

ISOFLAVONE PHYTOESTROGENS

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Abstract. Isoflavones, rich in soy bean, are currently receiving much attention because of their potential role in preventing and treating cancer and other human chronic diseases. Data from epidemiological reports and laboratories have shown that isoflavones have multi-biological and pharmacological effects in animals and humans. These include estrogenic and antiestrogenic effects, cell signalling conduction, as well as cell growth and death. Based on these properties, soy protein and isoflavones have been associated with reduced incidences of breast and prostate cancers, cardiovascular diseases or osteoporosis, and exhibit some other favorable effects. The mechanism through which isoflavones may exert the above-mentioned functions are not only based on the estrogenic properties of isoflavones, but also on their role as protein tyrosine-kinase inhibitors, as regulators of gene transcription, modulators of transcription factors, antioxidants, as well as by altering some enzyme activities.

INTRODUCTION

Interest in the physiological role of bioactive compounds present in plants has increased dramatically over the last decade. Of particular interest in relation to human health are the class of compounds known as the phytoestrogens, which embody several groups of non-steroidal estrogens including isoflavones & lignans that are widely distributed within the plant kingdom [11, 22].

Phytoestrogens (plant estrogen) is a generic name used to define classes of compound that have high structural similarity to estrogen. They are either of plant origin or derived from "in vivo" metabolism of precursors present in plants and/or eaten by mammals. In plants, phytoestrogens protect against ultraviolet radiation effects, control normal plant growth and protect from stress [3, 22, 24, 26, 28, 29].

Phytoestrogens, like synthetic estrogenic and anti-estrogenic compounds, bind receptors in mammals. They significantly differ from the synthetic estrogen-receptor modulators in their ability to undergo metabolism [14]. This remarkable characteristic of high turnover and short half-life provides that the phytoestrogens are stored in tissues. Phytoestrogens are able to selectively mimic the effects of estrogen in certain tissues. Data indicates that phytoestrogens may offer protection against a wide range of human conditions, including breast, bowel, colon, prostate, brain, and other cancers; cardiovascular diseases; osteoporosis and menopausal symptoms [1, 16, 18, 25, 27]. Molecular and cellular biology experiments, animal studies and human clinical trials suggest that isoflavones in particular may confer specific health benefits related to cardiovascular diseases, cancer, osteoporosis, menopausal symptoms and other diseases. Thus, populations consuming diets rich in phytoestrogens were shown to be more protected against breast cancer, heart disease, menopausal symptoms and osteoporosis than populations consuming less of these products [6, 9, 17, 21].

BIOCHEMICAL CLASSIFICATION OF PHYTOESTROGENS

Phytoestrogens are natural plant substances with a structural and functional similarity to genuine

17 β -estradiol. They are mainly absorbed by daily nutrition and are principally categorized in two biochemical classes [12]:

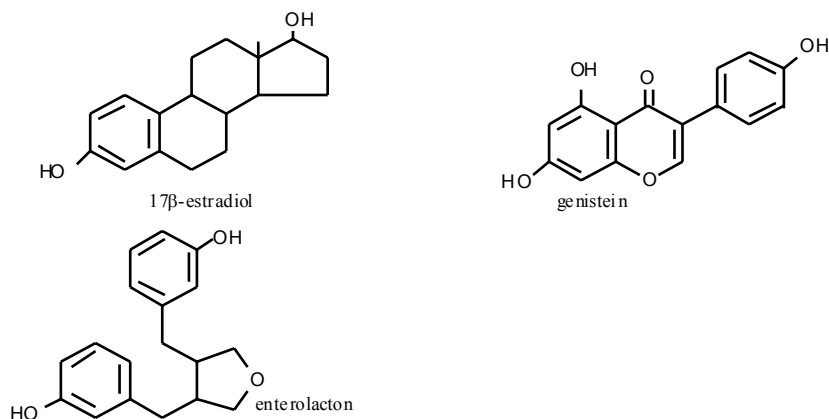
- **Lignans:** enterolacton and enterodiol;
 - **Isoflavones:** genistein, daidzein, biochanin-A, formononetin and glycitin;
Less significant are: coumestanes (coumestrol), lactones (ceralenone), sterols (sitosterole-A, B).
Very high concentrations are found in the following plants (in decreasing concentration) [3]:
 - **Isoflavones:** soy, red clover, legumes, vegetables;
 - **Lignans:** fruits, berries, full grain products, green tea, line seeds, vegetables
- The highest concentration of the phytoestrogens genistein and daidzein are found in soy bean as well as other soy products, including for example tofu, soy milk, miso, soyflour and refined soyprotein [8, 12, 13].

