Phytoestrogens and breast cancer – promoters or protectors?

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Abstract

The majority of breast cancers are oestrogen dependent and in postmenopausal women the supply of oestrogens in breast tissue is derived from the peripheral conversion of circulating androgens. There is, however, a paradox concerning the epidemiology of breast cancer and the dietary intake of phytoestrogens that bind weakly to oestrogen receptors and initiate oestrogen-dependent transcription. In Eastern countries, such as Japan, the incidence of breast cancer is approximately one-third that of Western countries whilst their high dietary intake of phytoestrogens, mainly in the form of soy products, can produce circulating levels of phytoestrogens that are known experimentally to have oestrogenic effects. Indeed, their weak oestrogenicity has been used to advantage by herbalist medicine to promote soy products as a natural alternative to conventional hormone replacement therapy (HRT). Such usage could increase in light of recent evidence that long-term HRT usage may be associated with an increased risk of breast cancer with a consequent reduction in prescription rates. So, are phytoestrogens safe as a natural alternative to HRT and could they be promoters or protectors of breast cancer? If they are promoters, then we must assume that it is due to their oestrogenic effect. If they are protectors, then other actions of phytoestrogens, including their ability to inhibit enzymes that are responsible for converting androgens and weak oestrogens into oestradiol, must be considered. This paper addresses these questions by reviewing the actions of phytoestrogens on oestrogen receptors and key enzymes that convert androgens to oestrogens in relation to the growth of breast cancer cells. In addition, it compares the experimental and epidemiological evidence pertinent to the potential beneficial or harmful effects of phytoestrogens in relation to the incidence/progression of breast cancer and their efficacy as natural alternatives to conventional HRT.

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Introduction

Breast cancer is not a disease of the modern world and its oldest recorded description was found in an Egyptian seven papyri written between 3000 and 1500 BC. Although the term 'cancer' did not exist at that time (the origin of the word being credited to Hippocrates, 460–370 BC), the 'Edwin Smith' papyrus described details of eight cases of tumors or ulcers of the breasts that are consistent with modern descriptions of breast cancer (Diamondopoulos 1996). Little is known about the epidemiology of breast cancer until 20th century although, interestingly, in 1713 an Italian doctor and a founder of occupational medicine, Bernardino Ramazzini, reported the virtual absence of cervical cancer and relatively high incidence of

breast cancer in nuns (Gallucci 1985). He (prophetically) speculated whether this was in some way related to their celibate life-style. Today, the epidemiology of breast cancer is closely monitored and in both industrialized and developing countries the incidence of breast cancer is steadily rising. It is the commonest form of cancer among women in the US and almost all of Europe and in the US the incidence of breast cancer amongst caucasians rose from 100 cases per 100 000 women in 1983 to over 130 in 2002 (National Cancer Institute, www.cancer.gov/cancertopics/types/breast). The corresponding figures for women in England were under 80 in 1983 to 120 in 2003 (National Statistics online www.statistics.gov.uk).

Apart from age (>50) and a family history of breast cancer, many of the known risk factors for

this disease relate to a woman's reproductive history, such as early menarche, nulliparity or a late pregnancy, and late menopause as well as prolonged oral contraceptive use and hormone replacement therapy (HRT; McPherson *et al.* 2000). More recently, dietary factors and exposure to endocrine-disrupting chemicals have been built into the list of risk factors although definitive evidence is still lacking (Mitra *et al.* 2004). That said, high consumption of phytoestrogens, which are very weak mimics of natural oestrogens, are associated with a lower incidence of breast cancer (Limer & Spiers 2004).

Phytoestrogens, HRT, and breast cancer

Phytoestrogens are plant-derived chemicals that have oestrogenic activity, combining with oestrogen receptors and initiating oestrogen-dependent transcription (Kuiper *et al.* 1998). Their affinities for the oestrogen receptor, however, are at least 1000–10 000 times lower than that of oestradiol and this is an important factor when considering dietary intake of these chemicals and their subsequent circulating concentrations.

There are several different groups of phytoestrogens that are classified according to their chemical structure (see Fig. 1). The most widely studied are the isoflavones, present in high concentrations in soy products and red clover, followed by the flavones and then coumestans (Table 1). Lignans that are widely distributed in fruits and vegetables have been investigated to a more limited extent, despite the fact that the vegetarian diets have been linked with a reduced incidence of breast cancer. More recently, there has been increasing interest in the stilbene, resveratrol

found in grape skins and red wine, and 8-prenylnaringenin present in hops and beer (Table 1).

