β : estrogen receptor binding is only part of the isoflavone story. *J Nutr* 130: 656S-657S, 2000.

- BEAGLEHOLE R: International trends in coronary heart disease mortality, morbidity and risk factors. *Epidemiol Rev* **12**: 1-15, 1990.
- BELCHER SM, ZSARNOVSZKY A: Estrogenic actions in the brain: estrogen, phytoestrogens and the rapid intracellular signaling mechanisms. *J Pharmacol Exp Ther* **299**: 408-414, 2001.
- BERRINO F, BELLATI C, SECRETO G, CAMERINI E, PALA V, PANICO S, ALLEGRO G, KAAKS R: Reducing bioavailable sex hormones through a comprehensive change in diet: the diet and androgens (DIANA) randomized trial. *Cancer Epidemiol Biomarkers Prev* **10**: 25-33, 2001.
- BINGHAM SA, ATKINSON C, LIGGINS J, BLUCK L, COWARD A: Phyto-oestrogens: where are we now? *Br J Nutr* **79**: 393-406, 1998.
- BLAIR HC, JORDAN SE, PETERSON TG, BARNES S: Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. *J Cell Biochem* **15**: 629-637, 1996.
- BROOKS JD, THOMPSON LU: Mammalian lignans and genistein decrease the activities of aromatase and 17 β -hydroxysteroid dehydrogenase in MCF-7 cells. *J Steroid Biochem Mol Biol* **94**: 461-467, 2005.
- BROWN NM, SETCHELL DR: Animal Models Impacted by Phytoestrogens in Commercial Chow: Implications for Pathways Influenced by Hormones. *Lab Invest* **81**: 735-747, 2001.
- CARUSIO N, WANGENSTEEN R, FILIPPELLI A, ANDRIANTSITOHAINA R: Oral administration of polyphenolic compounds from cognac decreases ADP-induced platelet aggregation and reduces chronotropic effect of isoprenaline in rats. *Physiol Res* **57**: 517-524, 2008.
- CASSIDY A, BINGHAM S, SETCHELL K: Biological effects of isoflavones in young women: importance of the chemical composition of soybean products. *Br J Nutr* **74**: 587-601, 1995.
- CELEC P, OSTATNÍKOVÁ D, CAGÁNOVÁ M, ŽUCHOVÁ S, HODOSY J, PUTZ Z, BERNADIČ M, KÚDELA M: Endocrine and cognitive effects of short-time soybean consumption in women. *Gynecol Obstet Invest* **59**: 62-66, 2005.
- CELEC P, OSTATNÍKOVÁ D, HODOSY J, PUTZ Z, KÚDELA M: Increased one week soybean consumption affects spatial abilities but not sex hormone status in men. *Int J Food Sci Nutr* **58**: 424-428, 2007.
- ČÍŽ M, PAVELKOVÁ M, GALLOVÁ L, KRÁLOVÁ J, KUBALA L, LOJEK A: The influence of wine polyphenols on reactive oxygen and nitrogen species production by murine macrophages RAW 264.7. *Physiol Res* **57**: 393-402, 2008.
- COLDITZ GA, HANKINSON SE, HUNTER DJ, WILETT WC, MANSON JE, STAMPHER MJ: The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. *N Engl J Med* **332**: 1589-1593, 1995.
- CORNWELL T, COHICK W, RASKIN I: Dietary phytoestrogens and health. *Phytochemistry* **65**: 995-1016, 2004.
- DAVIS SR, MURKIES AL, WILCOW G: Phytoestrogens in clinical practice. *Integr Med* **1**: 27-34, 1998.
- DEWELL A, HOLLENBECK CB, BRUCE B: The effects of soy-derived phytoestrogens on serum lipids and lipoproteins in moderately hypercholesterolemic postmenopausal women. *J Clin Endocrinol Metab* **87**: 118-121, 2002.
- DOUGLAS G, ARMITAGE JA, TAYLOR PD, LAWSON JR, MANN GE, POSTON L: Cardiovascular consequences of life-long exposure to dietary isoflavones in the rat. *J Physiol Lond* **571**: 477-487, 2006.
