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Review

The spectrum of phytoestrogens in nature: our knowledge is expanding

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ABSTRACT

The classical phytoestrogens, so far known, constitute a group of plant-derived compounds which include mainly isoflavones, lignans, coumestanes, stilbenes and the flavonoids quercetin and kaempherol. The discovery of many more novel estrogen-like compounds in the plant kingdom demonstrates that the spectrum of phytoestrogens in nature is expanding. The classical as well as the novel phytoestrogens show a complex mode of action via interaction with the nuclear estrogen receptor isoforms ERα and ERβ, exhibiting either estrogen-agonist or estrogen-antagonist effects. Their final biological activity, assessed by cell culture assay systems, animal studies and clinical trials, depends on multiple factors such as the chemical structure of the phytoestrogen, the kind of tissue and cell type, the intrinsic estrogenic status, the route of administration, the metabolism as well as the time and the level of exposure. They are characterized by high tissue specificity and dose-dependent activity. However, although phytoestrogen intake as food or dietary supplements, in particular soya products and the isoflavones genistein and daidzein, has been associated with "health promoting effects", some data indicate increased disease risk. Evidently, phytoestrogen supplementation should be viewed with caution until further studies satisfactorily delineate the effects of individual phytoestrogens on human health and disease.

Key words: Breast cancer, Estrogen receptor alpha, Estrogen receptor beta, Colon cancer, Coronary heart disease, Flavonoids, Osteoporosis, Phytoestrogens, Prostate cancer, SERMs

INTRODUCTION

Estrogens are steroid hormones with a complex mode of action, characterized by high tissue specificity and dose-dependent activity. They exert pleiotropic effects on a diverse range of tissues, such as ovary, testis, prostate, breast, uterus, bone, liver, immune system, cardiovascular and central nervous system.[1] Estrogens promote breast and endometrial cancer in women and exacerbate autoimmune diseases, whereas the loss of estrogens during menopause has been correlated with osteoporosis, coronary heart disease, depression and neurodegeneration. Compounds which antagonize the estrogenic effects (antagonists) in some tissues, such as breast and uterus, while mimicking

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the estrogens effects (agonists) in other tissues, such as bone, brain and cardiovascular cells, are known as selective estrogen receptor modulators (SERMs).[2]

The substitution during menopause of endogenous estrogens with exogenous estrogens, widely known as hormone replacement therapy (HRT), is of enormous importance. However, the Women's Health Initiative study raised serious concerns about the safety of HRT, resulting in low compliance of women due to the fear of breast and uterus cancer development.[3] SERMs are considered as a new alternative postmenopausal therapy, since they improve bone function with minimal risk for breast and uterine cancer. A large body of evidence has demonstrated that endogenous estrogens and SERMs mediate their effects by modulating the intracellular receptor subtypes estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), both members of the nuclear receptor superfamily. The binding of estrogens or SERMs to estrogen receptors (ERα and ERβ) initiates a series of events resulting in transcriptional regulation of specific genes, RNA and protein synthesis.

Phytoestrogens are a large family of plant-derived estrogens possessing significant estrogen agonist/antagonist activity. These, naturally occurring molecules, include the isoflavonoids, the lignans, the coumestanes, the stilbens and the flavonoids quercetin and kaempherol. Their effects, mediated via the estrogen receptor subtypes ERα and ERβ, have been shown to be cell type/tissue specific and dose-dependent. The so far known phytoestrogens may act as "natural" SERMs and may possibly be considered for the prevention of postmenopausal osteoporosis and cardiovascular disease without an adverse effect on breast and uterus. However, since data are still controversial, further clinical studies are needed to delineate satisfactorily the health effects of phytoestrogens in specific population groups.[4]

Current data demonstrate that many more novel compounds are continuously being identified in nature exhibiting estrogenic/ antiestrogenic properties, indicating that our knowledge about phytoestrogens in nature is expanding.

This review outlines the major categories of the so far known phytoestrogens, provides the current knowledge regarding their overall biological effects and briefly describes their estrogen-like mediated mechanism of action as well as their potential in triggering other signaling pathways. The review presents the laboratory evidence for the "novel" plant-derived compounds and their modulatory activity on the ER subtypes. In order to have a better understanding of the molecular mechanisms by which phytoestrogens modulate estrogen receptor-mediated activity, it was considered essential to briefly explicate the structure of estrogen receptors and their encoding respective genes as well as some key stages in the estrogen signaling cascade.

In this review the in vitro and in vivo assays, assessing the estrogenic/antiestrogenic potential of phytochemicals, are also presented and their advantages and disadvantages are discussed.

ESTROGEN RECEPTORS

Structure

Estrogens exert their effects via their respective receptors estrogen receptor alpha (ERα) and beta (ERβ), which are members of the nuclear receptor superfamily.[5,6] Estrogen receptors have been identified in all types of human cells and have distinct tissue expression patterns.[1] Target organs with a high estrogen receptor content are those related to reproductive functions such as the mammary gland, the ovaries and the uterus, known to be "classical" targets for estrogens. Bone, the cardiovascular system, the brain, the immune system and the liver are characterized by lower ER content.

The primary structure of the estrogen receptors has been fully characterized and their respective genes, ESR1 and ESR2 have been cloned and sequenced.[7,8] The encoded protein molecules contain six functional domains (Figure 1): the transcriptional regulation (A/B) domain (AF-1) which is highly immunoreactive, the DNA binding domain (C) which is responsible for binding to specific DNA sequences known as estrogen response elements (ERE), the domain D (between C and E domains, hinge region) and the domains E and F responsible for ligand binding, dimerization and transcriptional activation (AF-2). ERα and ERβ differ in size (595 and 530 amino acids, respectively). ERα and ERβ exhibit high homology in their DNA binding domain (96%), partial

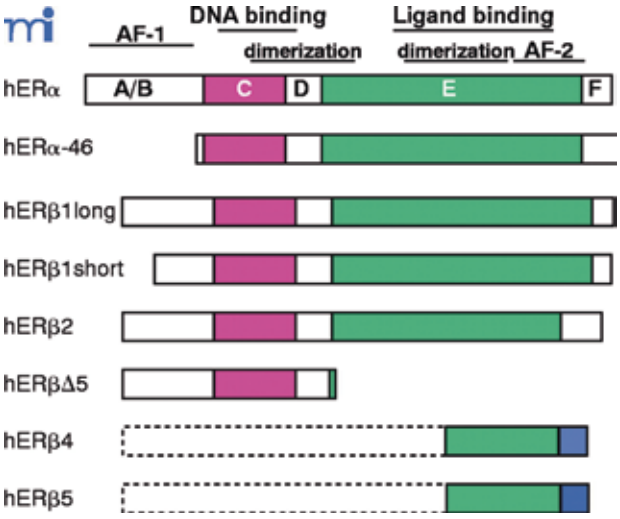

Figure 1. Schematic representation of human estrogen receptor α and β and their respective isoforms (Matthews J, Gustafsson JA, 2003 Molecular Interventions 3:281-292), used by kind permission.

homology in the ligand binding domain (53%), but very low in their A/B domain (30%) located in the amino-terminal part of the molecules. Due to high homology in the C domain, ERα and ERβ are likely to bind to various ligands with similar specificity and affinity. In ERα and ERβ the conformation of the α-helix 12 (H12) strongly depends on the nature of the ligand and evidently different ligands induce different receptor conformations, thus affecting the agonist/antagonist potency of ligands.[9] Differential splicing of the ERα and ERβ genes results in various ERα and ERβ splicing variants lacking certain exons. Moore et al (1998) described five ERβ isoforms (ERβ 1-5), ERβ1 corresponding to the previously described ERβ-530.[10] These isoforms are co-expressed with the wild type receptors in various tissues and pertinent data indicate that they are biologically active. Their role in estrogen-dependent diseases is thus under investigation.[1]

Mechanisms of estrogen action

Estrogens exert their effects on estrogen responsive cells via activation of estrogen receptor (ER) and, directly or indirectly, regulation of transcription of target genes involved in the cell function. There follows a brief description of the mechanisms of estrogen action, as reviewed by Nilsson et al (2001).[9] The term ER hereinafter refers to both ERα and ERβ subtypes.

