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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 17 β -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-1940s, 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

This review is based on a plenary lecture presented by A.J.V. at the Phytoestrogen Workshop, organized by the Departments of Pharmacy and Chemistry of the University of Helsinki and the Society for Medicinal Plant Research. The workshop was held in Helsinki on 05.07.2002 in honor of the birthdays of Prof. (emer.) Dr. Max von Schantz and Prof. (emer.) Herman Adlercreutz

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Bibliography

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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 -resorcylic 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 con-

ducting 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-hydroxyphenyl)propanoic 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].

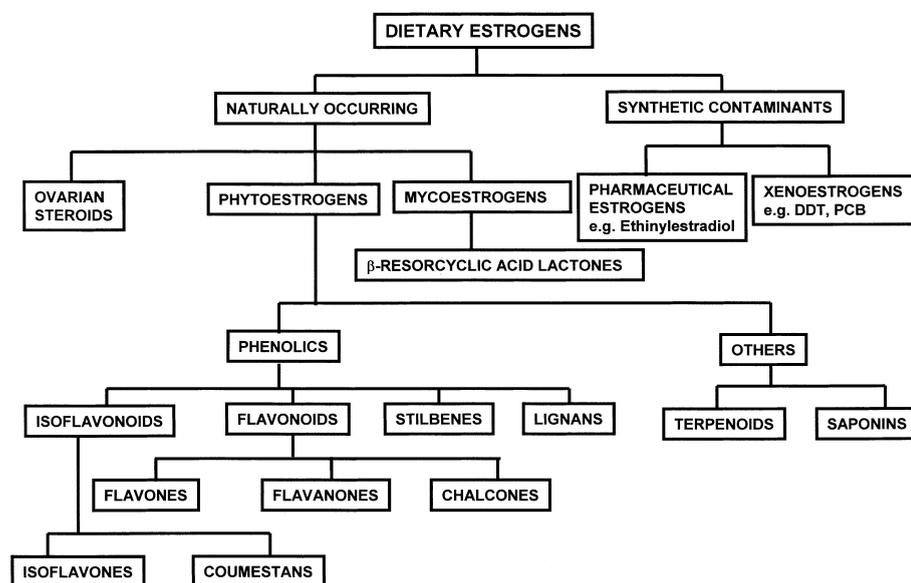
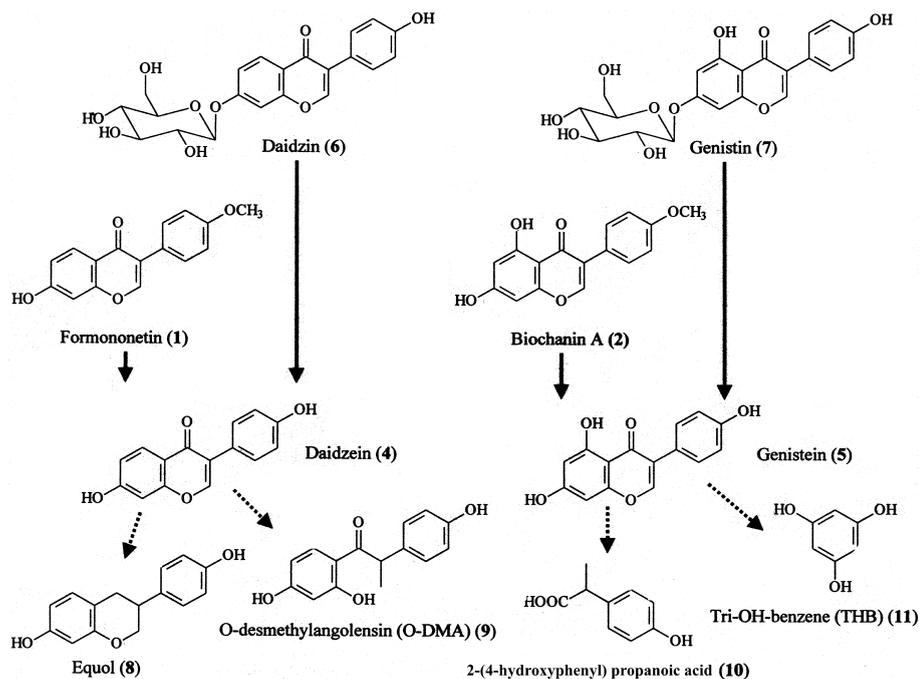


Fig. 1 Classification scheme of dietary estrogens.

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 (C₆-C₂-C₆) (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].

