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Phytoestrogens: Recent Developments

Abstract

Phytoestrogens are polyphenolic non-steroidal plant compounds with estrogen-like biological activity. Based on their chemical structure, phytoestrogens can be classified into four main groups, i.e., isoflavonoids, flavonoids, stilbenes, and lignans. For each group, the chemistry, dietary sources and biotransformation of the most interesting compounds will be discussed. Since phytoestrogens are structurally very similar to the estrogen 17β-estradiol, they may exhibit selective estrogen receptor modulating activities. Therefore, special attention will be given to the hormonal effects of various isoflavonoids, including genistein, daidzein, coumestrol and equol, several prenylated flavonoids, especially 8-prenylnaringenin, and the stilbene resveratrol. Furthermore, their non-hormonal effects will be discussed briefly. Finally, the latest developments on the potential protective properties of phytoestrogens and phytoestrogen-containing foods against hormone-dependent breast and prostate cancers and cardiovascular diseases, and as estrogen replacement therapy for postmenopausal women will be discussed.

Key words
Phytoestrogens · isoflavones · resveratrol · lignans · hop · estrogen receptor

Introduction

Phytoestrogens are polyphenolic non-steroidal plant compounds with estrogen-like biological activity. The estrogenic properties of certain plants have been recognized for more than fifty years. In the mid-1940 s, an infertility syndrome in sheep had been attributed to the ingestion of clover containing high levels of the isoflavones formononetin (**1**) and biochanin A (**2**) [1], [2]. More recently, an increasing number of epidemiological and experimental studies has suggested that the consumption of phytoestrogen-rich diets may have protective effects on estrogen-related conditions, such as menopausal symptoms [3], and estrogen-related diseases, such as prostate [4] and breast cancers [5], osteoporosis [6], and cardiovascular diseases (CVD) [7]. However, concerns have been raised about the potential dangers of consuming high levels of these compounds [8]. Consequently, phytoestrogens are currently under active investigation for their role on human health.

This review will discuss the recent literature on phytoestrogens, focusing on their biological effects and biotransformation, as well as on their epidemiological and experimental studies in animals and humans. In this way, a previous review in this journal on the *in vitro* evaluation methods of phytoestrogens will be complemented [9].

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History
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Chemistry, Dietary Sources, and Biotransformation of Phytoestrogens

Many structurally diverse compounds, originating from both industrial and natural sources, have been reported to possess estrogenic activity (Fig. **1**) [10]. Humans are exposed through the food chain to a variety of xenobiotic estrogen-like chemicals, such as DDT, polychlorinated biphenyls (PCBs), and diethylstilboestrol (DES) (**3**). Recently, much attention has been focused on these xenoestrogens for their long-term effects on the endocrine system. In addition, pharmaceutical estrogens, such as ethinylestradiol, can also be classified as synthetic estrogen-like compounds.

Without discussing the ovarian steroids, most of the natural estrogen-like compounds are produced by plants. These phytoestrogens are a diverse group of polyphenolic non-steroidal plant compounds that bind to human estrogen receptors (ERs) and exert the characteristics of endogenous steroidal estrogens. Based on their chemical structure, phytoestrogens can be classified into four main groups, i.e., isoflavonoids, flavonoids, stilbenes, and lignans (Fig. **1**), while β-resorcyclic acid lactones, which are produced by molds that contaminate cereal crops, are classified as mycoestrogens. To be complete, some terpenoids and saponins have also been reported to exert similar effects, although the number of publications on these compounds as phytoestrogens is rather limited. A striking example are the triterpenoids present in *Cimicifuga racemosa* extracts, which are considered to be at least partly responsible for the SERM activities of these extracts [11].

Isoflavonoids

Isoflavones are the most studied group of phytoestrogens and are found almost exclusively in the family Leguminosae [12]. Soybeans are a very rich source of isoflavones and contain approximately 2 grams of isoflavones per kilogram fresh weight [13]. However, it must be emphasized that the isoflavone content of soy products can greatly vary between different soybean varieties [14] and through soybean processing [15]. Consequently, not all soy protein sources are equal with respect to their isoflavone content and this should be taken into account when conducting epidemiological and nutritional studies. For example, soy protein concentrates from which meat analogues are made, have low concentrations of isoflavones if prepared by water extraction, and almost no isoflavones are present if prepared by alcohol extraction [16]. Furthermore, soybean oil contains no isoflavones and soy sauce has little or no isoflavones [17].

A large number of isoflavones has been identified from plants, with daidzein (**4**) and genistein (**5**) as the principal isoflavones. They occur in plants as the inactive glycosides daidzin (**6**) and genistin (**7**) and their respectively 4′-methyl ether derivatives, formononetin and biochanin A (Fig. **2**). Despite the high stability of the β-glycosides genistin and daidzin during processing [18], these precursors can be metabolized in the digestive tract by the enzymes of the normal microflora to their corresponding aglycones, genistein and daidzein [19], [20]. The gastrointestinal microflora can further metabolize daidzein to the potent estrogen equol (**8**), but this biotransformation has a high inter-individual variability [21]. Another metabolite produced by daidzein is *O*-demethylangolensin (O-DMA) (**9**) [22]. Metabolism of genistein by the microflora yields the end-products 2-(4-hydroxyphenylpropanoic acid (**10**) and 1,3,5-trihydroxybenzene (**11**) [23].

