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REVIEW ARTICLE

Phytoestrogens: a Review of the Present State of Research

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Phytoestrogens are a diverse group of plant-derived compounds that structurally or functionally mimic mammalian estrogens and show potential benefits for human health. The number of articles published on phytoestrogens has risen dramatically in the past couple decades. Further research continues to demonstrate the biological complexity of phytoestrogens, which belong to several different chemical classes and act through diverse mechanisms. This paper discusses the classification of phytoestrogens, methods of identification, their proposed mechanisms of action and botanical sources for phytoestrogens. The effects of phytoestrogens on breast and prostate cancers, cardiovascular disease, menopausal symptoms and osteoporosis will also be examined including research on benefits and risks. Copyright © 2003 John Wiley & Sons, Ltd.

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INTRODUCTION

Phytoestrogens are plant-derived compounds that structurally or functionally mimic mammalian estrogens and therefore are considered to play an important role in the prevention of cancers, heart disease, menopausal symptoms and osteoporosis (Setchell, 1998; Adlercreutz, 2002; Kronenberg and Fugh-Berman, 2002). Estrogens influence the growth and functioning of female and male reproductive tissues, maintain the skeletal and central nervous system, provide cardioprotective effects in the cardiovascular system, and protect against colon cancer and aging skin (Gruber *et al.*, 2002; Ruggiero and Likis, 2002). Considering the numerous effects estrogens have on the human body, it is not surprising to consider the potential of phytoestrogens for human health.

People are seeking complementary and alternative practices of health care as well as treatments seen as more 'natural' as a way to complement biomedical health care (Eisenberg *et al.*, 1993; Eisenberg *et al.*, 1998). Many women turn to phytoestrogens as an alternative to hormone replacement therapy (HRT) and estrogen replacement therapy (ERT) because of their undesirable side effects, such as increased risk of breast and endometrial cancer and irregular bleeding (Brzezinski and Debi, 1999; Wade *et al.*, 1999; Wagner *et al.*, 2001). Concerns about ERT and HRT are being further confirmed by the recent suspension of the Women's Health Initiative (WHI) study testing the risks and benefits of HRT on healthy postmenopausal women (Rossouw *et al.*, 2002). This study was the first randomized double-blind clinical trial to test the health benefits and risks of combined estrogen and progestin (Prempro™, composed of conjugated estrogens plus medroxyprogesterone acetate) on healthy postmenopausal women. The study was halted because the health risks exceeded the health benefits. Although phytoestrogens are being considered as an alternative to HRT, the present literature shows conflicting evidence for their use (Glazier and Bowman, 2001; Kang *et al.*, 2002).

There is a rapidly growing body of literature on phytoestrogens. In evaluating the literature on phytoestrogens two of the more cited reviews include those by Price and Fenwick (1985) and Kurzer and Xu (1997). Other more recent reviews are those by Adlercreutz (2002, 2003), Ibarreta and coauthors (2001) and Fitzpatrick (2003). Some reviews are general (Knight and Eden, 1996; Potter and Steinmetz, 1996; Adlercreutz, 1998a; Bingham *et al.*, 1998; Setchell, 1998; Tham *et al.*, 1998; Whitten and Naftolin, 1998; Anderson *et al.*, 1999; Messina, 1999) others are specific to health conditions, such as phytoestrogens as an alternative for hormone replacement therapy (Scheiber and Rebar, 1999; Glazier and Bowman, 2001; Kronenberg and Fugh-Berman, 2002; Wuttke *et al.*, 2003a; Wuttke *et al.*, 2003b); phytoestrogens and breast cancer (Barnes, 1998; Cline and Hughes, 1998; Adlercreutz *et al.*, 2000b; Glazier and Bowman, 2001; DeLemos, 2001; Messina and Loprinzi, 2001; This *et al.*, 2001; Wagner *et al.*, 2001; Adlercreutz, 2002; Adlercreutz, 2003; Peeters *et al.*, 2003); phytoestrogens and cardiovascular disease (Clarkson and Anthony, 1998; Van der Schouw *et al.*, 2000; Wroblewski Lissin and Cooke, 2000; Kris-Etherton *et al.*, 2002); phytoestrogens and chronic renal disease

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(Ranich *et al.*, 2001); phytoestrogens and prostate cancer (Moyad, 1999; Adlercreutz, 2002; Morrissey and Watson, 2003); phytoestrogens and colon cancer (Messina and Bennink, 1998; Adlercreutz, 2002); phytoestrogens and obesity and diabetes (Bhathena and Velasquez, 2002); and phytoestrogens in pediatrics (Zung *et al.*, 2001). Additional reviews present the phytoestrogen content and activity of foods, herbs and spices (Reinli and Block, 1996; Mazur, 1998; Zava *et al.*, 1998). Other reviews have focused on phytoestrogens and their effect on domestic animals (Adams, 1995; Lundh, 1995).

Soy (*Glycine max* (L.) Merr., Fabaceae) has received considerable attention as a source for phytoestrogens as shown by numerous review articles (Messina *et al.*, 1994; Anderson *et al.*, 1995; Setchell, 1998; Vincent and Fitzpatrick, 2000; Sirtori, 2001; Wagner *et al.*, 2001; Fitzpatrick, 2003), yet other botanical sources, such as red clover (*Trifolium pratense* L., Fabaceae) (Saloniemi *et al.*, 1995; Fugh-Berman and Kronenberg, 2001) and black cohosh (*Actaea racemosa* L. syn. *Cimicifuga racemosa* (L.) Nutt. Ranunculaceae) are also being studied (Foster, 1999; Liu *et al.*, 2001a; Wuttke *et al.*, 2003c).

In addition to reviews in English, there are reviews in French (Drapier-Faure, 2001), German (Huber, 2000), Japanese (Kinjo, 2000) and Polish (Badowski and Urbanek-Karlowska, 2001). Of historical interest are the early reviews of phytoestrogens by Bradbury and White (1954) and Farnsworth and coworkers (1975a,b).

The scope of this paper will take a botanical view in discussing the recent advances in the state of knowledge of phytoestrogens. Identification and classification of phytoestrogens will be presented as well as botanical sources of phytoestrogens and their proposed mechanisms of action. The effects of phytoestrogens on breast and prostate cancers, cardiovascular disease, menopausal symptoms and osteoporosis will also be examined including research on benefits and risks.

PHYTOESTROGENS

Phytoestrogens defined functionally are substances that promote estrogenic actions in mammals and structurally are similar to mammalian estrogen 17β-estradiol (E_2) (Price and Fenwick, 1985; Knight and Eden, 1996). Other mammalian endogenous estrogens are estriol and estrone, which are weakly estrogenic compared with their mammalian counterpart, E_2 (Gruber *et al.*, 2002) (Fig. 1). The diverse biological activity of phytoestrogens is due in part to their ability to act estrogenically as estrogen agonists and antiestrogenically as antagonists. As estrogen agonists, phytoestrogens mimic endogenous estrogens and cause estrogenic effects. As estrogen antagonists, they may block or alter estrogen receptors (ER) and prevent estrogenic activity, causing antiestrogenic effects (Brzezinski and Debi, 1999).

As estrogen agonists and antagonists, phytoestrogens can also be classified as selective estrogen receptor modulators (SERMs) (Brzezinski and Debi, 1999). SERMs are non-steroidal chemicals with a similar structure to E_2 and an affinity toward estrogen receptors (Riggs and Hartmann, 2003). They are unique in that they can function as agonists or antagonists depending on the tissue, ER and concentration of circulating

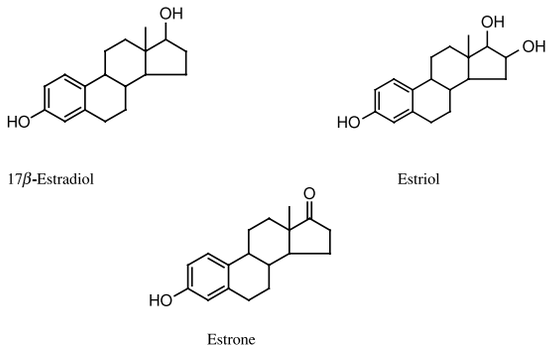


Figure 1. Structures of mammalian endogenous estrogens: 17β-estradiol, estriol and estrone.

endogenous estrogens (Gruber *et al.*, 2002). Tamoxifen and raloxifene are well-known SERMs. Tamoxifen has been used in clinical practice for breast cancer patients because it acts as an estrogen antagonist in breast tissue, slowing cancer cell proliferation and an estrogen agonist in bone tissue and in the cardiovascular system to prevent osteoporosis and heart disease. However, tamoxifen has shown estrogenic activity in the uterus and therefore may increase the risk of endometrial cancer (Poulet *et al.*, 1997; Pukkala *et al.*, 2002).

Mechanistically phytoestrogens have been shown to bind to two types of estrogen receptors: estrogen receptor α (ERα), which was cloned in 1986, and estrogen receptor β (ERβ) cloned in rats (Kuiper *et al.*, 1996) and in humans (Mosselman *et al.*, 1996). The two receptors differ in their tissue distribution and affinity to ligands, yet there is some overlap. In rats, ERα and ERβ both are clearly expressed in ovary and uterus tissue (Kuiper *et al.*, 1997). ERβ has been shown to have ligand specificity toward phytoestrogens and is distributed in humans in ovary, spleen, testis and thymus tissue (Mosselman *et al.*, 1996) and in rats in bladder, brain, lung, ovary, prostate, testis and uterus tissue (Kuiper *et al.*, 1997). Phytoestrogens show a lower binding affinity than E_2 and some show a higher binding affinity for ERβ than for ERα, which may suggest different pathways for their actions and explain tissue-specific variability of phytoestrogenic action (Kuiper *et al.*, 1998; Setchell, 1998). The complexity of phytoestrogens and ERs appears to be further compounded because different transcriptional activities *in vitro* are activated depending on the ligands, as well as the environment of the promoter region of specific genes for translated ERα and ERβ receptors (Paech *et al.*, 1997). Recent research in teleost fish (Atlantic croaker *Micropogonias undulates*) identified a third estrogen receptor, ERγ, which is found in various tissues (Hawkins *et al.*, 2000).

MECHANISMS OF ACTION

Both genomic and nongenomic mechanisms have been proposed to explain phytoestrogenic effects on human health (Anderson *et al.*, 1999). Phytoestrogens are able to interact with enzymes and receptors, and because of their stable structure and low molecular weight they can pass through cell membranes (Adlercreutz, 1998b).

These interactions allow them to bind to ERs, induce specific estrogen-responsive gene products, stimulate ER-positive breast cancer cell growth (Kurzer and Xu, 1997), interfere with steroid hormone metabolism or action (Adlercreutz, 1998b) and alter ER structure and affect transcription (Santti *et al.*, 1998). Some genomic mechanisms of action include estrogenic and antiestrogenic effects on ERs, while other effects may not involve direct interaction with ERs (Messina and Loprinzi, 2001). Nongenomic effects that do not involve ERs include: induction of cancer cell differentiation, inhibition of tyrosine kinase and DNA topoisomerase activities, suppression of angiogenesis and antioxidant effects of phytoestrogens (Kurzer and Xu, 1997). Other effects can take place at the cellular and molecular level and potentially influence the biosynthesis and metabolism of steroids and fatty acids, the serum steroid carrier proteins (sex steroid binding proteins and α-fetoprotein), and the intracellular and transmembrane transfer of hormones to a membrane and to nuclear receptors (Benassayag *et al.*, 2002). Phytoestrogens inhibit the enzymes needed for hormone conversions, which may reduce cancers by lowering the biological activity of sex hormones in target organs (Adlercreutz, 1998b). As estrogen-like compounds, some phytoestrogens are able to induce estrus in mammals (Mineta *et al.*, 2001).

The different activities and the bioavailability of phytoestrogens vary depending on such factors as the form of administration, dosage, individual metabolism and the ingestion of other pharmacological substances (Kelly *et al.*, 1995; Xu *et al.*, 1995; Wiseman, 1999). Target tissue, concentration dependency, number and type of ER, and the presence or absence of endogenous estrogens also influence the effect of phytoestrogens (Glazier and Bowman, 2001).

Not only do phytoestrogens differ in their biological activity, but they also differ structurally because they come from diverse chemical classes, which may affect their influence on tissues and receptors (Lieberman, 1996). Due to the diversity of chemicals that show estrogenic effects, it appears that estrogenic activity is often emphasized over chemical structure in defining phytoestrogens.

IDENTIFICATION OF PHYTOESTROGENS

The ability of plant substances to cause estrus in animals was documented in the mid-1920s (Costello and Lynn, 1950; Bradbury and White, 1954). The Allen-Doisy technique was one of the first bioassays to detect estrogenic activity in ovariectomized rats and mice (Allen and Doisy, 1923). In this bioassay the uterine weight of the test animal was used to measure estrogenic activity. A review of plants tested with this technique has been published and includes a variety of plants from different botanical families such as beets (*Beta vulgaris* L., Chenopodiaceae), parsley (*Petroselinum crispum* (Mill.) Nyman ex A. W. Hill, Apiaceae), plum and cherry (*Prunus* spp., Rosaceae), potato (*Solanum tuberosum* L., Solanaceae) and rice (*Oryza sativa* L., Poaceae) (Bradbury and White, 1954). Later, steroidal estrogens were detected in plants, such as estrone found in pomegranate seeds (*Punica granatum* L., Punicaceae) and the seeds of the date palm (*Phoenix dactylifera* L., Arecaceae), although the presence of these compounds has been questioned (Heftmann, 1967). Although phytoestrogens were originally noticed because they induced estrus in animals, not all plants that show estrogenic activity induce estrus. In addition, there are plant substances that induce estrus that are not phytoestrogens. Since the early 1920s additional *in vivo* and *in vitro* methods have been developed to test estrogenic and antiestrogenic activity of plants.

Some *in vivo* tests used to evaluate estrogenic activity are: the degree of cornification of vaginal epithelium in rats and mice, uterotrophic assays that measure uterine wet weight in immature or ovariectomized rats or mice, and proliferative effects in the female genital tract (Galey *et al.*, 1993; Diel *et al.*, 2002). Estrogenic activity based on bioassay methods should be viewed with caution when extrapolating to humans because phytoestrogens have been shown to metabolize differently in different animal species (Reinli and Block, 1996). In addition, many variables can affect the results in animal studies such as the model species, dosage, length of study and routes of administration (Yang and Bittner, 2002). In clinical and epidemiological studies, the same principle applies as studies vary by type of intervention (extracts, purified compounds), dosage, age of subject, and length of study, among other variables.

Some *in vitro* assays used to investigate estrogenic activity are: the receptor binding assay in which the binding affinity to the ERs is measured, E-screen assay which tests the ability of a substance to stimulate growth of estrogen sensitive cells such as human breast cancer cells, the reporter gene assay which measures the ability of a substance to activate transcription via an estrogen sensitive promoter, and the analysis of gene expression which evaluates the regulation of estrogen sensitive genes in cell culture (Diel *et al.*, 1999; Mueller, 2002). Some assays are more sensitive to phytoestrogens than others (Dixon-Shanies and Shaikh, 1999). Other examples of *in vitro* assays are the transient gene expression or co-transfection assay (Miksicek, 1993; Miksicek, 1994) and the Ishikawa cell line, an organ specific model that uses estrogen responsive human endometrial adenocarcinoma cells (Wober *et al.*, 2003). Measuring quantitative structure–activity relationships (QSAR) has been recommended as an initial screening technique for estrogen receptor binding affinity prior to *in vitro* and *in vivo* assays (Hu and Aizawa, 2003).

In the mid-1980s the primary method for phytoestrogen analysis was gas chromatography coupled with a mass spectrometer (GC-MS), which involved extensive purification procedures for either plants or physiological samples before analysis (Wang *et al.*, 2002). It is still valuable for physiological samples such as urine and blood containing low concentrations of phytoestrogens although it is labor intensive and new techniques have been developed (Wang *et al.*, 2002).

High pressure liquid chromatography (HPLC) was first used to detect flavonoids in 1976 by Fisher and Wheaton (1976). It is one of the most common analytical methods used for phytoestrogen identification because limited sample preparation is involved and both glycosides and aglycones can be analysed directly (Wang *et al.*, 2002). HPLC coupled with a mass spectrometer (LC-MS) is especially useful for this type of analysis. Franke and coworkers (1995) have

developed reversed-phase HPLC methods that are tailored for phytoestrogen analysis. Acid hydrolysis is part of sample preparation, which converts glycosides into their respective aglycones. The solvent system they used was a linear gradient of acetonitrile and acetic acid and water with a photodiode array (PDA) detector. This group recently reported a new technique for determining phytoestrogens (Franke *et al.*, 2002). One drawback of using LC-MS is the ineffective isomer differentiation and the inability to produce molecular ions for some flavonoids (Wang *et al.*, 2002). Additional reports discuss methods used to detect polyphenolic phytoestrogens in foods and biological fluids (Joannou *et al.*, 1995; Wilkinson *et al.*, 2002). Other non-chromatographic methods have also been used for phytoestrogen analysis, such as immunoassay techniques, deconvolution spectroscopy and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Wang *et al.*, 2002).

Comparing phytoestrogen levels and the content of foodstuffs and plants is not always possible because various methods are used to extract and quantify phytoestrogens. Different varieties of plants and brands of food as well as diverse forms of processing need to be considered when evaluating phytoestrogen levels (Mazur, 1998). In addition, individual plants may show different levels of phytoestrogens due to microbial or insect damage prior to analysis and seasonal variation (Price and Fenwick, 1985). Instability and easy degradation of some phytoestrogens during processing and analysis may explain varying analytical results (Ibarreta *et al.*, 2001).

CLASSIFICATION OF PHYTOESTROGENS

There are several classes of phytoestrogens: steroidal estrogens, found in few plants and the more ubiquitous phenolic estrogens, isoflavones, coumestans and lignans which will be the focus of this review. Other classes of phytoestrogens that have been reported include: anthraquinones (Matsuda *et al.*, 2001), chalcones (Rafi *et al.*, 2000), flavones (Milligan *et al.*, 1999), prenylflavonoids (Kitaoka *et al.*, 1998) and saponins (Chan *et al.*, 2002). Examples of plant steroids are estrone found in palm (*Elaeis guineensis* Jacq., Arecaceae) and β-sitosterol which is found in almost all plants (Farnsworth *et al.*, 1975a,b; Duke, 1992). Studies have reported conflicting evidence about estrone found in the seeds of pomegranate (Miksicek, 1994) and the presence of estrone in palm kernel residue could not be confirmed by further investigations (Jacobsohn *et al.*, 1965). The steroidal plant compounds reported in earlier investigations have been questioned due to the limited technology available at the time to isolate and identify compounds (Price and Fenwick, 1985).