The outcomes of the Million Women Study and the Women's Health Initiative showing an increased incidence of breast cancer (Beral 2003, Chlebowski *et al.* 2003) as well as coronary heart disease, stroke, and venous thromboembolism raised alarms about the use of HRT to treat menopausal women (Hickey *et al.* 2005). These findings were eagerly reported in the media and, together with the clinical evidence, had a negative impact on HRT prescribing. In fact, within months of these publications, there was a significant decrease in HRT prescriptions, particularly the combined conjugated equine oestrogens and progestagen products, and such declines have been reported in several countries (Lawton *et al.* 2003, Kim *et al.* 2005, Low Dog 2005, Usher *et al.* 2006). This could result in an increased use of 'natural' plant-derived alternatives to HRT that contain phytoestrogens. This raises two questions: are 'natural' alternatives potential promoters of breast cancer or could they actually protect against the growth of breast cancer?

Oestrogen synthesis in breast cancer

The majority of breast cancers (~70%) express oestrogen receptors and in these cases oestrogen is considered to promote tumor growth. Hence, treatments subsequent to surgery, radiotherapy, and/or chemotherapy are directed towards reducing oestrogenic effects within breast tissue (Smith & Chua 2006). The incidence rate of breast cancer continues to increase with age despite the loss of ovarian hormones in postmenopausal women. This apparent paradox has been resolved by the fact that extragonadal sites, including breast, brain, muscle, skin, bone, and adipose tissue can synthesize potent androgens and oestrogens from relatively inactive circulating steroid precursors derived from the adrenal cortex and to a much lesser extent the ovaries (Simpson *et al.* 2005). Indeed, after the menopause, nearly 100% oestrogens are formed in peripheral tissues (Labrie 2003) and exert their effects locally in a paracrine or intracrine manner.

In breast tissues, enzymes are present to convert inactive circulating precursors, such as androstenedione, dehydroepiandrosterone (DHEA) and its sulfated form DHEA-S, and oestrone sulfate (E₁S) into biologically active androgens and oestrogens (see Fig. 2).

In fact, postmenopausal breast cancer illustrates the importance of local oestrogen biosynthesis. In postmenopausal women, the concentration of 17 β -oestradiol (E₂) present in breast tumors is at least 20-fold

Figure 1 Chemical structures of the major classes of phytoestrogens compared with the structures of testosterone and 17 β -oestradiol.

Table 1 Chemical classification of the most widely investigated phytoestrogens and their major dietary sources

Flavones	Flavanones	Isoflavones	Coumestans	Lignans ^a	Stilbenes
Apigenin Quercetin Chrysin 7-Hydroxyflavone Mostly red/yellow fruits and vegetables	Flavanone Naringenin 8-Prenyl-naringenin Citrus fruit, hops	Geinstein Biochanin A Daidzein Formononetin Legumes, particularly soy bean and clover	Coumestrol Bean shoots, alfalfa, clover sunflower seeds	Enterolactone Enterodiol Most cereal, fruits and vegetables	Resveratrol Grape skins, red wine

^aEnterolactone and enterodiol are metabolized in the gut from the plant lignans secoidolariciresinol and matairesinol.

higher than that in the circulation, but in premenopausal women with carcinomas, this ratio was only 5 (Nakata *et al.* 2003, Pasqualini & Chetrite 2005).

Aromatase

The aromatase enzyme, CYP19, is a key enzyme in the conversion of androgens to oestrogens and over 60% of breast carcinomas express this enzyme (Lipton *et al.* 1992, Miller 1991) with higher levels of mRNA expression and activity compared with non-malignant tissue (Bulun *et al.* 1993, Utsumi *et al.* 1996, Chetrite *et al.* 2000). It has been reported that aromatase activity is highest in intratumoral stromal cells rather than the epithelial component in breast cancer (Purohit *et al.*

1995) and, whilst some immunohistochemical studies have supported this observation, others have shown that aromatase was immunolocalized either in both cells types or predominantly in carcinoma cells (see Suzuki *et al.* 2005a).

17 β -Hydroxysteroid dehydrogenases (HSDs)

These enzymes catalyze the interconversion of relatively inactive 17 β -keto steroids (e.g. androstenedione and oestrone) and active 17 β -hydroxysteroids, such as testosterone and oestradiol. To date, 12 isozymes of 17 β -HSD have been cloned, but in breast tissue the important isozymes are 17 β -HSD1, 5, and 7 (see Fig. 3). Oxidative 17 β -HSD2 activity that converts

Figure 2 Steroid synthesis in intratumoral stromal and carcinoma cells and the potential sites at which flavones and isoflavones may inhibit the production of biologically active oestrogens. Androgens taken up by carcinoma cells include androstenedione and DHEA, the latter of which is converted to androstenedione by 3 β -HSD1. Androstenedione is converted to oestrone by aromatase. Oestrone sulphate is converted to oestrone by ETS and 17 β -HSD. ETS, oestrogen sulfatase; EST, oestrogen sulfotransferase/SULT1E1; HSD, hydroxysteroid dehydrogenase.