Recently, several caco-2 cell line [24], [25], [26] and animal [27], [28] studies have examined the intestinal absorption of isoflavone glycosides. The results obtained from the caco-2 cell line, which is used as an *in vitro* model of the human intestinal epithelium, suggested that isoflavone aglycones are taken up into enterocytes more efficiently than their corresponding glycosides. Similar results were obtained in isolated rat intestinal perfusion models [27], [28], but the question still remains whether isoflavone glycosides can be absorbed intact from the human intestinal tract. A recent study showed that isoflavone glycosides were not absorbed intact across the enterocyte of healthy adults and thus hydrolysis of the sugar moiety was required for the absorption of isoflavone glycosides [29]. It was also suggested that isoflavone aglycones are absorbed through non-ionic passive diffusion from the jejunum. After absorption, the isoflavones are readily conjugated with glucuronic acid and to a lesser extent with sulfates, and are then excreted in urine [30].

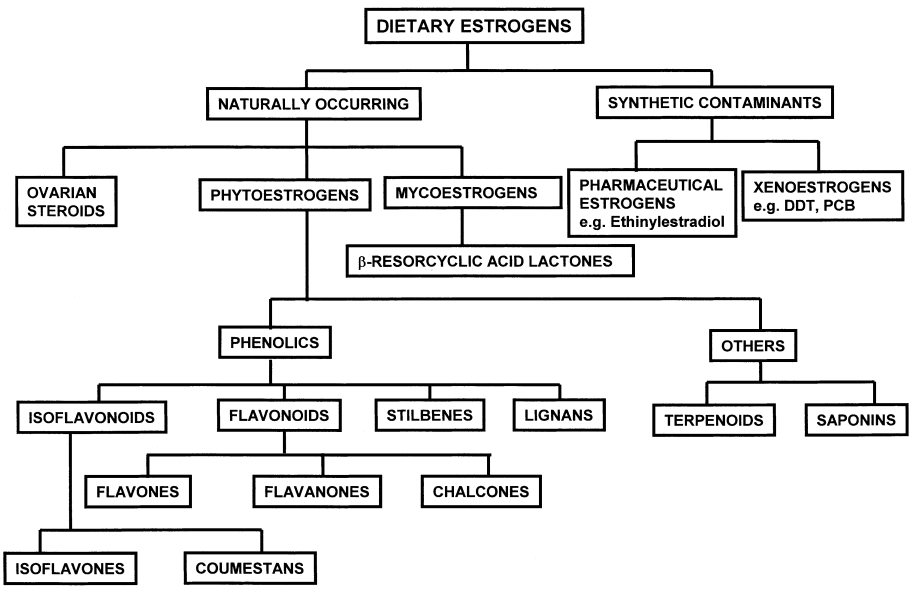


Fig. **1** Classification scheme of dietary estrogens.

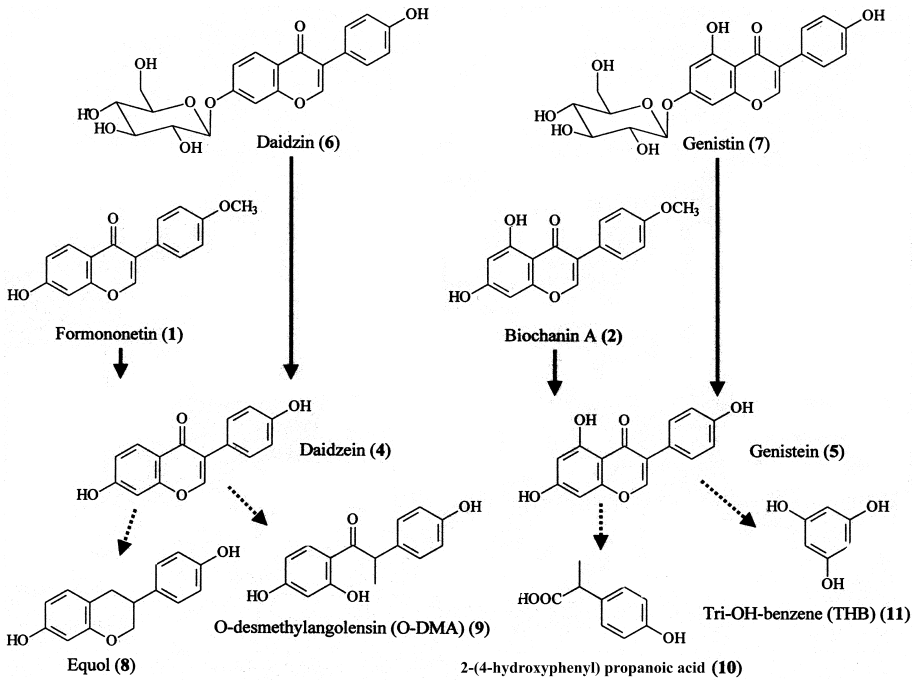


Fig. **2** Formation and biotransformation of the isoflavones genistein and daidzein.