- DE JONGE FH, LOUWERSE AL, OOMS MP, EVERS P, ENDERT E, VAN DE POLL NE: Lesions of the SDN-POA inhibit sexual behavior of male Wistar rats. *Brain Res Bull* **23**: 483-492, 1989.
- DE KLEIJN MJ, VAN DER SCHOUW YT, WILSON PW, GROBBEE DE, JACQUES PF: Dietary intake of phytoestrogens is associated with a favorable metabolic cardiovascular risk profile in postmenopausal U.S. women: the Framingham study. *J Nutr* **132**: 276-282, 2002.
- DUARTE J, ANDRIAMBELOSON E, DIEBOLT M, ANDRIANTSITOHAINA R: Wine polyphenols stimulate superoxide anion production to promote calcium signaling and endothelial-dependent vasodilatation. *Physiol Res* **53**: 595-602, 2004.
- DUFFY R, WISEMAN H, FILE SE: Improved cognitive function in postmenopausal women after 12 weeks of consumption of soya extract containing isoflavones. *Pharmacol Biochem Behav* **75**: 721-729, 2003.
- DUNCAN AM, PHIPPS WR, KURZER MS: Phyto-oestrogens. *Best Pract Res Clin Endocrinol Metab* **17**: 253-271, 2003.

- EVANS BA, GRIFFITHS K, MORTON MS: Inhibition of 5 alpha-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. *J Endocrinol* **147**: 295-302, 1995.
- FABIAN CJ, KIMLER BF, MAYO MS, KHAN SA: Breast-tissue sampling for risk assessment and prevention. *Endocr Relat Cancer* **12**: 185-213, 2005.
- FRITZ WA, ELTOUM IE, COTRONEO MS, LAMARTINIÈRE CA: Genistein alters growth but is not toxic to the rat prostate. *J Nutr* **132**: 3007-3011, 2002.
- FU XD, SIMONCINI T: Non-genomic sex steroid in the vascular system. *Semin Reprod Med* **25**: 178-186, 2007.
- GELLER SE, STUDEE L: Soy and red clover for midlife and aging. *Climacteric* **9**: 245-263, 2006.
- GEROSA F, TOMMASI M, BENATI C, GANDINI G, LIBONATI M, TRIDENTE G, CARRA G, TRINCHIERI G: Differential effects of tyrosine kinase inhibition in CD69 antigen expression and lytic activity induced by rIL-2, rIL-12, and rIFN-alpha in human NK cells. *Cell Immunol* **150**: 382-390, 1993.
- GILANI GS, NIMAL RATNAYAKE WM, MUELLER R, MAZZA G: Effects of source of protein and supplementary extracted isoflavones and anthocyanins on longevity of stroke-prone spontaneously hypertensive (SHRSP) rats. *J Toxicol Sci* **34**: 335-341, 2009.
- GINGERICH S, KRUKOFF TL: Estrogen modulates endothelial and neuronal nitric oxide synthase expression via an estrogen receptor beta-dependent mechanism in hypothalamic slice cultures. *Endocrinology* **146**: 2933-2941, 2005.
- GOODERHAM MH, ADLERCREUTZ H, OJALA ST, WÄHÄLÄ K, HOLUB BJ: A soy protein isolate rich in genistein and daidzein and its effects on plasma isoflavone concentrations, platelet aggregation, blood lipids and fatty acid composition of plasma phospholipid in normal men. *J Nutr* **126**: 2000-2006, 1996.
- GUERRERO JA, LOZANO ML, CASTILLO J, BENAVENTE-GARCÍA O, VICENTE V, RIVERA J: Flavonoids inhibit platelet function through binding to the thromboxane A₂ receptor. *J Thromb Haemost* **3**: 369-376, 2005.
- HAMPL R, OSTATNÍKOVÁ D, CELEC P, PUTZ Z, LAPČÍK O, MATUCHA P: Short-term effect of soy consumption on thyroid hormone levels and correlation with phytoestrogen level in healthy subjects. *Endocr Regul* **42**: 53-61, 2008.
- HARRIS HA: Estrogen receptor-β: recent lessons from in vivo studies. *Mol Endocrinol* **21**: 1-13, 2007.