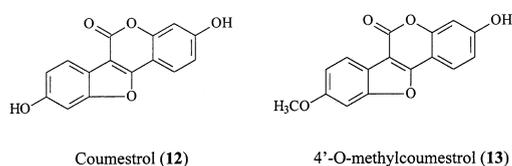


Fig. 3 The chemical structures of coumestrol and 4'-O-methylcoumestrol.

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.

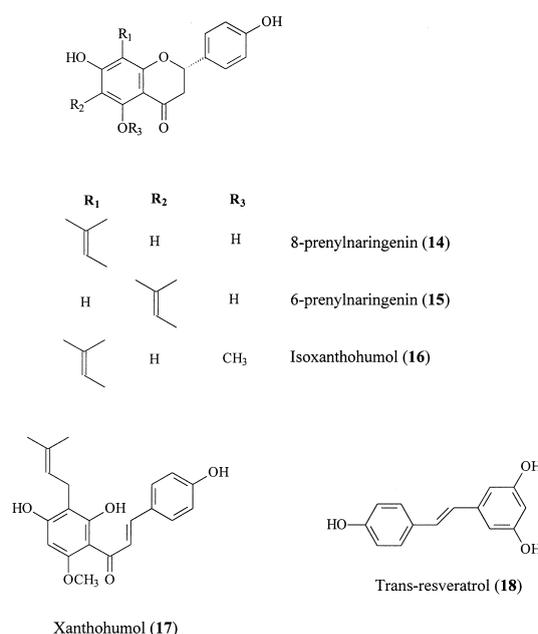


Fig. 4 Chemical structures of several prenylated flavonoids and the stilbene *trans*-resveratrol.

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,

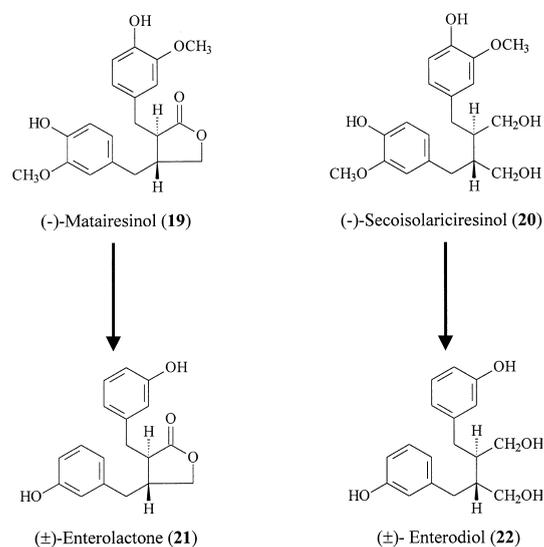


Fig. 5 Metabolism of lignans by the normal microflora.

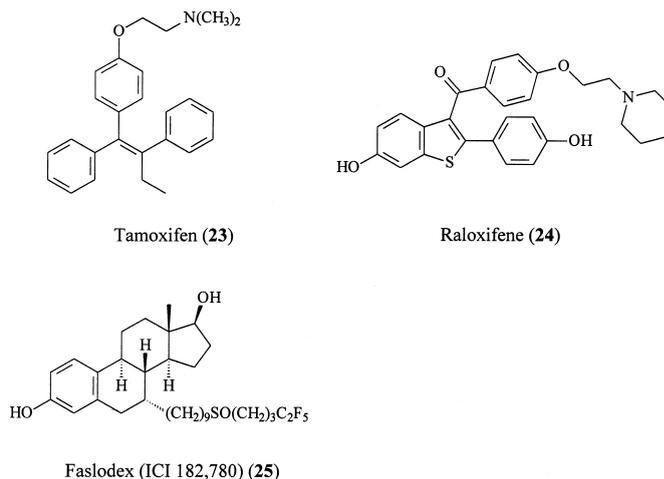


Fig. 6 Chemical structures of the synthetic SERMs tamoxifen, raloxifene, and faslodex.

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 $_{\alpha}$ and ER $_{\beta}$, 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 $_{\alpha}$ has moderate to high expression in uterus, testis, ovary, and kidney, while ER $_{\beta}$ is expressed mainly in prostate, uterus, ovary, testis, bone, lung, and brain [56], [57].