Coumestans, with coumestrol (**12**) and 4′-*O*-methylcoumestrol (**13**) as the major members, exhibit a close structural similarity to isoflavones (Fig. **3**) [31]. Their main dietary sources are alfalfa, soybean, and clover sprouts. Little is known about their metabolism in humans; however, their metabolism in a variety of animals is extensively reviewed elsewhere [32].

Flavonoids

The female flowers of hops (*Humulus lupulus* L.) are used in the brewing industry to add flavor and bitterness to beer. Recently, several prenylated flavonoids have been identified in hops and beer, including the flavanones 8-prenylnaringenin (**14**), 6-prenylnaringenin (**15**), and isoxanthohumol (**16**), and the chalcone xanthohumol (**17**) (Fig. **4**) [33], [34], [35]. 8-Prenylnaringenin, also named hopein, has been characterized as a very potent phytoestrogen [35], [36] and large quantities of this phytoestrogen are now available through synthesis, starting from the commercially available naringenin [37].

Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) (**18**), which exists as *cis*- and *trans*-isomers, is a secondary plant metabolite belonging to the class of stilbenes (C_6-C_2-C_6) (Fig. **4**). This phytoalexin is synthesized by plants, including grapevines (*Vitis vinifera*), in response to injury and fungal attack [38]. The extraction of resveratrol from natural sources is time-consuming and yields low amounts of the compound. Therefore, research on its biological properties really started when *trans*-resveratrol was synthesized [39].

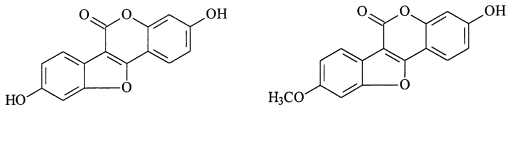


Fig. **3** The chemical structures of coumestrol and 4′-*O*-methylcoumestrol.

In contrast to the flavonoids, resveratrol is not widely distributed in the plant kingdom. The compound can be found mainly in grapes, peanuts, and pines. Therefore, red wine is one of the major dietary sources of resveratrol. Red wine contains much greater amounts of resveratrol than white wine, since resveratrol is concentrated in the grape skin and the manufacturing process of red wine includes prolonged contact with grape skins. Several scientists are claiming that resveratrol is the wine component responsible for the "French Paradox", i.e., the low incidence of heart diseases among the French people, who eat a relatively high-fat diet [40]. However, there is no consensus on the so-called French Paradox, since other scientists believe that proanthocyanidins are at least partly responsible for it.

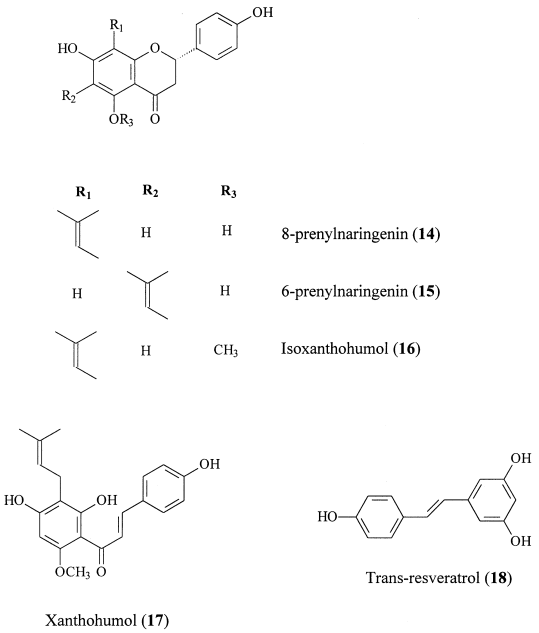


Fig. **4** Chemical structures of several prenylated flavonoids and the stilbene *trans*-resveratrol.

Until now, data on the absorption and metabolism of resveratrol are still scarce. In a rat intestinal perfusion model, it was demonstrated that the majority of the absorbed resveratrol was conjugated to yield resveratrol glucuronide [41]. In a study on rats, resveratrol was bioavailable when administered in a solution of hydroxypropyl-β-cyclodextrin [42], but underwent extensive first-pass glucuronidation. Recently, it was shown that resveratrol is absorbed much more efficiently than (+)-catechin and quercetin in humans after oral consumption [43]. Nevertheless, further studies are needed to confirm these results.

Lignans

Lignans, i.e., a group of dimeric phenylpropanoids, are mainly found in oilseeds, such as flaxseed, but they are also present in whole cereals, grains, vegetables, and fruits [44]. Matairesinol (**19**) and secoisolariciresinol (**20**) have been identified as two primary plant precursors of mammalian lignans (Fig. **5**). They are converted after ingestion by intestinal bacterial flora to the biologically active metabolites enterolactone (**21**) and enterodiol (**22**), respectively [45]. Both the parent compounds and the metabolites are measurable in various body fluids, such as urine, feces, and plasma [46], [47].