Direct action of estrogen receptors on the genome

Endogenous estrogens, synthetic estrogen analogs or phytoestrogens enter the cells, where they bind to the estrogen receptor (Figure 2). The conformation of the bound receptor depends strongly on the nature of the ligand. Binding results in the release of the receptor associated proteins, such as hsp90, dimerization of the receptor (ERα and ERβ may be present as homodimers or heterodimers), rapid nuclear localization of the activated ER-estrogen complex and binding of the dimer to its specific DNA sequences (EREs). The DNA bound complex interacts with other coactivator proteins such as p160 and the proteins of the CBP/P300 family, as well as other transcription factors, resulting in the remodeling of chromatin and initiation of transcription by the basal transcriptional machinery and RNA polymerase.[11,12] The result is either induction or repression of genes and increase or decrease in protein synthesis, respectively. The chromatin decompaction is a key step required for the initiation of transcriptional activation and requires histone acetyltransferases (HATs).[13] Many coactivator proteins required for ER activity are HATs (p160 family, p300, CBD).[14] Another group of enzymes, known as histone deacetylases (HDACs), deacetylate histones to promote ER-mediated transcriptional repression.[14,15]

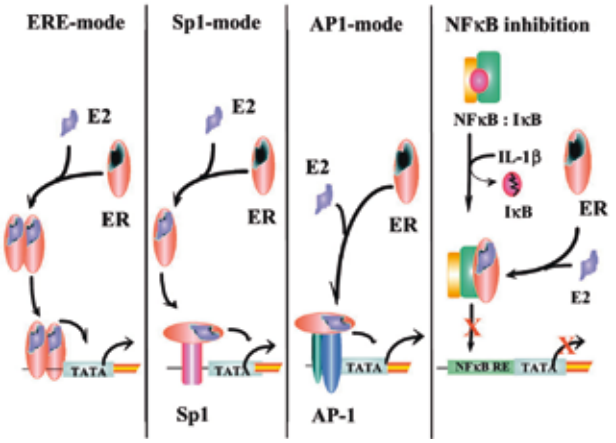

Figure 2. The genomic classical (via EREs) and non-classical (via AP-1 and viaNF-kB response elements) actions of the estrogen receptor (Nilsson et al, 2001. Physiol Rev 81: 1535-1565), used with kind permission.

An alternative pathway of ERα and ERβ action has been reported by which the receptors influence gene transcription, not through the ERE-directed classical pathway, but independently of ER binding to its consensus response elements. This is accomplished by interaction of the receptors with the transcription factor AP-1 (c-fos/c-jun dimers) by way of protein-protein interactions, thus modulating the binding of AP-1 to AP-1 sites on the DNA[9,16] (Figure 2). Current research indicates that binding of SERMS to ERα transactivates the transcription through binding to EREs, whereas binding of SERMs to ERβ stimulates the AP-1 pathway, demonstrating that ERα and ERβ respond differently to certain ligands at an AP-1 element.[17] Of note, some antiestrogens, such as tamoxifen, are strong agonists of AP-1 dependent transcription in certain tissues, whereas in other tissues they act as antiestrogen via the classical EREs. The estrogenic effects of tamoxifen resulting in tissue growth and differentiation, as for example in uterus and endometrium, may be attributed to the AP-1 mediated pathway, a major route via which estrogens promote growth and differentiation effects.[18]

Estrogen receptor interacts with another important transcription factor, nuclear factor-kB (NF-kB), resulting in suppression of genes, such as the interleukin-6 gene, or activation of genes, such as the serotonin receptor 1A gene[16,19,20] (Figure 2). This suggests that estrogen receptor regulates the NF-kB mediated gene expression in a cell type-specific manner. Interleukin-6, a cytokine transcriptionally up-regulated by NF-kB, regulates bone metabolism and endothelial cell function, therefore its estrogen-mediated repression may have important implications in osteoporosis, inflammatory processes and atherosclerosis.[20-22] Evidently, the interaction of ER with the transcription factor NF-kB as well as with the AP-1 is subtype and tissue specific and is considered to be involved in the inhibitory effects of estrogens on proinflammatory cytokine activity.[21]

In conclusion, the two ER subtypes exhibit distinct interactions with common or specific cofactors, bind in a variable manner to a variety of ligands and may act via different response elements exhibiting altered specificity and affinity. These characteristics and the differential expression of ERα and ERβ may be responsible for tissue-specific responses to estrogenic compounds.[9,23]

Cross talk between estrogen and other signaling pathways; Estrogen-independent activation of ER

Phosphorylation is one of the most frequent post-translational modifications of proteins on serine and threonine residues and less on tyrosines. Phosphorylation is regulated by cellular enzymes (kinases and phosphatases), which transfer a phosphate group from ATP onto target proteins or remove it correspondingly. Phosphorylation of the estrogen receptors is influenced by the cognate ligand and it affects receptor functions positively or negatively, such as transcriptional activity, stability and nucleocytoplasmic shuttling.[24]

Available data indicate that in several cell types extracellular signals such as insulin, IGF-I, EGF and TGF-β, via the mitogen activated protein kinases (MAPK), phosphorylate the AF-1 region of ERα and ERβ resulting in transactivation of the receptors in the absence of the ligand.[25] Studies have shown that ER is phosphorylated by growth factors resulting in the initiation of ERE-mediated gene expression.[26,27] For full activity of ERα AF-1, the phosphorylation of Ser-118 is very important, whereas for ERβ this effect is mediated via Ser-124. Phosphorylation may also take place at tyrosine residues of ERα, especially at Tyr-537 which regulates the ligand binding step.[9]

ERα and ERβ are also activated by neurotransmitters (dopamine) as well as by regulators of the general cellular phosphorylation state such as protein kinase A (PKA) and protein kinase C (PKC), which also phosphorylate ERs.[28,29] The cyclins, cyclin A and D1, which are regulatory subunits of cyclin-dependent kinases (CDKs), have also been shown to be ER activators.[30-32] The presence of factors other than the classical hormone, activating ERs, accompanied by an increased receptor phosphorylation, may modulate the activity of ERs in response to diverse physiological signals.[33,34] The estrogen-independent activation of estrogen receptors may be important when the concentration of growth factors is locally increased or when estrogens are too low.

Membrane and mitochondrial estrogen receptors

Accumulated evidence strongly supports the presence and importance of plasma membrane estrogen receptors in a variety of cells. The membrane ER is possibly similar to the nuclear receptor, which has translocated to a particular location in the membrane, known as caveolae, allowing its cross talk with other signaling molecules.