Figure 3 Enzymes involved in the major steroidogenic pathways that can generate biologically active oestrone and oestradiol. HSD, hydroxysteroid dehydrogenase; Arom, aromatase. *17β-HSD types 3 and 5 convert androstenedione to testosterone and type 2 catalyzes the reverse reaction; **17β-HSD types 1, 7, and 12 convert oestrone to oestradiol and types 2 and 4 catalyze the reverse reaction.

testosterone to androstenedione and oestradiol to oestrone (E_1) is predominant in normal breast tissue, but the reductive activity of 17β-HSD1 that drives the reverse reaction (E_1 to E_2) is dominant in breast cancers (Spiers *et al.* 1998, Miettinen *et al.* 1999). 17β-HSD1 expression has been immunohistochemically detected in breast carcinoma cells of over 55% patients (Sasano *et al.* 1996, Suzuki *et al.* 2000) and in postmenopausal breast cancers 17β-HSD1, mRNA expression was significantly higher than in premenopausal breast cancers as were the intratumoral $E_2:E_1$ ratios (Miyoshi *et al.* 2001). Together, results suggest that 17β-HSD1 may play an important role in maintaining high intratumoral oestradiol levels in postmenopausal breast cancers. Interestingly, 17β-HSD type 12, an isoform of 17β-HSD3 that is important in producing testosterone in the testis, has recently been characterized. 17β-HSD12, like type 1, converts oestrone to oestradiol and recent evidence suggests that this isozyme is the major oestrogenic 17β-HSD enzyme in the ovary (Luu-The *et al.* 2006). It is also highly expressed in mammary tissue though its potential role in breast cancer has not yet been addressed.

Sulfatase and sulfotransferase

Steroid sulfatase (STS) is an enzyme that hydrolyzes several steroids, including E_1S and DHEA-S. In breast tissue, it catalyzes the biologically inactive oestrone-3-sulfate to oestrone, which can be further metabolized to oestradiol by 17β-HSD1. STS activity is

detected in the majority of breast cancers and activity has been correlated with the level of STS mRNA and protein expression in breast cancer cells (Suzuki *et al.* 2003, Pasqualini 2004). This is higher in carcinoma tissue compared with normal breast tissue (Chetrite *et al.* 2000, Utsumi *et al.* 2000, Chetrite *et al.* 2005), and both immunohistochemical studies and real-time reverse transcriptase (RT)-PCR have localized STS expression in carcinoma cells but not in intratumoral stromal cells (see Suzuki *et al.* 2005b). The importance of E_1S and STS in postmenopausal breast cancer is highlighted by the following facts: E_1S is the most abundant circulating oestrogen in postmenopausal women, it has a considerably longer half-life in plasma compared with oestrone, levels of E_1S in breast tumors are considerably higher than in the plasma, and the activity of STS is 50–200 times that of aromatase (Pasqualini *et al.* 1996, Pasqualini & Chetrite 2005). STS mRNA expression is negatively correlated with the clinical outcome of breast cancer patients (Utsumi *et al.* 2000, Miyoshi *et al.* 2003) and it has been proposed that the sulfatase pathway is more important than the aromatase route, since aromatase mRNA expression has no diagnostic value (Reed *et al.* 2005).

Oestrogen sulfotransferase (EST, SULT1E1) that converts oestrogens to inactive oestrogen sulfates is present in both normal and cancerous breast tissue, and EST immunoreactivity was detected in the carcinoma cells of 44% patients with breast cancer (Suzuki *et al.* 2003). Despite the fact that the concentration of E_1S

in breast cancer tissue is 7–11 times greater than in plasma (Pasqualini *et al.* 1996), its significance in relation to the formation of active oestrogens in breast carcinoma cells is poorly understood.

3 β -Hydroxysteroid dehydrogenase

In relation to breast cancer, this enzyme has received little attention. There are two isoforms, 1 and 2; the latter being mainly expressed in the adrenal glands and gonads and type 1 being expressed in the placenta and other tissues, including skin and breast, where it is considered mainly to convert DHEA to androstenedione (Rheume *et al.* 1991). 3 β -HSD activity and immunoreactivity have been detected in breast carcinoma cells in a minority of breast cancer tissues (Gunasegaram *et al.* 1998) and thus, this enzyme may be important in increasing the local concentration of androstenedione, a substrate for oestrogen synthesis.

Phytoestrogens as enzyme inhibitors

There is growing evidence that phytoestrogens could have a protective effect on the initiation or progression of breast cancer by inhibiting the local production of oestrogens from circulating precursors in breast tissue. Indeed, *in vitro* experiments have shown that phytoestrogens inhibit the activity of key steroidogenic enzymes involved in the synthesis of oestradiol from circulating androgens and oestrogen sulfate.