Estrogen Receptors and Selective Estrogen Receptor Modulators (SERMs)

Estrogens are key regulators in a wide variety of target tissues, such as the male and female reproductive systems, bone tissue, and the cardiovascular and central nervous systems [48]. Estrogens are used for prevention and treatment of postmenopausal symptoms and as contraceptives, while estrogen antagonists are used in the treatment of hormone-dependent breast cancers. Consequently, it was believed that the administration of the estrogen antagonist tamoxifen (**23**) in breast cancer patients would lead to a decrease in bone mineral density (Fig. **6**). However, in a 24-month placebo-controlled study in breast cancer patients, the opposite was found [49]. The study indicated that tamoxifen could act as an agonist in bone and as an antagonist in the breast,

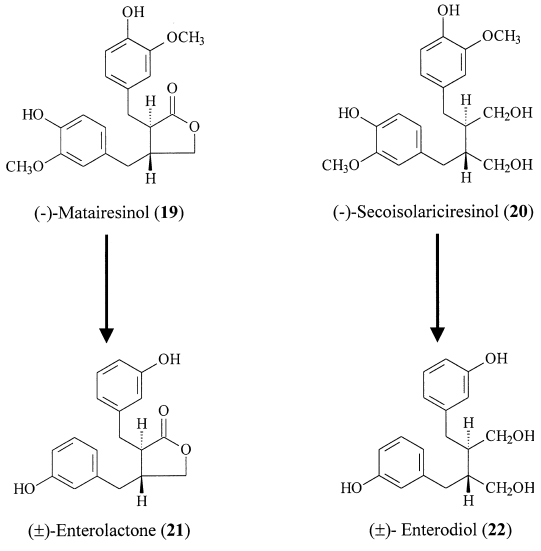


Fig. **5** Metabolism of lignans by the normal microflora.

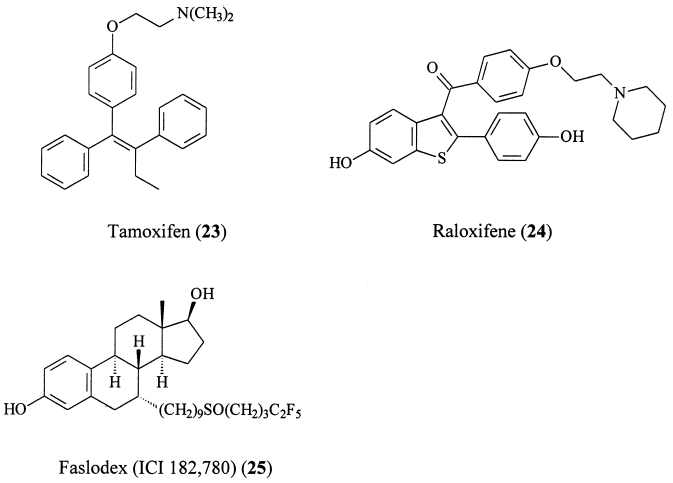


Fig. **6** Chemical structures of the synthetic SERMs tamoxifen, raloxifene, and faslodex.

and it is therefore termed as a selective estrogen receptor modulator or SERM. SERMs, such as tamoxifen (**23**), raloxifene (**24**), and faslodex (ICI 182,780) (**25**), are compounds that bind to estrogen receptors and modulate agonist or antagonist responses depending on the target tissue (Table **1**) [48], [50], [51]. Unfortunately, tamoxifen also exhibits stimulatory effects on the endometrium, but raloxifene, an SERM approved for osteoporosis prevention, does not stimulate the endometrium [52]. The biological effects of SERMs were better understood after the finding in 1996 of a second subtype of ER [53], [54]. So, to date, two estrogen receptors (ER) have been identified, i.e., ER_α and ER_β, which have eight exons encoding for six functional domains, designated A-F. Both ER subtypes differ in the ligand independent transactivation domain AF1 at the amino terminus and the ligand binding domain at the carboxy terminus [55], as well as in the tissue distribution [56], [57]. Studies of their tissue distribution and/or their relative levels indicated that ER_α has moderate to high expression in uterus, testis, ovary, and kidney, while ER_β is expressed mainly in prostate, uterus, ovary, testis, bone, lung, and brain [56], [57].

Animal estrogens are exclusively steroidal compounds, with 17β-estradiol (**26**) as the principal physiological estrogen in most species, including humans. 17β-Estradiol contains a phenolic group at position 3 and a secondary alcohol group at position 17 of a steroidal skeleton, separated from each other by a hydrophobic rather inflexible structure of about 1.2 nm. As shown in Fig. **7**, phytoestrogens are polyphenolic non-steroidal plant compounds that are structurally similar to 17β-estradiol and thus may act as estrogen agonists or antagonists. In this review, the SERM activity of phytoestrogens will be limited to isoflavonoids, prenylated flavonoids, and the stilbene resveratrol.