Animal estrogens are exclusively steroidal compounds, with 17 β -estradiol (**26**) as the principal physiological estrogen in most species, including humans. 17 β -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 17 β -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 17 β -estradiol and some SERMs on selected target tissues

	Bone	Breast	Uterus	Vasculature
17 β -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: 17 β -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 $_{\alpha}$ and ER $_{\beta}$ 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 promoter is analyzed. Several studies have used these assays together with the competition binding assay to compare the estrogenic activity of isoflavonoids on ER $_{\alpha}$ and ER $_{\beta}$ [56], [59], [60], [61], [62]. They clearly demonstrated that coumestrol was the most active isoflavonoid and bound almost as strongly as 17 β -estradiol to both ER $_{\alpha}$ and ER $_{\beta}$, but genistein induced transcription as strongly as coumestrol [61]. The isoflavones tested, including genistein, daidzein, and equol, exhibited a greater binding affinity to ER $_{\beta}$ than to ER $_{\alpha}$

[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 17 β -estradiol and coumestrol strongly increased the progesterone receptor (PR) mRNA expression and slightly down-regulated the ER $_{\alpha}$ mRNA expression [63]. Genistein and SERMs such as raloxifene and faslodex strongly decreased the ER $_{\alpha}$ protein levels. The study concluded that coumestrol exerts molecular properties which are very similar to those of 17 β -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 $_{\beta}$ than for ER $_{\alpha}$, but are 10² to 10⁵ 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 5 α -reductase [64], 17 β -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].

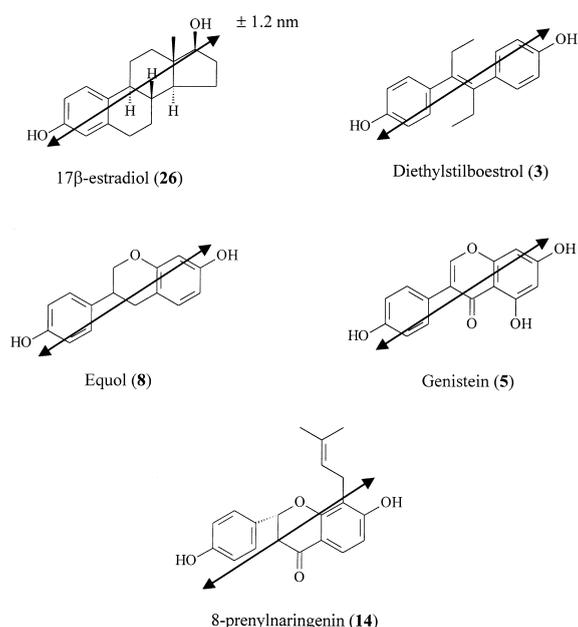


Fig. 7 A comparison of the chemical structures of 17 β -estradiol, diethylstilbestrol, and some phytoestrogens.

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 17 β -estradiol for binding to both ER $_{\alpha}$ and ER $_{\beta}$. 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: 17 β -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 $_{\alpha}$ and ER $_{\beta}$ [77]. The latter finding contrasts with other phytoestrogens, such as genistein and coumestrol, which have a higher binding affinity for ER $_{\beta}$ than for ER $_{\alpha}$. 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 μ M it increased the *in vitro* growth of MCF-7 cell lines, whereas at concentrations of 0.1 and 1 μ M it had no effect. At a concentration of 10 μ M resveratrol inhibited binding of 17 β -estradiol to ER and activated transcription of estrogen-response reporter genes transfected into human breast cancer cells [79]. In another study, resveratrol antagonized 17 β -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 ex-

amined more in detail in two other studies [77], [81]. It was reported that in the absence of 17 β -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 17 β -estradiol [81]. In mouse mammary glands, grown in culture, resveratrol inhibited the formation of 7,12-dimethylbenz[*a*]anthracene-induced and 17 β -estradiol-promoted atypical ductal hyperplasia. In another study, it was shown that resveratrol exhibited 17 β -estradiol antagonist activity for ER $_{\alpha}$ with select estrogen response elements (EREs), while resveratrol showed no 17 β -estradiol antagonist activity with ER $_{\beta}$ [77]. These results indicated that resveratrol differentially affects the transcriptional activity of ER $_{\alpha}$ and ER $_{\beta}$ 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 17 β -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 Cu²⁺-catalyzed oxidation of low-density lipoproteins (LDL) has been related to its high Cu²⁺ chelating capacity [39]. Furthermore, it has been demonstrated that resveratrol inhibits membrane lipid peroxidation mainly by scavenging peroxy 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 $_{\beta}$, 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].