BIOCHEMISTRY OF PHYTOESTROGENS

Due to the positioning of both hydroxyl groups in a special dimensional order phytoestrogens have a high sterical identity to 17 β -estradiol and are therefore able to bind at the estradiol receptor. Moreover, lignans and isoflavones possess a distinct antioxidative capacity through the prevalence of two phenyl groups (radical scavengers).

Phytoestrogens bind with different relative binding affinity (RBA) to the estradiol receptor (ER) with special preference of the estradiol receptor β (ER β). Opposite to the RBA of 17 β -estradiol of 100 the RBA of genistein is 5 on ER α and 36 on ER β . Other phytoestrogens, for example coumestane (coumestrole), will bind even with a significantly higher affinity to the ER-protein [26].

Structures of 17 β -estradiol and phytoestrogens [24]:



The organ specific distribution of the ER with an even higher concentration of ER β in prostate gland, ovaries, lung, bladder, kidneys, uterus and testis points to a special local efficacy of phytoestrogens in these organs. The effect of phytoestrogens is only partially based on the relatively good binding of the substances at the estradiol receptor. After consumption, the extremely high concentration of isoflavones and lignans is even higher than 17 β -estradiol. ER will be blocked for the genuine estradiol. It is of utmost meaning, that phytoestrogens after binding on ER, will only develop a relatively weak estrogenic activity [24, 26].

ABSORPTION AND METABOLISM

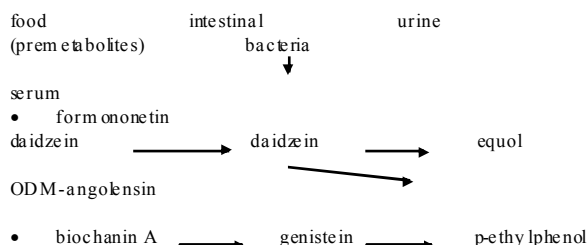
The principal isoflavones found in soy proteins and soy foods are daidzein, genistein and glycitein. Each of them is found in four chemical forms: the unconjugated form, or aglycone; the conjugated form, or glucoside (daidzin, genistin and glycitin); acetylglucoside; and malonylglucoside. The soy isoflavones daidzein and genistein primarily appear in the form of their glucosides, daidzin and genistin, respectively. Processing and fermentation of the soybean is known to influence the forms of isoflavones. The bioavailability and biological activities of different isoflavones also differ to some extent. Moreover, the estrogenic potency of equol is higher than its precursor, daidzein [22].

After ingestion, the conjugated form of isoflavones is hydrolyzed by intestinal β -glucosidases, which release the principal bioactive aglycones, daidzein and genistein. These compounds may be absorbed or further metabolized in the distal intestine with the formation of specific metabolites, such as equol and p-ethylphenol [22, 24].

The aglycones along with any bacterial metabolites are absorbed from the intestinal tract and transported via the portal venous system to the liver, where the isoflavones and their metabolites are efficiently conjugated with glucuronic acid (95%), and to a lesser extent are found as sulfate conjugates. They are then excreted in the urine or in the bile. Some isoflavones undergo enterohepatic recycling. It has been proposed that intestinal metabolism is essential for their subsequent absorption and bioavailability in the body. Andlauer et al. reported that genistin was partly absorbed without previous cleavage. Piskula et al. also demonstrated that both aglycones and their glucosides are absorbed very fast. These results contradict the above assumption. The results from Izumi et al. showed that the isoflavone aglycones were absorbed faster and in greater amounts than their glucosides in humans. The peak concentrations of isoflavones in blood are seen generally 4-8 h after dietary intake. Most of the daidzein and genistein are excreted in urine within the first 24 h after food intake. The rate of urinary excretion of daidzein was greater than that of genistein throughout the postmeal period [22].