- HERMENEGILDO C, OVIEDO PJ, GARCÍA-PÉREZ MA, TARÍN JJ, CANO A: Effects of phytoestrogens genistein and daidzein on prostacyclin production by human endothelial cells. *J Pharmacol Exp Ther* **315**: 722-728, 2005.
- HODGSON JM, PUDDEY IB, BEILIN LJ, MORI TA, BURKE V, CROFT KD, ROGERS PB: Effects of isoflavonoids on blood pressure in subjects with high-normal ambulatory blood pressure levels: a randomized controlled trial. *Am J Hypertens* **12**: 47-53, 1999.
- HOGERVORST E, SADJIMIM T, YESUFU A, KREAGER P, RAHARDJO TB: High tofu intake is associated with worse memory in elderly Indonesian men and women. *Dement Geriatr Cogn Disord* **26**: 50-57, 2008.
- HSIEH CY, SANTELL RC, HASLAM SZ, HELFERICH WG: Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. *Cancer Res* **58**: 3833-3838, 1998.
- HULKA BS: Epidemiology of susceptibility to breast cancer. *Prog Clin Biol Res* **395**: 159-174, 1996.
- IACOPINO A, CHRISTAKOS S, GERMAN D, SONSALLA PK, ALTAR CA: Calbindin-D28K-containing neurons in animal models of neurodegeneration: possible protection from excitotoxicity. *Brain Res Mol Brain Res* **13**: 251-261, 1992.
- INGRAM D, SANDERS K, KOLYBABA M, LOPEZ D: Case-control study of phyto-oestrogens and breast cancer. *Lancet* **350**: 990-994, 1997.
- JAYAGOPAL V, ALBERTAZZI P, KILPATRICK ES, HOWARTH EM, JENNINGS PE, HEPBURN DA, ATKIN SL: Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. *Diabetes Care* **25**: 1709-1714, 2002.
- JENDEKOVÁ L, KOJŠOVÁ S, ANDRIANTSITOHAINA R, PECHÁŇOVÁ O: The time-dependent effect of Provinols on brain NO synthase activity in L-NAME-induced hypertension. *Physiol Res* **55** (Suppl. 1): S31-S37, 2006.

- JICK H, DERBY LE, MYERS MW, VASILAKIS V, NEWTON KM: Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens. *Lancet* **349**: 981-983, 1996.
- JOY S, SIOW RC, ROWLANDS DJ, BECKER M, WYATT AW, AARONSON PI, COEN CW, KALLOI, JACOB R, MANN GE: The isoflavone Equol mediates rapid vascular relaxation: Ca²⁺-independent activation of endothelial nitric-oxide synthase/Hsp90 involving ERK1/2 and Akt phosphorylation in human endothelial cells. *J Biol Chem* **281**: 27335-27345, 2006.
- KANAZAWA T, TANAKA M, UEMURA T, OSANAI T, ONODERA K, OKUBO K, METOKI H, OIKE Y: Anti-atherogenicity of soybean protein. *Ann N Y Acad Sci* **676**: 202-214, 1993.
- KAO YC, ZHOU C, SHERMAN M, LAUGHTON CA, CHEN S: Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoestrogens: a site-directed mutagenesis study. *Environ Health Perspect* **106**: 85-92, 1998.
- KARVAJ M, BENO P, FEDOR-FREYBERGH PG: Positive effect of flavonoids to cardiovascular and central nervous system. *Neuro Endocrinol Lett* **28** (Suppl 4): 1-3, 2007.
- KAWAKITA S, MAROTTA F, NAITO Y, GUMASTE U, JAIN S, TSUCHIYA J, MINELLI E: Effect of an isoflavones-containing red clover preparation and alkaline supplementation on bone metabolism in ovariectomized rats. *Clin Interv Aging* **4**: 91-100, 2009.
- KLEJDUS B, MIKELOVÁ R, PETRLOVÁ J, POTĚŠIL D, ADAM V, STIBOROVÁ M, HODEK P, VACEK J, KIZEK R, KUBÁŇ V: Evaluation of isoflavone aglycon and glycoside distribution in soy plants and soybeans by fast column high-performance liquid chromatography coupled with a diode-array detector. *J Agric Food Chem* **53**: 5848-5852, 2005.