Phytoestrogens and aromatase

Amongst the phytoestrogens, the flavones and flavonones are the most potent inhibitors of aromatase. Early studies showed that apigenin and quercetin were potent inhibitors of aromatase in human placental microsomes and subsequent studies confirmed apigenin as a relatively potent inhibitor of the enzyme, along with chrysin, 7-hydroxyflavone, and hesperetin with IC₅₀s in the order of 0.3–3.0 μ M (Le Bail *et al.* 1998a, Jeong *et al.* 1999). Isoflavones were inactive (Le Bail *et al.* 1998a). In the human adrenocortical cell line, H295R, flavones were consistently more potent inhibitors than flavonones with IC₅₀ values for apigenin, 7-hydroxyflavone, and chrysin ranging from 4 to 20 μ M. These values, however, were between 100 and 1000 times higher than the IC₅₀ for the steroidal aromatase inhibitor, 4-hydroxyandrostenedione (Sanderson *et al.* 2004). Apigenin was a potent inhibitor of aromatase activity in primary cultures of human granulosa cells, significant inhibition being observed at 0.1 μ M, but only when testosterone was used as the substrate with higher doses being required to inhibit the

conversion of androstenedione to oestradiol (Lacey *et al.* 2005). This could be attributed to the higher affinity of androstenedione compared with testosterone for aromatase (Luu-The *et al.* 2001).

Overall, the isoflavones have weak inhibitory activity on aromatase in both cell-free and whole-cell preparations (Le Bail *et al.* 2000, Lacey *et al.* 2005), although Almstrup *et al.* (2002) reported that formononetin, biochanin A, and extract of red clover flowers inhibited aromatase activity at low concentrations (<1 μ M) but had oestrogenic activity at higher doses. In contrast, genistein has been reported to increase the aromatase activity in H295R cells and isolated rat follicles (Sanderson *et al.* 2004, Myllymäki *et al.* 2005), and this was paralleled by an increase in promoter-specific aromatase transcripts (Sanderson *et al.* 2004).

Similarly, lignans have weak inhibitory activity on aromatase in placental microsomes (Adlercreutz *et al.* 1993), human pre-adipocytes (Wang *et al.* 1994), human granulosa luteal cells (Lacey *et al.* 2005), and MCF-7 cells (Brooks & Thompson 2005) with IC₅₀s being >10 μ M, whilst coumestrol inhibited aromatase activity in pre-adipocytes with a K_i value of 1.3 μ M (Wang *et al.* 1994). More recently, Wang *et al.* (2006) showed that the polyphenol in red wine, resveratrol, inhibited aromatase activity in MCF-7 breast cancer cells stably transfected with CYP19 confirming a previous report that red wine inhibited aromatase activity (Eng *et al.* 2001). The IC₅₀ of resveratrol was 25 μ M and kinetic analysis indicated that both competitive and non-competitive inhibition might be involved. Similarly, flavonoid components of the hop (*Humulus lupulus* L.) were also shown to inhibit aromatase as well as 20 μ l/ml concentrations of different beers and lagers (Monteiro *et al.* 2006).

The ability of flavones to inhibit aromatase activity rather than the isoflavones has been attributed to the differences in their chemical structure. In a site-directed mutagenesis study, Kao *et al.* (1998) showed that when the 4'-hydroxyphenol group is attached to C2 (as in the flavones and flavonones), the A and C rings of these phytoestrogens mimic the D and C rings of the androgens substrates. In contrast, when the 4'-hydroxyphenol group is attached to C3 (as in the isoflavones), there is limited binding with aromatase; although this confirmation increases binding with the oestrogen receptor such that the A and C rings of the phytoestrogen mimic the A and B rings of oestrogen (see Fig. 1).

Phytoestrogens and 17 β -HSDs

The isoflavones have been shown to exert inhibitory effects on 17 β -HSD type 1 – the enzyme that converts

oestrone to oestradiol. In both cell-free preparations and T47-D breast cancer cells, genistein and daidzein inhibited the conversion of oestrone to oestradiol between 1 and 10 μM with weaker or no effects of biochanin A (Mäkelä *et al.* 1995, Le Bail *et al.* 2000, Brooks & Thompson 2005), whilst in human granulosa luteal cells only high doses of genistein ($\geq 10 \mu\text{M}$), but not biochanin A, inhibited 17 β -HSD type 1 (Whitehead & Lacey 2003). This contrasts the relatively potent effects of biochanin A on recombinant 17 β -HSD type 5 that reduces androstenedione to testosterone (Krazeisen *et al.* 2001). In MCF-7 and MDA-MB-231 breast cancer cell lines, 100 nM genistein stimulated the oxidation of oestradiol to oestrone and increased the levels of 17 β -HSD2, which was confirmed by western blots (Brueggemeier *et al.* 2001). The flavones have also been reported to inhibit 17 β -HSDs and, in this respect, chrysin, apigenin, and naringenin have been shown to inhibit 17 β -HSD1 at micromolar doses whilst quercetin was without effect (Mäkelä *et al.* 1995, Le Bail *et al.* 2000, Whitehead & Lacey 2003). In contrast, quercetin was a relatively potent inhibitor of 17 β -HSD type 5 (Krazeisen *et al.* 2001). Coumestrol has been reported to inhibit both 17 β -HSD types 1 and 5 in purified enzyme preparations (Mäkelä *et al.* 1995, Krazeisen *et al.* 2001) and more recent studies on MCF-7 cells showed that both enterolactone and enterodiol inhibited the conversion of oestrone to oestradiol with significant inhibition observed at 10 and 1 μM respectively (Brooks & Thompson 2005).