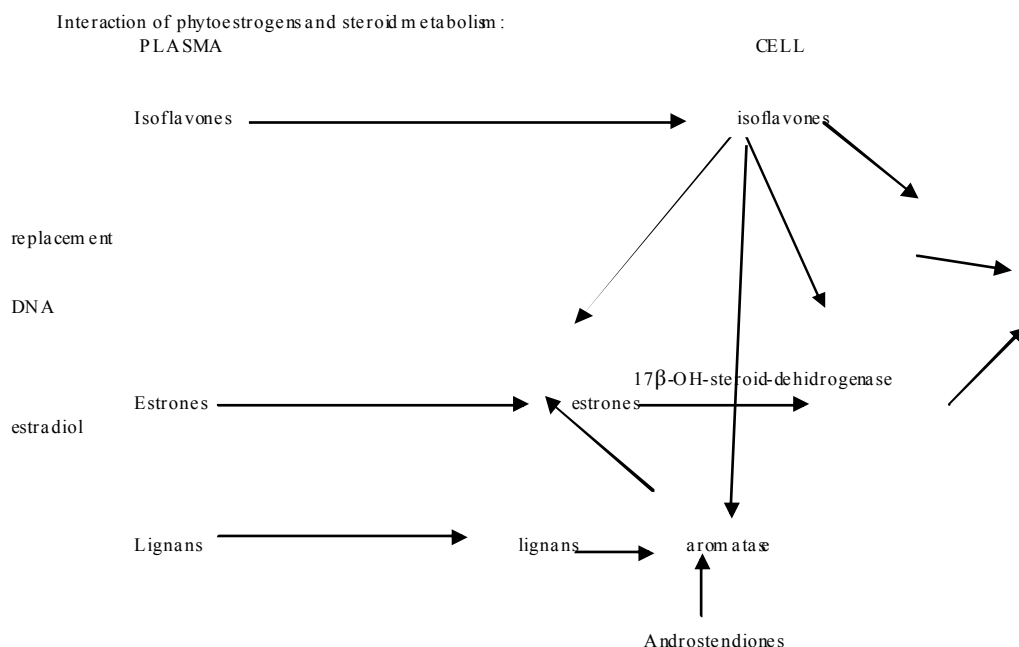
The presence of different populations of microflora in the human gut may influence the bioavailability of isoflavone phytoestrogens and causes wide inter-individual variation in isoflavone metabolite excretion [15]. The reasons for the considerable inter-individual variation in isoflavone metabolism following the consumption of soybean isoflavones have not been fully elucidated. Recent data from a human intervention study of soy-containing food (low or high in isoflavones) showed that proportion of energy from fat affects phytoestrogen excretion in the urine. Dietary fat intake decreases the capacity of gut microbial flora to synthesize equol [22].

Metabolism of isoflavones [26]:



Isoflavones can be detected in many tissues of animals and humans. Daidzein concentration was found to be high in plasma, liver, lung and kidney at about 30 $\mu\text{g/g}$ wet weight; to be moderate in skeletal muscle, spleen and heart at about 15-20 $\mu\text{g/g}$ wet weight; and to be low in brain and testis at about 2-5 $\mu\text{g/g}$ wet weight. Tissues including brain, liver, mammary, ovary, prostate, testis, thyroid and uterus showed significant dose-dependent increases in total genistein concentration. The liver contains the highest amounts of genistein while brain tissue accumulates less genistein, as compared to other tissues. Janning et al. found that the daidzein levels were usually three- to fivefold higher in the liver and kidney than in plasma [22].

Plasma concentrations of 50-800 ng/mL were found for daidzein, genistein and equol in adults consuming modest quantities of soy foods containing ~50 mg/d of isoflavones. In response to the consumption of soy foods, blood isoflavone concentrations can reach < 6 $\mu\text{mol/L}$ [22].



CLINICAL EFFECTS OF PHYTOESTROGENS

Hormonal effects

Isoflavones are structurally similar to mammalian endogenous estrogens, and thus may act as estrogen antagonist or agonists, depending on the isoflavone concentration, or the tissue of action. They act mainly by binding to the ER β , which was found to be expressed in many tissues, including the hypothalamus, pituitary gland, lung and thymus [6, 11, 22].