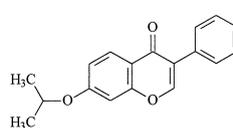
Phytoestrogens and CVD

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-1940s, 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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References

- Bennetts HW, Underwood EJ, Shier FL. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Australian Veterinary Journal* 1946; 22: 2–12
- Adams NR. Detection of the effects of phytoestrogens on sheep and cattle. *J. Animal Sci* 1995; 73: 1509–15
- Baird D, Umbach D. Dietary intervention study to assess estrogenicity of dietary soy, among postmenopausal women. *Clin Endocr* 1995; 80: 1685–90
- Denis L, Morton MS, Griffiths K. Diet and its preventive role in prostatic disease. *Eur Urol* 1999; 35: 377–87
- Lee HP, Gourley L, Duffy SW. Dietary effects on breast-cancer risk in Singapore. *Lancet* 1991; 337: 1197–200
- Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr* 1998; 68: 1375S–9S
- Bakhit RM, Klein BP, Essex-Sorlie D. Intake of 25 g of soybean protein with or without soybean fibre alters plasma lipids in men with elevated cholesterol concentrations. *J Nutr* 1994; 124: 213–22
- Abe T. Infantile leukaemia and soybeans: a hypothesis. *Leukaemia* 1999; 13: 317–20
- Diel P, Smolnikar K, Michna H. *In vitro* test systems for the evaluation of the estrogenic activity of natural products. *Planta Med* 1999; 65: 197–203
- Murkies AL, Wilcox G, Davis SR. Phytoestrogens. *J Clin Endocr Metab* 1998; 83: 297–303
- Wuttke W, Jarry H, Westphalen S, Christoffel V, Gorkow C, Seidlova-Wuttke D. Phytoestrogens: an alternative to standard hormone replacement therapy? *Gynäkologie* 2002; 35: 1007–20
- King A, Young G. Characteristics and occurrence of phenolic phytochemicals. *J Am Diet Assoc* 1999; 99: 213–8
- Reinli K, Block G. Phytoestrogen content of foods. *Nutr Cancer* 1996; 26: 123–48