The binding of 17β-estradiol (E2) to cell surface proteins triggers downstream signaling cascades resulting in calcium flux, adenylate cyclase activation and cAMP generation, phospholipase C activation and IP3 generation. Given that these signals are emanating from the activation of G protein related pathways and because G proteins and ER exist in caveolae, it is likely that an interaction may take place within this membrane domain. Alternatively, E2 via ER may activate another G-protein coupled receptor (GPCR) in the membrane, thus affecting G protein activation.[35,36]

Additional signal transduction pathways, being rapidly responsive to E2 and originating from the membrane, involve the stimulation/inhibition (being dependent on the ERα or ERβ subtype) of phosphoinositol-3 hydroxy kinase (PI3K) and the family of MAP kinases, such as extracellular-regulated kinase (ERK), p38β isoform, as well as the c-Jun N-terminal kinase (JNK).[37] Of note, the signaling from the membrane can also extend to other intracellular transcription factors such as AP-1 or NF-kB, resulting in gene repression or activation. In conclusion, signals emanating from the membrane are transduced to the nuclear compartment and regulate important functions, such as cell growth and survival, migration, angiogenesis and apoptosis.[1,35,36]

Finally, current data documenting the presence of ER subtypes in the mitochondria of animal and human cells implicate a role of ER signaling in energy production, thus further emphasizing the complexity of ER-signaling.[38,39]

ERα and ERβ expression

ERa and ERβ are co-expressed in many tissues. ERα is primarily expressed in the uterus, liver, heart, kidney, whereas ERβ is primarily expressed in the ovary, prostate, lung, gastrointestinal tract, bladder, central nervous system and hematopoietic system.[1,9]

Characterization of mice lacking either ERα or ERβ or both (αERKO, βERKO and αβERKO) has yielded valuable information about the unique role of each subtype in estrogen action in vivo.[40] Briefly, results showed that ERα is required for normal mammary gland development and mediates estrogen-stimulated increase in bone formation, while ERβ's role in bone is less clear. ERα is required for protection of vascular and endothelial cells against injury as well as for cardioprotection in a myocardial ischemia model. ERα appears to play a role in adipocyte growth and proliferation, the ERβ also possibly involved in adipogenesis.[1] ERα and ERβ are expressed in various brain areas and ER-mediated effects are considered to provide neuroprotection. In a mouse model of brain injury, estradiol treatment protects wild-type and ERβ-null mice from brain injury, while this protection is abolished in ERα-null mice, thus suggesting an important role for ERα.[41] However, recent data indicate that ERβ-mediates decreased microglia activation, suggesting a role of ERβ in neuroprotection.[42] On the other hand, ERα or ERβ selective agonists have been shown to protect hippocampal neurons against glutamate-induced cell death and enhanced bcl-2 expression in hippocampal neurons, suggesting that activation of either ERα or ERβ can promote neuroprotection.[43] Taken together, as ERβ is highly expressed in the brain compared to the reproductive organs in females, ERβ-selective agonists may be of benefit and protect, at least in part, from some neurodegeneration leading pathways.[44]

Accumulating evidence supports the key role of ER subtypes in some cancers, the ERβ expression being significantly reduced in breast, prostate and colon cancer.[1] In ovarian cancer, ERα is expressed in tumors of both epithelial and stromal origin, whereas ERβ is abundantly expressed predominantly in granulosa cell-derived tumors and to a lesser extent in epithelial origin tumors.[45] Malignant ovarian tumors originating from epithelial surface constitute about 90% of ovarian cancers and express low levels of ERβ compared to normal tissues. Recent data point out that restoration of ERβ in ovarian cancer cells leads to inhibition of proliferation and enhancement of apoptosis, thus suggesting that ERβ may play a possible

tumor-suppressor role in ovarian carcinogenesis.[46] Taken together, several lines of evidence indicate that ERβ exerts an inhibitory action on ERα-mediated gene expression and in many instances opposes the actions of ERα.[23]

Since many phytoestrogens bind to ERβ with higher affinity than ERα and ~~because~~ their potential to transactivate ERβ is higher than ERα, phytoestrogens may be of benefit in tissues in which ERβ-mediating signaling plays a significant role.[44]

PHYTOESTROGENS

Structure/Classification

The main classes of phytoestrogens are the isoflavones (genistein, daidzein, glycitein, equol and biochanin A), the lignans (enterolactone, enterodiol), the coumestanes (coumestrol), the flavonoids (quercetin, kaempferol), the stilbenes (resveratrol) and the mycotoxins (zearalenol)[47] (Figure 3). They are either of dietary plant origin or ingested as precursors and then metabolized in vivo by the microflora of the human gut. Indeed, equol is produced from daidzein by the intestinal microflora, while the mammalian lignans, enterodiol and enterolactone, are formed from plant-lignan glycoside precursors by the activity of the gut microflora in the proximal colon.[48,49] Of note, in some studies myco-estrogens are not included in the group of phytoestrogens because they are not fungal-derived but plant devired estrogens.[49] The aforementioned compounds are all polyphenols and structurally resemble the natural estrogens.

Polyphenols are present in all plants, while there are >8000 phenolic structures identified in nature which may be categorized in more than 10 classes. These may be simple molecules (such as phenolic acids) or highly polymerized (i.e. tannins). Flavonoids, the most common polyphenols in the plant kingdom, contain more than 5000 compounds subdivided into six major classes such as flavones (e.g. apigenin, luteolin), flavonols (e.g. quercetin, kaempherol), flavanones (e.g. naringenin, hesperidin), flavanols (e.g. catechins, epicatechin, gallocatechin), anthocyanidins (e.g. cyanidin) and isoflavones (e.g. genistein, daidzein).[50]

Flavonoids do not exhibit an estrogenic effect; however, the isoflavones (genistein, daidzein) and some flavones, flavanones and flavonols (apigenin, kaempferol and naringenin) activate estrogen-receptor-mediated signaling.

Absorption and Bioavailability

Exploration of the bioavailability of isoflavones (the most common form of phytoestrogens), particularly genistein and daidzein, revealed that it depends on the gut microflora activity. Data show that, despite the considerable degradation of isoflavones in the gut, their plasma concentrations are significant. Isoflavones are mainly present as inactive glycosides and, after removal of the sugar residue in the gut by bacteria (beta-glycosidases), they become active compounds, taken up by enterocytes and entering the peripheral circulation.[51] Following absorption, they are reconjugated in the liver, mainly to glucuronic acid and to a lesser degree to sulphuric acid.[52,53] There is a large variability in genistein and daidzein metabolism in humans, which has been attributed to variable individual intestinal flora and transit time. In the Japanese, genistein has been found in micromolar concentrations, but in western populations, levels are much lower.[49] Of note, Setchel and Cassidy (1999) have shown that in an adult, consumption of 50 mg/day of isoflavones may give rise to plasma concentrations ranging from 200-3000 nM.[54] Several other studies assessing plasma concentrations of phytoestrogens have demonstrated that the plasma levels in humans range from 10nm-10μM.[55] With regard to lignans, they are metabolized to enterolactone and enterodiol in the gut. In the Finnish population, consumption of wholegrain bread (200g/day) has resulted in variable plasma concentrations of enterolactone (15-41.4 nmol/l).[49]

A recent study assessing the effect of an 8-week dietary soy intervention on urinary isoflavone excretion among young girls (8-14 years old) revealed a very high urinary isoflavone excretion as compared to adult women. Such data suggest less intestinal degradation and/or greater absorption of isoflavones in young people, emphasizing that further investigations into the pharmacokinetics and age at consumption of isoflavones are needed.[56]

Soy infant formulas are widely used and may result in a daily exposure of infants to isoflavones (genistein,