- KREIJKAMP-KASPERS S, KOK L, GROBBEE DE, DE HAAN EH, ALEMAN A, VAN DER SCHOUW YT: Dietary phytoestrogen intake and cognitive function in older women. *J Gerontol A Biol Sci Med Sci* **62**: 556-562, 2007.
- KRITZ-SILVERSTEIN D, VON MUHLEN D, BARRET-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.
- KUIPER GG, GUSTAFSSON JA: The novel estrogen receptor-beta subtype: potential role in the cell- and promoter-specific actions of estrogens and anti-estrogens. *FEBS Lett* **410**: 87-90, 1997.
- KUIPER GG, LEMMEN JG, CARLSSON B, CORTON JC, SAFE SH, VAN DER SAAG PT, VAN DER BURG B, GUSTAFSSON JA: Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* **139**: 4252-4263, 1998.
- KURZER MS: Hormonal effects of soy in premenopausal women and men. *J Nutr* **132**: 570-573, 2002.
- LAPČÍK O, HAMPL R, AL-MAHARIK N, SALAKKA A, WÄHÄLÄ K, ADLERCREUTZ H: A novel radioimmunoassay for daidzein. *Steroids* **62**: 315-320, 1997.
- LEPHART ED: Dimorphic expression of calbindin-D28K in the medial basal hypothalamus from perinatal male and female rats. *Brain Res Dev Brain Res* **23**: 281-284, 1996.
- LEPHART ED, THOMPSON JM, SETCHELL KD, ADLERCREUTZ H, WEBER KS: Phytoestrogens decrease brain calcium-binding proteins but not alter hypothalamic androgen metabolizing enzymes in adult male rats. *Brain Res* **859**: 123-131, 2000.
- LEPHART ED, LUND TD, HORVATH TL: Brain androgen and progesterone metabolizing enzymes: biosynthesis, distribution and function. *Brain Res Brain Res Rev* **37**: 25-37, 2001.
- LEPHART ED, WEST TW, WEBER KS, RHEES RW, SETCHELL KD, ADLERCREUTZ H, LUND TD: Neurobehavioral effects of dietary soy phytoestrogens. *Neurotoxicol Teratol* **24**: 5-16, 2002.
- LEPHART ED, RHEES RW, SETCHELL KD, BU LH, LUND TD: Estrogens and phytoestrogens: brain plasticity of sexually dimorphic brain volumes. *J Steroid Biochem Mol Biol* **85**: 299-309, 2003.
- LEPHART ED, SETCHELL KD, LUND TD: Phytoestrogens: hormonal action and brain plasticity. *Brain Res Bull* **65**: 193-198, 2005.
- LEVIN ER: Cell localization, physiology, and nongenomic actions of estrogen receptors. *J Appl Physiol* **91**: 1860-1867, 2001.
- LIU D, HOMAN LL, DILLON JS: Genistein acutely stimulates nitric oxide synthesis in vascular endothelial cells by a cyclic adenosine 5'-monophosphate-dependent mechanism. *Endocrinology* **145**: 5532-5539, 2004.

- LIU L, GUO XE, ZHOU YQ, XIA JL: Prevalence of dementia in China. *Dement Geriatr Cogn Disord* **15**: 226-230, 2003.
- LOW YL, TAYLOR JI, GRACE PB, DOWSETT M, SCOLLEN S, DUNNING AM, MULLIGAN AA, WELCHAA, LUBEN RN, KHAW KT, DAY NE, WAREHAM NJ, BINGHAM SA: Phytoestrogen exposure correlation with plasma estradiol in postmenopausal women in European Prospective Investigation of Cancer and Nutrition-Norfolk may involve diet-gene interactions. *Cancer Epidemiol Biomarkers Prev* **14**: 213-220, 2005.
- LOW YL, DUNNING AM, DOWSETT M, FOLKERT E, DOODY D, TAYLOR J, BHANIANI A, LUBEN R, KHAW KT, WAREHAM NJ, BINGHAM SA: Phytoestrogen exposure is associated with circulating sex hormone levels in postmenopausal women and interacts with ESR1 and NR1I2 gene variants. *Cancer Epidemiol Biomarkers Prev* **16**: 1009-1016, 2007.