Phytoestrogens have been categorized based on their chemical structures, which resemble E_2. Estrogen receptors bind with steroidal as well as numerous non-steroidal compounds. An aromatic ring and a hydroxyl group is important for binding effectiveness and the remainder of the ER will accept hydrophobic groups (Anstead *et al.*, 1997). Important features that enable chemicals to bind to an ER are the steric and hydrophobic properties of a compound, as well as the hydrogen bonding between the phenolic hydroxyl group and the ER binding site (Hu and Aizawa, 2003). Estrogenic flavonoids are similar in structure to E_2. They are composed of a planar ring system that includes a *p*-hydroxy-substituted aromatic ring that is approximately 12 Å away from a second in-plane hydroxyl group (Hu and Aizawa, 2003). Two ring structures separated with two carbon atoms as well as spacing between hydrophobic and hydrogen bond interactions are also important in binding affinity to ERs (Brzozowski *et al.*, 1997). Other characteristics for ER-binding affinity of a chemical are the degree and size of branching of the alkyl group and its location on the phenolic ring and the distribution range of electron density on the A ring (Hu and Aizawa, 2003).

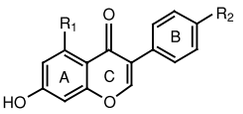
The biological activity of individual phytoestrogens varies and is often reported as less active than mammal or synthetic estrogens (Knight and Eden, 1996; Tham *et al.*, 1998). Differences in estrogenic activity of similarly classified chemicals may be due to the structural features or deviations in those structures. Some phytoestrogenic compounds may show different estrogenicity due to the bioassay employed (Messina and Loprinzi, 2001) and others may not show estrogenic activity in bioassays because only their metabolized derivatives are hormonally active (Miksicek, 1994). These metabolized derivatives may be active aglycons produced by the removal of the sugar moiety during metabolism or xenobiotics produced through metabolic processes (Adlercreutz *et al.*, 1987; Xu *et al.*, 1995). More research is needed to elucidate the structural-activity relationship that is required for a natural product to be active as a phytoestrogen.

Isoflavones

Isoflavones are the most well known of the phytoestrogens. The recognition of 'clover disease' in Australian sheep in the 1940s led to the investigation of estrogenic activity of isoflavones (Kingsbury, 1964). The sheep whose diet was predominately subterranean clover (*Trifolium subterraneum* L., Fabaceae) suffered from a reproductive disorder that reduced the lambing rates and involved abnormal lactation, changes in the sex organs, permanent infertility, prolapsed uterus and maternal dystocia (Bennetts *et al.*, 1946). Isoflavones are present in green clover and are not present at senescence (Adams, 1995).

Naturally occurring isoflavones that have shown estrogenic activity are: the aglycones, daidzein (4′,7-dihydroxyisoflavone) and genistein (4′,5,7-trihydroxyisoflavone); the glycosides, daidzin and genistin; and biochanin A and formononetin, 4′-methyl ethers of daidzein and genistein (Price and Fenwick, 1985; Kurzer and Xu, 1997) (Fig. 2). In plants, they can often be found as glycosides (Ibarreta *et al.*, 2001). In processing, isolation and analysis, these compounds are readily degraded chemically or enzymically to the aglycone (Price and Fenwick, 1985). Glycitein is another isoflavone reported in soy that has also shown estrogenic activity (Song *et al.*, 1999).

After mammals consume isoflavones, daidzein and genistein are metabolized in the gastrointestinal tract. Biochanin A and formononetin can metabolize to genistein and daidzein respectively. Daidzein may be



Isoflavone	R_1	R_2
Biochanin A	OH	OCH_3
Daidzein	H	OH
Formononetin	H	OCH_3
Genistein	OH	OH

Figure 2. The structures of isoflavones: biochanin A, daidzein, formononetin and genistein.

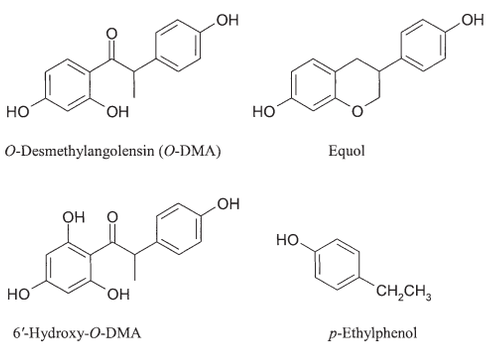


Figure 3. Structures of metabolized isoflavones. Formononetin is metabolized to daidzein which metabolizes into dihydrodaidzein, and then to *O*-desmethylangolensin (*O*-DMA) and equol. Biochanin A is metabolized to genistein which metabolizes into dihydrogenistein 6′-hydroxy-*O*-DMA and *p*-ethylphenol.

further metabolized to dihydrodaidzein and then to *O*-desmethylangolensin (*O*-DMA) and equol (Fig. 3) (Kurzer and Xu, 1997). Equol is not metabolized equally in all humans (Kelly *et al.*, 1995) and an individual's ability to transform soy isoflavones into equol may offer an explanation for the varied results of present phytoestrogen studies (Setchell *et al.*, 2002). Genistein metabolizes to dihydrogenistein and then to 6′-hydroxy-*O*-DMA (Kurzer and Xu, 1997) and hormonally inert *p*-ethylphenol in sheep and humans (Price and Fenwick, 1985; Ibarreta *et al.*, 2001) (Fig. 3). These new compounds produced from metabolism may have different biological effects than the original isoflavones digested (Naftolin and Stanbury, 2002).

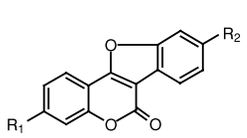
Isoflavones are primarily found in the Fabaceae family, which has food legumes such as soy, peanut (*Arachis hypogaea* L.) and clover (*Trifolium* spp.). Soy seeds show high levels of formononetin and biochanin A (both 729 μg/g dry weight) (Ibarreta *et al.*, 2001). Other food sources of isoflavones are oilseeds and nuts, such as the sunflower seed (*Helianthus* spp., Asteraceae) and walnut (*Juglans nigra* L., Juglandaceae) from different botanical families (Mazur, 1998; Sirtori, 2001). Isoflavones have also been found in the Iridaceae and the Euphorbiaceae family (Dewick, 1993). They are primarily extracted from soy and red clover (Messina, 1999). Raw soybeans contain 1.2–4.2 mg/g dry weight of isoflavones, while high protein soy ingredients like soy flour contain 1.1–1.4 mg/g dry weight (Kurzer and Xu, 1997).

Of all the isoflavones, genistein has received the most attention. Estrogenic and antiestrogenic effects of geinstein, including effects independent of ERs, have been discussed in relation to breast cancer (Bouker and Hilakivi-Clarke, 2000; Brownson *et al.*, 2002) and other conditions (Dixon and Ferreira, 2002). Several studies have tested genistein with breast cancer cell lines and the results tend to show cell proliferation at low doses and inhibition at high concentrations (Anderson *et al.*, 1999). Genistein has been shown as a powerful antioxidant (Ruiz-Larrea *et al.*, 1997). However, other researchers have shown that genistein and other phytoestrogens have low antioxidant ability, suggesting that phytoestrogens may not have such a significant effect as antioxidants (Mitchell *et al.*, 1998).

Coumestans

Coumestans are another group of plant phenols that show estrogenic activity. Coumestrol was first reported in 1957 by Bickoff and coworkers as a new phytoestrogen that was isolated from ladino clover (*Trifolium repens* L., Fabaceae), strawberry clover (*Trifolium fragiferum* L., Fabaceae) and alfalfa or lucerne (*Medicago sativa* L., Fabaceae) (Bickoff *et al.*, 1957). Their presence in fodder crops has been associated with disrupting reproductive performances of livestock (Price and Fenwick, 1985; Adams, 1995). For example, feeding cattle haylage containing 37 ppm (mg/kg) or more of coumestrol resulted in negative estrogenic effects such as udder development, bulling of steers and prolapsed vagina, cervix and rectum (Lookhart, 1980). However, few coumestans isolated from plants have shown uterotropic activity (Price and Fenwick, 1985; Kurzer and Xu, 1997). The main coumestans with phytoestrogenic effects are coumestrol and 4′-methoxycoumestrol (Fig. 4). Coumestrol and genistein have higher binding affinities to ERβ than the other phytoestrogen compounds (Whitten and Naftolin, 1998). *In vitro* coumestrol has been reported to inhibit bone resorption and to stimulate bone mineralization (Tsutsumi, 1995).

Coumestans are less common in the human diet than isoflavones (Ibarreta *et al.*, 2001), yet similar to isoflavones, in that they are also found in legumes, particularly food plants such as sprouts of alfalfa and mung bean (*Vigna radiata* (L.) R. Wilczek, Fabaceae) (Lookhart, 1980; Adams, 1995; Mazur, 1998) and they are especially high in clover (Franke *et al.*, 1995). Soy sprouts also show high levels of coumestrol (71.1 μg/g wet weight) (Ibarreta *et al.*, 2001).



Coumestan	R_1	R_2
Coumestrol	OH	OH
4′-Methoxycoumestrol	OH	OCH_3

Figure 4. The structures of coumestans: coumestrol and 4′-methoxycoumestrol.

The coumestrol content in plant material has been reported to vary according to plant variety, stage of growth, cutting, presence of disease, location and insect/fungal attacks (Price and Fenwick, 1985). Attacks by insects and fungus may cause plants, for example alfalfa, to produce coumestrol or 4′-methoxycoumestrol as phytoalexins (Adams, 1995). Phytoalexins are a diverse class of compounds that are often secondary metabolites formed as a result of injury or disruption, which aid plants by protecting them from insects, bacteria and viruses (Ibarreta *et al.*, 2001).

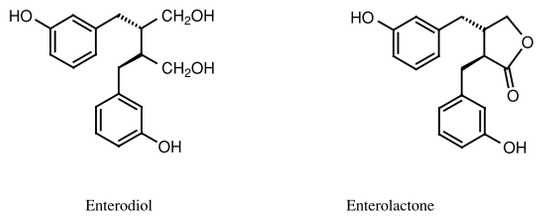


Figure 6. Structures of mammalian lignans: enterodiol and enterolactone. Plant lignans secoisolariciresinol and matairesinol are converted by human gut bacteria to enterodiol and enterolactone, respectively.

Lignans

Lignans were first identified in plants and later in biological fluids of mammals. As a class of compounds they contain a dibenzylbutane skeleton and in plants they aid in the formation of lignin used to construct the plant cell wall (Setchell and Adlercreutz, 1988). A cyclic pattern observed in the urinary excretion of these phenolic compounds by humans and animals during the menstrual cycle initiated interest in their physiological role (Adlercreutz *et al.*, 1987). They were thought to be a new class of endogenous hormones. These compounds were elucidated simultaneously by different researchers and identified as unique mammalian lignans (Setchell *et al.*, 1980; Stitch *et al.*, 1980).

The most well known phytoestrogenic lignans are secoisolariciresinol and matairesinol (Fig. 5) which are converted by bacterial action in the gut into enterodiol and enterolactone (Fig. 6), mammalian lignans not found in plants (Setchell and Adlercreutz, 1988). Enterodiol can be further metabolized to enterolactone (Borriello *et al.*, 1985). The removal of the sugar moiety through metabolism by intestinal bacteria is common in isoflavones and lignans (Adlercreutz *et al.*, 1987). Mammalian lignans, like isoflavones, have a low molecular weight and are considered chemically, biochemically and biologically unique and stable molecules because they have phenolic groups in the *meta* position of the aromatic rings (Adlercreutz, 1998a). Recently, other enterolactone precursors have been identified: arctigenin, 7-hydroxymatairesinol, lariciresinol, pinoresinol and syringaresinol (Fig. 5) (Meagher *et al.*, 1999; Heinonen *et al.*, 2001). The last three are found in cereals, specifically whole-grain rye products. However, more studies are needed to evaluate the estrogenic activity of these compounds.

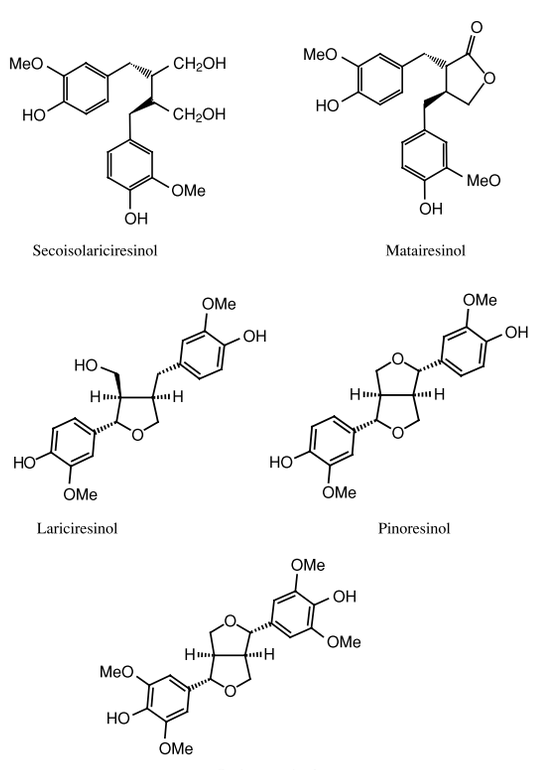


Figure 5. Structures of some plant lignans: secoisolariciresinol, matairesinol, lariciresinol, pinoresinol and syringaresinol.

Lignans are widespread in foodstuff such as cereals, fruits and vegetables and have not been studied as thoroughly as isoflavones and coumestans (Ibarreta *et al.*, 2001). Lignans are commonly found in rye bread (*Secale cereale* L., Poaceae) and oilseeds such as flaxseed (*Linum usitatissimum* L., Linaceae) (Thompson *et al.*, 1991). Flaxseed contains the most abundant amount of lignans with about 0.8 mg of secoisolariciresinol/g dry weight (Kurzer and Xu, 1997) and when crushed and defatted the phytoestrogen content rises (Mazur, 1998). Lignans are also found in brewed green and black tea and coffee (Mazur *et al.*, 1998). Grains and cereals show high levels of lignans in the aleurone and pericarp/testa layers (Mazur, 1998). Pumpkin seeds (*Cucurbita pepo* L., Cucurbitaceae) contain secoisolariciresinol and trace amounts of lariciresinol (Sicilia *et al.*, 2003). Secoisolariciresinol is found in many food and beverage categories, while matairesinol is found in smaller amounts and only trace amounts in food legumes (Mazur, 1998).

Large amounts of lignans have also been reported in coniferous trees, such as the heartwood of Norway spruce (*Picea abies* (L.) H. Karst, Pinaceae), which has shown greater concentrations of lignans than flax (Saarinen *et al.*, 2000). Hydroxymatairesinol (HMR) was first identified in spruce and was reported as a novel precursor to enterolactone. Additional testing with this compound showed potent antioxidant activity and decreased breast tumors in the rat dimethylben[*a*]anthracene-induced mammary tumor model; however, HMR did not show estrogenic activity in the uterine growth assay or through transcriptional responses via ERα or ERβ (Saarinen *et al.*, 2000). These results suggest that the antitumor activity of HMR may involve nongenomic mechanisms of activity.

A case-controlled study on metabolized lignan conducted with Finnish men showed a reduced risk of myocardial infarction associated with higher concentrations of serum enterolactone (Vanharanta *et al.*, 1999). Adlercreutz (2003) points out that purified lignans

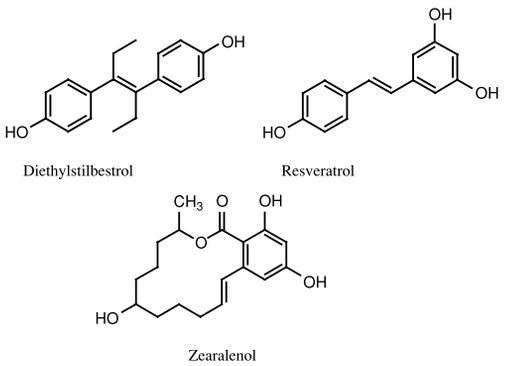


Figure 7. Structures of other estrogenic compounds: diethylstilbestrol, resveratrol and zearalenol.

consumed neonatally or prepubertally may have a protective effect on breast tissue by increasing the differentiation of proliferative terminal end bud structures also observed during pregnancy and lactation.

Other estrogenic compounds

Other compounds that have shown estrogenic activity are: resveratrol (*trans*-3,4′,5-trihydroxystilbene), diethylstilbestrol (DES) (4,4′-dihydroxy-*trans*-α, β-diethylstilbene) and mycotoxins such as zearalenol (Fig. 7). Resveratrol, a stilbene, is found in a variety of plants and functions as a phytoalexin to protect against fungal infections. Other sources of resveratrol are the skin of grapes (*Vitis vinifera* L., Vitaceae), wine, as well as other botanicals such as hu-chang (*Polygonum cuspidatum* Siebold & Zucc., Polygonaceae) (Bagchi *et al.*, 2001). Resveratrol has been reported to bind to ERs and stimulate the growth of human breast cancer cells (Ghem *et al.*, 1997) and to increase ovarian weight and disrupt estrous cycles in gonadally intact Sprague-Dawley rats (Henry and Witt, 2002).

In addition to plant estrogens, xenoestrogens and mycotoxins when ingested have an estrogenic response similar to endogenous estrogens. Pesticides and industrial chemicals (i.e. polychlorinated biphenyls), sometimes referred to as endocrine disrupters, are examples of xenoestrogens found in the environment (Belcher and Zsarnovszky, 2001; Ibarreta *et al.*, 2001). Diethylstilbestrol is a synthetic stilbene that has also shown strong estrogenic effects in humans (Setchell, 1998). Mycotoxins are secondary metabolites produced by molds such as *Fusarium* often found on stored crops (Kurzer and Xu, 1997; Ibarreta *et al.*, 2001). Resorcyclic acid lactone compounds, such as zearalenone and zearalenol and their derivatives, have been referred to as mycoestrogens, a type of mycotoxin, because they show estrogenic activity but they are not inherent to food plants (Fig. 7).

Zearalenone is a widely distributed mycoestrogen that has strong estrogenic effects. It is primarily produced by *Fusarium graminearum, F. culmorum* and *F. crookwellense* and is usually associated with corn but can also be found in other cereals such as barley and wheat (IARC, 1993). Reproductive problems such as vulvovaginitis (vaginal swelling), prolonged estrus, reduced sex drive, infertility and abortions have been shown in animals consuming contaminated feed as well as testicular atrophy and enlarged mammary glands in male swine (Schoental, 1985; Visconti and Pascale, 1998). In some cases farmers have exploited the potential of mycotoxins and used it to promote fattening of sheep and cattle (Yang and Bittner, 2002). Methods are being developed to detect mycotoxins in foodstuffs and feedstuffs to safeguard both animal and human health (Rosenberg *et al.*, 1998). The United States Food and Drug Administration (FDA) has not established advisory limits nor action levels for zearalenone, although if this mycotoxin is present usually other mycotoxins for which the FDA has set advisory levels are also present, such as deoxynivalenol produced by *Fusarium* spp.

New sources of estrogenic compounds are attractive for human health because of the numerous effects that endogenous estrogens have on the human body. Yet some synthetic estrogenic compounds have raised concern for human health because of the negative effects they can have on the body. Although phytoestrogens come from plants and are therefore considered 'natural', they may also have negative effects on health that have not been clearly identified. At present much of the research on phytoestrogens is evaluating the beneficial and adverse effects of these compounds on different health conditions.