- 14 Wang H, Murphy PA. Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year and location. *J Agr Food Chem* 1994; 42: 1674–7
- 15 Wang H, Murphy PA. Isoflavone content in commercial soybean foods. *J Agr Food Chem* 1994; 42: 1666–73
- 16 Anderson RL, Wolf WJ. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. *J Nutr* 1995; 206: 575S–86S
- 17 Fukutake M, Takahashi M, Ishida K, Kawamura H, Sugimura T, Wakabayashi K. Quantification of genistein and genistin in soybeans and soybean products. *Food Chem Toxicol* 1996; 34: 457–61
- 18 Coward L, Smith M, Kirk M, Barnes S. Chemical modification of isoflavones in soyfoods during cooking and processing. *J Nutr* 1998; 68: 1486S–91S
- 19 Day AJ, Dupont MS, Ridley S, Rhodes M, Rhodes MJC, Morgan MRA, Williamson G. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver β -glucosidase activity. *FEBS Lett* 1998; 436: 71–5
- 20 Hur HG, Rafii F. Biotransformation of the isoflavonoids biochanin A, formononetin, and glycitein by *Eubacterium limosum*. *FEMS Microbiol Lett* 2000; 192: 21–5
- 21 Kelly GE, Joannou GE, Reeder AY, Nelson C, Waring MA. The variable metabolic response to dietary isoflavones in humans. *Proc Soc Exp Biol Med* 1995; 208: 40–3
- 22 Heinonen S, Wähälä K, Adlercreutz H. Identification of isoflavone metabolites dihydrodaidzein, dihydrogenistein, 6'-OH-O-DMA, and cis-4-OH-equol in human urine by gas chromatography-mass spectroscopy using authentic reference compounds. *Anal Biochem* 1999; 274: 211–9
- 23 Coldham NG, Darby C, Hows M, King LJ, Zhang AQ, Sauer MJ. Comparative metabolism of genistein by human and rat gut microflora: detection and identification of the end-products of metabolism. *Xenobiotica* 2002; 32: 45–62
- 24 Oitate M, Nakaki R, Koyabu N, Takanaga H, Matsuo H, Ohtani H, Sawada Y. Transcellular transport of genistein, a soybean-derived isoflavone, across human colon carcinoma cell line (Caco-2). *Biopharm Drug Dispos* 2001; 22: 23–9
- 25 Murota K, Shimizu S, Miyamoto S, Izumi T, Obata A, Kikuchi M, Terao J. Unique uptake and transport of isoflavone aglycones by human intestinal caco-2 cells: comparison of isoflavonoids and flavonoids. *J Nutr* 2002; 132: 1956–61
- 26 Walle UK, French KL, Walgren RA, Walle T. Transport of genistein-7-glucoside by human intestinal caco-2 cells: potential role for MRP2. *Res Commun Mol Pathol Pharmacol* 1999; 103: 45–56
- 27 Andlauer W, Kolb J, Fürst P. Isoflavones from tofu are absorbed and metabolized in the isolated rat small intestine. *J Nutr* 2000; 130: 3021–7
- 28 Andlauer W, Kolb J, Stehle P, Fürst P. Absorption and metabolism of genistein in isolated rat small intestine. *J Nutr* 2000; 130: 843–6
- 29 Setchell KDR, Brown NM, Zimmer-Nechemias L, Brashear WT, Wolfe BE, Kirschner AS, Heubi JE. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am J Clin Nutr* 2002; 76: 447–53
- 30 Lampe JW, Martini MC, Kurzer MS, Adlercreutz H, Slavin JL. Urinary lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. *Am J Clin Nutr* 1994; 60: 122–8
- 31 Davis SR, Dalais FS, Simpson ER, Murkies AL. Phytoestrogens in health and disease. *Recent Prog Horm Res* 1999; 54: 185–211
- 32 Price KR, Fenwick GR. Naturally-occurring estrogens in foods: a review. *Food Addit Comtam* 1985; 2: 73–106
- 33 Stevens JF, Ivancic M, Hsu VL, Deinzer ML. Prenylflavonoids from *Humulus lupulus*. *Phytochemistry* 1997; 44: 1575–85
- 34 Stevens JF, Taylor AW, Deinzer ML. Quantitative analysis of xanthohumol and related flavonoids in hops and beer by liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 1999; 832: 97–107
- 35 Milligan SR, Kalita JC, Heyerick A, Rong H, De Cooman L, De Keukeleire D. Identification of a potent phytoestrogen in hops (*Humulus lupulus* L.) and beer. *J Clin Endocr Metab* 1999; 83: 2249–52
- 36 Milligan SR, Kalita JC, Pocock V, Van de Kauter V, Stevens JF, Deinzer ML et al. The endocrine activities of 8-prenylnaringenin and related hop (*Humulus lupulus* L.) flavonoids. *J Clin Endocr Metab* 2000; 85: 4912–5