Thus, there is limited data on the effects of phytoestrogens on the reductive and oxidative reactions of 17 β -HSDs, although these may be very important in the generation of oestradiol in oestrogen-producing tissues. In both human granulosa cells and breast cancer cells, the production of oestradiol is several fold higher when androstenedione is used as a substrate compared with testosterone and the conversion of oestrone to oestradiol, which requires only 17 β -HSD type 1, is higher than either the conversion of androstenedione or testosterone to oestradiol (Whitehead & Lacey 2003 and unpublished results). Thus, the activity of 17 β -HSDs exceeds that of aromatase and may be more important in the local generation of oestradiol from oestrone and E_1S than from androgen substrates.

Phytoestrogens, oestrone sulfatase and sulfotransferase

Oestrone sulfatase (ETS) that converts E_1S to oestrone may be more important for generating oestradiol in breast tissue compared with the aromatase pathway (Kirk *et al.* 2001, Reed *et al.* 2005). Despite this,

comparatively few studies have investigated the effects of phytoestrogens on either ETS or oestrogen sulfotransferase (EST/SULT1E1), the latter catalyzing the sulfation of oestrone. Early studies reported that quercetin, genistein, daidzein, and other flavonoids inhibited hepatic ETS, with quercetin being the most potent phytoestrogen (Huang *et al.* 1997). In contrast, daidzein was reported to have no effect on ETS although sulfoconjugates of this phytoestrogen inhibited ETS (Wong & Keung 1997, Harris *et al.* 2004). The effects of several dietary flavonoids, including quercetin, genistein, daidzein, and equol, on sulfotransferases have also been investigated and they inhibited hepatic oestrogen/androgen sulfotransferase with $\text{IC}_{50\text{s}}$ in the nanomolar range (Ghazali & Waring 1999, Mesia-Vela & Kauffman 2003, Harris *et al.* 2004). Although the reported inhibitory potency of these compounds varied between studies, taken together the evidence shows that phytoestrogens have an overall inhibitory activity on enzymes involved in the interconversion of E_1S and oestrone. In addition, dietary flavonoids can be sulfated by several human sulfotransferases, including oestrogen sulfatase (Harris *et al.* 2004). This sulfation may further influence the bioavailability of endogenous oestrogens and alter the ratio of active oestrogens and inactive oestrogen sulfates.

Oestrogen receptors and breast cancer

Two oestrogen receptors (ERs) coded on different genes have been identified – $\text{ER}\alpha$ and $\text{ER}\beta$ – although numerous mRNA splice variants exist for these receptors in both diseased and normal tissue (Moore *et al.* 1998, Flouriot *et al.* 2000, Deroo & Korach 2006). Like other steroid receptors, they are transcription factors and have a similar domain structure (Fig. 4). The N-terminal A/B domains are completely distinct between the two receptors, the DNA-binding C domain is highly conserved, whilst the C-terminal E/F domains are similar, sharing about 50% homology. That said, the ligand-binding cavity in the E domain is highly conserved in the two receptors (Kuiper *et al.* 1996, Mosselman *et al.* 1996).

There are two activation domains in the oestrogen receptors, an N-terminal activation function (AF-1) and a C-terminal activation function (AF-2) and these regions act synergistically to recruit co-activators or co-repressors and thus regulate gene transcription. The AF-1 region of the receptor is involved in protein–protein interactions (e.g. phosphorylation) and can activate transcriptional activity of target genes in the

Figure 4 (1) Functional domains of the ER α and ER β . Activation function-1 (AF-1) can recruit co-regulators for gene transcription independent of ligand binding to the E/F domain of the receptors. The AF-1 has only weak activity in ER β receptors compared with α receptors. The ability of AF-2 to recruit co-regulators is dependent on ligand binding and is equally active in both ER α and ER β . (2) Homodimers of ER β have greater transcriptional activity at response elements of the DNA (e.g. AP-1) other than the specific oestrogen-response element (ERE). This contrasts the binding of ER α homodimers to the ERE. Dimerization of ER β with ER α is thought to silence ER α . (3) Many phytoestrogens bind preferentially to ER β , dimers of which may bind to consensus sites, such as AP-1 and activate/inhibit genes that regulate tumor growth. Binding to ER β may also induce heterodimerization with ER α and hence silence its activation of genes stimulating cell proliferation.

absence of oestrogen or other oestrogenic ligands (Onate *et al.* 1998, Tremblay *et al.* 1999). Co-activator binding to AF-1 leads to either partial or no activation of ER α , whilst full activation of this receptor requires recruitment of co-activators to both AF-1 and AF-2 regions (Tzukerman *et al.* 1994, Benecke *et al.* 2000). This requires binding of oestrogenic ligands (see Bardin *et al.* 2004, Koehler *et al.* 2005). The transcriptional activity of AF-1 in ER β is weak or negligible compared with ER α whilst that of AF-2 is similar in both receptors (Barkhem *et al.* 1998, Cowley & Parker 1999, Delaunay *et al.* 2000). Thus, when only AF-2 activity is required, the transcriptional activities of the two receptors are similar but when both AF-1 and AF-2 are active, then the activity of ER α greatly exceeds that of ER β (McInerney *et al.* 1998, Cowley & Parker 1999).