Biological Effects of Isoflavonoids

Isoflavonoids as SERMs

A great number of isoflavonoids has been tested in a competition binding assay to assess their relative binding affinities [58]. The estrogen receptor relative binding affinities of the isoflavonoids

Table **1** Comparison between the biological effects of 17β-estradiol and some SERMs on selected target tissues

	Bone	*Breast*	*Uterus*	*Vasculature*
17β-Estradiol (**26**)	+	+	+	+
Tamoxifen (**23**)	+	–	(partial) +	+
Raloxifene (**24**)	+	–	–	+
Genistein (**5**)	+	+/–	No effect	+

+, agonist activity; –, antagonist activity.
According to Hall et al. [48] and Tikkanen et al. [51].

tested decreased in the following order: 17β-estradiol (control) > coumestrol > genistein > equol > daidzein > biochanin A. In this assay the binding of a compound to a receptor is determined, but it cannot distinguish between agonistic and antagonistic activity [9]. In addition, the study mentioned above did not make a difference between ER_α and ER_β binding affinities.

Besides the receptor binding assay, there are several *in vitro* test systems, including cell-proliferation assays and gene reporter assays, to evaluate the estrogenic activity of natural compounds [9]. In the so-called "E-screen" the ability of a compound to stimulate the proliferation of human estrogen-dependent breast cancer cell lines, such as MCF-7 and T47-D, is measured. In a reporter gene assay, the capability of a compound to activate the transcription of an estrogen-sensitive promotor is analyzed. Several studies have used these assays together with the competition binding assay to compare the estrogenic activity of isoflavonoids on ER_α and ER_β [56], [59], [60], [61], [62]. They clearly demonstrated that coumestrol was the most active isoflavonoid and bound almost as strongly as 17β-estradiol to both ER_α and ER_β, but genistein induced transcription as strongly as coumestrol [61]. The isoflavones tested, including genistein, daidzein, and equol, exhibited a greater binding affinity to ER_β than to ER_α

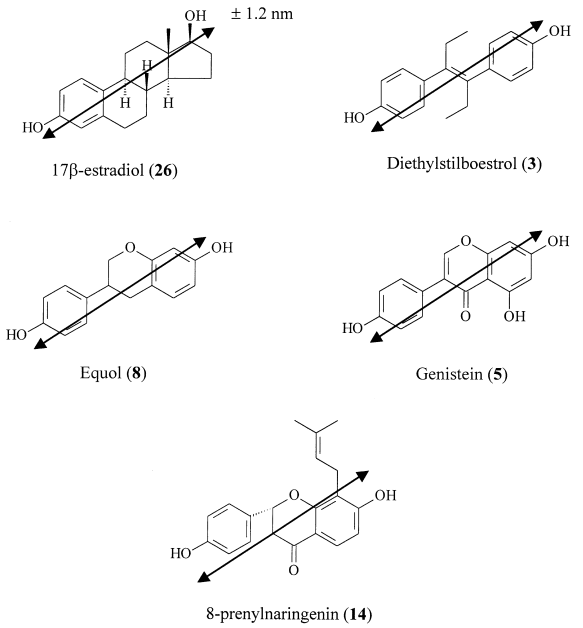


Fig. **7** A comparison of the chemical structures of 17β-estradiol, diethylstilbestrol, and some phytoestrogens.

[56], [59], [60], [61], [62]. However, the concentration required for induction was almost the same for both ERs and was much higher than expected from the binding affinity [60]. Isoflavone glycosides, such as daidzin, glycitin, and genistin, bound weakly to both receptors and estrogen receptor-dependent transcriptional expression was poor [60]. Interestingly, genistin stimulated the growth of MCF-7 cells more strongly than genistein. Formononetin and biochanin A exhibited a significantly lower binding affinity and transcription induction compared to their non-methylated forms daidzein and genistein, respectively [59], [61]. Consequently, metabolization of formononetin and biochanin A by microflora is necessary to obtain phytoestrogenic activity.

A recent study on MCF-7 cell lines showed that 17β-estradiol and coumestrol strongly increased the progesterone receptor (PR) mRNA expression and slightly down-regulated the ER_α mRNA expression [63]. Genistein and SERMs such as raloxifene and faslodex strongly decreased the ER_α protein levels. The study concluded that coumestrol exerts molecular properties which are very similar to those of 17β-estradiol, whereas the molecular properties of genistein are comparable to those of the SERMs raloxifene and faslodex.

In conclusion, isoflavones have a relatively greater binding affinity for ER_β than for ER_α, but are 10^2 to 10^5 times less active than steroidal estrogens. They are, however, frequently present in the human body in much higher quantities than endogenously produced estrogens. Additionally, methylation or glycosidation of isoflavones generally decreased their binding affinity to ER and their estrogen-dependent transcription expression.

Other biological effects of isoflavonoids

Some isoflavonoids are able to inhibit several key enzymes in estrogen and androgen biosynthesis, including 5α-reductase [64], 17β-hydroxysteroid oxidoreductase [65], and aromatase [66], and can stimulate the synthesis of sex hormone-binding globulins (SHBG) [67]. Furthermore, several isoflavonoids have been reported to exert other non-hormonal effects *in vitro*, including inhibition of tyrosine kinases [68], DNA topoisomerases I and II [69], and anti-angiogenesis [70] and antioxidant activity [71]. In addition to the SERM activity of some isoflavonoids, all these non-hormonal effects may contribute to their potential preventive effects against certain types of cancer. However, it must be emphasized that many of these non-hormonal effects have been shown with very high concentrations, which can hardly be obtained *in vivo* [72].