- 37 Gester S, Metz P, Zierau O, Vollmer G. An efficient synthesis of the potent phytoestrogens 8-prenylnaringenin and 6-(1,1-dimethylallyl)-naringenin by europium(III)-catalyzed Claisen rearrangement. *Tetrahedron* 2001; 57: 1015–8
- 38 Langcake P, Pryce RJ. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection and injury. *Physiol Plant Pathol* 1976; 9: 77–86
- 39 Frémont L. Biological effects of resveratrol. *Life Sci* 2000; 66: 663–73
- 40 Soleas GJ, Diamandis EP, Goldberg DM. Resveratrol: a molecule whose time has come? And gone? *Clin Biochem* 1997; 30: 91–113
- 41 Andlauer W, Kolb J, Siebert K, Fürst P. Assessment of resveratrol bioavailability in the perfused small intestine of the rat. *Drug Exp Clin Res* 2000; 26: 47–55
- 42 Marier JF, Vachon P, Gritsas A, Zhang J, Moreau JP, Ducharme MP. Metabolism and disposition of resveratrol in rats: extent of absorption, glucuronidation, and enterohepatic recirculation evidenced by a linked-rat model. *J Pharmacol Exp Ther* 2002; 302: 369–73
- 43 Soleas GJ, Yan J, Goldberg DM. Ultrasensitive assay for three polyphenols (catechin, quercetin, and resveratrol) and their conjugates in biological fluids utilizing gas chromatography with mass selective detection. *J Chromatogr B* 2001; 757: 161–72
- 44 Thompson LU, Robb P, Serraino M, Cheung F. Mammalian lignan production from various foods. *Nutr Cancer* 1991; 16: 43–52
- 45 Borriello SP, Setchell KDR, Axelson M, Lawson AM. Production and metabolism of lignans by the human faecal flora. *J Appl Bacteriol* 1985; 58: 37–43
- 46 Adlercreutz H, Fotsis T, Kurzer MS, Wähälä K, Mäkelä T, Hase T. Isotope dilution gas chromatographic-mass spectrometric method for the determination of unconjugated lignans and isoflavonoids in human feces, with preliminary results in omnivorous and vegetarian women. *Anal Biochem* 1995; 225: 101–8
- 47 Morton MS, Chan PS, Cheng C, Blacklock N, Matos-Ferreira A, Abranches-Monteiro Let al. et allignans and isoflavonoids in plasma and prostatic fluid in men: samples from Portugal, Hong Kong, and the United Kingdom. *Prostate* 1997; 32: 122–8
- 48 Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J Biol Chem* 2001; 276: 36869–72
- 49 Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC et al. Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. *New Engl J Med* 1992; 326: 852–6
- 50 McDonnell DP. The molecular pharmacology of SERMs. *TEM* 1999; 10: 301–11
- 51 Tikkanen MJ, Adlercreutz H. Dietary soy-derived isoflavone phytoestrogens: could they have a role in coronary heart disease prevention? *Biochem Pharmacol* 2000; 60: 1–5
- 52 Mitlak BH, Cohen FJ. Selective estrogen receptor modulators: a look ahead. *Drugs* 1999; 57: 653–63
- 53 Kuiper G, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* 1996; 93: 5925–30
- 54 Mosselman S, Polman J, Dijkema R. ER β : identification and characterization of a novel human estrogen receptor. *FEBS Lett* 1996; 392: 49–53
- 55 Dechering K, Boersma C, Mosselman S. Estrogen receptors α and β : two receptors of a kind? *Curr Med Chem* 2000; 7: 561–76
- 56 Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β . *Endocrinology* 1997; 138: 863–70
- 57 Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G et al. Human estrogen receptor β -gene structure, chromosomal localization, and expression pattern. *J Clin Endocrinol Metab* 1997; 82: 4258–65
- 58 Branham WS, Dial SL, Moland CL, Hass BS, Blair RM, Fang H et al. Phytoestrogens and mycoestrogens bind to the rat uterine estrogen receptor. *J Nutr* 2002; 132: 658–64
- 59 Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 1998; 139: 4252–63
- 60 Morito K, Hirose T, Kinjo J, Hirakawa T, Okawa M, Nohara T et al. Interaction of phytoestrogens with estrogen receptors α and β . *Biol Pharm Bull* 2001; 24: 351–6
- 61 Morito K, Aomori T, Hirose T, Kinjo J, Hasegawa J, Ogawa S et al. Interaction of phytoestrogens with estrogen receptors α and β (II). *Biol Pharm Bull* 2002; 25: 48–52
- 62 Schmitt E, Dekant W, Stopper H. Assaying the estrogenicity of phytoestrogens in cells of different estrogen sensitive tissues. *Toxicol in vitro* 2001; 15: 433–9