HUMAN HEALTH AND PHYTOESTROGENS

Investigators have proposed the hypothesis that lowered cardiovascular disease, osteoporotic fractures, rates of breast cancer and hot flushes in Asian populations are related to a diet rich in soy, in other words phytoestrogens (Adlercreutz, 1998a; Clarkson and Anthony, 1998; Adlercreutz *et al.*, 2000b; Wagner *et al.*, 2001). Therefore, diet has been evaluated especially in relation to phytoestrogen content. However, when evaluating this relationship, confounding factors such as lifestyle, diet, socio-cultural and morphological differences that distinguish Asian and Western populations must be considered in the analysis (This *et al.*, 2001). Several studies have discussed the potential effects of phytoestrogens in treating breast cancer, endometrial cancer, liver disease and prostate cancer (Fotsis *et al.*, 1993; Strom *et al.*, 1999; Adlercreutz *et al.*, 2000b; Messina and Loprinzi, 2001; Lei *et al.*, 2002). Additional research has shown that intestinal bacteria are seen as important in the metabolism of phytoestrogens and have the ability to refine phytoestrogens into compounds similar in structure to E_2 that protect against cancer (Xu *et al.*, 1995). Some of the proposed mechanisms by which phytoestrogens may inhibit cancer cells are: inhibition of DNA topoisomerase, suppression of angiogenesis, induction of differentiation in cancer cell lines and induction of apoptosis (Glazier and Bowman, 2001).

As studies continue to evaluate the biological effects of phytoestrogens on human health, the complexity is more evident as estrogenic and antiestrogenic effects are observed as well as a variety of mechanisms of action (Kronenberg and Hughes, 1999). More well designed clinical trials are needed to assess the beneficial effects of phytoestrogens on health (Naftolin and Stanbury, 2002).

Breast cancer

In western countries, breast cancer is the most common cancer affecting women (Parkin, 2001). Historically, the risk of breast cancer was much higher in American women than in Asian women prior to the influence of the western diet in Asian cultures (Bouker and Hilakivi-Clarke, 2000). Epidemiological studies of breast cancer and the dietary intake of soy and lignan have recently been reviewed (Peeters *et al.*, 2003), as well as the mechanisms of phytoestrogenic action in breast tissue (Adlercreutz, 2003). One task has been to find an estrogen replacement therapy for women at risk for breast cancer or who have survived breast cancer. A diet rich in phytoestrogens has been suggested as a preventative agent against breast cancer although there is conflicting evidence (Adlercreutz *et al.*, 2000b; Wagner *et al.*, 2001). Phytoestrogens act as weak estrogens and exhibit estrogenic activity in a low-estrogen environment; therefore it has been postulated that they show antiestrogenic activity in a high-estrogen environment (Messina and Loprinzi, 2001). This explanation suggests that prior to menopause when there is a high-estrogen environment phytoestrogens may protect against breast cancer and after menopause when there is a low-estrogen environment they may stimulate breast cancer (Anderson *et al.*, 1999). This theory is highly debated and many studies show conflicting evidence about the action of phytoestrogens and breast cancer.

Five *in vitro* studies of phytoestrogens on mammary cells have been reviewed and summarized by This and coauthors (2001). Biphasic effects of genistein in varying concentrations of culture medium were demonstrated on mammary cells (MCF-7 cancer cells). At physiological doses of 100 nM/L to 1 μM/L, genistein stimulates cellular proliferation. In the presence of physiological doses of E_2, genistein competes for the binding site of E_2 and slightly inhibits cellular proliferation. At doses greater than 10 μM/L genistein inhibits cellular proliferation most likely because of the inhibition of tyrosine kinase activity of growth factor receptors. Therefore the activity of genistein is highly dependent on its concentration and the concentration of estradiol in the culture medium (This *et al.*, 2001). Black cohosh, dong quai (*Angelica sinensis* (Oliv.) Diels, Apiaceae), ginseng (*Panax* spp., Araliaceae) and licorice root (*Glycyrrhiza glabra* L., Fabaceae) were tested on cell proliferation of MCF-7 human breast cancer cells and dong quai and ginseng were observed to stimulate the growth of MCF-7 cells (Amato *et al.*, 2002).

An *in vivo* study reported that newborn female rats treated with genistein and then exposed to a carcinogen showed an increased latency and lowered incidence and number of induced mammary tumors (Lamartiniere *et al.*, 1995). This antitumor activity was further confirmed by studies reviewed by Barnes (1997) that showed a reduction in the number of tumors observed in animals treated with genistein during neonatal and prepubertal periods as opposed to a later period (Barnes, 1997).

However, other studies have shown contradictory evidence and suggest that breast cancer patients should avoid soy. A commonly reported study found that dietary genistein may stimulate the growth of estrogen-dependent tumors in humans with low estrogen levels (Hsieh *et al.*, 1998). Ovariectomized athymic nude mice were implanted with MCF-7 cells and then either given a control diet, a diet with genistein, or a diet supplemented with estradiol (Hsieh *et al.*, 1998). The tumors were measured and the diets with genistein were observed to stimulate tumor growth. Another study with women in need of surgery for benign and malignant breast tumors, who were treated with 45 mg of isoflavones for 2 weeks, showed higher rates of breast cancer cell proliferation (McMichael Philips *et al.*, 1998).

There are many different mechanisms that have been suggested for the action of phytoestrogens on breast tissue. There are several isoforms of ER that may play a role in ERβ heterodimerization with ERα resulting in decreased estrogenic effects (Adlercreutz, 2002). Additional mechanisms proposed are: inhibition of tyrosine as well as other protein kinases, inhibition of angiogenesis, alteration of growth-factor activity and binding proteins (Adlercreutz, 2002).

In a case-controlled study, women (n = 144) who excreted high concentrations of phytoestrogens, equol and enterolactone, showed a significant lowered risk of breast cancer (Ingram *et al.*, 1997). Evidence from an epidemiological study suggests that a diet low in fat and high in soy proteins, vegetable oils and vegetables rich in carotene is associated with a lowered risk of breast cancer in premenopausal women (Lee and Gourley, 1991). Some methodologies do not determine the exact quantities of phytoestrogens ingested and therefore are problematic for comparisons. Plasma estrogens and breast cancer have been studied more than urinary estrogens because plasma estrogens are easier to identify (Kurzer, 2002). In evaluating various studies, methodologies must be considered when extrapolating conclusions. Studies often differ by subject age, timing and dose of administration, length of study and form of analysis. More research is needed to evaluate the different binding effects of phytoestrogens to ERα and ERβ for breast cancer.

Cardiovascular disease

The leading cause of death in women in industrialized nations is coronary heart disease (CHD). In menopause the risk of CHD greatly increases and it is proposed that this is due to the loss of estrogen (Wroblewski Lissin and Cooke, 2000). Lipid profiles, vascular reactivity, cellular proliferation and thrombosis are factors that affect CHD and on which phytoestogens have shown beneficial effects (Anderson *et al.*, 1999).

Mechanisms suggested to explain the prevention of cardiovascular disease and the reduction of atherosclerosis are: improvement of plasma lipid concentrations, reduction of thrombus formation such as inhibition of platelet action, improvement of systemic arterial compliance and antioxidant activity (Van der Schouw *et al.*, 2000). Phytoestrogens also bind to ERs and activate them, which allows homodimerization and interaction with estrogen response elements (ERE) that are regulatory sites on DNA molecules situated within target gene promoters (Van der Schouw *et al.*, 2000). Target gene transcription may be positively or negatively regulated by the DNA-bound receptor depending on the cellular and promoter context. Several mechanisms of action reported to explain the hypocholesterolemic effects of phytoestrogens include:

increased bile acid secretion, which aids removal of low density lipoprotein (LDL); affected hepatic metabolism coupled with increased removal of LDL by hepatocytes; and enhanced thyroid function (Wroblewski Lissin and Cooke, 2000). LDL shows increased oxidative resistance when isoflavones are incorporated into LDL cholesterol (Vincent and Fitzpatrick, 2000). Additional mechanisms of action have been suggested to explain the effects on plasma lipid concentrations including: action on ERs, reduction of endogenous cholesterol synthesis, and increased activity of cholesterol receptors (Glazier and Bowman, 2001). The isoflavone, genistein, has been shown to act as a protein tyrosine kinase inhibitor, which may explain decreased platelet action (Tham *et al.*, 1998).

Studies suggest that isoflavones as antioxidants may affect atherogenesis by reducing the oxidation of LDL (Ruiz-Larrea *et al.*, 1997; Wagner *et al.*, 1997). Kurzer and Xu (1997) reviewed antioxidant effects of isoflavones from *in vitro* and *in vivo* studies and reported that soy isoflavones act as antioxidants by directly or indirectly enhancing the activities of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase enzymes. Hwang and coworkers (2001) reported that extracts of soy, alfalfa and acerola cherry (*Malpighia glabra* L., Malpighiaceae) may synergistically interact to prevent LDL oxidation. Most of the research has been conducted with soy isoflavones and limited research has been conducted to evaluate the effects of lignans and coumestans on cardiovascular disease.

Prostate cancer

Prostate cancer is one of most common cancers for men in the United States, however, little is known about its etiology. *In vitro* studies using human prostate cancer cells have shown the inhibition of cell growth with high concentrations of phytoestrogens (Adlercreutz *et al.*, 2000a). Rats consuming soy and rye bran had delayed growth of implanted prostate tumors (Landstöm *et al.*, 1998). Further testing with the same phytoestrogens increased apoptosis of the tumors and reduced tumor growth in nude mice implanted with human prostate tumors. However, estrogen has shown controversial effects, such as growth of prostate cancer and benign prostatic hyperplasia and therefore phytoestrogens may have similar effects (Adlercreutz *et al.*, 2000a). Adlercreutz (2002) reviewed some of the more recent studies on prostate cancer and stated that findings support the hypothesis that soy consumption prevents prostate cancer, yet more studies are needed.

The interaction of phytoestrogens with ERs and ER isoforms such as ERβ2 or ERβcx in the prostate as well as other receptors can lead to a variety of biological actions (Morrissey and Watson, 2003). The relative binding affinity for different ERs and the formation of the ER complex, which can bind to EREs may explain the actions observed in the prostate. Other factors that affect whether phytoestrogens have a positive or negative effect on the prostate may be the ER co-factors and phosphorylation status (Morrissey and Watson, 2003). In addition, the effects on growth factor receptors have been suggested because of the inhibition of growth-factor-mediated stimulation of cell proliferation (Adlercreutz, 2002). Other mechanisms may be due to the regulation of steroid receptor pathways, antiandrogenic effects, inhibition of 5α-reductase, 17β-hydroxysteroid dehydrogenase and aromatase activity, inhibition of tryrosine specific protein kinases and DNA topoisomerase II and antioxidant activity (Morrissey and Watson, 2003).

Several epidemiological studies suggest the beneficial use of phytoestrogens in reducing prostate cancer (Severson *et al.*, 1989; Jacobsen *et al.*, 1998; Strom *et al.*, 1999). A human study of 83 cases and 107 controls used a dietary questionnaire to evaluate phytoestrogen consumption for prostate cancer risk (Strom *et al.*, 1999). The results showed slightly protective effects on prostate cancer risk with greater consumption of phytoestrogens. Severson and coworkers (1989) showed that increased tofu consumption was associated with a decreased risk of prostate cancer in men of Japanese ancestry living in Hawaii. Another study reported that Adventist men who consumed soy milk daily were at lower risk for prostate cancer (Jacobsen *et al.*, 1998). However, this study had a small number of cases and therefore the confidence interval was wide. An herbal mixture including licorice and ginseng as well as six other herbs has shown estrogenic activity and was effective in two cases of hormone-refractory prostate cancer (De la Taille *et al.*, 2000). Other studies have evaluated alternative therapies, such as soy, black cohosh, vitamin E and red clover for their potential use in alleviating hot flashes for prostate cancer patients (Moyad, 2002). A recently published randomized cross-over study on soy food consumption and serum prostate specific antigen (PSA) in men with hyperlipidemia showed lowered LDL, no significant effects on serum PSA, and a reduced calculated risk for CHD (Jenkins *et al.*, 2003).

There is a paucity of research on the effects of phytoestrogens and prostate cancer. Although the present studies show a positive association between phytoestrogens and prostate cancer risk, more clinical studies are needed to confirm this hypothesis.

Menopausal symptoms

The symptoms associated with menopause cause many women to seek medical solutions. Hormone replacement therapy has proven effective in the reduction of hot flushes, yet it is still controversial if HRT may be associated with increased risks of breast and endometrial cancer. Initial findings from the WHI randomized controlled trial in which women received a daily dose of conjugated equine estrogen (0.625 mg) and medroxyprogesterone acetate (2.5 mg) have shown an increased risks to benefits ratio (Rossouw *et al.*, 2002). The investigators detected increased risks for invasive breast cancer and CHD with the consumption of the combined hormone preparation after 5.2 years of average follow-up. Due to controversial evidence on HRTs, alternative therapies have been sought, such as phytoestrogens and SERMs.

The mechanisms of action are still poorly understood because of the complex biological actions observed in phytoestrogens. As discussed before, the target tissue, the kinds of ERs and the concentration of endogenous estrogens are all factors that affect phytoestrogen activity at the cellular level. Other non-receptor mechanisms may also explain the biological effects of

phytoestrogens on menopausal symptoms, such as antioxidation, blocking of enzymes involved in the biosynthesis of estrogen, inhibition of protein kinase which is part of intracellular signaling, and inhibition of 5α-reductase and aromatase (Vincent and Fitzpatrick, 2000). The reduction of bioavailability levels of free sex steroids such as estrogen may also be the result of phytoestrogens stimulating sex hormone-binding globulin (SHBG) synthesis (Vincent and Fitzpatrick, 2000).

Some botanicals used in Western countries for menopausal syndromes are black cohosh, dong quai, ginseng, red clover, hops (*Humulus lupulus* L., Cannabaceae), oil of evening of primrose (*Oenothera biennis* L., Onagraceae) and chasteberry or chaste tree berry (*Vitex agnus-castus* L., Verbenaceae) (Liu *et al.*, 2001b; Kronenberg and Fugh-Berman, 2002). Several reviews have discussed studies conducted on phytoestrogens and menopausal symptoms and still much contradictory evidence exists as to the benefits of phytoestrogens (Glazier and Bowman, 2001; Merritt, 2001; Kang *et al.*, 2002; Kronenberg and Fugh-Berman, 2002; Wuttke *et al.*, 2003b). More research is needed in this area, especially studies examining the long-term effects of phytoestrogens on endometrial tissue and bone loss.

Osteoporosis/bone health

Osteoporosis is often associated with women in menopause. The evidence supports ERT in the prevention of osteoporosis in postmenopausal women and therefore phytoestrogens have been evaluated for their effects on bone mineral density. Researchers hypothesize that a diet rich in isoflavones has a protective effect on bone (Tham *et al.*, 1998). Ipriflavone, a synthetic isoflavone derivative (7-isopropoxy-isoflavone), has been used extensively in animal and human studies to evaluate bone health and phytoestrogens with beneficial results (Scheiber and Rebar, 1999). Vincent and Fitzpatrick (2000) reviewed three animal studies and concluded that genistein has a biphasic effect, lower doses improved bone mineral as opposed to high doses, on bone mineral density in ovariectomized rats. Van der Shouw and coworkers (2000) reviewed three studies in bone mineral density with phytoestrogen consumption that were conducted with postmenopausal women. Two of the studies showed an increase in bone mineral density and the third study, a 10-year follow-up study conducted in the Netherlands, reported a loss of bone associated with higher urinary equol and enterolactone excretion.

Kurzer and Xu (1997) reviewed several other studies that include possible mechanisms of action to explain the beneficial effect of phytoestrogens on bone loss. These mechanisms include preventing urinary calcium loss, beneficial effects on osteoblasts, and influences on the secretion of calcitonin which suppresses bone resorption. In addition, soy foods are considered a good source of calcium. Estrogen receptors have been found in osteoblasts, which may cause alteration in some protein production. The phytoestrogen–ER complex (formed by the binding of phytoestrogens and the ER) may bind to EREs and inhibit or suppress specific gene expression (Anderson *et al.*, 1999). More long-term studies are needed on bone density and fracture rates to determine safety, efficacy and appropriate dosage.

Cognition

Cognition and memory functioning have been reported to decrease around menopause, and therefore studies have investigated the association of ERT and cognition, as well as phytoestrogens and cognition (Vincent and Fitzpatrick, 2000). However, limited studies are available on the effects of phytoestrogens on cognitive functioning. A study with female rats proposed that soy phytoestrogens function as estrogen agonists because they increased choline acetyltransferase and nerve growth factor messenger RNA in the frontal cortex and hippocampus (Pan *et al.*, 1999). Other human studies have also suggested improved memory with dietary phytoestrogens (File *et al.*, 2001; File *et al.*, 2002).

Contradictory evidence has also been reported. One review paper summarizes two studies on tofu consumption in Hawaii that suggest increased cognitive dysfunction (Sirtori, 2001). The mechanisms are not understood, but it has been suggested that phytoestrogens act as estrogen agonists and may increase spine density and synapse formation in the hippocampus of adults (File *et al.*, 2003). In addition, phytoestrogens may interact with the transcription of neurotrophin genes (File *et al.*, 2003). More research is needed to understand the effect phytoestrogens might have on cognitive functioning.

BOTANICAL SOURCES STUDIED FOR PHYTOESTROGENS

Plant compounds can be limited to specific botanical families that may assist researchers in identifying other species that contain the same compounds. This concept is referred to as chemotaxonomy. For example, there is a higher concentration of phytoestrogens in legume plants even though they are also found in grains, vegetables and fruits distributed across the plant kingdom. The most common phytoestrogens found in legumes are isoflavones. Isoflavones are part of the isoflavonoids, which are almost exclusively limited in distribution to the Fabaceae family and more specifically to the subfamily Papilionaceae (Dewick, 1993). The other subfamilies in Fabaceae, Mimosaceae and Caesalpiniaceae, have shown very few plants with isoflavonoids. A few isolated cases of isoflavonoid derivatives have been reported in other plant families in the dicotyledons. Of the monocotyledons, species of *Iris* (Iridaceae) are a major source of isoflavonoids. Of the gymnosperm, the genera *Juniperus* and *Podocarpus* have been reported to produce isoflavonoids (Dewick, 1993).

Phylogenetic analysis uses chemical, molecular and traditional systematic data (such as plant anatomy) to suggest evolutionary relationships of plant taxa (Daly *et al.*, 2001). This type of analysis provides a useful tool that can guide researchers in more effectively locating specific plant compounds because it elucidates chemotaxonomies (Daly *et al.*, 2001).