Biological Effects of Prenylated Flavonoids

Prenylated flavonoids as SERMs

Several prenylated flavonoids have been studied for their estrogenic activity [35], [36], [73], [74]. All these studies concluded that 8-prenylnaringenin exerts *in vitro* a very high estrogenic activity. In a competition binding assay, it was demonstrated that 8-prenylnaringenin competed strongly with 17β-estradiol for binding to both ER_α and ER_β. The relative binding affinities were significantly higher than the most active isoflavonoids coumestrol and genistein [36]. No significant difference in relative binding affinity was observed between 2(*S*)- and 2(*R*)-enantiomers of 8-prenylnaringenin [73]. Movement of the prenyl unit from position 8 to 6 resulted in loss of the activity [36], [73]. Xanthohumol and isoxanthohumol showed no affinity for both ERs [36].

In an MCF-7 cell line proliferation assay, the estrogenic activity of 8-prenylnaringenin was found to be 25 times higher than that of the isoflavone genistein [73]. It was suggested that the high activity of 8-prenylnaringenin is related to its lipophilicity, resulting in a higher permeability of cellular membranes compared to genistein. In an estrogen-inducible yeast (*Saccharomyces cerevisiae*) assay the estrogenic potency of a series of isoflavonoids and prenylated flavonoids decreased in the following order: 17β-estradiol (control) > 8-prenylnaringenin > coumestrol > genistein > daidzein >> 6-prenylnaringenin [35].

Other biological effects of prenylated flavonoids

Prenylated flavonoids are less studied than isoflavonoids, but two studies attracted our attention. First, it was found that 8-prenylnaringenin up-regulated the function of the E-cadherin/catenin complex in human mammary carcinoma cells [75]. Down-regulation of elements of the E-cadherin/catenin complex at transcriptional or posttranslational levels is a common feature of carcinoma cells. Nevertheless, further studies are required to demonstrate its potential anti-cancer activity *in vivo*. Second, prenylation of naringenin antagonized the pro-oxidant effect of naringenin on LDL oxidation, but the antioxidant activity of the prenylated flavanones is still lower than that of the flavonol quercetin [76].

Biological Effects of Resveratrol

Resveratrol as SERM

trans-Resveratrol is structurally similar to DES and binds equally to both ER_α and ER_β [77]. The latter finding contrasts with other phytoestrogens, such as genistein and coumestrol, which have a higher binding affinity for ER_β than for ER_α. Although resveratrol can exist as a *cis*- or *trans*-isomer, the *trans*-isomer exerted a higher activity in estrogen-dependent human breast cancer cell lines [78]. At concentrations of 10 and 25 μM it increased the *in vitro* growth of MCF-7 cell lines, whereas at concentrations of 0.1 and 1 μM it had no effect. At a concentration of 10 μM resveratrol inhibited binding of 17β-estradiol to ER and activated transcription of estrogen-response reporter genes transfected into human breast cancer cells [79]. In another study, resveratrol antagonized 17β-estradiol-stimulated growth and inhibited transcription of PR in MCF-7 cells [80]. These results suggest that resveratrol acts as a mixed estrogen agonist/antagonist, which was examined more in detail in two other studies [77], [81]. It was reported that in the absence of 17β-estradiol, resveratrol weakly induced ER-dependent transcriptional events in some mammary tumor cell lines, whereas down-regulation was observed when resveratrol was co-administered with 17β-estradiol [81]. In mouse mammary glands, grown in culture, resveratrol inhibited the formation of 7,12-dimethylbenz[*a*]anthracene-induced and 17β-estradiol-promoted atypical ductal hyperplasia. In another study, it was shown that resveratrol exhibited 17β-estradiol antagonist activity for ER_α with select estrogen response elements (EREs), while resveratrol showed no 17β-estradiol antagonist activity with ER_β [77]. These results indicated that resveratrol differentially affects the transcriptional activity of ER_α and ER_β in an ERE sequence-dependent manner.

Dose-response studies revealed that orally administered resveratrol had minimal *in vivo* effects on estrogen target tissues in growing Sprague-Dawley rats, including no effects on uterine growth, body weight, serum cholesterol, and radial bone growth [82]. In contrast, resveratrol antagonized the serum cholesterol-lowering effect of 17β-estradiol. It was concluded that resveratrol has little or no estrogenic activity on estrogen target tissues and may even be an estrogen antagonist [82].

Other biological effects of resveratrol

Resveratrol exerts a wide variety of biological effects, including inhibition of platelet aggregation [83], modulation of lipoprotein metabolism [84], and antioxidant activity [39], [85]. The inhibitory activity of resveratrol on Cu^{2+}-catalyzed oxidation of low-density lipoproteins (LDL) has been related to its high Cu^{2+} chelating capacity [39]. Furthermore, it has been demonstrated that resveratrol inhibits membrane lipid peroxidation mainly by scavenging peroxyl radicals within the membrane [85]. Although it is less active than the chain-breaking antioxidant α-tocopherol, its capacity of spontaneously entering the lipid environment may allow resveratrol to exert a significant antioxidant activity *in vivo*. The biological effects mentioned above suggest a possible role for resveratrol in the prevention of atherosclerosis and CVD [40].