- 63 Diel P, Olf S, Schmidt S, Michna H. Molecular identification of potential selective estrogen receptor modulator (SERM) like properties of phytoestrogens in the human breast cancer cell line MCF-7. *Planta Med* 2001; 67: 510–4
- 64 Evans BA, Griffiths K, Morton MS. Inhibition of 5 α -reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. *J Endocrinol* 1995; 147: 295–302
- 65 Mäkelä S, Poutanen M, Kostian ML, Lehtimäki N, Strauss L, Santti R, Vihko R. Inhibition of 17 β -hydroxysteroid oxidoreductase by flavonoids in breast and prostate cancer cells. *Proc Soc Exp Biol Med* 1998; 217: 310–6
- 66 Adlercreutz H, Bannwart C, Wahala KT. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J Steroid Biochem Mol Biol* 1993; 44: 147–53
- 67 Mousavi Y, Adlercreutz H. Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture. *Steroids* 1993; 58: 301–4
- 68 Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N et al. Genistein, a specific inhibitor of tyrosine-specific protein kinase. *J Biol Chem* 1987; 262: 5592–5
- 69 Okura A, Arakawa H, Oka H, Yoshinari T, Monden Y. Effect of genistein on topoisomerase activity and on the growth of [Val12]Ha-ras-transformed NIH 3T3 cells. *Biochem Biophys Res Commun* 1988; 157: 183–9
- 70 Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R, Schweigerer L. Genistein, a dietary derived inhibitor of angiogenesis. *Proc Natl Acad Sci USA* 1993; 90: 2690–4
- 71 Cos P, Calomme M, Sindambiwe JB, De Bruyne T, Cimanga K, Pieters L et al. Cytotoxicity and lipid peroxidation-inhibiting activity of flavonoids. *Planta Med* 2001; 67: 515–9
- 72 Strauss L, Santti R, Saarinen N, Streng T, Joshi S, Mäkelä S. Dietary phytoestrogens and their role in hormonally dependent disease. *Toxicol Lett* 1998; 102–103: 349–54
- 73 Kitaoka M, Kadokawa H, Sugano M, Ichikawa K, Taki M, Takaishi S et al. Prenylflavonoids: A new class of non-steroidal phytoestrogens (part 1). Isolation of 8-isopentenylnaringenin and an initial study on its structure-activity relationship. *Planta Med* 1998; 64: 511–5
- 74 Zierau O, Gester S, Schwab P, Metz P, Kolba S, Wulf M, Vollmer G. Estrogenic activity of the phytoestrogens naringenin, 6-(1,1-dimethylallyl)naringenin and 8-prenylnaringenin. *Planta Med* 2002; 68: 449–51
- 75 Rong H, Boterberg T, Maubach J, Stove C, Depypere H, Van Slambrouck S et al. 8-Prenylnaringenin, the phytoestrogen in hops and beer, up-regulates the function of the E-cadherin/catenin complex in human mammary carcinoma cells. *Eur J Cell Biol* 2001; 80: 580–5
- 76 Miranda CL, Stevens JF, Ivanov V, McCall M, Frei B, Deinzer ML, Buhler DR. Antioxidant and prooxidant actions of prenylated and nonprenyated chalcones and flavanones *in vitro*. *J Agric Food Chem* 2000; 48: 3876–84
- 77 Bowers JL, Tyulmenkov VV, Jernigan SC, Klinge CM. Resveratrol acts as a mixed agonist/antagonist for estrogen receptors α and β . *Endocrinology* 2000; 141: 3657–67
- 78 Basly JP, Marre-Fournier F, Le Bail JC, Habrioux G, Chulia AJ. Estrogenic/antiestrogenic and scavenging properties of (E)- and (Z)-resveratrol. *Life Sci* 2000; 66: 769–77
- 79 Gehm BD, McAndrews JM, Chien PY, Jameson JL. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. *Proc Natl Acad Sci USA* 1997; 94: 14138–43
- 80 Lu RQ, Serrero G. Resveratrol, a natural product derived from grape, exhibits antiestrogenic activity and inhibits the growth of human breast cancer cells. *J Cell Physiol* 1999; 179: 297–304
- 81 Bhat KPL, Lantvit D, Christov K, Mehta RG, Moon RC, Pezzuto JM. Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. *Cancer Res* 2001; 61: 7456–63
- 82 Turner RT, Evans GL, Zhang M, Maran A, Sibonga JD. Is resveratrol an estrogen agonist in growing rats? *Endocrinology* 1999; 140: 50–4
- 83 Pace-Asciak CR, Hahn S, Diamandis EP, Soleas G, Goldberg DM. The red wine phenolics *trans*-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clin Chim Acta* 1995; 235: 207–14
- 84 Goldberg DM, Hahn S, Parkes JG. Beyond alcohol: beverage consumption and cardiovascular mortality. *Clin Chim Acta* 1995; 237: 155–87
- 85 Tadolini B, Juliano C, Piu L, Franconi F, Cabrini L. Resveratrol inhibition of lipid peroxidation. *Free Rad Res* 2000; 33: 105–14
- 86 Pervaiz S. Resveratrol – from the bottle to the bedside? *Leukemia Lymphoma* 2001; 40: 491–8
- 87 Hsieh TC, Juan G, Darzynkiewicz Z, Wu JM. Resveratrol increases nitric oxide synthase, induces accumulation of p53 and p21 (WAF1/CIP1), and suppresses cultured bovine pulmonary artery endothelial cell proliferation by perturbing progression through S and G2. *Cancer Res* 1999; 59: 2596–601
- 88 Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM. Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles county. *Brit J Cancer* 1991; 63: 963–6
- 89 Messina M, Persky V, Setchell KDR, Barnes S. Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. *Nutr Cancer* 1994; 21: 113–31
- 90 Stark A, Madar Z. Phytoestrogens: a review of recent findings. *J Pediatr Endocr Met* 2002; 15: 561–72
- 91 Adlercreutz H. Phyto-estrogens and cancer. *Lancet Oncol*, 2002; 3: 364–73
- 92 Wu AH, Wan P, Hankin J, Tseng CC, Yu MC, Pike MC. Adolescent and adult soy intake and risk of breast cancer in Asian-Americans. *Carcinogenesis* 2002; 23: 1491–6
- 93 Shu XO, Jin F, Dai Q, Wen WQ, Potter JD, Kushi LH et al. Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. *Cancer Epidem Biomar* 2001; 10: 483–8
- 94 Lamartiniere CA. Protection against breast cancer with genistein: a component of soy. *Am J Clin Nutr* 2000; 71: 1705S–7S
- 95 Hulten K, Winkvist A, Lenner P, Johansson R, Adlercreutz H, Hallmans G. An incident case-referent study on plasma enterolactone and breast cancer risk. *Eur J Nutr* 2002; 41: 168–76
- 96 Jacobsen BK, Knutsen SF, Fraser GE. Does high soy milk intake reduce prostate cancer incidence? The adventist health study (United States). *Cancer Cause Control* 1998; 9: 553–7
- 97 Nagata C, Inaba S, Kawakami N, Nakizoe T, Shimizu H. Inverse association of soy product intake with serum androgen and estrogen concentrations in Japanese men. *Nutr Cancer* 2000; 36: 14–8
- 98 Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A. Phyto-estrogens: where are we now? *Brit J Nutr* 1998; 79: 393–406
- 99 Messina M, Bennink M. Soyfoods, isoflavones and risk of colonic cancer: a review of the *in vitro* and *in vivo* data. *Bailliere Clin Endocrinol Met* 1998; 12: 707–28
- 100 Gardner CD, Newell KA, Cherin R, Haskell WL. The effect of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolemic postmenopausal women. *Am J Clin Nutr* 2001; 73: 728–35
- 101 Crouse JR, Morgan T, Terry JG, Ellis J, Vitols M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. *Arch Intern Med* 1999; 159: 2070–6
- 102 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* 2001; 86: 3053–60
- 103 Glazier MG, Bowman M. A review of the evidence for the use of phytoestrogens as a replacement for traditional estrogen replacement therapy. *Arch Intern Med* 2001; 161: 1161–72
- 104 Hodgson JM, Puddey IB, Beilin LJ, Mori TA, Croft KD. Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: a randomized controlled trial in humans. *J Nutr* 1998; 128: 728–32
- 105 Simons LA, von Konigsmark M, Simons J, Celemajer DS. Phytoestrogens do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women. *Am J Cardiol* 2000; 85: 1297–301
- 106 Erdman JW. Soy protein and cardiovascular disease: a statement for healthcare professionals from the Nutrition Committee of the AHA. *Circulation* 2000; 102: 2555–9
- 107 Kirk EA, Sutherland P, Wang SA, Chait A, LeBoeuf RC. Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. *J Nutr* 1998; 128: 954–9
- 108 Agnusdei D, Crepaldi G, Isaia G, Mazzuoli G, Ortolani S, Passeri M, Bufalino L et al. A double blind, placebo-controlled trial of ipriflavone for prevention of postmenopausal spinal bone loss. *Calcif Tissue Int* 1997; 61: 142–7