- LUND TD, WEST TW, TIAN LY, BU LH, SIMMONS DL, SETCHELL KD, ADLERCREUTZ H, LEPHART ED: Visual spatial memory is enhanced in female rats (but inhibited in males) by dietary soy phytoestrogens. *BMC Neurosci* **2**: 20, 2001.
- MAHN K, BORRÁS C, KNOCK GA, TAYLOR P, KHAN IY, SUGDEN D, POSTON L, WARD JP, SHARPE RM, VIÑA J, AARONSON PI, MANN GE: Dietary soy isoflavone induced increases in antioxidant and eNOS gene expression lead to improved endothelial function and reduced blood pressure in vivo. *FASEB J* **19**: 1755-1757, 2005.
- MANN GE, ROWLANDS DJ, LI FY, DE WINTER P, SIOW RC: Activation of endothelial nitric oxide synthase by dietary isoflavones: role of NO in Nrf2-mediated antioxidant gene expression. *Cardiovasc Res* **75**: 261-274, 2007.
- MARTIN D, SONG J, MARK C, EYSTER K: Understanding the cardiovascular actions of soy isoflavones: potential novel targets for antihypertensive drug development. *Cardiovasc Hematol Disord Drug Targets* **8**: 297-312, 2008.
- MCEWEN B: Estrogen actions throughout the brain. *Recent Prog Horm Res* **57**: 357-384, 2002.
- MCEWEN BS, ALVES SE: Estrogen actions in the central nervous system. *Endocr Rev* **20**: 279-307, 1999.
- MCMICHAEL-PHILLIPS DF, HARDING C, MORTON M, ROBERTS SA, HOWELL A, POTTEN CS, BUNDRED NJ: Effects of soy-protein supplementation on epithelial proliferation in the histologically normal human breast. *Am J Clin Nutr* **68** (6 Suppl): 1431S-1435S, 1998.
- MERTENS WC, HILBERT V, MAKARI-JUDSON G: Contralateral breast cancer: factor associated with stage and size at presentation. *Breast J* **10**: 304-312, 2004.
- MERZ-DEMLOW BE, DUNCAN AM, WANGEN KE, XU X, CARR TP, PHIPPS WR, KURZER MS: Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. *Am J Clin Nutr* **71**: 1462-1469, 2000.
- 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.
- MORABITO N, CRISAFULLI A, VERGARA C, GAUDIO A, LASCO A, FRISINA N, D'ANNA R, CORRADO F, PIZZOLO MA, CINCOTTA M, ALTAVILLA D, IENTILE R, SQUADRITO F: Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. *J Bone Miner Res* **17**: 1904-1912, 2002.
- MORITO K, HIROSE T, KINJO J, HIRAKAWA T, OKAWA M, NOHARA T, OGAWA S, INOUE S, MURAMATSU M, MASAMUNE Y: Interaction of phytoestrogens with estrogen receptors alpha and beta. *Biol Pharm Bull* **24**: 351-356, 2001.
- NAGATA C, TAKATSUKA N, INABA S, KAWAKAMI N, SHIMIZU H: Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. *J Natl Cancer Inst* **90**: 1830-1835, 1998.
- NESTEL P, FUJII A, ZHANG L: An isoflavone metabolite reduces arterial stiffness and blood pressure in overweight men and postmenopausal women. *Atherosclerosis* **192**: 184-189, 2007.
- OSTATNÍKOVÁ D, CELEC P, HODOSY J, HAMPL R, PUTZ Z, KÚDELA M: Short-term soybean intake and its effect on steroid sex hormones and cognitive abilities. *Fertil Steril* **88**: 1632-1636, 2007.
- OSTERLUND M, KUIPER GGJM, GUSTAFSSON JA, HURD YL: Differential distribution and regulation of estrogen receptor alpha and beta mRNA within the female rat brain. *Brain Res Mol Brain Res* **54**: 175-180, 1998.

- PALOMARES MR, HOPPER L, GOLDSTEIN L, LEHMAN CD, STORER BE, GRALOW JR: Effect of soy isoflavones on breast proliferation in postmenopausal breast cancer survivors. *Breast Cancer Res Treat* **88** (Suppl 1): 4002, 2004.