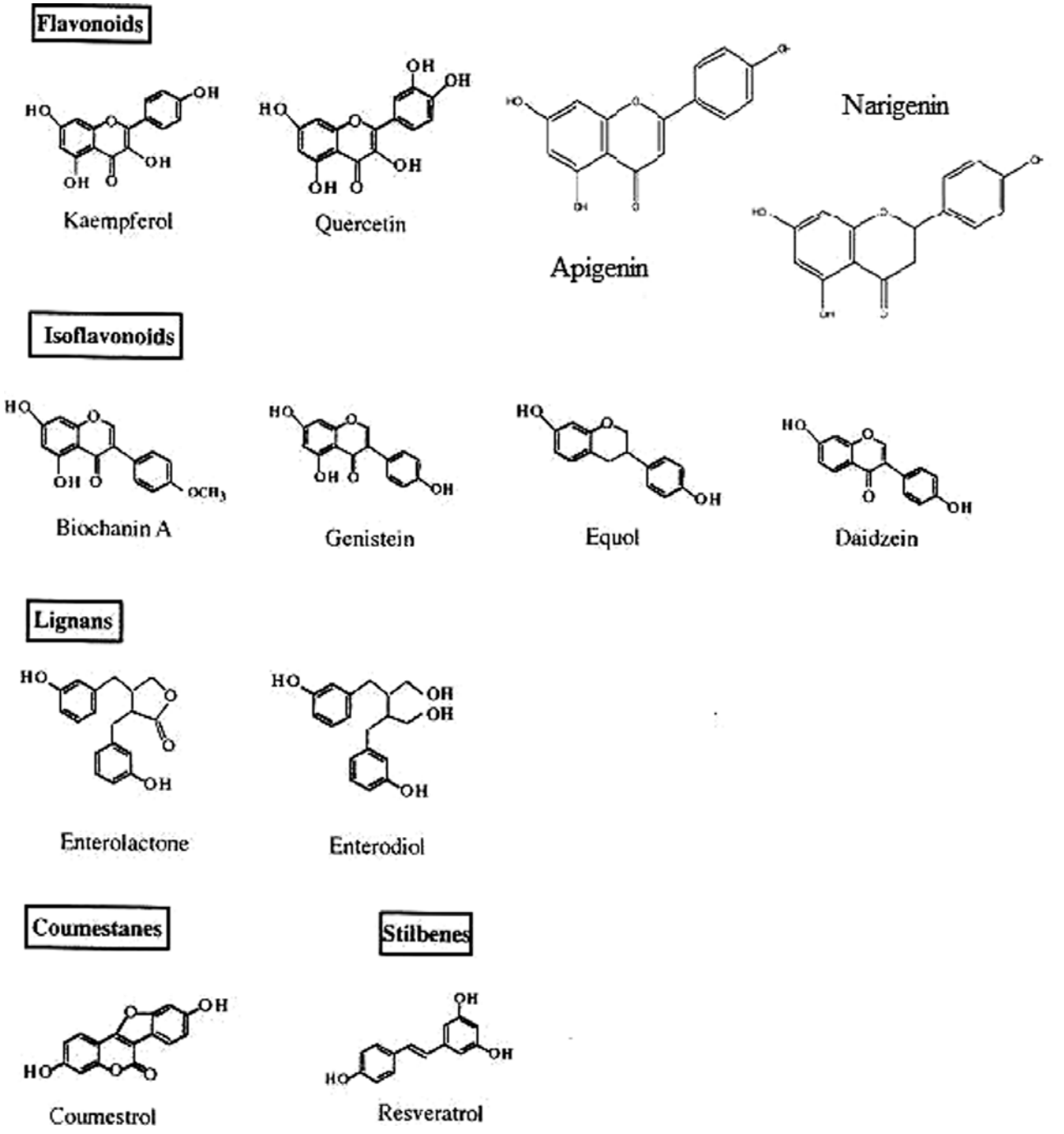

Figure 3. The chemical structure of the so far known phytoestrogens.

daidzein and equol) that is 6- to 11-fold higher on a body weight basis from the dose that has hormonal effects in adults consuming soy foods. The mean plasma concentration of total genistein in infants fed soy-based formula has been reported to be 2,500 pmol/ml (684 ng/ml) as compared to 276 pmol total genistein/ml blood concentration found in Asians on a traditional diet that is high in soy content.[57,58] It has been reported that the circulating concentration of isoflavones in infants fed soy-based formulas were 13,000-22,000 times higher than plasma estradiol concentrations in early life and may exert biological

effects, whereas the contribution of isoflavones from breast milk and cow milk was negligible.[57] In addition, it has been shown that infants fed soy-based formula maintain a high steady state plasma concentration of isoflavones, which may possibly be explained by reduced intestinal biotransformation, as evidenced by low or undetectable concentrations of equol and other metabolites.[59]

In a recent report, isoflavones in breastfed infants, whose mothers consumed soy for 2 to 4 days, were significantly increased in the infants urine (from 29.8 to 111.6nmol/mg creatinine) and in breast milk increased from 5.1 to 70.7nmol/L.[60] Of note, in some countries such as Israel, the soy-based formula is widespread, with rates far beyond clinical indications (in case of suspicion of cow's milk allergy).[61] Since there is a broad spectrum of infant foods becoming available, there may be a high exposure to dietary isoflavones during the first year of life. Due to the lack of definitive evidence of hormonal activities of isoflavones at doses relevant to the soy-fed infants, further studies in experimental animals and human populations exposed to isoflavone-containing products and in particular soy-based infant formulas are necessary.[62]

Flavonoids are mainly found conjugated with sugars (glycosides) in plants and occasionally as aglycones. Evidence supports that the aglycone form of flavonoids as well as their glycosides show a considerable degree of intestinal absorption, ranging from 10-60%, depending on the type and the dietary source of the flavonoid. For example, the absorption of querquetin from tea has been shown to be half that of onions. Given that querquetin in onions is found mainly as a glycoside moiety, it has been suggested that the glycosidic form of flavonoids may enhance absorption.[63] Current evidence indicates that many of the metabolites of phytoestrogens and flavonoids formed, either in the gut or in liver, may be also biologically active and may mediate estrogen-signaling.[64,65] Taken together, the bioavailability of dietary substances determines their activity in vivo. However, the relevance of in vitro studies to the in vivo situation should be considered with caution. Although metabolism in peripheral tissues also takes place, most of the conversion seems to occur at the level of the gut and liver, therefore the target tissue levels may be analogous to those in plasma.

Intake

Several studies have investigated the dietary intake of polyphenols and phytoestrogens; results, however, are contradictory. Numerous factors, such as light, environmental conditions, plant genetics, ripeness and species variety, affect the formation and the content of flavonoids and phytoestrogens in plants. Moreover, different methods assessing the content of polyphenols in plants have given variable results.[63] The most widely studied phytoestrogens are the isoflavones genistein and daidzein from soy food (mainly consumed in the Orient), whereas lignans are mainly consumed by Europeans, since foods such as cereal brans, legumes and vegetables (known to be rich in lignans) play important role in the European diet. Soya beans contain 560-3810mg/kg isoflavones, depending on variety and growing conditions. Soya milk, bean sprouts etc., contain 13-2030mg isoflavones/kg, depending on the starting raw material and final water content. Soy foods, however, are now widely available to European consumers and soya-based European foods contain 38-3000mg isoflavones/kg, depending on the source of soya and dilution with other ingredients.[66] In the literature, various reports point out that the estimated daily intake of flavonoids differs among countries, ranging from 20-100mg/day.[63] Dietary assessment of phytoestrogens gives estimates varying between an intake ranging from 50mg to 200 mg of phytoestrogen/day in a traditional Japanese diet.[67,68] In Asian countries, an average daily dietary intake of soy and isoflavones is estimated to be 50g and 20-150mg, respectively, while in western countries the intake is lower, i.e. 1g daily and 2mg daily, respectively.[69] It must be underlined that women consuming the commercially available phytoestrogen preparations are taking doses ranging from 50-150mg aglycones/day.[70]

In view of the above, it becomes evident that when screening phytochemicals to assess their possible estrogenic activity, the levels of the compounds tested must be at a range that is thought to be achievable in humans in vivo. In this regard, many factors should be taken into account, such as the daily intake of the phytochemical, the rates of metabolism and clearance as well as the plasma levels, factors which are known

to vary considerably between individuals.

Phytoestrogens are modulators of ERα and ERβ-mediated activity

A large body of evidence demonstrates various features of phytoestrogens: estrogen-like action, estrogen receptor binding, ER-transactivation, estrogen-dependent target genet expression or cellular growth effects. Different studies, however, provide variable results regarding the estrogen-like potencies of the phytoestrogens. This may be due to the fact that the potencies of several phytoestrogens have been tested in various cell systems using different techniques (i.e. radioligand binding assays, transactivation assays, target gene expression) under different conditions (such as different dosages, in presence or in absence of estradiol), so that results are not comparable.