Based on the phylogenetic tree of Daly and coworkers (2001), plants that have phytoestrogens tend to be concentrated in an upper evolutionary group that consists of the botanical orders, Fagales, Cucurbitales, Rosales, Fabales and Malpighiales). Cucurbitales contains Cucurbitaceae (pumpkin), Rosales contains Cannabaceae

Table 1. Plants with estrogen receptor binding activity and activity in estrogen dependent breast cancer cell lines

Order	Family (Subfamily)	Botanical name (Common name)	Phytoestrogen (Class)	Reference
Rosales	Cannabaceae	*Humulus lupulus* (hops)	8-Prenylnaringenin (flavone)	(Hesse *et al.*, 1981; Eagon *et al.*, 1997; Zava *et al.*, 1998; Dixon-Shanies and Shaikh, 1999; Milligan *et al.*, 1999; Liu *et al.*, 2001b)
	Moraceae	*Maclura pomifera*	(sterol or isoflavone)	(Maier *et al.*, 1995)
		Morus microphylla	Not identified	(Maier *et al.*, 1995)
	Rosaceae	*Prunus africanum syn. Pygeum africanum*	Not identified	(Mathé *et al.*, 1995)
Fabales	Fabaceae (Caesalpiniaceae)	*Senna obtusifolia* syn. *Cassia obtusifolia*	Not identified	(Matsuda *et al.*, 2001)
			Genistein, daidzein, formononetin, biochanin A, glycitein (isoflavone), coumestrol (coumestan)	(Zava *et al.*, 1998; Song *et al.*, 1999; Boue *et al.*, 2003)
	Fabaceae (Papilionaceae)	*Glycine max* (soy)	Glabridin (flavonoid), glabrene (isoflavene), isoliquiritigenin (flavonoid), licochalcone-A, 2′,4′,4′-three hydroxyl chalcone (chalcone)	(Zava *et al.*, 1998; Rafi *et al.*, 2000; Tamir *et al.*, 2000, 2001)
		Glycrrhiza glabra (licorice)		
		Medicago sativa (alfalfa)	Coumestrol (coumestan), apigenin (flavone), daidzein, genistein (isoflavone), secoisolariciresinol (lignan)	(Mazur, 1998; Boue *et al.*, 2003)
		Phaseolus vulgaris (bean)	Daidzein, genistein (isoflavone), secoisolariciresinol (lignan)	(Mazur, 1998; Boue *et al.*, 2003)
		Pueraria lobata (kudzu)	Puerarin, daidzin, genistin, daidzein and genistein (isoflavone), secoisolariciresinol (lignan)	(Mazur, 1998; Boue *et al.*, 2003)
		Trifolium pratense (red clover)	Genistein, daidzein, formononetin, biochanin A (isoflavone), coumestrol (coumestan)	(Zava *et al.*, 1998; Liu *et al.*, 2001b; Boue *et al.*, 2003)
		Vigna radiata (mung bean)	Coumestrol (coumestan), (isoflavone)	(Boue *et al.*, 2003)
Malpighiales	Linaceae	*Linum usitatissimum* (flax)	Matairesinol, isolariciresinol (lignan)	(Adlercreutz *et al.*, 1992)
Brassicales	Brassicaceae	*Brassica oleracea* (cabbage, brussel sprout)	Secoisolariciresinol (lignan)	(Mazur, 1998; Ju *et al.*, 2000)
Apiales	Apiaceae	*Angelica sinensis* (dong quai)	Not identified	(Eagon *et al.*, 1997; Dixon-Shanies and Shaikh, 1999)
	Araliaceae	*Eleutherococcus senticosus* (Siberian ginseng)	Not identified	(Pearce *et al.*, 1982)
		Panax notoginseng (San-chi or Tien-chan ginseng)	Ginsenosides Rg1 (saponin)	(Chan *et al.*, 2002)
Solanales	Solanaceae	*Mandragora autumnalis* (mandrake)	Not identified	(Zava *et al.*, 1998)
Lamiales	Lamiaceae	*Leonurus cardiaca* (motherwort)	Not identified	(Zava *et al.*, 1998)
		Thymus vulgaris (thyme)	Not identified	(Zava *et al.*, 1998)
	Verbenaceae	*Verbena officinalis* (vervain)	Not identified	(Zava *et al.*, 1998)
		Vitex agnus-castus (chasteberry)	Not identified	(Dixon-Shanies and Shaikh, 1999; Liu *et al.*, 2001b)

Table 1. (*Continued*)

Order	Family (Subfamily)	Botanical name (Common name)	Phytoestrogen (Class)	Reference
Gentianales	Rubiaceae	*Coffea arabica* (coffee)	Matairesinol, secoisolariciresinol (lignan)	(Kitts, 1987; Mazur *et al.*, 1998)
		Uncaria tomentosa (cat's claw)	Not identified	(Salazar and Jayme, 1998)
Caryophyllales	Polygonaceae	*Fallopia multiflora* syn. *Polygonum multiflorum*	(anthraquinone)	(Matsuda *et al.*, 2001)
		Polygonum cuspidatum (hu-chang)	Emodin, emodin 8-*O*-β-D-glucopyranoside (anthraquinone)	(Matsuda *et al.*, 2001)
		Rheum coreanum	(anthraquinone)	(Matsuda *et al.*, 2001)
		Rheum officinale	(anthraquinone)	(Matsuda *et al.*, 2001)
		Rheum palmatum	(anthraquinone)	(Matsuda *et al.*, 2001)
		Rheum rhabarbarum	(anthraquinone)	(Matsuda *et al.*, 2001)
		Rheum tanguticum	(anthraquinone)	(Matsuda *et al.*, 2001)
		Rheum undulatum	(anthraquinone)	(Matsuda *et al.*, 2001)
		Rumex acetosella (sheep sorrel)	Not identified	(Zava *et al.*, 1998)
		Rumex crispus (yellow dock)	Not identified	(Zava *et al.*, 1998)
Ranunculales	Papaveraceae	*Sanguinaria canadensis* (bloodroot)	Not identified	(Zava *et al.*, 1998)
Zingiberales	Zingiberaceae	*Curcuma longa* (tumeric)	Not identified	(Zava *et al.*, 1998)
Poales	Poaceae	*Avena sativa* (oat)	Secoisolariciresinol (lignan)	(Uusitupa *et al.*, 1992; Mazur, 1998)
Arecales	Arecaceae	*Serenoa repens* (saw palmetto)	Not identified	(Di Silverio *et al.*, 1992)
Liliales	Liliaceae	*Aloe arborescens*	Not identified	(Matsuda *et al.*, 2001)
		Aloe ferox	Not identified	(Matsuda *et al.*, 2001)
		Dracaena loureiroi	5,7-Dihydroxy-3-(4-hydroxylbenzyl)-4-chromanone (homoisoflavone), 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone (retrodihydrochalcone)	(Ichikawa *et al.*, 1997)
Asparagales	Agavaceae	*Yucca* sp.	Not identified	(Zava *et al.*, 1998)
Magnoliales	Annonaceae	*Anaxagorea luzonensis*	8-Isopentenylnaringenin (prenylflavonoid)	(Kitaoka *et al.*, 1998)

(hops) and Fabales contains Fabaceae (soy, licorice, red clover) and Malpighiales (flax). Other plant species that have shown estrogenic activity are found in different orders located at basal positions from the initial group discussed: Myrtales (evening primrose), Apiales (dong quai, ginseng) and Lamiales (chasteberry). Although estrogenic activity has been reported for these plant species, specific phytoestrogens have not been identified. Chemotaxonomy offers an innovative way to select potential plant sources for further testing. For example, the phylogenetic relationships suggest that research should be conducted in plant families found within the initial group discussed above. Although the majority of studies on phytoestrogens have been conducted on soy, other botanical species have also been included in this discussion.

Soy

Soy or soybean (*Glycine max*) belongs to the Fabaceae family and has long been used as a food plant. Of the bean plants it has one of the highest levels of protein and oil (Duke, 1981). Medicinally it has been reported in ancient Chinese herbals for the healthy functioning of the heart, kidneys, liver and stomach (Duke and Ayensu, 1985). It was domesticated in China around the eleventh century BC and there are many varieties. The Chinese distinguish the different varieties by color. Black seeds are associated with medicine and have been used for strength and vigor as well as in mixtures for post-partum and sexual disorders (Li, 1973). The black bean sprouts have been used as a laxative, for rheumatism and hair growth. A 'bean relish' made of salted fermented beans has been highly valued in Chinese medicine and has multiple uses including colds, headaches, hemorrhaging in abortion, threatened abortion, difficult labor, irritability and fever (Li, 1973). In the 1940s, genistin, the genistein glycoside, was first reported in soybean oil meal and was later shown to have estrogenic activity in mice (Cheng *et al.*, 1953). Soy has been well studied for phytoestrogens and numerous articles have been published on the benefits and risks of soy over the last few years, therefore we will present some of the more recent research.

It is unclear whether soy protein with trace amounts of isoflavones (phytoestrogen-extracted soy protein), phytoestrogen-intact soy protein, or a combination of both causes the beneficial cholesterol effects seen in animal studies (Van der Schouw *et al.*, 2000). Consumption of soy protein has shown a decrease in lipid peroxidation compared with casein consumption in postmenopausal cynomolgus monkeys and lowered atherosclerosis in rabbits (Wagner *et al.*, 1997; Van der Schouw *et al.*, 2000). Isoflavone-intact soy protein has lowered LDL and raised high density lipids (HDL) cholesterol suggesting that the active components are found in the extractable protein portion (Clarkson and Anthony, 1998). When acetylcholine was administered to rhesus monkeys fed intact isoflavones, their arteries dilated while the group fed isoflavones extracted from soy constricted them (Honoré *et al.*, 1997). These results are supported by another study with rhesus female monkeys that observed that a diet of soy with intact isoflavones sustained normal coronary blood flow after an intracoronary infusion of collagen possibly due to platelet aggregation being inhibited or because platelets release vasoconstrictors (Williams and Clarkson, 1998). Two studies showed that a diet of soy protein inhibited atherosclerotic plaque formation as opposed to animal protein, yet more research is needed to determine if the benefits are mediated by soy protein or phytoestrogens (Van der Schouw *et al.*, 2000). Further investigations are needed to elucidate the effects of phytoestrogen dietary supplements, which are often isolated from soy and then encapsulated and sold in tablets or capsules.

A meta-analysis of 38 studies reported that a diet containing an average of 47 g soy protein decreased total cholesterol by roughly 9.3%, LDL cholesterol by 12.9%, and triglycerides by 10.5% and increased high-density lipoprotein (HDL) cholesterol by 2% (Anderson *et al.*, 1995). Another review reported that several human studies found evidence that a diet of soy protein reduces LDL (Wroblewski Lissin and Cooke, 2000). In a randomized clinical trial conducted for 9 weeks with hypercholesterolemic men and women, increased isoflavone doses (25 mg, 42 mg or 58 mg) in soy protein showed a lower LDL and total cholesterol concentration suggesting a dose-response relationship (Crouse *et al.*, 1998). A placebo-controlled crossover trial with menopausal and perimenopausal women showed improved systemic arterial compliance with the daily consumption of soy isoflavone supplements (Nestel *et al.*, 1997). However, four small studies showed no statistically significant association between a soy isoflavone diet and an increase in HDL cholesterol for pre-menopausal women; and the effects on LDL cholesterol were conflicting (Cassidy *et al.*, 1995). Further findings showed that isoflavone pills improved arterial compliance but did not show effects on plasma lipids (Clarkson and Anthony, 1998).

A reduction of the relative risk of breast cancer in premenopausal women who consume a soy rich diet has been reported (Ingram *et al.*, 1997) while others indicate a moderate and non-significant reduction of this risk (Petrakis *et al.*, 1996; Hargreaves *et al.*, 1999). Kurzer (2002) reviewed hormonal effects, including concentrations of reproductive hormones in the blood, menstrual cycle lengths and menstrual phase lengths, of soy in premenopausal women and reported that lowered risk of breast cancer is often associated with a longer menstrual cycle, reduced estrogens, increased sex-hormone binding globulin and increased excretion of 2 to 16 α-hydroxy estrogens. Time exposure has also been proposed to explain the benefits of soy. Limited evidence exists to suggest that soy consumption in adult life is protective against breast cancer, however, high levels of consumption (Peeters *et al.*, 2003) or consumption throughout life (Adlercreutz, 2002) could potentially reduce the risk of breast cancer.

A detailed discussion of soy for women who have survived breast cancer has been presented (Messina and Loprinzi, 2001). The avoidance of soy for breast cancer patients has been reported in one study that showed increased breast nipple aspirate fluid (NAF) secretion and breast cell hyperplasia in postmenopausal women on ERT who consumed daily 80 mg of soy isoflavones over 5 months (Petrakis *et al.*, 1996). A drawback of this study is the lack of a control group and fluid secretion continued to increase in the patients even after they ceased their consumption of soy. Presently soy

isoflavones have not been reported to show significant effects on endometrial tissue (Vincent and Fitzpatrick, 2000; Balk *et al.*, 2002).

Several studies have reviewed the use of soy for HRT (Burke *et al.*, 2000; Kronenberg and Fugh-Berman, 2002). A double-blind parallel multi-center randomized placebo controlled trial of 104 postmenopausal women who took 60 g of isolated soy protein daily (76 mg of isoflavones) versus placebo reported that the mean number of daily hot flushes were significantly reduced in the group consuming soy ($p < 0.01$) (Albertazzi *et al.*, 1998). Other studies have also shown positive effects with soy extracts (Scambia *et al.*, 2000; Faure *et al.*, 2002; Han *et al.*, 2002). However, negative and mixed results of soy for menopausal symptoms have also been reported (Quella *et al.*, 2000; St. Germain *et al.*, 2001). These studies often differ by soy product, dose, length of study and subjects therefore making it difficult to compare the results. More studies are needed to elucidate the effects and safety of soy on women with a history of breast cancer and osteoporosis.

Black cohosh

Black cohosh (*Actaea racemosa*, *Cimicifuga racemosa*) is a North American plant in the buttercup family (Ranunculaceae) that grows in Eastern North America, from southern Maine to Georgia (Ramsey, 1997). Black cohosh, also known as baneberry, black snakeroot, bugbane and rattleweed is used for various women's health conditions. Native Americans used the roots and rhizomes for a variety of indications such as stimulation of menstrual flow, dysmenorrhea, suppression of cough, treatment of diarrhea, childbirth and rheumatism (Foster, 1999). The 19th century American Eclectic physicians recognized black cohosh to be 'very efficacious in maladies of the female reproductive organs' (King and Newton, 1852).

Knowledge of this useful herb soon reached Europe, where it has been a popular herbal medicine for menopausal symptoms for over 50 years. A standardized black cohosh extract (Remifemin™) was developed in Germany, and this product has been studied both in animals and short-term clinical trials of menopausal women. According to these studies the extract appears to provide relief for hot flashes (Jarry *et al.*, 1985; Stoll, 1987).

A historical study using the Allen-Doisy bioassay did not detect estrogenic activity in black cohosh, which was part of the Lydia E. Pinkham mixture (Costello and Lynn, 1950). More recent studies reported that formononetin was detected in a methanol extract of a black cohosh sample that previously bound to an ER *in vitro* (Jarry and Harnischfeger, 1985; Jarry *et al.*, 1985), although this is surprising based on chemotaxonomy. Others have reported detecting additional flavonoids, such as kaempferol (Schmitz, 1993) and biochanin A, genistein 4′-methyl ether (McCoy and Kelly, 1996). The authors speculated that black cohosh may work through estrogen-like compounds (Jarry *et al.*, 1985), but to date this has not been established. Others have looked for and not found formononetin in black cohosh extracts (Struck *et al.*, 1997; Kennelly *et al.*, 2002).

In vitro data on black cohosh in breast cancer cell lines is limited and the case is being made in both the scientific and popular press that the biological activity of black cohosh is not estrogenic, but rather, through some SERM or non-steroidal compound that has clinical effects similar to some of the activities of estrogen. Inconsistent results are reported for *in vitro* studies using black cohosh extracts and estrogen receptor binding activity and stimulation of breast cancer cells (Jarry and Harnischfeger, 1985; Jarry *et al.*, 1985; Düker *et al.*, 1991; Nesselhut *et al.*, 1993; Harnischfeger and Cillien, 1996; Zava *et al.*, 1998; Dixon-Shanies and Shaikh, 1999). Fukinolic acid, a black cohosh constituent, showed increased cell proliferation of an estrogen dependent MCF-7 cell system with reference to estradiol (Kruse *et al.*, 1999). Another study tested estrogenic and antiestrogenic effects of black cohosh extracts on proliferation of MCF-7 cells and on gene expression using ethanol and isopropanol extracts and concluded that black cohosh contains antiestrogenic compounds because the extract antagonized estradiol induced activities in the different experiments (Zierau *et al.*, 2002). Another recent investigation found that black cohosh extract did not display significant competitive binding to ERα nor ERβ (Liu *et al.*, 2001a). Furthermore, in this same study black cohosh did not show estrogen activity, as indicated by alkaline phosphatase (AP) activity with cultured Ishikawa (endometrial) cells. In addition, black cohosh extracts do not have any estrogenic activity in a number of assays where ER-positive breast cancer cells were used (personal communication, Dr Ruth Lupu).

Kronenberg and Fugh-Berman (2002) reviewed randomized, controlled trials of Remifemin™ for menopausal symptoms conducted in Germany and the United States. Three of the four trials report black cohosh helpful for hot flashes, although more research is needed to examine long-term effects. A recent study reported beneficial effects of combined administration of tamoxifen and black cohosh extract (CR BNO 1055) for hot flushes in women surviving breast cancer (Hernández Muñoz and Pluchino, 2003). A double-blind placebo-controlled study with postmenopausal women demonstrated that CR BNO 1055 was comparable to conjugated estrogens in treating climacteric complaints and maintaining bone metabolism, and did not increase endometrial thickness (Wuttke *et al.*, 2003c).

Investigators propose that black cohosh contains unidentified compounds that produce estrogenic effects (Kronenberg and Fugh-Berman, 2002; Wuttke *et al.*, 2003c). An additional hypothesis is that the compounds in black cohosh interact with a third estrogen receptor, ERγ, yet to be identified in humans but recently reported in teleost fish (Jarry *et al.*, 2003). Metabolic conversion of precursor molecules, serotonin receptor blocking activity and dopaminergic compounds have also been suggested as potential mechanisms of action for black cohosh (Jarry *et al.*, 2003). Currently, black cohosh is in a clinical trial at Columbia University and the University of Illinois at Chicago for the treatment of menopausal hot flashes, as well as assessment of cognitive function and bone metabolism.

Most studies have reported that black cohosh extract is free of significant side effects. However, the length of these studies is not sufficient to insure safety with respect to uterine tissue and function. Further studies are needed to elucidate the mechanism of action of black cohosh. In addition, studies are needed to determine

whether users of black cohosh may benefit from decreased risk of fractures and cardioprotective effects, as purported of traditional ERT, as well as to clarify whether black cohosh extract stimulates breast cancer cells *in vivo*, *in vitro* and in women.

Red clover

Red clover (*Trifolium pratense*) in the Fabaceae family is a herb that is indigenous to Europe and parts of the Middle East and has naturalized to North America (Mabberley, 1997). It is well known as animal fodder. Humans have rarely consumed red clover, although it has been used medicinally. In the beginning of the twentieth century a 'Trifolium compound' that included red clover blossoms along with other botanicals was marketed by pharmaceutical companies for venereal disease, although there was little evidence to support this use (Foster and Tyler, 1999). Traditionally it has been reported by Native American Iroquis as a gynecological aid for 'the change of life' (Herrick, 1995). The Celtics and Romans employed red clover as a sedative. It has also been used to purify blood, treat skin conditions and for bronchial asthma because it reduces muscle spasm and is a decongestant (Keville, 1999).