- PAN W, IKEDA K, TAKEBE M, YAMORI Y: Genistein, daidzein and glycitein inhibit growth and DNA synthesis of aortic smooth muscle cells from stroke-prone spontaneously hypertensive rats. *J Nutr* **131**: 1154-1158, 2001.
- PATISAUL HB: Phytoestrogen action in the adult and developing brain. *J Neuroendocrinol* **17**: 57-64, 2005.
- PECHÁŇOVÁ O, REZZANI R, BABÁL P, BERNÁTOVÁ I, ANDRIANTSITOHAINA R: Beneficial effects of Provinols: cardiovascular system and kidney. *Physiol Res* **55** (Suppl 1): S17-S30, 2006.
- PECHÁŇOVÁ O, ŠIMKO F: The role of nitric oxide in the maintenance of vasoactive balance. *Physiol Res* **56** (Suppl 2): S7-S16, 2007.
- PETERSON G, BARNES S: Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine kinase autophosphorylation. *Prostate* **22**: 335-345, 1993.
- PETRAKIS NL, BARNES S, KING EB, LOWENSTEIN J, WIENCKE J, LEE MM, MIIKE R, KIRK M, COWARD L: Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women. *Cancer Epidemiol Biomarkers Prev* **5**: 785-794, 1996.
- PIETRAS RJ, SZEGO CM: Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. *Nature* **265**: 69-72, 1977.
- PILŠÁKOVÁ L, RIEČANSKÝ I, OSTATNÍKOVÁ D, JAGLA F: Missing evidence for the effect one-week phytoestrogen-rich diet. *Neuro Endocrinol Lett* **30**: 125-130, 2009.
- POLKOWSKI K, MAZUREK AP: Biological properties of genistein. A review of in vitro and in vivo data. *Acta Pol Pharm* **57**: 135-155, 2000.
- POTTER SM, BAUM JA, TENG H, STILLMAN RJ, SHAY NF, ERDMAN JW Jr: Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr* **68**: 1375-1379, 1998.
- PUZSEROVÁ A, CSIZMÁDIOVÁ Z, ANDRIANTSITOHAINA R, BERNÁTOVÁ I: Vascular effects of red wine polyphenols in chronic stress-exposed Wistar-Kyoto rats. *Physiol Res* **55** (Suppl 1): S39-S47, 2006.
- REZZANI R, TENGATTINI S, BONOMINI F, FILIPPINI F, PECHÁŇOVÁ O, BIANCHI R, ANDRIANTSITOHAINA R: Red wine polyphenols prevent cyclosporine-induced nephrotoxicity at the level of the intrinsic apoptotic pathway. *Physiol Res* **58**: 511-519, 2008.
- RIVAS M, GARAY RP, ESCANERO JF, CIA P Jr, CIA P, ALDA JO: Soy milk lowers blood pressure in men and women with mild to moderate essential hypertension. *J Nutr* **132**: 1900-1902, 2002.
- SACKS FM, LICHTENSTEIN A, VAN HORN L, HARRIS W, KRIS-ETHERTON P, WINSTON M; AMERICAN HEART ASSOCIATION NUTRITION COMMITTEE: Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee. *Circulation* **113**: 1034-1044, 2006.
- SAMMAN S, LYONS WALL PM, CHAN GS, SMITH SJ, PETOCZ P: The effect of supplementation with isoflavones on plasma lipids and oxidisability of low density lipoprotein in premenopausal women. *Atherosclerosis* **147**: 277-283, 1999.
- SANDERS TA, DEAN TS, GRAINGER D, MILLER GJ, WISEMAN H: Moderate intakes of intact soy protein rich in isoflavones compared with ethanol-extracted soy protein increase HDL but do not influence transforming growth factor beta(1) concentrations and hemostatic risk factors for coronary heart disease in healthy subjects. *Am J Clin Nutr* **76**: 373-377, 2002.
- SEOK YM, BAEK I, KIM YH, JEONG YS, LEE IJ, SHIN DH, HWANG YH, KIM IK: Isoflavone attenuates vascular contraction through inhibition of the RhoA/Rho-kinase signaling pathway. *J Pharmacol Exp Ther* **326**: 991-998, 2008.