In addition to the classical mechanism of activated oestrogen receptors regulating gene transcription through binding to the consensus oestrogen response element (ERE) on the DNA, they can also regulate

transcription by binding to other promoter elements on the DNA, such as AP-1-binding sites (Webb *et al.* 1995), cAMP response element (Sabbah *et al.* 1999), and SF1-response element (Vanacker *et al.* 1999) to which other transcription factors bind. Interestingly, there is also some evidence that the potency of the two different ERs on non-ERE-binding sites versus ERE-binding sites differs (Paech *et al.* 1997, Cowley & Parker 1999). For example, ER β is more potent overall than ER α on AP-1-binding sites whilst the contrary occurs on EREs.

There is a distinct tissue expression of ER α and ER β and of their co-regulators (Mueller & Korach 2001) and thus oestrogenic ligands will have different effect in different tissues. In addition, selective oestrogen receptor modulators (SERMs), such as tamoxifen and raloxifen can exhibit tissue-specific oestrogenic activity. Thus, they are both antagonists in breast tissue but agonists in bone, while only raloxifen is a pure antagonist in the uterus (Yamamoto *et al.* 2003). This is because the binding of a SERM results in

specific conformational change in the receptor and this determines which co-activators or co-repressors are recruited (Shang & Brown 2002). Another factor influencing selectivity of ER ligands is that the specific co-regulators recruited also depend on whether the activated oestrogen receptors bind to the ERE promoter or other transcription-binding sites (Klinge *et al.* 2004, Koehler *et al.* 2005). Finally, different ligands to these two receptors will attract different co-activators (McKenna *et al.* 1999, Moggs & Orphanides 2001). Of these co-activators, steroid receptor co-activator 1 (SRC1) has been widely investigated. Interestingly, studies have shown that genistein binding to ER β enhanced interactions between SRC1 and SRC3 but had minimal effects on other transcription factors (Wong *et al.* 2001). Thus, phytoestrogens may not only bind to ERs but may also modulate the recruitment of specific co-activators through which specific gene transcription could be achieved.

A comparison of the relative binding affinities of the two receptor subtypes showed that oestradiol binds with equal affinity and yet tamoxifen and the pure

anti-oestrogen, ICI 164,384, bind with twice the affinity to ER β , diethylstilbestrol with half the affinity, and phytoestrogens with up to five times the affinity for ER β (Kuiper *et al.* 1997). However, the two oestrogen receptors may either form homo- or heterodimers and thus their biological activity may depend on the specific dimerization of the receptors.

There are further complications in unraveling the diverse actions of activated oestrogen receptors (Fig. 5). First, growth factor-stimulated protein-kinase signaling pathways can modulate ER transcription by phosphorylating ER or ER-associated co-regulators and affecting subcellular localization of co-factors (Tremblay *et al.* 1999, Watanabe *et al.* 2001). Thus, several steroid responses are functionally linked to c-Src or tyrosine kinase receptors that can induce ligand-independent stimulation of steroid receptor-mediated transcription (Shupnik 2004). On the other hand, membrane-associated ERs which may be activated by growth factors, can interact with cell signaling pathways, such as the mitogen-activated protein extracellular kinase/extracellular signal-regulated kinase (MEK/ERK) and phosphatidylinositol

Figure 5 Activation and interaction of oestrogen receptors with cell-signaling pathways and inhibition by phytoestrogens. Growth factors activate cell-signaling pathways, (1) such as ERKs and PI3K/AKT (3). They also activate membrane-associated ERs (2). In turn activated kinases phosphorylate and activate oestrogen receptors (4). Activated cytosolic ERs may also modulate the activity of cell-signaling pathways (dotted lines). Genistein is a potent tyrosine kinase inhibitor and inhibits signal transduction of growth factors. Apigenin inhibits PI3K and further inhibits cell signaling.

kinase (PI3K) pathways and thus initiate non-genomic effects (Razandi *et al.* 2004, Levin 2005, Marino *et al.* 2006). Both steroids and growth factors stimulate proliferation of steroid-dependent tumor cells and interaction of these pathways can occur at several levels. This is important in relation to the possible effects of phytoestrogens because although these compounds are all weakly oestrogenic, some are also inhibitors of cell signaling pathways (see below).