Isoflavones have been shown to possess an estrogenic hormone function. They have been shown to induce specific estrogen-responsive gene products and stimulate the genital tract of female animals. In rodents and rats, isoflavones were found to stimulate mammary and uterine growth. But compared to estradiol, the isoflavone estrogenic effects are weak. In the mouse uterine growth assay, genistein and daidzein are roughly 100,000 times less effective than estradiol [14, 22].

The hormonal actions of isoflavones might explain epidemiological observations of lowered risk for chronic diseases and menopausal symptoms in populations that consume soy. However, effects of soy consumption on hormonal metabolism have been inconsistent among most studies, probably as a result of methodological differences in subjects characteristics, study design, isoflavone form, dosage, and length of diet period [22].

Soy isoflavones appear to affect the menstrual cycle and concentrations of reproductive hormones in premenopausal women. Cassidy et al. found that premenopausal women consuming 60 g textured soy-bean (containing 45 mg isoflavones) experienced a 2.5 d increase in the length of their follicular phase whereas no change was noted in women fed on a similar amount of soybeans from which the isoflavones had been chemically removed. Particularly noteworthy is the finding that serum follicle stimulating hormone and luteinizing hormone levels decreased significantly in response to the consumption of soybean isoflavones. In postmenopausal women, the effects of soy isoflavones on endogenous estrogen metabolism were shown to be less pronounced than in premenopausal women [22].

Genistein and daidzein suppressed glucocorticoid and stimulated androgen production in cultured human adrenal cortical cells [22].

At high dosages, isoflavones may act as antagonists of estrogen. They have generally been reported to have lower binding affinity for estrogen receptors and a lower potency in producing estrogenic effects compared with 17β -estradiol. Thus, when isoflavones displace 17β -estradiol molecules, it can reduce the function of real estrogen. At concentrations 100-1000 times that of estradiol (the probably levels in human plasma after regular consumption), isoflavones may be able to compete effectively with endogenous mammalian estrogens, bind the ERs, and prevent estrogen-stimulated growth in mammals. This may also result in interference with the release of gonadotropins and interruption of the feedback-regulating system of the hypothalamus-pituitary-gonadal axis [22, 24].

Regulating sex hormone receptors at the transcriptional level

At pharmacological concentrations, genistein decreases ER α mRNA levels in the rat uterus. Daidzein is capable of down-regulating androgen receptor and ER α mRNA expression significantly in rat uteri [22].

No changes were detected in the pituitary indicating the possible central effects of daidzein on the neuroendocrine system. Kuiper et al. found genistein and daidzein stimulated estrogen-dependent receptor gene activity at concentrations ranging from 10-1000 nM in cell cultures. Interestingly, some results indicated soy isoflavones increase nerve growth factor mRNA and brain-derived neurotrophic factor mRNA in rats [22, 24].

Influence cell signalling

Isoflavones, particularly genistein, can regulate the cell signalling conduction from receptor expression to cytoplasmic downstream signalling [22].

Genistein inhibits tyrosine protein kinase activity. Many of the peptide growth factor signal transduction pathways that were implicated in certain cancers involve the action of tyrosine kinases. Therefore, a circulating tyrosine kinase inhibitor, such as genistein, may have beneficial effects in the treatment of cancer. Recently, genistein has been shown to alter ion channel function of culture cells [22].

Cell proliferation, animal growth and development

Most isoflavone studies on cell proliferation were performed using estrogen-dependent human breast carcinoma MCF-7 cells. The results displayed biphasic effects: stimulation of growth at low concentrations and inhibition at high concentrations. Genistein and biochanin A, at 0.1-10 μ M, induced cell DNA synthesis 150-235%, while at 20-90 μ M, inhibited DNA synthesis by 50%. The growth inhibitory effects of daidzein might be mediated through a block at the G1 stage of the cell cycle.

Isoflavones can freely pass the placental barrier. In humans the isoflavone concentrations in the neonate are similar to those in maternal plasma. Research has reported that isoflavones at concentrations found in a standard natural-ingredient diet may affect the sexual differentiation of female rats in utero.