- SETCHELL KD, BROWN NM, DESAI P, ZIMMER-NECHEMIAS L, WOLFE BE, BRASHEAR WT, KIRSCHNER AS, CASSIDY A, HEUBI JE: Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *J Nutr* **131**: 1362-1375, 2001.
- SHIH CD: Activation of estrogen receptor beta-dependent nitric oxide signaling mediates the hypotensive effects of estrogen in the rostral ventrolateral medulla of anesthetized rats. *J Biomed Sci* **16**: 60, 2009.

- SHIN JI, LEE YK, KIM YM, HWANG JT, PARK OJ: Possible link between NO concentrations and COX-2 expression in systems treated with soy-isoflavones. *Ann N Y Acad Sci* **1095**: 564-573, 2007.
- SIMERLY RB: Organization and regulation of sexually dimorphic neuroendocrine pathways. *Behav Brain Res* **92**: 195-203, 1998.
- SIMONCINI T, RABKIN E, LIAO JK: Molecular basis of cell membrane estrogen receptor interaction with phosphatidylinositol 3-kinase in endothelial cells. *Arterioscler Tromb Vasc Biol* **23**: 198-203, 2003.
- SIMONS LA, VON KONIGSMARK M, SIMONS J, CELERMAJER DS: Phytoestrogens do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women. *Am J Cardiol* **85**: 1297-1301, 2000.
- SLOW RC, LI FY, ROWLANDS DJ, DE WINTER P, MANN GE: Cardiovascular targets for estrogens and phytoestrogens: transcriptional regulation of nitric oxide synthase and antioxidant defense genes. *Free Radic Biol Med* **42**: 909-925, 2007.
- SLEMENDA C, HUI SL, LONGCOPE C, JOHNSTON CC: Sex steroids and bone mass. A study of changes about the time of menopause. *J Clin Invest* **80**: 1261-1269, 1987.
- SQUADRITO F, ALTAVILLA D, CRISAFULLI A, SAIITTA A, CUCINOTTA D, MORABITO N, D'ANNA R, CORRADO F, RUGGERI P, FRISINA N, SQUADRITO G: Effect of genistein on endothelial function in postmenopausal women: a randomized, double-blind, controlled study. *Am J Med* **114**: 470-476, 2003.
- TEEDE HJ, DALAIS FS, KOTSOPOULOS D, LIANG YL, DAVIS S, McGRATH BP: Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. *J Clin Endocrinol Metab* **86**: 3053-3060, 2001.
- TEEDE HJ, McGRATH BP, DESILVA L, CEHUN M, FASSOULAKIS A, NESTEL PJ: Isoflavones reduce arterial stiffness: a placebo-controlled study in men and postmenopausal women. *Arterioscler Thromb Vasc Biol* **23**: 1066-1071, 2003.
- TRAVIS RC, SPENCER EA, ALLEN NE, APPLEBY PN, RODDAM AW, OVERVAD K, JOHNSEN NF, OLSEN A, KAAKS R, LINSEISEN J, BOEING H, NÖTHLINGS U, BUENO-DE-MESQUITA HB, ROSMM, SACERDOTE C, PALLI D, TUMINO R, BERRINO F, TRICHOPOULOU A, DILIS V, TRICHOPOULOS D, CHIRLAQUE MD, ARDANAZ E, LARRANAGA N, GONZALEZ C, SUÁREZ LR, SÁNCHEZ MJ, BINGHAM S, KHAW KT, HALLMANS G, STATTIN P, RINALDI S, SLIMANI N, JENAB M, RIBOLI E, KEY TJ: Plasma phyto-oestrogens and prostate cancer in the European Prospective Investigation into Cancer and Nutrition *Br J Cancer* **100**: 1817-1823, 2009.
- TSUCHIHASHI R, SAKAMOTO S, KODERA M, NOHARA T, KINJO J: Microbial metabolism of soy isoflavones by human intestinal bacterial strains. *J Nat Med* **62**: 456-460, 2008.
- TURHAN NO, BOLKAN F, ILTEMIR D, ARDICOGLU Y: The effect of isoflavones on bone mass and bone remodeling markers in postmenopausal women. *Turk J Med Sci* **38**: 145-152, 2008.