Given the conflicting results in the literature, Mueller et al (2004) employed a human endometrial Ishikawa cell line that stably expresses human ERα or ERβ to determine the potencies of the best known phytoestrogens in one comparable system for both ERα and ERβ activity.[64] They found that the soy-derived genistein, coumestrol and equol displayed a preference for transactivation of ERβ-ERE responses compared to ERα-ERE responses and were 10- to 100-fold less potent than diethylstilbestrol. Resveratrol, enterolactone and its human metabolite 6-OH enterolactone and human metabolites of daidzein were weak agonists to both ERα and ERβ. Interestingly, they showed that phytoestrogens affect the transcriptional activity of ERα and ERβ in an ERE sequence-dependent manner,[64,71] thus implying a degree of promoter dependency. Their ligand binding measurements revealed that genistein, coumestrol and equol had high binding affinity to ERα and ERβ and with a distinct preference for ERβ.[64] Quercetin, a common phytoestrogen, has been shown to induce ERE-dependent transactivation in MCF-7 cells through both ERα and ERβ but with a higher capacity, like genistein, to stimulate ERβ responses as compared to the stimulation ERα responses.[72] It must be stressed that Kostelac et al (2003) revealed that the isoflavones genistein and daidzein, its metabolite equol, and coumestrol were able to modulate the binding of both ERα and ERβ to estrogen-response elements (EREs).[73] Of note, genistein and daidzein preferentially activated the binding of ERβ to ERE, whereas coumestrol and equol showed only a slight preference in the binding of ERβ to ERE compared to the binding of ERα to ERE. Such data lead to the conclusion that phytoestrogens differ not only in their ERE-transactivation potencies and their ER-binding affinities, but also in their ability to increase the ER binding onto DNA-response elements (EREs).

On the other hand, Harris et al (2005), in MCF-7 cells transfected with ERα or ERβ, evaluated the dose-dependent responses of various phytoestrogens in the absence and presence of 17β-estradiol.[55] Their results revealed that genistein, daidzein, apigenin and coumestrol had a differential and robust transactivation of ERα- and ERβ-induced transcription, with up to 100-fold stronger activation of ERβ. Equol, naringenin and kaempferol were weaker agonists. In the presence of 0.5 nM 17β-estradiol, the addition of genistein, daidzein and resveratrol superstimulated the system, while kaempferol and quercetin acted as antagonists at the highest doses. In the same line, Muller et al (2004) have shown that in the presence of diethylstilbestrol, resveratrol displayed a biphasic activity: at low doses it increased the DES-induced activity of ERα and ERβ, but at high doses it inhibited the activity of ERα and ERβ.[64]

It is of interest that the ability of phytoestrogens to modulate cellular proliferation is also dose-dependent and estrogenic status-dependent. Resveratrol, genisteina and quercetin have shown biphasic modulation with regard to proliferation of ER positive breast cancer cell lines. They stimulate proliferation at low concentration (physiologically relevant), the ER possibly involved in adverse cell proliferative effects.[74-76] However, they inhibit proliferation at concentrations higher than 50-60μM, the inhibitory cellular growth effects considered to be exerted by way of ER-independent pathways. Of note, Shao et al (2000) showed that genistein's anti-proliferative effects are more pronounced in the presence of estradiol, implying that the "good estrogen" action of genistein is relevant to chemoprotection.[77]

Animal studies have also shown that phytoestrogens exert variable effects depending on the absence or presence of endogenous estradiol. For example, a group of immature, ovariectomized Sprague-Dawley

rats ingesting a commercially available, closed-formula diet containing "high amounts" of genistein and daidzein (21mg genistein and 14mg daidzein per 100g of rat feed) failed to exhibit a uterotrophic response to administered estradiol. This finding suggests that the response was already stimulated to nearly maximal levels by phytoestrogens before the animals were treated with estradiol.[78] However, in another study involving immature, intact Sprague-Dawley females exposed to higher doses of genistein (37.5 or 75mg per 100g body weight of a modified AIN-76 diet), no detectable increase in uterine weight was observed when compared to controls fed the same diet.[79] This result implies that, at least with respect to this assay, intact females are much less responsive than ovariectomized females to dietary phytoestrogens. Such findings emphasize the importance of variable dosages and the impact of estrogenic status in the overall estrogenic effect of phytoestrogens.

Phytoestrogens, similar to estrogens, have been shown to act via several membrane-initiated signaling mechanisms in cell lines expressing the membrane version of ERα (mERα), resulting in the activation of ERK kinases.[80-82] Phytoestrogens have been shown not only to exert direct effects on ER activity but also to affect the formation of endogenous 17β-estradiol, thus regulating indirectly estrogen-signaling. Indeed, some inhibit aromatase, an enzyme that catalyzes the conversion of testosterone to estradiol, thus protecting against breast cancer via ER-independent mechanisms.[83,84] Metabolites of genistein and daidzein isolated from human urine (i.e. glucuronides, sulfates) have also demonstrated interactions with estrogen receptor α and β, indicating that metabolism of isoflavones not only by enteric bacteria but also by hepatic enzymes may affect estrogenic activity.[65]

In conclusion, the so far known phytoestrogens and some of their human metabolites, produced either by enteric bacteria or hepatic enzymes, are selective estrogen receptor modulators, exhibiting variable potencies towards ERα- or ERβ-mediated responses, the ERβ-mediated activity preferentially induced. Finally, most phytoestrogens modulate many other signalling cascades in various cell types involving MAP kinase and NF-kapa B signalling pathways, AP-1-mediated signalling, cell cycle regulation, apoptosis and other nuclear receptor-mediated signalling.[85-89]

In view of the above data, care should be taken when defining the estrogenic/antiestrogenic of a compound. The intrinsic estrogenic status and the dose should be considered, especially in the context of using a compound to prevent symptoms associated with estrogen deficiency during menopause or to prevent hyperestrogenic effects in breast and endometrial cancer. Evidently, it is essential to assess the phytoestrogens' effects at multiple levels, in vitro and in vivo, in order to obtain a full picture which may be relevant to different physiological or pathological states in humans (i.e. menopause, premenopause, breast cancer).

NOVEL ESTROGEN-LIKE COMPOUNDS OF PLANT ORIGIN

Apart from the known phytoestrogens, accumulating evidence attests to the fact that there are many more phytoestrogens in nature. A detailed presentation of the recently identified novel phytoestrogens in nature is given below. Their chemical structures are shown in Figure 4.

Halabalaki et al (2000) isolated three novel arylobenzofurans, namely ebenfuran I, ebenfuran II and ebenfuran III, from the plant Onobrychis ebenoides. All three compounds showed high binding affinity for the native ER.[90] Further investigation by Papoutsi et al (2004) revealed that ebenfuran II, at low concentrations (10^{-6}-10^{-9}M), demonstrated a selective estrogen receptor modulator profile, being antiestrogenic in MCF-7 breast cancer cells and estrogenic in osteoblasts (KS483 cell line), without any stimulatory activity in uterine cells, implying that it may be considered for the prevention and treatment of breast cancer and osteoporosis.[91] Acteoside and martynoside, which are plant phenylpropanoid glycosides, have been shown to antagonize both ERα and ERβ-mediated signaling.[92] Papoutsi et al (2006), by using the appropriate end-point assays, demonstrated that martynoside was anti-estrogenic in MCF-7 cells, induced mineralization in osteoblasts and inhibited endometrial cell proliferation, thus implying its potential as natural SERM.[92]

Ellagic acid, a dietary polyplenol, present in abundance in strawberries, has shown significant antiestrogenic activity in MCF-7 breast cancer cells at low