Resveratrol has also been reported to promote apoptosis of human tumor cells [86] and to induce the expression of the tumor suppressor p53 [87], suggesting a potential anti-cancer activity.

Effects of Phytoestrogens in Humans

Recently, several epidemiological and experimental studies in animals and humans have suggested that the consumption of foods rich in phytoestrogens may have protective effects on estrogen-related conditions, such as menopausal symptoms, and estrogen-related diseases, such as prostate and breast cancers, osteoporosis, and CVD. In this review, the latest developments on the potential protective properties of phytoestrogens and phytoestrogen-containing foods against hormone-dependent breast and prostate cancers and CVD, and as estrogen replacement therapy (ERT) for postmenopausal women will be discussed more in detail. Additionally, the potential dangers of consuming high levels of these compounds will be discussed.

Phytoestrogens and cancers

Breast cancer

Epidemiological studies have indicated that the incidence and mortality of breast cancer in the Western world is much higher compared to Asian countries. When Asian people emigrated to the USA, it was shown that the first-generation female migrants had a lower risk of breast cancer, but the protection was lost in the second generation with an increasingly Western diet [88]. It was therefore suggested that certain phytochemicals present in Asian diets can affect cancer incidence. Until now, most of the research has been focused on phytoestrogens and more particularly on isoflavones. First, the average daily dietary intake of soy and isoflavones in Asian populations has been estimated to be respectively 50 g/day and 30 mg/day, while in the Western populations the intake is limited to respectively 1 g/day and 1 mg/day [89], [90]. Second, these phytochemicals display estrogen-like activity and high affinity binding to the ERs, suggesting a role in hormone-dependent diseases, such as breast cancer.

Nowadays, there is an increasing number of human (and animal) studies demonstrating that a high soy intake during childhood is associated with a reduced breast cancer risk [91], [92], [93], [94]. However, there is no convincing evidence to suggest that soy or isoflavone consumption in Western countries during adult life is protective against breast cancer [91]. Soy consumption before puberty may have the same risk-lowering effect as an early pregnancy. It is suggested that phytoestrogens promote cell differentiation in the mammary gland, resulting in enhancement of mammary gland maturation [94]. Further studies must confirm these results and must provide evidence that the isoflavones present in soy are responsible for the health effects of soy.

The plant-lignan glycosides matairesinol and secoisolariciresinol are converted after ingestion by intestinal bacterial flora to the biologically active enterolactone and enterodiol, respectively. In several human studies a very low plasma enterolactone concentration was associated with an increased breast cancer risk [91], [95]. Consumption of fiber-rich whole-grain bread may stimulate the production of enterolactone, but in rats and humans, an increase in dietary fat intake decreases the urinary excretion of lignans, despite constant grain-fiber intake. Although obesity is negatively associated with plasma enterolactone in women, the effect of fat intake on breast-cancer risk may be indirect, via production of mammalian lignans [91]. This emphasizes the importance of the gut microflora, but the question still remains if the mammalian lignan enterolactone is protective or is just a biomarker of a healthy diet.

Prostate cancer

Asian men have a lower incidence of prostate cancer compared to men from Western countries. As discussed above for breast cancer, it was also suggested for prostate cancer that soy intake could be a protective diet factor. Several recent human studies support the hypothesis that soy intake prevents prostate cancer [91], [96]. Although the recent studies are encouraging, it is still premature to make recommendations on phytoestrogen intake and prostate cancer prevention or management.

The mechanism of phytoestrogen action is still unknown, but most studies suggest that the protective effects could be related to a reduction in androgen production, e.g., through inhibition of 5α-reductase. A recent study has shown an inverse association of soy product intake with serum androgen and estrogen concentrations in Japanese men [97]. Another possible mechanism of action is the binding of phytoestrogens, such as genistein, with the ER_β, which is the predominant ER in the prostate.

Colon cancer

In contrast to the breast and prostate cancers, colon cancer does not have a strong association with hormone status [98]. In a recent review article, it was stated that soy and isoflavonoids do not seem to protect against colon cancer, but lignans or lignan-rich food can inhibit colon-cancer development in animal models [91]. Therefore, the relationship between intake of lignans and colonic cancer risk warrants further investigation [99].

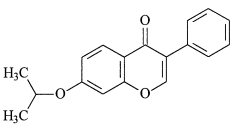
Phytoestrogens and CVD

Recently, double-blind clinical trials have shown that consumption of soy protein compared to other proteins such as casein can lower total and LDL-cholesterol levels [100], [101], [102], [103]. The effect is variable, but is generally greater in hypercholesterolemic than in normocholesterolemic subjects. In contrast, several double-blind, placebo-controlled clinical trials using isoflavone supplements alone have not shown a beneficial effect on serum lipids [104], [105]. A recent consensus paper indicated that both soy protein and isoflavones may be needed for lowering serum cholesterol concentrations [106]. Soybeans are an excellent source of proteins since, in contrast to animal proteins, soybeans contain no cholesterol. In addition, soybeans are low in saturated fat. Soy isoflavones may exert its effect by up-regulating LDL-receptor activity [107]. In conclusion, although soy proteins may reduce the lipid values, it is now essential to start some clinical studies to investigate the effect of soy on CVD prevention.