PHARMACOLOGICAL AND THERAPEUTIC EFFECTS

Anti- cancer

Epidemiological data suggest that a diet rich in isoflavones provides protection against several forms of cancer, particularly those that are hormone-dependent, such as breast, prostate, and lung cancer [2, 22, 27].

In vitro data have demonstrated that isoflavones inhibit cancer cell growth, including prostate cancer cells and MCF-7 human breast cancer cell line. There are literally hundreds of "in vitro" studies showing that genistein inhibits the growth of a wide range of both hormone-dependent and hormone-independent cancer cells. The concentration of genistein required to inhibit angiogenesis "in vitro" was reported to be higher than the genistein concentration likely to be achieved "in vivo" [22, 24, 26].

In animal studies, neonatal injections of pharmacologic doses of genistein have been shown to suppress the development of dimethylbenzanthracene-induced mammary adenocarcinomas in rats. So far, there have been very few human studies to reveal direct evidence that soy intake or isoflavones may protect against breast cancer [22].

Xu et al. suggested that isoflavones may exert cancer-preventive effects by decreasing estrogen synthesis and altering metabolism away from genotoxic metabolites toward inactive metabolites [22].

It has become apparent that the anti-cancer mechanisms of isoflavones are not exclusively via the estrogen receptor. In vitro studies have revealed that numerous mechanisms may be involved. One is via inhibiting protein tyrosine kinase [23]. Other anticancer mechanisms of isoflavones may include inhibition of 3β -hydroxysteroid dehydrogenase, 17β -hydroxysteroid dehydrogenase, 5α -reductase and aromatase, followed by affecting the level of active steroid hormones. Isoflavones also inhibited DNA topoisomerase I and II activity, which were predicted to cause DNA damage [24, 26]. The transcription factor p53 currently has become the most important tumor suppressor. It has been shown that genistein induced the up-regulation of p53 protein. More recently, it was suggested that genistein may inhibit cell growth by both increased expression and production of transforming growth factor (TGF) β 1 signaling pathways [22].

Another mechanism to partially explain the anti-cancer activity of isoflavones involves their ability to inhibit angiogenesis, or new blood vessel growth, which is required for tumor growth [10, 22].

Certain references describe adverse effects of isoflavones in relation to breast cancer. The isoflavones dose is crucial to increase or decrease cancer risk. In vitro studies have demonstrated that genistein enhanced the proliferation of estrogen-dependent human breast cancer cells at concentrations as low as 10 nM, with a concentration of 100 nM achieving proliferative effects similar to those of 1 nM estradiol. At higher concentration, genistein inhibits MCF-7 cell growth [22].

Soy diets containing varying amounts of genistein stimulated the growth of estrogen-dependent (MCF-7) tumors in a dose-dependent manner [22]. Peeters et al. estimated that the real protective effect of phytoestrogens may be smaller than expected or only limited to premenopausal women [22]. Moreover, soy intake in premenopausal women may increase breast cancer risk by elevating the levels of prolactin. Preliminary data suggested positive relations between estrogenic effects, plasma prolactin levels, and breast cancer risk [22].

Lowering the risk of cardiovascular diseases

Many investigations have demonstrated that soy protein inhibits cardiovascular diseases and reduces atherosclerosis risk in animals and humans [27]. The beneficial effects of soy are thought to be mediated by many mechanisms. Most researchers consider that these effects result from a reduction of plasma low density lipoprotein (LDL) cholesterol and triglyceride concentrations [7, 24]. Soy protein may also inhibit platelet activation and aggregation and reduce the amount of serotonin in the platelets [20, 22, 26].

The hypocholesterolemic effects of soy protein may function by influencing lipid metabolism through altering lipid-related gene expression. The results of Tikkanen et al. showed that intake of soy protein containing 60 mg isoflavones per day may provide protection against oxidative modification of LDL [22].

Currently, the mechanism associated with soy's beneficial effects on cardiovascular health are not fully understood. It is possible that soy substances other than isoflavones such as saponins, phytic acid, protein components, amino acid composition or a protein-isoflavone interaction may be involved in the multi-various processes. The ability of saponins to lower cholesterol in some species is especially well-known [19, 22].