- VERA R, SANCHEZ M, GALISTEO M, VILLAR IC, JIMENEZ R, ZARZUELO A, PEREZ-VIZCAINO F, DUARTE J: Chronic administration of genistein improves endothelial dysfunction in spontaneously hypertensive rats: involvement of eNOS, calveolin and calmodulin expression and NADPH oxidase activity. *Clin Sci (Lond)* **112**: 183-191, 2007.
- WANG HJ, MURPHY PA: Isoflavone composition in American and Japanese soybeans in Iowa: effects of variety, crop year, and location. *J Agric Food Chem* **42**: 1674-1677, 1994.
- WANG J, ELTOUM IE, LAMARTINIERE CA: Genistein alters growth factor signaling in transgenic prostate model (TRAMP). *Mol Cell Endocrinol* **219**: 171-180, 2004.
- WANGEN KE, DUNCAN AM, XU X, KURZER MS: Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. *Am J Clin Nutr* **73**: 225-231, 2001.
- WASHBURN S, BURKE GL, MORGAN T, ANTHONY M: Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. *Menopause* **6**: 7-13, 1999.
- WATSON CS, ALYEA RA, JENG YJ, KOCHUKOV MY: Nongenomic actions of low concentration estrogens and xenoestrogens on multiple tissues. *Mol Cell Endocrinol* **274**: 1-7, 2007.
- WATSON MA, TAYLOR H, LEPHART ED: Androgen-dependent modulation of calbindin D28K in hypothalamic tissue during prenatal development. *Neurosci Res* **32**: 97-101, 1998.

-
- WEBER KS, JACOBSON NA, SETCHELL KDR, LEPHART ED: Brain aromatase and 5 α reductase, regulatory behaviors and testosterone levels in adult rats on phytoestrogen diets. *Proc Soc Exp Biol Med* **22**: 131-135, 1999.
- WEBER KS, SETCHELL KD, STOCCO DM, LEPHART ED: Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5 α -reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. *J Endocrinol* **170**: 591-599, 2001.
- WELTY FK, LEE KS, LEW NS, ZHOU JR: Effect of soy nuts on blood pressure and lipid levels in hypertensive, prehypertensive, and normotensive postmenopausal women. *Arch Intern Med* **167**: 1060-1067, 2007.
- WHITE L, PETROVITCH H, ROSS GW, MASAKI K, ABBOTT RD, TENG EL, RODRIGUEZ BL, BLANCHETTE PL, HAVLIK RJ, WERGOWSKA G, CHIU D, FOLEY DJ, MURDAUGH C, CURB JD: Prevalence of dementia in older Japanese-American men in Hawaii: The Honolulu-Asia aging Study. *JAMA* **276**: 955-960, 1996.
- WHITE LR, PETROWITCH H, ROSS GW, MASAKI K, HARDMAN J, NELSON J, DAVIS D, MARKESBERY W: Brain aging and midlife tofu consumption. *J Am Coll Nutr* **19**: 242-255, 2000.
- WISEMAN H, CASEY K, CLARKE DB, BARNES KA, BOWEY E: Isoflavone aglycon and glucoconjugate content of high- and low-soy U.K. foods used in nutritional studies. *J Agric Food Chem* **50**: 1404-1410, 2002.
- YAMORI Y: Food factors for atherosclerosis prevention: Asian perspective derived from analyses of worldwide dietary biomarkers. *Exp Clin Cardiol* **11**: 94-98, 2006.
- YANG G, SHU XO, JIN F, ZHANG X, LI HL, LI Q, GAO YT, ZHENG W: Longitudinal study of soy food intake and blood pressure among middle-aged and elderly Chinese women. *Am J Clin Nutr* **81**: 1012-1017, 2005.
- ZENEBE W, PECHÁŇOVÁ O, ANDRIANTSITOHAINA R: Red wine polyphenols induce vasorelaxation by increased nitric oxide bioactivity. *Physiol Res* **52**: 425-432, 2003.
- ZIEGLER RG, HOOVER RN, PIKE MC: Migration patterns and breast cancer risk in Asian-American women. *J Natl Cancer Inst* **85**: 1819-1827, 1993.
-