Phytoestrogens as estrogen replacement therapy (ERT)

ERT is recommended for postmenopausal women to prevent menopausal symptoms, osteoporosis, and CVD [103]. Despite these benefits, however, there are still concerns that ERT may cause cancer of the breast. Consequently, there is a growing interest among patients and researchers in phytoestrogens as an alternative to the conventional ERT.

The best results for osteoporosis prevention were obtained for ipriflavone (7-isopropoxyisoflavone, Fig. **8**) (**27**), suggesting that it is a useful and safe alternative to ERT in treating existing low bone mass or osteoporosis in postmenopausal women [108], [109]. Nevertheless, one study questioned the efficacy and safety of ipriflavone for prevention of postmenopausal bone loss. [110]. Ipriflavone is a synthetic isoflavone, derived from daidzein. It does not seem to act through direct estrogen receptor activity and is therefore not strictly a phytoestrogen. However, approximately 10% of the ingested dose is converted back to daidzein in the body [111]. Clinical studies of the effects of phytoestrogens



Ipriflavone (**27**)

Fig. **8** Chemical structure of the synthetic ipriflavone or 7-isopropoxyisoflavone.

and phytoestrogen-containing foods on bone health have produced mixed results. One clinical study indicated that flaxseed supplementation had no effect on biomarkers of bone metabolism in postmenopausal women [112], while two clinical studies administering isoflavone preparations to postmenopausal women reported a bone loss prevention effect [113], [114].

Clinical studies of the effects of isoflavone-containing soy preparations on the incidence of hot flashes in postmenopausal women provided mixed results. Some clinical studies showed a significant but small reduction of hot flashes [115], [116], while one study administering a soy preparation to breast cancer survivors did not improve their incidence of hot flashes [117]. As stated by Glazier and Bowman, it must be emphasized that a 15% reduction in hot flashes would mean a reduction of 1 hot flash per day in patients experiencing 12 hot flashes per day [103]. So, if supplementation of phytoestrogens will cause a statistically significant improvement on the number of hot flashes, this does not mean that the patient's quality of life will improve.

Finally, a human study in postmenopausal women supplemented with isoflavone-containing soy powder concluded that isoflavones do not have estrogenic effects on endometrial tissue, in contrast to 17β-estradiol [118].

Potential adverse effects

Concerns on the potential adverse effects of phytoestrogens have been originated from their structural similarity to DES. The best-known adverse effects are reported in animals. In the mid-1940 s, an infertility syndrome in sheep had been attributed to the ingestion of phytoestrogen-rich clover. Nevertheless, there are concerns about the long-term effects of phytoestrogens given to infants and young children [72]. It was found that infants fed on soy milk formulas had plasma isoflavone levels that are orders of magnitude greater than those of infants fed on human or cow's milk [119]. Further studies are needed to investigate if the soy formula-fed infants would display later in life a greater risk to breast or prostate cancer.

In a recent study, *s.c.* injection of genistein in ovariectomized mice decreased thymic weight and thymic and splenic $CD4^+$ $CD8^-$ T cell numbers and resulted in immune suppression and lymphocytopenia [120]. The latter was also seen in a human study on ipriflavone for the prevention of postmenopausal bone loss [110].

In conclusion, although the intake of phytoestrogens is higher in countries where the incidence of estrogen-related cancers is lower, it is unwise to exclude adverse effects of phytoestrogen supplementation. More in particular, supplementation with very high doses of pure phytoestrogens and supplementation of infants and pregnant women must be approached with caution.

Concluding Remarks

In view of the current data, phytoestrogens are generally accepted as beneficial rather than deleterious, particularly when consumed in food products. The consumption of phytoestrogen-containing food products, especially soy products, may contribute to a lower risk of developing CVD and prostate cancer in healthy people, while soy intake during childhood is associated with a reduced breast cancer risk. Although a large number of studies is encouraging, it is still premature to recommend specific amounts of dietary phytoestrogens for prevention of chronic diseases. First, the dosage and purity of commercial phytoestrogens and their possible adverse effects remain largely unknown. Second, large population-based studies have not been carried out nor are there randomized controlled clinical trials to standardize dosage and ensure safety and efficacy. Third, at this moment, most of the studies were performed with food products and not with a single phytoestrogenic compound. It is therefore a simplistic view to extract the health effects of a high soy consumption to the potential health effects of isoflavones present in soy. It may be that other components present in these food products contribute to these effects. Additionally, the lifestyle factors in Asian countries are quite different from these in Western countries and cannot simply be reduced to the intake of one food product.

In conclusion, until more information on phytoestrogens is gathered and fully understood, it is recommended to eat a balanced diet containing a wide variety of fruits, vegetables, including soy, and whole grain products.

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