Neuroprotective and neurotrophic efficacy of phytoestrogens in cultured hippocampal neurons

Epidemiological data from retrospective and case-control studies have indicated that estrogen replacement therapy (ERT) can decrease the risk of developing Alzheimer's disease. In addition ERT has been found to promote cellular correlates of memory and to promote neuronal survival both in vivo and in vitro [26]. Phytoestrogens have been proposed as potential alternatives to ERT. Six phytoestrogens, genistein, genistin, daidzein, daidzin, formononetin, equol, were tested for their neuroprotective efficacy against two toxic insults, glutamate excitotoxicity and beta-amyloid. Neuronal membrane damage was quantitatively measured by lactate dehydrogenase (LDH) release, and neuronal mitochondrial viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. Results of these studies demonstrated that all phytoestrogens induced a modest but significant reduction in LDH release following exposure to glutamate and beta-amyloid. In contrast, none of the phytoestrogens induced a significant increase in reduced MTT levels, which occurred in the presence of a full estrogen agonist, 17β -estradiol. Analysis of the neurotrophic potential of genistein and daidzein, two phytoestrogens that exerted a significant reduction in LDH release, demonstrated that neither of these molecules promoted hippocampal neuron process outgrowth. Results of these analyses indicate that although phytoestrogens exert a neuroprotective effect at the plasma membrane, they do not sustain neuron mitochondrial viability nor do they induce cellular correlates of memory as neurite outgrowth and synaptogenesis are putative mechanisms of memory. Data derived from these investigations would predict

that phytoestrogens could exert some neuroprotective effects analogous to that of antioxidants, but that these molecules are not functional equivalents to endogenously active 17β -estradiol or to estrogen replacement formulations and, therefore, would raise the concern that they may not reduce the risk of Alzheimer's disease or sustain memory function in postmenopausal women [29].

Immune system

It is well known that estrogen has an important effect on the immune system. For example, most autoimmune diseases are more common in women than in men and quite frequently begin under conditions when estrogen levels change dramatically, during puberty, menopause, pregnancy. The exact mechanism involved in these metabolic processes have yet to be determined. Daidzein, in vitro, has been proven to increase the activation of murine lymphocytes. In another in vitro study of Zhang et al., it was shown that isoflavone glucuronides might not only compete with endogenous estrogen to inhibit estrogen-dependent proliferation of cancer cells. They are also able to activate natural killer cells to potentially increase the immune defenses of the body against cancer at nutritionally relevant concentrations [22].

Isoflavones have also demonstrated an anti-inflammatory potential in various animal models, including chronic ileitis, inflammation-induced corneal neovascularization, and ischemia reperfusion injury. However, any potential anti-inflammatory benefit of an isoflavone diet needs to be balanced by the possibility that such dietary modifications may also be detrimental [22].

Cellular and molecular mechanisms of isoflavones effects

It has become apparent from the diversity of isoflavone properties that no single action can explain many of the effects of isoflavones. Isoflavones exert multifunctions through genomic and nongenomic mechanisms of cellular regulation. First, isoflavones have a similar structure to estradiol and are capable of binding to the two estrogen receptors, ER α and ER β . Secondly, isoflavones can interact with membrane proteins (receptors) and exert an effect that is expressed through secondary messengers in the cytoplasm [22].

Structurally, ER β is highly homologous to ER α in the DNA binding domain, but shows only 55% homology in the ligand binding domain. These structural differences lead to different relative binding affinities in ligand binding assays. Compared to ER α , isoflavones have a greater relative binding affinity to ER β , while estradiol binds to ER α and ER β with equal affinity. Studies by structural biologists demonstrated that genistein is completely buried within the hydrophobic core of the protein and binds in a manner similar to 17β -estradiol [22].

There are recent findings from cell lines that ER α and ER β can associate with the G-protein and protein kinase A (PKA) to activate many of the intracellular cascades. For example, genistein potentiated GHRH-stimulated cAMP accumulation in a concentration-dependent manner [22].