Whilst activation of ER α is known to promote growth in breast tumors, the specific functions of ER β are less well understood, although in cell lines activation of ER β inhibits cell proliferation. The ratio of ER α :ER β is increased in carcinogenesis (Rutherford *et al.* 2000, Skliris *et al.* 2003) and expression of ER β was associated with low breast tumor aggressiveness and improved disease-free survival rates compared with those with ER β -negative tumors. One theory for the effects of ER β on the growth of breast tumors is that it may dimerize with ER α and silence its activation functions and that loss of ER β in cancer cells could signal the stage of oestrogen-dependent tumor progression (Hall & McDonnell 1999, Pettersson *et al.* 2000, Lindberg *et al.* 2003). This may be significant as a possible protective effect of phytoestrogens on breast cancer since many phytoestrogens have a stronger affinity for ER β than ER α (Kuiper *et al.* 1998).

Whilst several variants of the full-length ER α have been identified, as well as a short isoform that lacks the N-terminal AF-1 region (Flouriot *et al.* 2000), ER β isoforms are comparatively more abundant and, in human breast tissue, the expression levels of the different isoforms are higher than that of the wild-type ER β (Saji *et al.* 2002, Fuqua *et al.* 2003). The long ER β 1 isoform is now considered to be the wild type (Xu *et al.* 2003), although the original ER β clone encoded a shorter protein with a truncated N-terminal region (Kuiper *et al.* 1996). Therefore, most studies investigating the affinity of phytoestrogens for ER β were carried out on the short form of this receptor (Kuiper *et al.* 1998).

The ER β gene yields other splice variants (numbered 2–5) that encode proteins with different C-terminal amino acids, and in breast tumors total ER β mRNA is significantly lower than in normal breast tissue (Girault *et al.* 2004, Park *et al.* 2006). Several studies have been undertaken to determine whether there is an altered expression pattern of the different isoforms of ER β – notably ER β 1, ER β 2 (ER β cx), and ER β 5 – in breast cancer, but results have not been consistent (Saji *et al.* 2002, Zhao *et al.* 2003, Davies *et al.* 2004, Esslimani-Sahla *et al.* 2005, Park *et al.* 2006). All ER β isoforms can inhibit the

transcriptional activity of ER α on ERE-containing reporters, but only human (h)ER β 1 can bind the oestrogen ligand and has transcriptional activity on its own (Peng *et al.* 2003).

Whilst phytoestrogens induce a stronger transactivation of ER β compared with ER α (though not compared with oestradiol) and can superstimulate ER activation against a background of oestradiol (Harris *et al.* 2005), no studies have addressed the interaction of phytoestrogens with the various isoforms of ER β . A recent study, however, investigated whether or not phytoestrogens could modulate the expression of ER β isoforms (Cappelletti *et al.* 2006). In breast cancer cell lines, both oestradiol and genistein upregulated total ER β , particularly ER β 2, whilst quercetin was without effect. Through modulation of ER β expression, genistein inhibited oestrogen-induced cell growth – perhaps by dimerizing with and hence silencing the growth-promoting effects of ER α ? Therefore, some phytoestrogens may increase the expression of ER β isoforms and this could be important in limiting the oestrogenic stimulation of growth. Clearly, studies are required to investigate the interaction of phytoestrogens with different ER β isoforms and their modulation of ER β expression.

Phytoestrogens and the growth of breast cancer cells *in vitro* and *in vivo*

In vitro studies

The growth of oestrogen-responsive breast cancer cell lines, notably MCF-7 cells, has been used to test the oestrogenicity of various phytoestrogens and xenoestrogens – the E-Screen – which measures metabolically active cells by their ability to cleave the yellow tetrazolium salt, MTT, to purple formazan crystals (Soto *et al.* 1995). This has subsequently been widely used in conjunction with other assays, such as the luciferase-reporter-gene assay, competitive binding assays, viability studies, DNA synthesis, and induction of oestrogen-responsive genes (Guttendorf & Westendorf 2001).

The growth-promoting effects of genistein and other isoflavones in soy products and extracts of red clover, such as daidzein and biochanin A and a metabolite of daidzein, equol, have been most widely investigated, presumably due to the epidemiological evidence linking high soy diets with a lower incidence of breast cancer (Bouker & Hilakivi-Clarke 2000). Overall, studies have shown that low doses ($\leq 10 \mu\text{M}$) of genistein, biochanin A, and daidzein stimulate *in vitro* growth of MCF-7 and T-47D cells, but not the

ER-negative MDA-MB 231/435 breast cancer cell lines, but at higher doses growth and survival of both ER-positive and ER-negative cell lines is inhibited (Zava & Duwe 1997, Hsieh *et al.* 1998, Le Bail *et al.* 1998b, Dixon-Shanies & Shaikh 1999, Hsu *et al.* 1999, Nakagawa *et al.* 2000, Dampier *et al.* 2001, de Lemos 2001, Schmitt *et al.* 2001).