The estrogenic activity of red clover was initially recorded in 1950 (Legg, 1950). Later the estrogenic compounds were identified as formononetin, biochanin A, daidzein and genistein (Mazur, 1998). Coumestrol has been reported in clover but the species was not specified (Franke *et al.*, 1995). Although *in vitro* data on red clover are limited, studies do support the plant's estrogenic activity. Red clover extracts tested in four *in vitro* assays showed consistent estrogenic effects with the highest binding potency for both ERs compared with other plant extracts tested (Liu *et al.*, 2001b). Genistein appeared as the most active component in AP induction ability and up-regulation of the progesterone receptor (*PR*) expression in Ishikawa cells and there was no evidence of antiestrogenic activity. This study supports the results of earlier work using the radioreceptor assay that showed red clover extracts bound to the ER of MCF-7 cells and to the *PR* of T47-D cell lines (Zava *et al.*, 1998). Two preparations of Menoflavon®, a red clover extract, showed a higher binding affinity to ERβ than ERα in a transactivation assay (Dornstauder *et al.*, 2001).

The *in vivo* estrogenic and antiestrogenic effects of red clover extract have been studied in the uterus, vaginal cells and mammary glands of ovariectomized Sprague-Dawley rats (Burdette *et al.*, 2002). Uterine weight and thickness were increased with the extract, although less than increases observed with E_2 and there was no evidence of antagonizing effects on E_2. Red clover extract did induce partial cornification of vaginal cells at high doses after 2 weeks. Lastly, no estrogenic or antiestrogenic effects of red clover extract were observed on the mammary gland (Burdette *et al.*, 2002).

Several human studies have evaluated red clover for a variety of health conditions such as breast cancer, cardiovascular disease and menopausal symptoms. Rimostil™, a red clover extract, has significantly increased HDL cholesterol in serum at different doses (28.5, 57 and 85.5 mg/day) and lowered levels of HDL cholesterol in serum after stopping the treatment; however, there was no control group in this study (Clifton-Bligh *et al.*, 2001). A double-blind placebo-controlled trial with postmenopausal women consuming isoflavones daily derived from red clover reported improved arterial compliance, although the study design has been questioned as well as the high dropout rate (Nestel *et al.*, 1999). On the other hand, a randomized double-blind study using purified isoflavones, predominately formononetin and biochanin A from red clover, did not significantly change the total plasma cholesterol, LDL cholesterol, HDL cholesterol or plasma triglyceride levels in postmenopausal women with slightly elevated plasma cholesterol levels (Howes *et al.*, 2000).

The effects of red clover on endometrial cancer have also been studied. A double-blind randomized, controlled study using purified isoflavones (50 mg daily) from red clover did not show reduced cell proliferation in the endometrium of 30 women ranging in age from 45 to 50 based on the detection of a Ki-67 antigen in endometrial biopsy specimens (Hale *et al.*, 2001). Small sample size, the type of isoflavone treatment and the limited timing of examination in the menstrual cycle may have affected the results.

Red clover has been reviewed for menopausal symptoms (Fugh-Berman and Kronenberg, 2001; Kronenberg and Fugh-Berman, 2002). A red clover extract, Promensil™, contains isoflavones in the aglycone form and was used in a randomized, double-blind, placebo-controlled trial with 30 menopausal women. Significant differences between control and placebo group were observed at week 8 and 12 with the control group showing reduced hot flush count (Van der Weijer and Barentsen, 2002).

One note of caution about red clover is the potential presence of coumarins that have been reported in some clover species, often in damaged plant tissue. These compounds act as anticoagulants and therefore should be evaluated in red clover extracts prior to consumption (Fugh-Berman and Kronenberg, 2001). Additional studies are needed to further elucidate the benefits or adverse effects of red clover as a source of phytoestrogens.

Flax

Flax (*Linum usitatissimum*) in the Linaceae family is an herb that is considered one of the oldest continuously cultivated plants. The Latin name means 'most useful' (Haggerty, 1999). As a fiber, it is well-known in both tropical and temperate regions and was found in the Egyptian tombs. Several European Pharmacopoeias have included it as a medicinal plant (Grieve, 1985). The seeds, also known as linseed, are medicinal and have been used to make flour; they produce mucilage when infused or boiled in water. Flax has been used for colds, coughs and fevers, to reduce inflammation and as a demulcent. Native American Cherokee used flax for fevers and 'violent colds, coughs and diseases of lungs' (Hamel and Chiltoskey, 1975) and the oil has been used as a laxative (Haggerty, 1999). In the Middle Ages the flowers were used for protection against sorcery (Grieve, 1985). Flax has been attractive as an oil seed because it contains polyunsaturated fatty acids such

as α-linolenic acid, which may lower cholesterol and have antioxidant effects for health.

Flaxseed is considered one of the richest sources of lignan phytoestrogens (Thompson *et al.*, 1991), although a recent study has reported Norway spruce as another rich source (Saarinen *et al.*, 2000). Several studies have developed techniques to identify and quantify phytoestrogens in flaxseed (Obermeyer *et al.*, 1995; Muir and Westcott, 2000; Charlet *et al.*, 2002), yet limited clinical studies have been conducted on the phytoestrogens in flax and their role in human health.

In rats flaxseed consumption has resulted in developmental reproductive changes (Tou *et al.*, 1998), changes in estrous cycle (Orcheson *et al.*, 1998) and menstrual cycle (Phipps *et al.*, 1993). One *in vivo* study with nude mice fed a diet supplemented with 10% flaxseed showed a decrease in tumor growth rate and metastasis as well as lowered extracellular levels of vascular endothelial growth factor (Dabrosin *et al.*, 2002). Another study testing spermatogenesis and testis structure in Sprague-Dawley rats showed no effect on maternal ingestion of flaxseed during lactation and gestation followed by postnatal consumption of the same diet as the mother (Sprando *et al.*, 2000).

In a randomized, crossover trial of two 3-week periods with defatted flaxseed lignans there was a reduction in LDL cholesterol, total cholesterol, and apolipoprotein B and A-1 and no significant effect on HDL cholesterol. However, consumption of flaxseed did reduce protein thiol groups compared with the controls, which may suggest increased oxidative activity (Jenkins *et al.*, 1999). A 12 week study of 145 women with climacteric complaints showed a reduction in menopausal symptoms (including hot flush and vaginal dryness) with the consumption of a diet rich in phytoestrogens (including soybean foods and flaxseed) (Brzezinski *et al.*, 1997). Before radical prostatectomy, a powdered flaxseed and low-fat diet was tested in a pilot trial, although beneficial, the estrogenic effects were not determined. Significant decreases in total testerosterone and free androgen were observed which may be due to reduced fat intake (Demark-Wahnefried *et al.*, 2001). Additional studies are needed to elucidate the estrogenic activity of lignans in flax.

Licorice

The licorice (*Glycyrrhiza glabra*) plant is a perennial, belonging to the Fabaceae family and indigenous to Eurasia. In Greek *Glycyrrhiza* means 'sweet root' (Wang and Nixon, 2001). The sweet yellow wood of the licorice root has been consumed for thousands of years in China for its health benefits and detoxification effects as well as its use as a flavoring and sweetening agent (Foster and Tyler, 1999; Wang and Nixon, 2001). The earliest recorded medicinal use is most likely around 2100 BC (Gibson, 1978). Medicinally it has been used as a demulcent and expectorant and has been shown to have antioxidant and antimicrobial activity. In the United States, licorice is added to tobacco as well as candies, toothpaste and beverages (Wang and Nixon, 2001). Other medicinal uses are as a tonic, to quench thirst, for asthma, fever and externally for burns and sores (Li, 1973; Gibson, 1978). Although it is not indigenous to North America, reports indicate that it was purchased by Native Americans and used for female troubles (Smith, 1928) as well as for coughs, asthma and as an expectorant (Hamel and Chiltoskey, 1975). The main components of licorice are glycyrrhizin (glycyrrhizinic acid), which is sweeter than sugar, and glycyrrhetinic acid. Both have been clinically used in the treatment of hyperlipidemia, allergic inflammation, atopic dermatitis and atherosclerosis (Tamir *et al.*, 2001).

The estrogenic activity of licorice was first published in 1950 (Costello and Lynn, 1950). Licorice has shown antiprogestin activity, which may be mediated by other compounds because purified glycyrrhizin did not bind to ER or *PR* (Zava *et al.*, 1998). However, in another study licorice extracts showed weak binding affinity to both the ERs as well as weak stimulation of *PR* expression (20 μg/mL) (Liu *et al.*, 2001b). Glabrene, glabridin, and isoliquiritigenin, 2′,4′,4′-three hydroxyl chalcone (ILC) found in the ethanol extract of licorice root show estrogenic activity (Tamir *et al.*, 2001). Glabrene, an isoflavene, has been reported as a new class of phytoestrogens and glabridin is a novel phytoestrogen that is lipophilic and structurally similar to E_2 (Tamir *et al.*, 2001). It has been isolated from licorice extract and appears to have ER mediated estrogen effects. Glabridin binds to human ER and *in vitro* showed biphasic effects on estrogen-dependent human breast cancer cells and increasing concentrations showed biphasic effects on the growth of anchorage-independent growth MCF-7 cells (Tamir *et al.*, 2000). Another compound in licorice, licochalcone-A, has shown estrogenic activity with ERs and induced apoptosis in MCF-7 and HL-60 cell lines (Rafi *et al.*, 2000).

Additional *in vivo* results demonstrated the ability of licorice root to double activity in skeletal and cardiovascular tissue (Tamir *et al.*, 2000) and its effect on reducing testosterone production in rats (Moyad, 2002). Daily consumption of 7 g of a licorice dietary supplement (0.5 g of glycyrrhizic acid) in seven men significantly reduced serum testosterone within 4 days (Armanini *et al.*, 1999). Health risks such as hypermineralocorticoidism involving potassium loss and sodium retention, edema and increased blood pressure have been associated with the human consumption of glycyrrhizinic acid and need to be considered when administering licorice (Stormer *et al.*, 1993; Wang and Nixon, 2001). More research is needed to evaluate the benefits and adverse effects of licorice as a source of phytoestrogens.

Hops

Hops (*Humulus lupulus*), a perennial climbing vine in the Cannabaceae family, has been extensively cultivated for its bitter properties found in the female flowers used in beer and medicine. Medicinally hops have been valued as a sedative, for inflammation and as a tonic (Foster and Tyler, 1999). The Native American Cherokee used the plant for inflamed kidneys, as a sedative, for pain relief and for breast and female complaints where the womb was debilitated (Hamel and Chiltoskey, 1975). Menstrual disturbances were frequently observed in women hops pickers and their estrogenic activity was associated with the plant (Verzele, 1986).

The following compounds have been identified as estrogenic: 8-prenylnaringenin, 6-prenylnaringenin,

xanthohumol and isoxanthohumol. The female flowers of hops are considered estrogenic. The estrogenic activity of hops is variable between 0 and 300 μg E_2 equivalents/g, which may be due to the physiological state of the plant or environmental effects (Verdeal and Ryan, 1979). This difference should be kept in mind when evaluating studies based on plant extracts versus isolated, purified compounds. The most potent phytoestrogen in hops is 8-prenylnaringenin, which is found in beer in low quantities (18 ng/mL) (Milligan *et al.*, 1999). Isoflavones have also been reported in beer, most notably formononetin, although they are thought to be from barley seeds before brewing (Lapcík *et al.*, 1998). Other studies have tested the presence of hops in dietary supplements used for breast enhancement (Coldham and Sauer, 2001). Further testing is needed *in vivo* and in humans to evaluate adequately the potential estrogenic activity of these hop-based dietary supplements.

Hops extract *in vitro* did show significant binding affinities to both ERα and ERβ, estrogenic activity with AP induction, and up-regulated *PR* expression in Ishikawa cells, however, it showed strong cytotoxicity (Liu *et al.*, 2001b). Hops extract has also acted as a growth inhibitor and showed significant antiproliferation effects in serum stimulated T-47D cells at 0.1% and 0.01% concentration (Dixon-Shanies and Shaikh, 1999) and stimulated T-47D and MCF-7 cells in depleted serum at 0.2% (Zava *et al.*, 1998). In various bioassays, 8-prenylnaringenin has shown estrogenic potency (Milligan *et al.*, 2000; Coldham and Sauer, 2001). However, hops showed little uterotrophic activity in prepubertal and adult ovx mouse bioassays (Coldham and Sauer, 2001). Presently, there is limited scientific evidence concerning the estrogenic activity and health benefits of hops.

Dong quai

Dong quai (*Angelica sinensis*) is an herb that belongs to the Apiaceae family and has been used extensively in traditional Chinese medicine for many years (Mei *et al.*, 1991). Dong quai has been referred to as the 'female ginseng' and is used for a variety of conditions such as a blood tonic and decongestant for body organs (Hardy, 2000). The root is used for women as a tonic often in combination with other herbs. Other women's conditions treated with dong quai are dysmenorrhea, irregular menstruation, anemia, constipation and abdominal pain (Zhu, 1987).

In vitro dong quai has acted as a growth inhibitor with breast cancer cell lines (Zava *et al.*, 1998; Dixon-Shanies and Shaikh, 1999) but has also been observed to stimulate the growth of MCF-7 cells (Amato *et al.*, 2002). Dong quai has shown weak binding affinity for ERα and ERβ and weak stimulation of *PR* expression (Liu *et al.*, 2001b).

A randomized, controlled trial using a combination of 60 mg soy isoflavones, 100 mg dong quai, 50 mg black cohosh taken by women who suffered from menstrual migraines reduced the frequency of attacks, and their severity after 1 month of initiation (Burke *et al.*, 2002). A double-blind randomized placebo controlled clinical trial with dong quai did not significantly reduce hot flushes or endometrium thickening in postmenopausal women over a 24-week period (Hirata *et al.*, 1997).

Further studies are needed to evaluate the active compounds and estrogenic effects of dong quai. Psolarens identified in dong quai may cause photosensitization and therefore health risks should also be considered (Hann *et al.*, 1991).

Other botanical sources

Other botanical sources tested for their estrogenic effects and potential health benefits are: evening primrose oil (*Oenothera biennis*, Onagraceae); chasteberry (*Vitex agnus-castus*, Verbenaceae); alfalfa (*Medicago sativa*, Fabaceae); and ginseng (*Panax* spp. and *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., Araliaceae) (Glazier and Bowman, 2001; Liu *et al.*, 2001b; Amato *et al.*, 2002; Kronenberg and Fugh-Berman, 2002). Coffee (*Coffea arabica* L., Rubiaceae) has also shown weak estrogenic activity (Kitts, 1987). Other plants that have shown ER binding activity and activity in estrogen dependent breast cancer cells are presented in Table 1 using phylogenetic relationships based on Daly and coworkers, 2001.

Evening primrose is a common herb in North America that has been used medicinally by the Native Americans (Grieve, 1985). A randomized controlled trial of oral gamolenic acid from evening primrose oil on hot flushes showed no significant benefits over the placebo group (Chenoy *et al.*, 1994). For centuries the dried ripe fruit of chasteberry tree has been used medicinally for its beneficial effects on female reproduction and to decrease sexual desire, specifically of monks, hence the name (Hardy, 2000). Chasteberry extract has shown growth inhibition with T-47D breast cells (Dixon-Shanies and Shaikh, 1999) and estrogenic activity based on *in vitro* assays (Liu *et al.*, 2001b). Several other studies have evaluated chasteberry for premenstrual syndrome with beneficial effects (Berger *et al.*, 2000; Loch *et al.*, 2000).

Alfalfa is a perennial herb that has a limited history of medicinal use although it is a common forage plant. A homeopathic physician Alexander L. Blackwood made the association that if alfalfa fattened cows it might work in the same way with humans and it was purported by homeopathic and eclectic physicians to 'increase the appetite and the flesh' (Foster and Tyler, 1999). Coumestrol was the first phytoestrogen identified in alfalfa (Bickoff *et al.*, 1957) and is considered one of the richest food sources for this phytoestrogen (Kurzer and Xu, 1997). Alfalfa did not show affinity to ER or *PR* binding (Zava *et al.*, 1998) and therefore may act through a different mechanism. More studies are needed on serum levels of phytoestrogens after alfalfa consumption to elucidate the effects of metabolism on estrogenic activity (Hwang *et al.*, 2001).

Ginseng has a long history of medicinal use in Asia as a tonic and stimulant. There are several different plants that are referred to as ginseng and all are in the Araliaceae family: *Panax ginseng* C.A. Mey. (Chinese or Korean ginseng), *P. quinquefolium* L. (American ginseng) and *Eleutherococcus senticosus* (Siberian ginseng). Case studies have shown postmenopausal vaginal bleeding with *Panax* sp. intake, which may suggest weak estrogenic effects (Punnonen and Lukola, 1980), although a randomized, double-blind, placebo-controlled

multicenter trial of postmenopausal women consuming Ginsana® (100 mg of standardized ginseng extract from *Panax ginseng*) did not show significant differences in menopausal symptoms between groups (Wiklund *et al.*, 1999).

Historically, Bradbury and White as well as Farnsworth and coworkers compiled extensive data about plant species with estrogenic activity and compounds. Some of these studies have been further confirmed with more advanced laboratory techniques. As techniques continue to be refined, they will provide researchers with higher resolution to detect phytoestrogenic activity and compounds. These methods coupled with phylogenetic analysis can offer effective ways to search for new sources of phytoestrogens in the plant kingdom.

ADVERSE EFFECTS OF PHYTOESTROGENS

The mechanisms and potencies of phytoestrogens are not completely clarified and they may be considered potential endocrine disrupters, and therefore caution should be exercised when taking them. Some concerns have been discussed about the risks associated with phytoestrogens such as increased plasma concentration of isoflavones in babies that ingest soymilk, the ability of non-hormonal secondary plant metabolites to modify sex steroid metabolism, and the effects of phytoestrogens on thyroid (Ibarreta *et al.*, 2001). In addition, the genetic toxicity potential of phytoestrogens has recently been reviewed (Kulling *et al.*, 2002). As discussed earlier, sheep consuming large amounts of clover showed infertility and reproductive disorders (Bennetts *et al.*, 1946; Adams, 1995). Cheetahs in captivity also had reduced fertility rates when consuming a feline diet composed of a soybean product, which was reversed when it was removed from the diet (Setchell *et al.*, 1987). Toxicities associated with herbal medicines that include phytoestrogens have also been presented in the literature (Sheehan, 1998).