Other diseases

Estrogen is used in hormone replacement therapy to prevent menopausal symptoms and osteoporosis in postmenopausal women. But estrogen has been proven to be associated with an incidence of breast and endometrial cancer. This relationship has severely hampered the clinical use of estrogen. Therefore, there is growing interest to use isoflavones as a potential alternative to the estrogens in hormone replacement therapy. Observational studies have shown a lower incidence of menopausal symptoms and osteoporosis in Asian women who have a diet rich in soy products [22, 26].

Decrease of climacteric complaints

In clinical studies, menopausal women who consumed isoflavone-enriched foods have alleviated symptoms associated with hot flashes. Another study has reported no beneficial effects of isoflavones on hot flashes. Thus, data are currently insufficient to draw definitive conclusions regarding the use of isoflavones for the treatment of menopausal symptoms [22, 26].

Prevention of osteoporosis

Osteoporosis is characterized by a loss of bone mass usually associated with aging, due to increased bone resorption and reduced bone formation. The beneficial effects of estrogen replacement therapy (ERT) on prevention of postmenopausal osteoporosis are well known. But more researchers have begun turning to isoflavones as an alternative therapy [26]. Data from animal studies suggest that isoflavones could prevent bone loss that occurs as a result of estrogen deficiency. Results from Yamaguchi's group have shown that daidzein and genistein stimulated osteoblastic bone formation and inhibited osteoclastic bone resorption. Data available from human studies about the effect of isoflavones on osteoporosis are limited. Potter et al. showed a dose

between 50 and 90 mg per day seems to be needed to show a skeletal benefit in postmenopausal women. Recently, Aikawa et al. reported soy isoflavones attenuated bone loss from the lumbar spine in perimenopausal women. Somekawa et al. revealed consumption of soy products is associated with increased bone mass in postmenopausal Japanese women. However, other investigations failed to find the bone-repairing effect of isoflavones in postmenopausal women. Therefore, the impact of genistein and daidzein on bone loss appears to be minimal. However, one or more of the isoflavone metabolites may prove to be a clinically useful agent in the prevention and treatment of osteoporosis [22].

Effects on the endometrium

Estrogen stimulates the endometrium, and there is a low but important risk of endometrial hyperplasia and carcinoma in the presence of unopposed estrogen. The effects of isoflavones on the endometrium have been assessed in animal and human studies. Foth and Cline treated surgically menopausal female macaques with isoflavone, estradiol, or placebo for 6 months. After 6 months, histopathologic, morphometric, and immunohistochemical assessments of endometrium were performed. 6 months of isoflavone therapy did not reduce proliferation of endometrium. Isoflavones did not have estrogenic effects on endometrial tissues [26].

Effects of isoflavones on alcohol pharmacokinetics and alcohol-drinking behavior.

Daidzin is efficacious in lowering blood alcohol levels and shortens sleep time induced by alcohol ingestion [24]. A study was conducted to test the antidipsotropic effect of daidzin and two other major isoflavonoids: daidzein and puerarin from *Pueraria lobata*. An alcohol-prefering rat model, the selectively-bred P line of rats, was used for this study. All three isoflavonoid compounds were effective in suppressing voluntary alcohol consumption by the P rats. The decrease in alcohol consumption was accompanied by an increase in water intake, so that the total fluid volume consumed daily remained unchanged. The effects of these isoflavonoid compounds on alcohol and water intake were reversible. Data demonstrate that isoflavonoid compounds are effective in suppressing the appetite for alcohol when taken orally [4].

CONCLUSIONS

Interest in the field of dietary estrogens has exploded in the past five years. The evidence showing that these nonsteroidal estrogens have an array of potent biological activities is indisputable, and animal and clinical studies are providing convincing evidence for potentially beneficial effects of a diet containing these compounds. The threshold intake of dietary estrogens necessary to achieve a biological effect in humans appear to be 30-50 mg/d, which is readily attainable by the inclusion of modest amounts of soy protein in the average Western diet. Although it may be difficult for adults to consume sufficiently large enough quantities of isoflavones for normal dietary sources to cause the type of deleterious effects previously experienced by several animal species, there is a distinct possibility of risk associated with the use of these compounds as uncontrolled over-the-counter pharmacologic agents, because estrogens exhibit biphasic responses that are highly dose-dependent.

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