- ¹⁰⁹ Scheiber MD, Rebar RW. Isoflavones and postmenopausal bone health: a viable alternative to estrogen therapy? *Menopause* 1999; 6: 233–41
- ¹¹⁰ Alexandersen P, Toussaint A, Christiansen C, Devogelaer JP, Roux C, Fechtenbaum J, Gennari C, Reginster JY. Ipriflavone in the treatment of postmenopausal osteoporosis: a randomized controlled trial. *JAMA* 2001; 285: 1482–8
- ¹¹¹ Petilli M, Fiorelli G, Benvenuti S, Frediani U, Gori F, Brandi ML. Interactions between ipriflavone and the estrogen receptor. *Calcif Tissue Int* 1995; 56: 160–5
- ¹¹² Lucas EA, Wild RD, Hammond LJ, Khalil DA, Juma S, Daggy BP, Stoecker BJ, Arjmandi BH. Flaxseed improves lipid profile without altering biomarkers of bone metabolism in postmenopausal women. *J Clin Endocrinol Metab* 2002; 87: 1527–32
- ¹¹³ Potter SM, Baum JA, Teng HY, Stillman RJ, Shay NF, Erdman JW. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr* 1998; 68: 1375S–9S
- ¹¹⁴ Clifton-Bligh PB, Baber RJ, Fulcher GR, Nery ML, Moreton T. The effect of isoflavones extracted from red clover (Rimostil) on lipid and bone metabolism. *Menopause* 2001; 8: 259–65
- ¹¹⁵ Scambia G, Mango D, Signorile PG, Angeli RA, Palena C, Gallo D et al. Clinical effects of a standardized soy extract in postmenopausal women: a pilot study. *Menopause* 2000; 7: 105–11
- ¹¹⁶ Albertazzi P, Pansini F, Bonaccorsi G, Zanotti L, Forini E, De Aloysio D. The effect of dietary soy supplementation on hot flashes. *Obstet Gynecol* 1998; 91: 6–11
- ¹¹⁷ Quella SK, Loprinzi CL, Barton DL, Knost JA, Sloan JA, LaVasseur BI et al. Evaluation of soy phytoestrogens for the treatment of hot flashes in breast cancer survivors: a north central cancer treatment group trial. *J Clin Oncol* 2000; 18: 1068–74
- ¹¹⁸ Duncan AM, Underhill KEW, Xu X, Lavalleur J, Phipps WR, Kurzer MS. Modest hormonal effects of soy isoflavones in postmenopausal women. *J Clin Endocrinol Metab* 1999; 84: 3479–84
- ¹¹⁹ Setchell KDR, Zimmer-Nechemias L, Cai J, Heubi JE. Exposure of infants to phyto-oestrogens from soy based infant formula. *Lancet* 1997; 350: 23–7
- ¹²⁰ Yellay S, Naaz A, Szewczykowski MA, Sato T, Woods JA, Chang J et al. The phytoestrogen genistein induces thymic and immune changes: a human health concern? *PNAS* 2002; 99: 7616–21