Several articles have discussed the potential risks involved with soy-based infant formulas (Murphy *et al.*, 1997; Fitzpatrick, 1998; Setchell *et al.*, 1998; Zung *et al.*, 2001). Some negative effects of phytoestrogens may be the result of receiving high levels of isoflavones during fetal development (Setchell *et al.*, 1998). Studies by Setchell *et al.* (1997) showed that infants fed soy-based formula have high concentrations of daidzein and genistein in their plasma, 13 000 to 22 000 times higher than E_2 in early life and proportionately higher than normal adult intake of isoflavones (Setchell *et al.*, 1997). This has raised concern about the health benefits and long-term effects that phytoestrogens may have on developing and mature neuronal function and the interaction of phytoestrogens with E_2 during perinatal development of the brain (Belcher and Zsarnovszky, 2001). Postnatal rats did show estrogenic activity, including induced permanent estrus with a 40 mg/kg dose of isoflavones, while no effects were observed at the 4 mg/kg dose which is predicted of infants fed soy formula (Lewis *et al.*, 2003). Some propose that health protection may be provided for infants that consume soy-based formulas (Murphy *et al.*, 1997). Zung and collaborators (2001) reviewed studies on soy-based formulas in relation to growth rate and concluded that there is no significant effect on growth, although with premature infants there are conflicting data. More studies conducted on infants who consume soy-based formulas are needed to further understand the beneficial and adverse effects of phytoestrogens.

As potential endocrine disrupters, phytoestrogens may act as antiestrogens and harm the reproductive health of males (Sharpe and Skakkebaek, 1993; Santti *et al.*, 1998). Reduced sperm quality, undescended testes and urogenital tract abnormalities were increased in the sons of mothers taking DES compared with those who did not take the miscarriage preventative drug (Sheehan, 1998). Animal studies conducted with DES resulted in male genital abnormalities during development, including cysts, testicular lesions and lack of growth of the seminal vesicles and therefore concern has been raised about the effects of phytoestrogens on male development (Santti *et al.*, 1998). High doses of genistein are shown to alter pituitary responsiveness and basal luteinizing hormone (LH) secretion in castrated postpubertal rats. Coumestrol consumption by rat pups suppressed testicular testosterone concentrations and resulted in abnormal sexual behavior in adulthood (Whitten and Naftolin, 1992). Studies in cultured human lymphoblastoid cells reported that coumestrol was mutagenic and clastogenic (Domon *et al.*, 2001). Recent work has shown that prenatal exposure to phytoestrogens may interact with platelet-derived growth factor during testis development (Thuillier *et al.*, 2003). However, Kurzer (2002) reviewed studies on adult men that showed no adverse effects on sperm quality with the consumption of soy isoflavones. A recent study evaluating the long-term reproductive effects of genistein (0–10 mg/kg per day) during gestation and lactation in mice showed no significant effect on sperm count, the number of motile sperm, or sperm mobility nor was there any effect on testicular gene expression (Fielden *et al.*, 2003). In adolescent boys, isoflavone supplementation showed no significant effect on bone turnover and growth (Jones *et al.*, 2003). Additional studies are needed to further evaluate the risks of phytoestrogen consumption in male development.

Concern has been associated with thymic weight and effects on the immune system. Ovariectomized adult mice injected with genistein produced dose-responsive decreases in thymic weight of up to 80% (Yellayi *et al.*, 2002). Other concerns related to phytoestrogens are their effect on thyroxine, insulin and glucagon (Ohno *et al.*, 1993). Hypothyroid cases were associated with infants fed soybean diets (Fort *et al.*, 1990). Thyroid hormone concentrations were observed in ovariectomized ewes fed red clover silage which showed changes in the area of the thyroid follicles and significantly higher concentrations of total T_3 and free T_3 in plasma, however, no differences were observed between the total T_4 and free T_4. These results suggest that men and women with thyroid conditions should use caution when consuming phytoestrogens (Madej *et al.*, 2002).

In the United States, regulation is limited on standardization, preparation and extraction methods of the phytoestrogen products being sold and marketed as nutritional supplements. As nutritional supplements these products are not supported by clinical trials and therefore should be administered and taken with this in mind (This *et al.*, 2001).

CONCLUSIONS

Research in phytoestrogens has increased dramatically in the past several years as seen by the numerous publications. However, many questions remain. Research is still needed to evaluate the safety of phytoestrogens on human systems, beneficial and harmful doses, gender differences in response to phytoestrogens, differences in the chemical classes of phytoestrogens and the effects phytoestrogens may have with other drugs or dietary products. Due to the functional and structural differences of phytoestrogens, their biological activities are also highly variable and there may be other effects that have not yet been studied.

Phytoestrogens are common in the human diet and able to exert many biological effects that have been observed in cell, animal and human systems. The majority of these effects are seen as beneficial to our health, although contradictory effects have been shown and further research is needed to confirm these findings. Estrogenic compounds and their activities are complex and often species specific. The complexity of phytoestrogens suggests that interpretations need to be made with caution.

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REFERENCES

Adams NR. 1995. Detection of the effects of phytoestrogens on sheep and cattle. *J Anim Sci* **73**: 1509–1515.

Adlercreutz H. 1998a. Epidemiology of phytoestrogens. *Baillieres Clin Endocrinol Metab* **12**: 605–623.

Adlercreutz H. 1998b. Evolution, nutrition, intestinal microflora, and prevention of cancer: a hypothesis. *Proc Soc Exp Biol Med* **217**: 241–246.

Adlercreutz H. 2002. Phyto-oestrogens and cancer. *Lancet Oncol* **3**: 364–373.

Adlercreutz H. 2003. Phytoestrogens and breast cancer. *J Steroid Biochem Mol Biol* **1803**: 1–6.

Adlercreutz H, Höckerstedt K, Bannwart C *et al.* 1987. Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *J Steroid Biochem* **27**: 1135–1144.

Adlercreutz H, Mazur W, Bartels P *et al.* 2000a. Phytoestrogens and prostate disease. *J Nutr* **130**: 658S–659S.

Adlercreutz H, Mazur W, Stumpf K *et al.* 2000b. Food containing phytoestrogens, and breast cancer. *BioFactors* **12**: 89–93.

Adlercreutz H, Mousavi Y, Clark J *et al.* 1992. Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. *J Steroid Biochem Mol Biol* **41**: 331–337.

Albertazzi P, Pansini F, Bonaccorsi G, Zanotti L, Forini E, De Aloysio D. 1998. The effects of dietary soy supplementation on hot flushes. *Obstet Gynecol* **91**: 6–11.

Allen E, Doisy EA. 1923. An ovarian hormone: preliminary report on its location, extraction and partial purification, and action in test animals. *JAMA* **81**: 819–821.

Amato P, Christophe S, Mellon PL. 2002. Estrogenic activity of herbs commonly used as remedies for menopausal symptoms. *Menopause* **9**: 145–150.

Anderson JJB, Anthony M, Messina M, Garner SC. 1999. Effects of phyto-oestrogens on tissues. *Nutr Res Rev* **12**: 75–116.

Anderson JW, Johnstone BM, Cook-Newell ME. 1995. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* **333**: 276–282.

Anstead GM, Carlson KE, Katzenellenbogen JA. 1997. The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site. *Steroids* **62**: 268–303.

Armanini D, Bonanni G, Palermo M. 1999. Reduction of serum testosterone in men by licorice. *N Engl J Med* **341**: 1158.

Badowski P, Urbanek-Karlowska B. 2001. Phytoestrogens in food – a review. *Rocz Panstw Zakl Hig* **52**: 203–212.

Bagchi D, Das DK, Tosaki A, Bagchi M, Kothari SC. 2001. Benefits of resveratrol in women's health. *Drugs Exp Clin Res* **XXVII**: 233–248.

Balk JA, Whiteside DA, Naus G, DeFerrari E, Roberts JM. 2002. A pilot study of the effects of phytoestrogen supplementation on postmenopausal endometrium. *J Soc Gynecol Investig* **9**: 238–242.

Barnes S. 1997. The chemopreventive properties of soy isoflavonoids in animal models of breast cancer. *Breast Cancer Res Treat* **46**: 169–179.

Barnes S. 1998. Phytoestrogens and breast cancer. *Baillieres Clin Endocrinol Metab* **12**: 559–579.

Belcher SM, Zsarnovszky A. 2001. Estrogenic actions in the brain: estrogen, phytoestrogens, and rapid intracellular signaling mechanisms. *J Pharmacol Exp Ther* **299**: 408–414.

Benassayag C, Perrot-Applanat M, Ferre F. 2002. Phytoestrogens as modulators of steroid action in target cells. *J Chromatogr B Analyt Technol Biomed Life Sci* **777**: 233–248.

Bennetts HW, Underwood EJ, Shier FL. 1946. A specific breeding problem of sheep on subterranean clover pastures in western Australia. *Aust Vet J* **22**: 2–12.

Berger D, Schaffner W, Schrader E, Meier B, Brattström A. 2000. Efficacy of *Vitex agnus castus* L. extract Ze 440 in patients with pre-menstrual syndrome (PMS). *Arch Gynecol Obstet* **264**: 150–153.

Bhathena SJ, Velasquez MT. 2002. Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am J Clin Nutr* **76**: 1191–1201.

Bickoff EM, Booth AN, Lyman RL, Livingston AL, Thompson CR, DeEds F. 1957. Coumestrol, a new estrogen isolated from forage crops. *Science* **126**: 969–970.

Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A. 1998. Phyto-oestrogens: where are we now? *Br J Nutr* **79**: 393–406.

Borriello SP, Setchell KDR, Axelson M, Lawson AM. 1985. Production and metabolism of lignans by the human faecal flora. *J Appl Bacteriol* **58**: 37–43.

Boue SM, Wiese TE, Nehls S *et al.* 2003. Evaluation of the estrogenic effects of legume extracts containing phytoestrogens. *J Agric Food Chem* **51**: 2193–2199.

Bouker KB, Hilakivi-Clarke L. 2000. Genistein: does it prevent or promote breast cancer? *Environ Health Perspect* **108**: 701–708.

Bradbury RB, White DE. 1954. Estrogens and related substances in plants. *Vitam Horm* **XII**: 207–233.

Brownson DM, Azios NG, Fuqua BK, Dharmawardhane SF, Mabry TJ. 2002. Flavonoid effects relevant to cancer. *J Nutr* **132**: 3482S–3489S.

Brzezinski A, Adlercreutz H, Shaoul R *et al.* 1997. Short-term effects of phytoestrogen-rich diet on postmenopausal women. *Menopause* **4**: 89–94.

Brzezinski A, Debi A. 1999. Phytoestrogens: the 'natural' selective estrogen receptor modulators? *Eur J Obstet Gynecol Reprod Biol* **85**: 47–51.

Brzozowski AM, Pike ACW, Dauter Z *et al.* 1997. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **389**: 753–758.

Burdette JE, Liu J, Lantvit D *et al.* 2002. *Trifolium pratense* (red clover) exhibits estrogenic effects *in vivo* in ovariectomized Sprague-Dawley rats. *J Nutr* **132**: 27–30.

Burke BE, Olson RD, Cusack BJ. 2002. Randomized, controlled trial of phytoestrogen in the prophylactic treatment of menstrual migraine. *Biomed Pharmacother* **56**: 283–288.

Burke GL, Vitolins MZ, Bland D. 2000. Soybean isoflavones as an alternative to traditional hormone replacement therapy: are we there yet? *J Nutr* **130**: 664S–665S.

Cassidy A, Bingham S, Setchell K. 1995. Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. *Br J Nutr* **74**: 587–601.

Chan RY, Chen WF, Dong A, Guo D, Wong MS. 2002. Estrogen-like activity of ginsenoside Rg1 derived from *Panax notoginseng*. *J Clin Endocrinol Metab* **87**: 3691–3695.

Charlet S, Bensaddek L, Raynaud S, Gillet F, Mesnard F, Fliniaux M-A. 2002. An HPLC procedure for the quantification of anhydrosecoisolariciresinol. Application to the evaluation of flax lignan content. *Plant Physiol Biochem* **40**: 225–229.

Cheng E, Story CD, Yoder L, Hale WH, Burroughs W. 1953. Estrogenic activity of isoflavone derivatives extracted and prepared from soybean oil meal. *Science* **118**: 164–165.

Chenoy R, Hussain S, Tayob Y, O'Brien PMS, Moss MY, Morse PF. 1994. Effect of oral gamolenic acid from evening primrose oil on menopausal flushing. *Br Med J* **308**: 501–503.

Clarkson TB, Anthony MS. 1998. Phytoestrogens and coronary heart disease. *Baillieres Clin Endocrinol Metab* **12**: 589–604.

Clifton-Bligh PB, Baber RJ, Fulcher GR, Nery M-L, Moreton T. 2001. The effect of isoflavones extracted from red clover (Rimostil) on lipid and bone metabolism. *Menopause* **8**: 259–265.

Cline JM, Hughes CL. 1998. Phytochemicals for the prevention of breast and endometrial cancer. *Cancer Treat Res* **94**: 107–134.

Coldham NG, Sauer MJ. 2001. Identification, quantitation and biological activity of phytoestrogens in a dietary supplement for breast enhancement. *Food Chem Toxicol* **39**: 1211–1224.

Costello CH, Lynn EV. 1950. Estrogenic substances from plants: I. *Glycyrrhiza*. *J Am Pharm Assoc* **39**: 177–180.

Crouse JR, Terry JG, Morgan TM *et al.* 1998. Soy protein containing isoflavones reduces plasma concentrations of lipids and lipoproteins. *Circulation* **97**: 816.

Dabrosin C, Chen J, Wang L, Thompson LU. 2002. Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts. *Cancer Lett* **185**: 31–37.

Daly DC, Cameron KM, Stevenson DW. 2001. Plant systematics in the age of genomics. *Plant Physiol* **127**: 1328–1333.

De la Taille A, Hayek OR, Burchardt M, Burchardt T, Katz AE. 2000. Role of herbal compounds (PC-SPES) in hormone-refractory prostate cancer: two case reports. *J Altern Complement Med* **6**: 449–451.

De Lemos MLD. 2001. Effects of soy phytoestrogens genistein and daidzein on breast cancer growth. *Ann Pharmacother* **35**: 1118–1121.

Demark-Wahnefried W, Price DT, Polascik TJ *et al.* 2001. Pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer before surgery: exploring the effects on hormonal levels, prostate-specific antigen, and histopathologic features. *Urology* **58**: 47–52.

Dewick PM. 1993. Isoflavonoids. In *The Flavonoids: Advances in Research Since 1986*. Harborne JB (ed.). Chapman & Hall: London 117–238.

Di Silverio F, D'Eramo G, Lubrano C *et al.* 1992. Evidence that *Serenoa repens* extract displays an antiestrogenic activity in prostatic tissue of benign prostatic hypertrophy patients. *Eur Urol* **21**: 309–314.

Diel P, Schmidt S, Vollmer G. 2002. *In vivo* test systems for the quantitative and qualitative analysis of the biological activity of phytoestrogens. *J Chromatogr B Analyt Technol Biomed Life Sci* **777**: 191–202.

Diel P, Smolnikar K, Michna H. 1999. *In vitro* test systems for the evaluation of the estrogenic activity of natural products. *Planta Med* **65**: 197–203.

Dixon RA, Ferreira D. 2002. Genistein. *Phytochemistry* **60**: 205–211.

Dixon-Shanies D, Shaikh N. 1999. Growth inhibition of human breast cancer cells by herbs and phytoestrogens. *Oncol Rep* **6**: 1383–1387.

Domon OE, McGarrity LJ, Bishop M, Yoshioka M, Chen JJ, Morris SM. 2001. Evaluation of the genotoxicity of the phytoestrogen, coumestrol, in AHH-1 $TK^{+/-}$ human lymphoblastoid cells. *Mutat Res* **474**: 129–137.

Dornstauder E, Jisa E, Unterrieder I, Krenn L, Kubelka W, Jungbauer A. 2001. Estrogenic activity of two standardized red clover extracts (Menoflavon®) intended for large scale use in hormone replacement therapy. *J Steroid Biochem Mol Biol* **78**: 67–75.

Drapier-Faure E. 2001. Phytoestrogens. Therapeutic use. *Gynecol Obstet Fertil* **29**: 141–147.

Duke JA. 1981. *Handbook of Legumes of World Economic Importance*. Plenum Press: New York.

Duke JA. 1992. *Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants*. CRC Press: Boca Raton.

Duke JA, Ayensu ES. 1985. *Medicinal Plants of China: Volume One*. Reference Publications: Michigan.

Düker E-M, Kopanski L, Jarry H, Wuttke W. 1991. Effects of extracts from *Cimicifuga racemosa* on gonadotropin release in menopausal women and ovariectomized rats. *Planta Med* **57**: 420–424.

Eagon CL, Elm MS, Teepe AG, Eagon PK. 1997. Medicinal botanicals: estrogenicity in rat uterus and liver. *Proc Am Assoc Cancer Res* **38**: 193.

Eisenberg DM, Davis SL, Ettner SL *et al.* 1998. Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. *JAMA* **280**: 1569–1575.

Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL. 1993. Unconventional medicine in the United States: prevalence, costs and patterns of use. *N Engl J Med* **328**: 246–252.

Farnsworth NR, Bingel AS, Cordell GA, Crane FA, Fong HHS. 1975a. Potential value of plants as sources of new antifertility agents I. *J Pharm Sci* **64**: 535–598.

Farnsworth NR, Bingel AS, Cordell GA, Crane FA, Fong HHS. 1975b. Potential value of plants as sources of new antifertility agents II. *J Pharm Sci* **64**: 717–754.

Faure ED, Chantre P, Mares P. 2002. Effects of a standardized soy extract on hot flushes: a multicenter, double-blind, randomized, placebo-controlled study. *Menopause* **9**: 329–334.

Fielden MR, Samy SM, Chou KC, Zacharewski TR. 2003. Effect of human dietary exposure levels of genistein during gestation and lactation on long-term reproductive development and sperm quality in mice. *Food Chem Toxicol* **41**: 447–454.

File SE, Duffy R, Wiseman H. 2002. Improved memory and frontal lobe function in post-menopausal women after 3 months' treatment with soya supplements. *Eur J Neuropsychopharmacol* **12**: S406.

File SE, Hartley DE, Alom N, Rattray M. 2003. Soya phytoestrogens change cortical and hippocampal expression of BDNF mRNA in male rats. *Neurosci Lett* **338**: 135–138.

File SE, Jarrett N, Fluck E, Duffy R, Casey K, Wiseman H. 2001. Eating soya improves human memory. *Psychopharmacology (Berl)* **157**: 430–436.

Fisher JF, Wheaton TA. 1976. A high-pressure liquid chromatographic method for the resolution and quantitation of naringin and naringenin rutinoside in grapefruit juice. *J Agric Food Chem* **24**: 898–899.

Fitzpatrick LA. 2003. Soy isoflavones: hope or hype? *Maturitas* **44**: S21–S29.

Fitzpatrick M. 1998. Comments on isoflavones in soy-based infant formulas. *J Agric Food Chem* **46**: 3396–3397.

Fort P, Moses N, Fasano M, Goldberg T, Lifshitz F. 1990. Breast and soy-formula feedings in early infancy and the prevalence of autoimmune thyroid disease in children. *J Am Coll Nutr* **9**: 164–167.

Foster S. 1999. Black cohosh: *Cimicifuga racemosa* a literature review. *HerbalGram* **45**: 35–50.