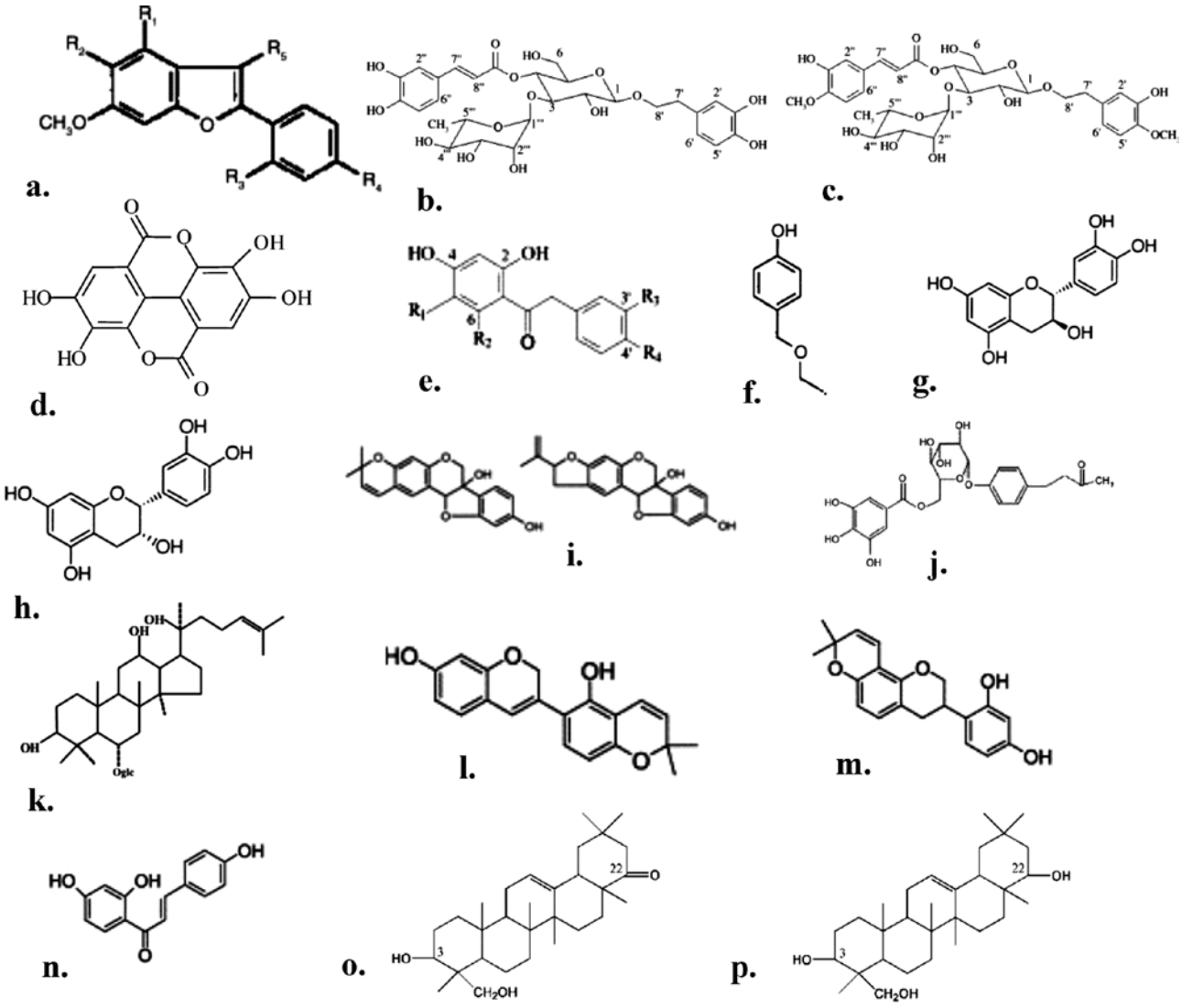

Figure 4. The chemical structure of some novel phytoestrogens. **a.** arylbenzofurans, **b.** acteoside, **c.** martynoside, **d.** ellagic acid, **e.** deoxybenzoines, **f.** ethoxymethylphenol, **g.** catechin, **h.** epicatechin, **i.** glyecollins, **j.** lindleyin, **k.** gRhi, **l.** glabrene, **m.** glabridin, **n.** isoliquitireginin chalone, **o.** soyasapogenol A, **p.** soyasapogenol B.

concentration (10^{-9}M-10^{-7}M) and estrogenic activity in osteoblasts (induced mineralization), without any effects on endometrium-derived epithelial cells. In this regard, ellagic acid may be considered as natural SERM.[93]

Deoxybenzoins, intermediates in the biosynthesis of isoflavones, are found in several plants and marine sources, such as Glycyrrhiza sp., Trifolium subterraneum and Ononis spinosa and have structural similarities to isoflavones. Fokialakis et al (2004) showed that deoxybenzoins constitute a new class of phytoestrogens exhibiting affinities for ERα and ERβ, grossly comparable to those of daidzein, while some demonstrated considerable selectivity and transcriptional bias towards ERβ. Their activity, in stimulating the proliferation of ER-positive breast cancer cells and regulating the expression of endogenous and stably transfected reporter genes, differed considerably, with some inhibiting cell proliferation, while effectively inducing gene expression at the same time.[94]

4-Ethoxymethylphenol (4EM) is another novel

phytoestrogen, found in Maclura pomifera that acts as an agonist of ERα and ERβ in MCF-7 breast cancer cells and in HeLa cells. The presence of coactivators SRC-1, CBP and E6-AP, enhanced, like estrogen, the 4EM-mediated transcription of ERα. The activity of 4EM was specific for ER and did not activate the transcription of the progesterone receptor.[95]

Tea catechins, which are flavonoids, include catechin, epicatechin, epicatechin gallate, epigallocatechin and the epigallocatechin gallate. Experimental evidence indicates that in HeLa cells, transiently transfected with an ERE-regulated luciferase reporter and an ERα or ERβ expression vector, tea catechins alone did not induce luc activity in either of ERs. However, high concentrations of ECG or EGCG ($5X10^{-6}$M) reduced the E2-induced estrogen effects, whereas lower concentrations increased significantly the E2-induced luc activity via ERα and ERβ. In view of the lower doses found in human plasma after tea drinking along with their estrogenic effects, the role of usual tea drinking might be helpful for the prevention of estrogen deficient diseases, such as menopausal disorders and osteoporosis.[96]

Phytoalexins constitute a chemically heterogeneous group of antimicrobial substances. They are synthesized de novo, accumulate in plants as a stress response and are products of a plant's secondary metabolism. The glyceollins represent a group of phytoalexins whose biosynthesis is increased in response to stress signals. Glyceollin accumulates in high concentrations in soybeans under stress conditions. The glyceollin isomers I-III are derived from the precursor daidzein in the glyceollin pathway. The glyceollin can represent as much as 56% of the total isoflavone composition in soybean. In vitro data show that glyceollins display only slight estrogenic activity, but they cause a dose-dependent suppression of E2-induced transactivation and MCF-7 proliferation. Glyceollins suppressed estrogen activity through both ERα and ERβ in transiently transfected HEK 293 cells. Such data imply that glyceollins, being unique antagonists on ER in both HEK 293 and MCF-7 cells, may represent an important component of the health effect of soybased foods.[97]

The phytochemical lindleyin isolated from Rhei rhizoma (rhubarb), a herbal medicine extract traditionally used in Chinese medicine, acts as an antagonist (10^{-5}M) equally to both ERα and ERβ, being a novel phytoestrogen. However, it had no effect on TRα or PPARγ, suggesting that the ER interactions are specific. Lindleyin has a phenolic backbone and is a glycoside obtained from Aeonium lindrleyi, a crassulacea endemic to the Canary Islands.[98]

Ginseng is a popular herbal medicine that has been used for over 2000 years in oriental countries. A component of ginseng, saponin, known as gingenoside (gingenoside-Rh1), has been shown to activate the transcription of the ERE-responsive luc-reporter gene in MCF-7 breast cancer cells and CV-1 cells at high concentration (50 μM), implying that this compound is a weak phytoestrogen, presumably by binding and activating the estrogen receptor.[99]