Foster S, Tyler VE. 1999. *Tyler's Honest Herbal: A Sensible Guide to the Use of Herbs and Related Remedies*, 4th edn. Haworth Herbal Press: New York.

Fotsis T, Pepper M, Adlercreutz H *et al.* 1993. Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis. *Proc Natl Acad Sci USA* **90**: 2690–2694.

Franke AA, Custer LJ, Cerna CM, Narala K. 1995. Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. *Proc Soc Exp Biol Med* **208**: 18–26.

Franke AA, Custer LJ, Wilkens LR *et al.* 2002. Liquid chromatographic-photodiode array mass spectrometric analysis of dietary phytoestrogens from human urine and blood. *J Chromatogr B Analyt Technol Biomed Life Sci* **777**: 45–59.

Fugh-Berman A, Kronenberg F. 2001. Red clover (*Trifolium pratense*) for menopausal women: current state of knowledge. *Menopause* **8**: 333–337.

Galey FD, Mendez LE, Whitehead WE, Holstege DM, Plumlee KH, Johnson B. 1993. Estrogenic activity in forages: diagnostic uses of the classical mouse uterine bioassay. *J Vet Diagn Invest* **5**: 603–608.

Ghem BD, McAndrews JM, Chien P-Y, Jameson JL. 1997. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. *Proc Natl Acad Sci USA* **94**: 14138–14143.

Gibson MR. 1978. *Glycyrrhiza* in old and new perspectives. *Lloydia* **41**: 348–354.

Glazier MG, Bowman MA. 2001. A review of the evidence for the use of phytoestrogens as a replacement for traditional estrogen replacement therapy. *Arch Intern Med* **161**: 1161–1172.

Grieve M. 1985. *A Modern Herbal: The Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folklore of Herbs, Grasses, Fungi, Shrubs and Trees with All Their Modern Scientific Uses*. Jonathan Cape: London.

Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. 2002. Production and actions of estrogens. *N Engl J Med* **346**: 340–352.

Haggerty WJ. 1999. Flax: ancient herb and modern medicine. *Herbalgram* **45**: 51–57.

Hale GE, Hughes CL, Robboy SJ, Agarwal SK, Bievre M. 2001. A double-blind randomized study on the effects of red clover isoflavones on the endometrium. *Menopause* **8**: 338–346.

Hamel PB, Chiltoskey MU. 1975. *Cherokee Plants and Their Uses–A 400 Year History*. N.C. Herald Publishing: Sylva, NC.

Han KK, Soares JM, Jr., Haidar MA, Rodrigues de Lima G, Baracat EC. 2002. Benefits of soy isoflavone therapeutic regimen on menopausal symptoms. *Obstet Gynecol* **99**: 389–394.

Hann SK, Park Y-K, Im S, Byun SW. 1991. Angelica-induced phytophotodermatitis. *Photodermatol Photoimmunol Photomed* **8**: 84–85.

Hardy ML. 2000. Herbs of special interest to women. *J Am Pharm Assoc* **40**: 234–242.

Hargreaves DF, Potten CS, Harding C *et al.* 1999. Two-week dietary soy supplementation has an estrogenic effect on normal premenopausal breast. *J Clin Endocrinol Metab* **84**: 4017–4024.

Harnischfeger G, Cillien N. 1996. Influence of *Cimicifuga racemosa* extract fractions on the proliferation of human carcinoma cells *in vitro* with regard to their estrogen receptor sensitivity *in* PM 62, Abstracts of the *44th Annual Congress of Gesellschaftfür Arzneipflanzenflorschung, Germany,* 40.

Hawkins MB, Thornton JW, Crews D, Skipper JK, Dotte A, Thomas P. 2000. Identification of a third distinct estrogen receptor and reclassification of estrogen receptors in teleosts. *Proc Natl Acad Sci USA* **97**: 10751–10756.

Heftmann E. 1967. Steroid hormones in plants. *Am Perfum Cosmet* **82**: 47–49.

Heinonen S, Nurmi T, Liukkonen K *et al.* 2001. *In vitro* metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *J Agric Food Chem* **49**: 3178–3186.

Henry LA, Witt DM. 2002. Resveratrol: phytoestrogen effects on reproductive physiology and behavior in female rats. *Horm Behav* **41**: 220–228.

Hernández Muñoz G, Pluchino S. 2003. *Cimicifuga racemosa* for the treatment of hot flushes in women surviving breast cancer. *Maturitas* **44**: S59–S65.

Herrick JW. 1995. *Iroquois Medical Botany*. Syracuse University Press: New York.

Hesse R, Hoffmann B, Karg H, Vogt H. 1981. Demonstration of phytoestrogens in feed plants and hops by means of receptor test. *Zentralbl Veterinarmed A* **28**: 422–454.

Hirata JD, Swiersz LM, Zell B, Small R, Ettinger B. 1997. Does dong quai have estrogenic effects in postmenopausal women? A double-blind, placebo-controlled trial. *Fertil Steril* **68**: 981–986.

Honoré EK, Williams JK, Anthony MS, Clarkson TB. 1997. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. *Fertil Steril* **67**: 148–154.

Howes JB, Sullivan D, Lai N *et al.* 2000. The effects of dietary supplementation with isoflavones from red clover on the lipoprotein profiles of post menopausal women with mild to moderate hypercholesterolaemia. *Atherosclerosis* **152**: 143–147.

Hsieh CY, Santell RC, Haslam SZ, Helferich WG. 1998. Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells *in vitro* and *in vivo*. *Cancer Res* **58**: 3833–3838.

Hu J-Y, Aizawa T. 2003. Quantitative structure–activity relationships for estrogen receptor binding affinity of phenolic chemicals. *Water Res* **37**: 1213–1222.

Huber J. 2000. Phytoestrogens and SERMs, alternatives to classical hormone therapy? *Ther Umsch* **57**: 651–654.

Hwang J, Hodis HN, Sevanian A. 2001. Soy and alfalfa phytoestrogen extracts become potent low-density lipoprotein antioxidants in the presence of acerola cherry extract. *J Agric Food Chem* **49**: 308–314.

IARC. 1993. Toxins derived from *Fusarium graminearum, F. culmorum* and *F. crookwellense*: zearalenone, deoxynivalenol, nivalenol and fusarenone x. *IARC Monogr Eval Carcinog Risks Hum* **56**: 397–444.

Ibarreta D, Daxenberger A, Meyer HHD. 2001. Possible health impact of phytoestrogens and xenoestrogens in food. *APMIS* **109**: 161–184.

Ichikawa K, Kitaoka M, Taki M *et al.* 1997. Retrodihydrochalcones and homoisoflavones isolated from Thai medicinal plant *Dracaena loureiri* and their estrogen agonist activity. *Planta Med* **63**: 540–543.

Ingram D, Sanders K, Kolybaba M, Lopez D. 1997. Case–control study of phyto-oestrogens and breast cancer. *Lancet* **350**: 990–994.

Jacobsen BK, Knutsen SF, Fraser GE. 1998. Does high soy milk intake reduce prostate cancer incidence? The Adventist Health Study (United States). *Cancer Causes Control* **9**: 553–557.

Jacobsohn GM, Frey MJ, Hochberg RB. 1965. The absence of steroid estrogens in plants. *Steroids* **6**: 93–99.

Jarry H, Harnischfeger G. 1985. Studies on the endocrine effects of the contents of *Cimicifuga racemosa*: 1. Influence on the serum concentration of pituitary hormones in ovariectomized rats. *Planta Med* **1**: 46–49.

Jarry H, Harnischfeger G, Düker E. 1985. Studies on the endocrine effects of the contents of *Cimicifuga racemosa* 2. *In vitro* binding of compounds to estrogen receptors. *Planta Med* **4**: 316–319.

Jarry H, Metten M, Spengler B, Christoffel V, Wuttke W. 2003. *In vitro* effects of the *Cimicifuga racemosa* extract BNO 1055. *Maturitas* **44**: S31–S38.

Jenkins DJA, Kendall CWC, D'Costa MA *et al.* 2003. Soy consumption and phytoestrogens: effect on serum prostate specific antigen when blood lipids and oxidized low-density lipoprotein are reduced in hyperlipidemic men. *J Urol* **169**: 507–511.

Jenkins DJA, Kendall CWC, Vidgen E *et al.* 1999. Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measures, and *ex vivo* androgen and progestin activity: a controlled crossover trial. *Am J Clin Nutr* **69**: 395–402.

Joannou GE, Kelly GE, Reeder AY, Waring M, Nelson C. 1995. A urinary profile study of dietary phytoestrogens. The identification and mode of metabolism of new isoflavonoids. *J Steroid Biochem Molec Biol* **54**: 167–184.

Jones G, Dwyer T, Hynes K, Dalais FS, Parameswaran V, Greenaway TM. 2003. A randomized controlled trial of phytoestrogen supplementation, growth and bone turnover in adolescent males. *Eur J Clin Nutr* **57**: 324–327.

Ju YH, Carlson KE, Sun J *et al.* 2000. Estrogenic effects of extracts from cabbage, fermented cabbage, and acidified brussel sprouts on growth and gene expression of estrogen-dependent human breast cancer (MCF-7) cells. *J Agric Food Chem* **48**: 4628–4634.

Kang HJ, Ansbacher R, Hammound M. 2002. Use of alternative and complementary medicine in menopause. *Int J Gynaecol Obstet* **79**: 195–207.

Kelly GE, Joannou GE, Reeder AY, Nelson C, Waring MA. 1995. The variable metabolic response to dietary isoflavones in humans. *Proc Soc Exp Biol Med* **208**: 40–43.

Kennelly EJ, Baggett S, Nuntanakorn P *et al.* 2002. Analysis of thirteen populations of black cohosh for formononetin. *Phytomedicine* **9**: 461–467.
Keville K. 1999. Herbs, therapeutic use. *Better Nutr* **61**: 34.
King J, Newton RS. 1852. *The Eclectic Dispensatory of the United States of America*. Derby & Co.: Cincinnati.
Kingsbury JM. 1964. *Poisonous Plants of the United States and Canada*. Prentice-Hall: Englewood Cliffs, NJ.
Kinjo J. 2000. Phytoestrogens. *Nippon Rinsho* **58**: 2434–2438.
Kitaoka M, Kadokawa H, Sugano M *et al.* 1998. Prenylflavonoids: a new class of non-steroidal phytoestrogen (Part 1). Isolation of 8-isopentenylnaringenin and an initial study on its structure–activity relationship. *Planta Med* **64**: 511–515.
Kitts DD. 1987. Studies on the estrogenic activity of a coffee extract. *J Toxicol Environ Health* **20**: 37–49.
Knight DC, Eden JA. 1996. A review of the clinical effects of phytoestrogens. *Obstet Gynecol* **87**: 897–904.
Kris-Etherton PM, Hecker KD, Bonanome A *et al.* 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am J Med Sci* **113**: 71S–88S.
Kronenberg F, Fugh-Berman A. 2002. Complementary and alternative medicine for menopausal symptoms: a review of randomized, controlled trials. *Ann Intern Med* **137**: 805–813.
Kronenberg F, Hughes C. 1999. Exogenous and endogenous estrogens: an appreciation of biological complexity. *Menopause* **6**: 4–6.
Kruse SO, Löhning A, Pauli GF, Winterhoff H, Nahrstedt A. 1999. Fukiic and piscidic acid esters from the rhizome of *Cimicifuga racemosa* and the *in vitro* estrogenic activity of fukinolic acid. *Planta Med* **65**: 763–764.
Kuiper GGJM, Carlsson BO, Grandien K *et al.* 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptor alpha and beta. *Endocrinology* **138**: 863–870.
Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J-A. 1996. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* **93**: 5925–5930.
Kuiper GGJM, Lemmen JG, Carlsson B *et al.* 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. *Endocrinology* **139**: 4252–4263.
Kulling SE, Lehmann L, Metzler M. 2002. Oxidative metabolism and genotoxic potential of major isoflavone phytoestrogens. *J Chromatogr B Analyt Technol Biomed Life Sci* **777**: 211–218.
Kurzer MS. 2002. Hormonal effects of soy in premenopausal women and men. *J Nutr* **132**: 570S–573S.
Kurzer MS, Xu X. 1997. Dietary phytoestrogens. *Annu Rev Nutr* **17**: 353–381.
Lapcík O, Hill M, Hampl R, Wähälä K, Adlercreutz H. 1998. Identification of isoflavonoids in beer. *Steroids* **63**: 14–20.
Lamartiniere CA, Moore J, Holland M, Barnes S. 1995. Neonatal genistein chemoprevents mammary cancer. *Proc Soc Exp Biol Med* **208**: 120–123.
Landstöm M, Zhang JX, Hallmans G *et al.* 1998. Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats. *Prostate* **36**: 151–161.
Lee HP, Gourley L. 1991. Dietary effects on breast-cancer risk in Singapore. *Lancet* **337**: 1197–1200.
Legg SP. 1950. The seasonal and species distribution of oestrogen in British pasture plants. *Biochem J* **46**: Proc xix–xx.
Lei B, Roncaglia V, Viganò R *et al.* 2002. Phytoestrogens and liver disease. *Moll Cell Endocrinol* **193**: 81–84.
Lewis RW, Brooks N, Milburn GM *et al.* 2003. The effects of the phytoestrogen genistein on the postnatal development of the rat. *Toxicol Sci* **71**: 74–83.
Li S. 1973. *Chinese Medicinal Herbs*. (Compiled by Shizhen; translated and researched by F. Porter Smith and G. A. Stuart). Georgetown Press: San Francisco.
Lieberman S. 1996. Are the differences between estradiol and other estrogens, naturally occurring or synthetic, merely semantical? *J Clin Endocrinol Metab* **81**: 850–851.
Liu A, Yang Z, Zhu M, Huo J. 2001a. Estrogenicity of black cohosh (*Cimicifuga racemosa*) and its effect on estrogen receptor level in human breast cancer MCF-7 cells. *Wei Sheng Yan Jiu* **30**: 77–80.
Liu J, Burdette JE, Xu H *et al.* 2001b. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *J Agric Food Chem* **49**: 2472–2479.
Loch E-G, Selle H, Boblitz N. 2000. Treatment of premenstrual syndrome with a phytopharmaceutical formulation containing *Vitex agnus castus*. *J Womens Health Gend Based Med* **9**: 315–320.
Lookhart GL. 1980. Analysis of coumestrol, a plant estrogen, in animal feeds by high-performance liquid chromatography. *J Agric Food Chem* **28**: 666–667.
Lundh T. 1995. Metabolism of estrogenic isoflavones in domestic animals. *Proc Soc Exp Biol Med* **208**: 33–39.
Mabberley DJ. 1997. *The Plant-Book*, 2nd edn. Cambridge University Press: Cambridge.
Madej A, Persson E, Lundh T, Ridderstråle Y. 2002. Thyroid gland function in ovariectomized ewes exposed to phytoestrogens. *J Chromatogr B Analyt Technol Biomed Life Sci* **777**: 281–287.
Maier CGA, Chapman KD, Smith DW. 1995. Differential estrogenic activities of male and female plant extracts from two dioecious species. *Plant Sci* **109**: 31–43.
Mathé G, Orbach-Arbouys S, Bizi E, Court B. 1995. The so-called phyto-estogenic action of *Pygeum africanum* extract. *Biomed Pharmacother* **49**: 339–340.
Matsuda H, Shimoda H, Morikawa T, Yoshikawa M. 2001. Phytoestrogens from the roots of *Polygonum cuspidatum* (Polygonaceae): structure-requirement of hydroxyanthraquinones for estrogenic activity. *Bioorg Mol Chem Lett* **11**: 1839–1842.
Mazur W. 1998. Phytoestrogen content in foods. *Baillieres Clin Endocrinol Metab* **12**: 729–742.
Mazur WM, Wähälä K, Rasku S, Salakka A, Hase T, Adlercreutz H. 1998. Lignan and isoflavonoid concentrations in tea and coffee. *Br J Nutr* **79**: 37–45.
McCoy J, Kelly W. 1996. Survey of *Cimicifuga racemosa* for phytoestrogenic flavonoids. In *212th American Chemical Society National Meeting, Orlando, FL*.
McMichael Philips DF, Harding C, Morton M, Roberts SA. 1998. Effects of soy-protein supplementation on epithelial proliferation in the histologically normal human breast. *Am J Clin Nutr* **68**: S1431–S1436.
Meagher LP, Beecher GR, Flanagan VP, Li BW. 1999. Isolation and characterization of the lignans, isolariciresinol and pinoresinol, in flaxseed meal. *J Agric Food Chem* **47**: 3173–3180.
Mei Q-B, Tao J-Y, Cui B. 1991. Advances in the pharmacological studies of radix *Angelica sinensis* (Oliv) Diels (Chinese Danggui). *Chin Med J* **104**: 776–781.
Merritt JC. 2001. Therapeutic options: hormone replacement therapy–soy therapy. *J Natl Med Assoc* **93**: 288–292.
Messina MJ. 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am J Clin Nutr* **70**: 439S–450S.
Messina M, Bennink M. 1998. Soyfoods, isoflavones and risk of colonic cancer: a review of the *in vitro* and *in vivo* data. *Baillieres Clin Endocrinol Metab* **12**: 707–728.
Messina MJ, Loprinzi CL. 2001. Soy for breast cancer survivors: a critical review of the literature. *J Nutr* **131**: 3095S–3108S.
Messina MJ, Persky V, Setchell K, Barnes S. 1994. Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. *Nutr Cancer* **21**: 113–131.
Miksicek RJ. 1993. Commonly occuring plant flavonoids have estrogenic activity. *Mol Pharmacol* **44**: 37–43.
Miksicek RJ. 1994. Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. *J Steroid Biochem Mol Biol* **49**: 153–160.
Milligan SR, Kalita JC, Heyerick A, Rong H, Cooman L, Keukeleire D. 1999. Identification of a potent phytoestrogen in hops (*Humulus lupulus* L.) and beer. *J Clin Endocrinol Metab* **83**: 2249–2252.
Milligan SR, Kalita JC, Pocock V *et al.* 2000. The endocrine activities of 8-prenylnaringenin and related hop (*Humulus lupulus* L.) flavonoids. *J Clin Endocrinol Metab* **85**: 4912–4914.
Mineta T, Norikoshi K, Yamashita J. 2001. Induction of persistent estrus by early postnatal exposure to isoflavone in mice. *Environ Sci* **8**: 515.
Mitchell JH, Gardner PT, McPhail DB, Morrice PC, Collins AR, Duthie GG. 1998. Antioxidant efficacy of phytoestrogens in chemical and biological model systems. *Arch Biochem Biophys* **360**: 142–148.

Morrissey C, Watson RWG. 2003. Phytoestrogens and prostate cancer. *Curr Drug Targets* **4**: 231–241.
Mosselman S, Polman J, Dijkema R. 1996. ERβ: identification and characterization of a novel human estrogen receptor. *FEBS Lett* **392**: 49–53.
Moyad MA. 1999. Soy, disease prevention, and prostate cancer. *Semin Urol Oncol* **17**: 97–102.
Moyad MA. 2002. Complementary/alternative therapies for reducing hot flashes in prostate cancer patients: reevaluating the existing indirect data from studies of breast cancer and postmenopausal women. *Urology* **59**: 20S–33S.
Mueller SO. 2002. Overview of *in vitro* tools to assess the estrogenic and antiestrogenic activity of phytoestrogens. *J Chromatogr B Analyt Technol Biomed Life Sci* **777**: 155–165.
Muir AD, Westcott ND. 2000. Quantitation of the lignan secoisolariciresinol diglucoside in baked goods containing flax seed or flax meal. *J Agric Food Chem* **48**: 4048–4052.
Murphy PA, Song T, Buseman G, Barua K. 1997. Isoflavones in soy-based infant formulas. *J Agric Food Chem* **45**: 4635–4638.
Naftolin F, Stanbury MG. 2002. Phytoestrogens: are they really estrogen mimics? *Fertil Steril* **77**: 15–17.
Nesselhut T, Schellhas C, Deitrich R, Kuhn W. 1993. Studies on mamma carcinoma cells regarding the proliferation potential of herbal medication with estrogen-like effects. *Arch Gynecol Obstet* **254**: 817–818.
Nestel PJ, Pomeroy S, Kay S *et al.* 1999. Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. *J Clin Endocrinol Metab* **84**: 895–898.
Nestel PJ, Yamashita T, Sasahara T *et al.* 1997. Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. *Arterioscler Thromb Vasc Biol* **17**: 3392–3398.
Obermeyer WR, Musser SM, Betz JM, Casey RE, Pohland AE, Page SW. 1995. Chemical studies of phytoestrogens and related compounds in dietary supplements: flax and chaparral. *Soc Exp Biol Med* **208**: 6–12.
Ohno T, Kato N, Ishii C *et al.* 1993. Genistein augments cyclic adenosine 3'5'-monophosphate (cAMP) accumulation and insulin release in min6 cells. *Endocr Res* **19**: 273–285.
Orcheson LJ, Rickard SE, Seidl MM, Thompson LU. 1998. Flaxseed and its mammalian lignan precursor cause a lengthening or cessation of estrous cycling in rats. *Cancer Lett* **125**: 69–76.
Paech K, Webb P, Kuiper GG *et al.* 1997. Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. *Science* **277**: 1508–1510.
Pan Y, Anthony M, Clarkson TB. 1999. Effect of estradiol and soy phytoestrogens on choline acetyltransferase and nerve growth factor mRNAs in the frontal cortex and hippocampus of female rats. *Proc Soc Exp Biol Med* **221**: 118–125.
Parkin DM. 2001. Global cancer statistics in the year 2000. *Lancet Oncol* **2**: 533–543.
Pearce PT, Zois I, Wynne KN, Funder JW. 1982. *Panax ginseng* and *Eleutherococcus senticosus* extracst – *in vitro* studies on binding to steroid receptors. *Endocrinol Jpn* **29**: 567–573.
Peeters PHM, Keinan-Boker L, Van der Schouw YT, Grobbee DE. 2003. Phytoestrogens and breast cancer risk. *Breast Cancer Res Treat* **77**: 171–183.
Petrakis NL, Barnes S, King EB *et al.* 1996. Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women. *Cancer Epidemiol Biomarkers Prev* **5**: 785–794.
Phipps WR, Martini MC, Lampe JW, Slavin JL, Kurzer MS. 1993. Effect of flax seed ingestion on the menstrual cycle. *J Clin Endocrinol* **77**: 1215–1219.
Potter JD, Steinmetz K. 1996. Vegetables, fruit and phytoestrogens as preventive agents. *IARC Sci Publ* **139**: 61–90.
Poulet FM, Roessler ML, Vancutsem PM. 1997. Initial uterine alterations caused by developmental exposure to tamoxifen. *Reprod Toxicol* **11**: 815–822.
Price KR, Fenwick GR. 1985. Naturally occurring oestrogens in foods – a review. *Food Addit Contam* **2**: 73–106.
Pukkala E, Kyyrönen P, Sankila R, Holli K. 2002. Tamoxifen and toremifene treatment of breast cancer and risk of subsequent endometrial cancer: a population-based case-control study. *Int J Cancer* **100**: 337–341.
Punnonen R, Lukola A. 1980. Oestrogen-like effect of ginseng. *Br Med J* **281**: 1110.
Quella SK, Loprinzi CL, Barton DL *et al.* 2000. Evaluation of soy phytoestrogens for the treatment of hot flashes in breast cancer survivors: a north central cancer treatment group trial. *J Clin Oncol* **18**: 1068–1074.
Rafi MM, Rosen RT, Vassail A *et al.* 2000. Modulation of bcl-2 and cytotoxicity by licochalcone-A, a novel estrogenic flavonoid. *Anticancer Res* **20**: 2653–2658.
Ramsey GW. 1997. *Cimicifuga*. In *Flora of North America. North of Mexico: Magnoliophyta: Magnoliidae and Hamamelidae,* Flomof North America Editorial Committee (ed.). Oxford University Press: New York, 177–181.
Ranich T, Bhathena SJ, Velasquez MT. 2001. Protective effects of dietary phytoestrogens in chronic renal disease. *J Ren Nutr* **11**: 183–193.
Reinli K, Block G. 1996. Phytoestrogen content of foods – a compendium of literature values. *Nutr Cancer* **26**: 123–148.
Riggs BL, Hartmann LC. 2003. Selective estrogen-receptor modulators–mechanisms of action and application to clinical practice. *N Engl J Med* **348**: 618–629.
Rosenberg E, Krska R, Wissiack R *et al.* 1998. High-performance liquid chromatography-atmospheric-pressure chemical ionization mass spectrometry as a new tool for the determination of the mycotoxin zearalenone in food and feed. *J Chromatogr A* **819**: 277–288.
Rossouw JE, Anderson GL, Prentice RL *et al.*, Writing Group for the Women's Health Initiative Investigators. 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principle results from the Women's Health Initiative randomized controlled trial. *JAMA* **288**: 321–333.
Ruggiero RJ, Likis FE. 2002. Estrogen: physiology, pharmacology, and formulations for replacement therapy. *J Midwifery Womens Health* **47**: 130–138.
Ruiz-Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP, Rice-Evans CA. 1997. Antioxidant activity of phytoestrogenic isoflavones. *Free Radic Res* **26**: 63–70.
Saarinen NM, Wärri A, Mäkelä SI *et al.* 2000. Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (*Picea abies*). *Nutr Cancer* **36**: 207–216.
Salazar EL, Jayme V. 1998. Depletion of specific binding sites for estrogen receptor by *Uncaria tomentosa*. *Proc West Pharmacol Soc* **41**: 123–124.
Saloniemi H, Wähälä K, Nykänen-Kurki P, Kallela K, Saastamoinen I. 1995. Phytoestrogen content and estrogenic effect of legume fodder. *Proc Soc Exp Biol Med* **208**: 13–17.
Santti R, Mäkelä S, Strauss L, Korkman J, Kostian M-L. 1998. Phytoestrogens: potential endocrine disruptors in males. *Toxicol Ind Health* **14**: 223–237.
Scambia G, Mango D, Signorile PG *et al.* 2000. Clinical effects of a standardized soy extract in postmenopausal women: a pilot study. *Menopause* **7**: 105–111.
Scheiber MD, Rebar RW. 1999. Isoflavones and postmenopausal bone health: a viable alternative to estrogen therapy, *Menopause* **6**: 233–241.
Schmitz U. 1993. Nicht Steroidales Anti-Estrogen und Alkaloide aus Rhizom *Cimicifuga racemosa* (Ranunculaceae) (Dissertation) Witten/Herdecke University, Germany.
Schoental R. 1985. Trichotehecenes, zearalenone, and other carcinogenic metabolites of *Fusarium* and related microfungi. *Adv Cancer Res* **45**: 217–290.
Setchell KDR. 1998. Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin Nutr* **68**: 1333S–1146S.
Setchell KDR, Zimmer-Nechemias L, Cai J, Heubi JE. 1998. Isoflavone content of infant formulas and the metabolic fate of these phytoestrogens in early life. *Am J Clin Nutr* **68**: 1453S–1461S.
Setchell KDR, Adlercreutz H. 1988. Mammalian lignans and phyto-oestrogens recent studies on their formation, metabolism and biological role in health and disease. In *Role of the Gut Flora in Toxicity and Cancer,* Rowland IR (ed.). Academic Press: San Diego, 315–345.
Setchell KDR, Brown NM, Lydeking-Olsen E. 2002. The clinical importance of the metabolite equol – a clue to the effectiveness of soy and its isoflavones. *J Nutr* **132**: 3577–3584.
Setchell KDR, Gosselin SJ, Welsh MB *et al.* 1987. Dietary estrogens – a probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology* **93**: 225–233.

Setchell KDR, Lawson AM, Mitchell FL, Adlercreutz H, Kirk DN, Axelson M. 1980. Lignans in man and in animal species. *Nature* **287**: 740–742.

Setchell KDR, Zimmer-Nechemias L, Cai J, Heubi JE. 1997. Exposure of infants to phyto-estrogens from soy-based infant formula. *Lancet* **350**: 23–27.

Severson RK, Nomura AMY, Grove JS, Stemmerman GN. 1989. A prospective study of demographics and prostate cancer among men of Japanese ancestry in Hawaii. *Cancer Res* **49**: 1857–1860.

Sharpe RM, Skakkebaek NE. 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* **341**: 1392–1395.

Sheehan DM. 1998. Herbal medicines, phytoestrogens and toxicity: risk: benefit considerations. *Proc Soc Exp Biol Med* **217**: 379–385.

Sicilia T, Niemeyer HB, Honig DM, Metzler M. 2003. Identification and stereochemical characterization of lignans in flaxseed and pumpkin seeds. *J Agric Food Chem* **51**: 1181–1188.

Sirtori CR. 2001. Risks and benefits of soy phytoestrogens in cardiovascular diseases, cancer, climacteric symptoms and osteoporosis. *Drug Saf* **24**: 665–682.

Smith HH. 1928. Ethnobotany of the Meskwaki Indians. *Bull Public Museum City Milwaukee* **4**: 175–326.

Song TT, Hendrich S, Murphy PA. 1999. Estrogenic activity of glycitein, a soy isoflavone. *J Agric Food Chem* **47**: 1607–1610.

Sprando RL, Collins TFX, Wiesenfeld P *et al.* 2000. Testing the potential of flaxseed to affect spermatogenesis: morphometry. *Food Chem Toxicol* **38**: 887–892.

St. Germain A, Peterson CT, Robinson JG, Alekel DL. 2001. Isoflavone-rich or isoflavone-poor soy protein does not reduce menopausal symptoms during 24 weeks of treatment. *Menopause* **8**: 17–26.

Stitch SR, Toumba JK, Groen MB *et al.* 1980. Excretion, isolation and structure of a new phenolic constituent of female urine. *Nature* **287**: 738–740.

Stoll W. 1987. Phytotherapy influences atrophic vaginal epithelium: Double-blind study – *Cimicifuga* vs. estrogenic substances. *Therapeuticum* **1**: 23–31.

Stormer FC, Reistad R, Alexander J. 1993. Glycyrrhizic acid in liquorice–evaluation of health hazard. *Fd Chem Toxic* **31**: 303–312.

Strom SS, Yamamura Y, Duphorne CM *et al.* 1999. Phytoestrogen intake and prostate cancer: a case-control study using a new database. *Nutr Cancer* **33**: 20–25.

Struck D, Tegtmeier M, Harnischfeger G. 1997. Flavones in extracts of *Cimicifuga racemosa*. *Planta Med* **63**: 289.

Tamir S, Eizenberg M, Somjen D, Izrael L, Vaya J. 2001. Estrogen-like activity of glabrene and other constituents isolated from licorice root. *J Steroid Biochem Mol Biol* **78**: 291–298.

Tamir S, Eizenberg M, Somjen D *et al.* 2000. Estrogenic and antiproliferative properties of glabridin from licorice in human breast cancer cells. *Cancer Res* **60**: 5704–5709.

Tham DM, Gardner CD, Haskell WL. 1998. Clinical Review 97: potential benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. *J Clin Endocrinol Metab* **83**: 2223–2235.

This P, De la Rochefordiere A, Clough K, Fourquet A, Magdelenat H, The Breast Cancer Group of the Institut Curie. 2001. Phytoestrogens after breast cancer. *Endocr Relat Cancer* **8**: 129–134.

Thompson LU, Robb P, Serraino M, Cheung F. 1991. Mammalian lignan production from various foods. *Nutr Cancer* **16**: 43–52.

Thuillier R, Wang Y, Culty M. 2003. Prenatal exposure to estrogenic compounds alters the expression pattern of platelet-derived growth factor receptors α and β in neonatal rat testis: identification of gonocytes as targets of estrogen exposure. *Biol Reprod* **68**: 867–880.

Tou JCL, Chen J, Thompson LU. 1998. Flaxseed and its lignan precursor, secoisolariciresinol diglycoside, affect pregnancy outcome and reproductive development in rats. *J Nutr* **128**: 1861–1868.

Tsutsumi N. 1995. Effect of coumestrol on bone metabolism in organ culture. *Biol Pharm Bull* **18**: 1012–1015.

Uusitupa MIJ, Ruuskaanen E, Makinen E *et al.* 1992. A controlled study on the effect of beta-glucan-rich oat bran on serum lipids in hypercholesterolemic subjects: relation to apolipoprotein E phenotype. *J Am Coll Nutr* **11**: 651–659.

Van der Schouw YT, Kleijn MJJ, Peeters PHM, Grobbee DE. 2000. Phyto-oestrogens and cardiovascular disease risk. *Nutr Metab Cardiovasc Dis* **10**: 154–167.

Van der Weijer PHM, Barentsen R. 2002. Isoflavones from red clover (Promensil®) significantly reduce menopausal hot flush symptoms compared with placebo. *Maturitas* **42**: 187–193.

Vanharanta M, Voutilainen S, Lakka TA, Van der Lee M, Adlercreutz H, Salonen JT. 1999. Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study. *Lancet* **354**: 2112–2115.

Verdeal K, Ryan DS. 1979. Naturally-occurring estrogens in plant foodstuffs–a review. *J Food Prot* **42**: 577–583.

Verzele M. 1986. Centenary review: 100 years of hop chemistry and its relevance to brewing. *J Inst Brew* **92**: 32–48.

Vincent A, Fitzpatrick LA. 2000. Soy isoflavones: are they useful in menopause? *Mayo Clin Proc* **75**: 1174–1184.

Visconti A, Pascale M. 1998. Determination of zearalenone in corn by means of immunoaffinity clean-up and high-performance liquid chromatography with fluorescence detection. *J Chromatogr A* **815**: 133–140.

Wade C, Kronenberg F, Kelly A, Murphy PA. 1999. Hormone-modulating herbs: implications for women's health. *J Am Med Womens Assoc* **54**: 181–183.

Wagner JD, Anthony MS, Cline JM. 2001. Soy phytoestrogens: research on benefits and risks. *Clin Obstet Gynecol* **44**: 843–852.

Wagner JD, Cefalu WT, Anthony MS, Litwak KN, Zhang L, Clarkson TB. 1997. Dietary soy protein and estrogen replacement therapy improve cardiovascular risk factors and decrease aortic cholesteryl ester content in ovariectomized cynomolgus monkeys. *Metabolism* **46**: 698–705.

Wang C-C, Prasain JK, Barnes S. 2002. Review of the methods used in the determination of phytoestrogens. *J Chromatogr B Analyt Technol Biomed Life Sci* **777**: 3–28.

Wang ZY, Nixon DW. 2001. Licorice and cancer. *Nutr Cancer* **39**: 1–11.

Whitten PL, Naftolin F. 1992. Effects of a phytoestrogen diet on estrogen-dependent reproductive processes in immature female rats. *Steroids* **57**: 56–61.

Whitten PL, Naftolin F. 1998. Reproductive actions of phytoestrogens. *Baillieres Clin Endocrinol Metab* **12**: 667–690.

Wilkinson AP, Wähälä K, Williamson G. 2002. Identification and quantification of polyphenol phytoestrogens in foods and human biological fluids. *J Chromatogr B Analyt Technol Biomed Life Sci* **777**: 93–109.

Wiklund IK, Mattsson L-Å, Lindgren R, Limoni C. 1999. Effects of a standardized ginseng extract on quality of life and physiological parameters in symptomatic postmenopausal women: a double-blind, placebo-controlled trial. Swedish Alternative Medicine Group. *Int J Clin Pharmacol Res* **19**: 89–99.

Williams JK, Clarkson TB. 1998. Dietary soy isoflavones inhibit *in-vivo* constrictor responses of coronary arteries to collagen-induced platelet activation. *Coron Artery Dis* **9**: 759–764.

Wiseman H. 1999. The bioavailability of non-nutrient plant factors: dietary flavonoids and phyto-oestrogens. *Proc Nutr Soc* **58**: 139–146.

Wober J, Weibwange I, Vollmer G. 2003. Stimulation of alkaline phosphate activity in Ishikawa cells induced by various phytoestrogens and synthetic estrogens. *J Steroid Biochem Mol Biol* **1782**: 1–7.

Wroblewski Lissin L, Cooke JP. 2000. Phytoestrogens and cardiovascular health. *J Am Coll Cardiol* **35**: 1403–1410.

Wuttke W, Jarry H, Becker T *et al.* 2003a. Phytoestrogens: endocrine disrupters or replacement for hormone replacement therapy? *Maturitas* **44**: S9–S20.

Wuttke W, Jarry H, Westphalen S, Christoffel V, Seidlová-Wuttke D. 2003b. Phytoestrogens for hormone replacement therapy? *J Steroid Biochem Mol Biol* **1789**: 1–15.

Wuttke W, Seidlová-Wuttke D, Gorkow C. 2003c. The *Cimicifuga* preparation BNO 1055 vs. conjugated estrogens in a double-blind placebo-controlled study: effects on menopause symptoms and bone markers. *Maturitas* **44**: S67–S77.

Xu X, Harris KS, Wang H-J, Murphy PA, Hendrich S. 1995. Bioavailability of soybean isoflavones depends upon gut microflora in women. *J Nutr* **125**: 2307–2315.

Yang CZ, Bittner GD. 2002. Effects of some dietary phytoestrogens in animal studies: review of a confusing landscape. *Lab Animal* **31**: 43–48.

Yellayi S, Naaz A, Szewczykowski MA *et al.* 2002. The phytoestrogen genistein induces thymic and immune changes: a human health concern? *Proc Natl Acad Sci USA* **99**: 7616–7621.

Zava DT, Dollbaum CM, Blen M. 1998. Estrogen and progestin bioactivity of foods, herbs, and spices. *Proc Soc Exp Biol Med* **217**: 369–378.

Zhu DPQ. 1987. Dong Quai. *Am J Chin Med* **XV**: 117–125.

Zierau O, Bodinet C, Kolba S, Wulf M, Vollmer G. 2002. Antiestrogenic activities of *Cimicifuga racemosa* extracts. *J Steroid Biochem Mol Biol* **80**: 125–130.

Zung A, Reifen R, Kerem Z, Zadik Z. 2001. Phytoestrogens: the pediatric perspective. *J Pediatr Gastroenterol Nutr* **33**: 112–118.