Consumption of Glycyrrhiza glabra, the licorice plant, can be traced back 6000 years. There is evidence that compounds isolated from the licorice root exhibit varying degrees of ER action in different estrogen responsive tissues. Briefly, results indicated that glabrene, isoliquiritigenin (ILC) and glabridin bind ER. The stimulatory effects of glabrene in vivo were tissue specific and similar to those of estradiol. The effects of concomitantly increasing concentrations of glabrene and ILC on the growth of breast tumor cells were variable, promoting ER-dependent growth at low concentrations (10nM-10μM) and exerting ER independent antiproliferative activity at concentrations >15 μM.[100,101]

Saponins are, inter alia, important components of soy. Recent evidence has shown that a soy sapogenol has the potential to induce estrogen receptor mediated signaling.[102]

BIOLOGICAL EFFECTS

Epidemiological, clinical and animal studies as well as studies in vitro have provided data on the effect of phytoestrogens on human health and disease. Accumulating evidence, based on epidemiological and animal studies, has indicated that phytoestrogens intake may have protective effects against various cancers. However, controversial results have also been reported.[49,103] Phytoestrogens, originating from various plant sources, have shown variable effects on

different types of cancer. It is noteworthy that many of the epidemiological and experimental studies have focused on isoflavones and soy products. It is only recently that interest is increasing in the protective effects of other classes of phytoestrogens, such as lignans.[49]

It has been shown that in adult women the consumption of phytoestrogen-rich foods such as soy, a source of isoflavones, is not or only slightly protective against breast cancer. No negative effects of soy on breast cancer have been reported, while it may be beneficial if consumed before puberty or during adolescence or at very high doses.[49,104,105] Such findings are in agreement with conclusions derived from studies of immigrants and other epidemiological studies.[49,106] On the other hand, a diet based on wholegrain products, low in lignans, resulting in low plasma enterolactone levels, has been associated with increased breast cancer risk. A high plasma enterolactone (30-70 nmol/l) is probably protective against breast cancer, at least in Nordic countries with high wholegrain intake.[49,107] In a recent prospective study, high genistein circulating levels were associated with reduced breast cancer risk in the Dutch population, whereas no effects of lignans on breast cancer risk were observed.[108] It is important to mention that animal and cell culture studies have demonstrated that certain phytoestrogens like genistein and resveratrol not only reduce but also stimulate estrogen-dependent growth such as uterine growth and breast cancer growth, depending on dose and timing of exposure.[49,76,109-113] Several other potential adverse health effects have also been reported.[114,115] However, several other lines of evidence indicate that genistein and resveratrol can protect against breast cancer in rat by regulating important mammary growth and differentiation pathways, the timing of exposure being critical for the mammary protective effects of genistein.[116] Taken together, the role of phytoestrogens in breast cancer is controversial and their overall clinical efficacy is questionable, while negative effects on the breast cannot be completely excluded.

Prostate cancer, one of the main causes of death among men in western societies, has been studied intensively in relation to phytoestrogen intake. Epidemiological, animal and cell culture studies have demonstrated that dietary phytoestrogens, mainly soy and soy-derived isoflavones, may play a protective role against prostate cancer.[49] In a recent prospective study in 43,509 Japanese men (age 45 to 74 years), who generally have a high intake of isoflavones, intakes of genistein, daidzein and soy food decreased risk of localized prostate cancer, whereas positive associations were seen between isoflavones and advanced prostate cancer.[117] Well-designed trials in human subjects, however, assessing the role of lignans in prostate cancer are not available, thus any definite conclusions for this class of phytoestrogens can not be drawn.[49,118]

Data on the effect of soy and isoflavones on colon cancer, based on experimental rat models, are conflicting, showing either no effect or even a stimulatory one. Ryebran or isolated lignans seem to protect against colon cancer and the formation of polyps.[49] The positive findings from animals, however, cannot be extrapolated to humans, thus, well-designed experimental studies are needed to define the impact of phytoestrogens on human colon cancer.

Several animal and in vitro studies indicate that phytoestrogens, mainly daidzein and genistein, prevent bone loss. Human studies, however, which are of short duration (6 months) and include a small number of subjects, have demonstrated that phytoestrogens exert only moderately beneficial effects on bone.[67,68] Based on data from the EU-funded project phytohealth, there is a suggestion, but no conclusive evidence, that phytoestrogens, primarily genistein and daidzein, given as soyabean-protein isolates, whole-soyabean foods or extracts, supplements or pure compounds, have a beneficial effect on bone health.[119] However, it is considered that long-lasting, well-designed clinical trials are needed to prove a specific role of phytoestrogens in the prevention of osteoporosis.

The phytoestrogen intake and the incidence of hot flushes and vaginal dryness in postmenopausal women has been studied extensively, yielding either favorable effects or no effect at all.[112,119,120]

Animal studies have led to the conclusion that isoflavones (in particular their metabolic product equol) lead to infertility (mainly in females) and endometrial hyperplasia in cattle, sheep and the guinea-pig. Despite the fact that phytoestrogens are implicated in adverse effects upon fertility in various animals,

there are no similar reports on humans consuming large amounts of these compounds (such as Asian women). In humans, intake of phytoestrogens has been reported to interfere with the regulation of the menstrual cycle in premenopausal women (increased length of the menstrual cycle and reduced LH, FSH and progesterone levels).[47]

There is growing evidence linking the intake of soy-based foods and genistein with a reduction of coronary heart disease. Many of the studies have shown that whole-soyabean foods exert favorable effects on cardiovascular disease, especially on serum lipoprotein profile, while isolated isoflavones seem to improve only the endothelial function.[67,70,119] The cardioprotective effect of the individual phytoestrogens, however, has yet to be substantiated by long-term controlled trials.[121] Finally, in cell cultures it has been shown that genistein may exert neuroprotective functions or induce neurotoxicity.[122-124] Recent in vivo findings strengthen the idea that soy induces neurodegeneration.[125] Epidemiological data demonstrate a positive correlation between tofu consumption and brain atrophy.[126] It is concluded that further studies are needed to delineate the effects of phytoestrogen on cognitive functions.

The variation between studies and the conflicting results obtained may be due to multiple factors, such as the soy product used or the type of the individual phytoestrogen used, their plant origin, the dose delivered, the time and duration of exposure, the metabolism, the route of administration, the bioavailability, as well as the intrinsic estrogenic status. Given that differences in the above parameters may result in variable and in many instances divergent effects, it becomes evident that, at present, definite conclusions on the health effects of individual phytoestrogens cannot be drawn. The studies conducted so far are limited to soya isoflavones and soya products. Further systematic research is needed to evaluate the clinical effects of each individual phytoestrogen at the physiological and pharmacological levels, taking also into account whether these substances would be delivered in the context of food or as dietary supplement or even as pharmaceutical agents.[127,128]

At present, although there are no approved health claims for phytoestrogens, many preparations in the form of plant extracts or mixtures, containing varying amounts of isolated phytoestrogens, are commercially available on the market in numerous forms as dietary supplements and as health food products.[70,129] Given that these products are not under any regulatory controls, their composition is not consistent, their components vary greatly and, since there have not been systematic dose-ranging studies, no dietary recommendations for each of these products can be made; therefore, their safety and clinical effectiveness are questionable. Evidently, the use of the so-called health-promoting phytoestrogen-enriched products should be viewed with caution.

TEST SYSTEMS ASSESSING PHYTOESTROGENS' ESTROGENIC ACTIVITY

In vitro test systems

The estrogenic/antiestrogenic potential of phytoestrogens is usually assessed by an array of in vitro test systems as described below:

1) Radiometric competitive receptor binding assay.

Given that the first step in the ER transduction cascade is binding of a ligand to ER, the measurement of ligand binding is an important assay characterizing the potential of a test compound to interact with the ER. The 'classic' ligand binding assay uses radioactively labeled estradiol, which competes with the test compound for ER binding sites. This competitive binding assay provides relative binding affinities of test compounds to ERα or ERβ compared to estrogens, which is usually unlabeled estradiol or diethylstilbestrol (DES). The use of a fluorescein labeled estradiol instead of radioactive label made it possible to use fluorescence polarization to measure binding affinities. Data indicate that relative binding affinity rankings of various compounds are identical in both assays. The advantage of the radioactive binding assay is that both quantification of ER binding sites of any tissue or cell sample expressing ER can be obtained, and binding affinities of compounds may also be determined. In contrast, fluorescence polarization requires the use of purified ER due to the high background fluorescence in crude extracts. The advantage of fluorescence polarization is that it is a high-throughput assay which can screen many

compounds for ER binding affinity within a day or less.

2) The luciferase reporter gene assay using cells lacking endogenous ER.

The binding of the liganded ER dimer to the promoter region of its target gene on DNA specific sites, i.e. the estrogen response element (ERE) is a crucial step in the ER signaling pathway. Transient transactivation assays or recombinant cell assays, in which cells are co-transfected with the cDNA for ERα, or ERβ and a reporter gene containing an ERE, are widely used to measure ligand-induced ER-mediated gene activation. In this assay, a mammalian cell line lacking endogenous ER is transfected with an expression plasmid carrying the cDNA of ERα or ERβ together with ERE linked to a luciferase reporter DNA. Addition of test compounds, candidates for estrogen-like activity, induces dose-dependent transcription of the reporter luciferase that can easily be monitored and quantified. It is a very sensitive assay whereby very weak to highly potent estrogens can be analyzed for their estrogenicity and antiestrogenicity.

3) The luciferase reporter gene assay using cells containing endogenous ER.

In the transient transactivation assays, exogenous receptor (ERα or ERβ) is forced into a cell accustomed to the lack of ER; therefore, some effects measured may not reflect the physiological response of the analyzed cell type. These assays measure the potential of a "candidate" estrogen to trigger ER signaling at the level of the ERα/ERE or ERβ/ERE pathway. To account for these limitations, cell lines with endogenous ER expression (like MCF-7) can be used to measure the potential of a "candidate" estrogen to transactivate the native ER/ERE pathway.

4) Cell lines with endogenous ER expression measuring the expression of endogenous ER-target genes (end point assays).

Cell lines with endogenous ER expression can also be used to measure the expression of endogenous ER target genes, i.e. the analysis of end-points markers, such as mRNA or protein expression. The measurement of endogenous ER target gene (or protein) expression represents a valuable physiological assay for tissue specific estrogenicity or antiestrogenicity. In this type of assay, all steps of the ER signaling transduction pathway are considered, i.e. ER ligand binding, ER expression, ER dimerization, nuclear translocation, nuclear binding, available co-activators, etc. It is important to mention that the right protein (or gene) marker has to be monitored dependent on the chosen cell line.

5) Proliferation assay using an established cell line that is known to respond to estrogens.

The proliferation assay assesses the growth promoting effects of a "candidate" estrogen, since estrogens are known stimulants of cellular growth, whereas antiestrogens arrest the growth of cells (depending on the cell line). This assay system measures the potential of a "candidate" estrogen to trigger generalized effects on growth, which are the reflection of complex transcriptional factors and may differ from the effects of the "candidate" estrogen on ligand-mediated regulation of a single target gene or protein.[130,131]

Available data regarding the estrogenic/antiestrogenic potency of compounds are predominantly obtained with ligand-binding, transactivation assays and proliferation assays. Evidence shows that 1) it is difficult to compare data about the estrogenic potential of compounds evaluated by different methods using different end points; 2) the relative estrogenic potency of compounds determined with different assays might vary greatly; and 3) it cannot be expected that the correlation of the results obtained is always good. It is important to mention that the estrogenic potencies of compounds determined by different assays are not comparable, whereas the sensitivity of the different assays represented by the EC50 values for the standard estrogen 17β-estradiol differ greatly (by two or three orders of magnitude), the cell free binding assays being the less sensitive.[132]

The aforementioned broad spectrum of in vitro test systems may be useful to elucidate the mechanism of action of the test phytoestrogens and to set priorities for further assessment. It is important that the combination of several in vitro test systems be required in order to predict validly the effects in vivo. However, the in vitro assays do not include either metabolites of compounds or aspects of the absorption of compounds, thus they may generate false-negative or false-positive effects. In vivo stud-

ies are only able to predict the action of a substance in the organism, since under in vivo conditions the substance is exposed to absorption processes and multiple metabolic transformation.

In vivo test systems

Various in vivo assays (animal models) are used to characterize the estrogenic potency of phytoestrogens and their mechanism of action. The animals used are usually immature, hypophysectomized or ovariectomized rats, mice or rabbits. The test phytoestrogen is given orally or subcutaneously. Among the most common animal models are several tumor models, the uterotrophic assay and the analysis of estrogen sensitive endpoints (such as morphological, histological, biochemical and molecular endpoints) in the uterus and other estrogen sensitive target tissues, such as the vagina, the mammary gland, the liver, the bone, the cardiovascular system and the brain.

The uterotrophic assay assesses the ability of phytoestrogens to stimulate uterine growth. However, this assay may not be exactly suitable for assessing estrogenicity, since there are compounds, like raloxifene, that exert tissue-specific estrogen-like activity without effects on the uterus.[133] Tumor models are mainly used to assess the potential chemopreventive properties of phytoestrogens. There are several approaches, such as spontaneous carcinogenesis, chemical carcinogen-induced tumor models and tumor models by xenotransplantation of tumor cells. Spontaneous carcinogenesis may be applied for prostate and the endometrium. Chemical carcinogen-induced tumor models involve the exposure of rats to DMBA or NMU for the development of mammary carcinomas. Finally, some tumor cell lines, if xenotransplanted to immunodeficient nude mice or rats, grow tumors at the ectopic site which eventually metastasize through blood or lymphatics. This model is usually used for breast, prostate and endometrial tumors.[134]

Additionally, there are more specific animal models to evaluate the possible beneficial effects of phytoestrogens in osteoporosis, atherosclerosis and neurodegeneration. These include the ovariectomized adult rat model for osteoporosis, the rabbit model for high-cholesterol induced atheromatosis and several animal models for brain injury.[135-145]

In conclusion, the use of a suitable panel of different in vitro test systems combined with a final assessment in animal models will predict the real estrogenic potential of phytoestrogens.

CONCLUSION

A high-phytoestrogen diet may result in various health effects; however, some of the results obtained are still controversial. It must be stressed that present data are limited in characterizing the biological effects of soy products and the isoflavones genistein, daidzein, while only a few data exist on lignans. Laboratory evidence indicates that apart from the so far known phytoestrogens, e.g. the isoflavones, lignans, coumestanes, the stilbenes and the flavonoids quercetin and kaempferol, there are many more phytoestrogens identified in nature; thus the spectrum of phytoestrogens is expanding and their biological role awaits determination. The experimental methodology followed is of high importance in defining the estrogenic effect of phytochemicals, the in vitro studies providing the first clues about the estrogenic potency, whereas the use of animal models indicates the real estrogenic potential of phytoestrogens. Many data have shown a wide range of hormonal activities of phytoestrogens (estrogenic/antiestrogenic via the ER subtypes). Overall, however, phytoestrogen biological activity depends on multiple factors, e.g. the chemical form of the phytoestrogen, the route of administration, the metabolism, the intrinsic estrogenic status and the time and the level of exposure. On the market there are several preparations containing varying concentrations of individual phytoestrogens of different origin and their use as dietary supplements may result in high levels of phytoestrogens, relative to those provided in whole foods. In addition, the idea that what is “natural” is also safe may not prove valid.

In this respect, the consequences of phytoestrogen intake may not necessarily be beneficial and in some cases may actually increase disease risk.

Because of the lack of well-designed, prospective studies in humans for individual phytoestrogens and due to the fact that the commercial preparations of phytoestrogens are not subjected to any regulatory controls, dietary recommendations for individual

phytoestrogens cannot be given. Caution is rather suggested for their consumption and each preparation should be thoroughly evaluated individually before it is used as a dietary supplement or as a therapeutic agent.

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