Of the flavones, apigenin and quercetin have been the most widely studied, and reports generally show that both compounds inhibit E₂-induced DNA synthesis and proliferation of ER-positive and ER-negative breast cancer cells (Wang & Kurzer 1998, Collins-Burow *et al.* 2000, Yin *et al.* 2001). There is some debate as to whether these effects are mediated through the ER (van der Woude *et al.* 2005) or through an ER-independent mechanism (Collins-Burow *et al.* 2000). Coumestrol, like genistein, has a bi-phasic effect on cell growth with increased proliferation at low doses and anti-proliferative activity at high doses ($\geq 10 \mu\text{M}$; Wang & Kurzer 1998, Dixon & Shaikh 1999, Schmidt *et al.* 2005). Lignans have also been investigated for their effects on cell growth. At low doses enterolactone stimulated but at concentrations above $10 \mu\text{M}$, it inhibited proliferation of MCF-7 cells (Wang & Kurzer 1997, Saarinen *et al.* 2005).

Resveratrol has been shown to have chemopreventive activity. Jang *et al.* (1997) reported that resveratrol inhibited a number of events associated with the initiation, promotion, and progression of cancer. Numerous other *in vitro* studies have confirmed these findings (Bhat & Pezzuto 2002, Kim *et al.* 2004, Le Corre *et al.* 2005, Lanzilli *et al.* 2006), although Matsumura *et al.* (2005) failed to show any inhibitory effect of resveratrol on the growth of MCF-7 cells except in the presence of oestradiol. In contrast, other studies have shown the resveratrol can have a bi-phasic effect on cells growth in both ER α -positive and ER α -negative cell lines (Basly *et al.* 2000). Finally, recent studies have shown that the effects of prenylnaringenin on MCF-7 cell growth were similar to genistein, in that low doses (10^{-8} – 10^{-6} M) stimulated growth with a sharp decline occurring at 10^{-5} M (Matsumura *et al.* 2005).

The mechanisms through which phytoestrogens may stimulate/inhibit growth of ER-positive breast cancer cells are somewhat controversial, although it is generally assumed that the growth-stimulatory properties of phytoestrogens are mediated through their ability to bind to oestrogen receptors (Matsumura *et al.* 2005). Such receptor binding may stimulate events in the G₁ to S phase entry in the cell cycle (Foster *et al.* 2001) or, like oestrogens, inhibit apoptosis (Schmidt *et al.* 2005). Growth inhibitory effects of high doses of phytoestrogens may involve inhibition of cell-cycle progression,

inhibition of growth factor cell signaling pathways (e.g. tyrosine kinase and/or PI3K) or antagonism of endogenous oestrogens. It is the authors' view that high concentrations of certain phytoestrogens are simply cytotoxic, as has been expressed by others (Maggiolini *et al.* 2001), since concentrations of certain phytoestrogens, such as genistein and prenylnaringenin show little or no dose inhibitory effects on cell growth with a steep reduction being observed at $10 \mu\text{M}$ and above (Matsumura *et al.* 2005).

It is beyond the scope of this review to address the evidence relating to the mechanisms of cell growth/inhibition of breast cancer cells *in vitro*, but it is interesting to note that phytoestrogens bind more strongly to ER β than ER α (Kuiper *et al.* 1998). In a recent study on MCF-7 cells transiently transfected with ER α or ER β , only coumestrol and genistein were shown to bind dose-dependently with ER α with an EC₅₀ of 10^{-6} and 5×10^{-7} M respectively (Harris *et al.* 2005). Other phytoestrogens tested showed very weak binding and usually only at the highest doses tested (10^{-6} and 10^{-5} M) such that IC_{50s}, in the dose range tested, could not be determined. All phytoestrogens investigated including daidzein, equol, apigenin, quercetin, and naringenin bound to ER β with IC_{50s} in the range of 10^{-9} – 10^{-6} M, genistein being most potent in this respect (3×10^{-9} M). In light of these data, it is tempting to speculate why genistein and coumestrol stimulate cell growth and DNA synthesis (Wang & Kurzer 1998). Unlike other phytoestrogens, they can activate the growth-promoting effects of ER α , but the caveat exists that the IC_{50s} for the ER α is 100 times higher than the IC_{50s} for the ER β and thus difficult to reconcile with the evidence that activation of the ER β inhibits growth and might silence ER α activation through dimerization (see above). However, the inability of apigenin and quercetin to bind to ER α , except at very high doses, could explain why these flavones only exert growth-inhibitory effects (Miodini *et al.* 1999, Yin *et al.* 2001) although other cellular mechanisms may exist (see below). In this respect, it is interesting to note that resveratrol binds with comparable affinity to both receptor subtypes, though with 7000-fold lower affinity than oestradiol (Bowers *et al.* 2000), and, whilst the growth-promoting activity of resveratrol at low doses appears to be dependent on ER α , growth-inhibitory effects may not be mediated by antagonizing this receptor (Basly *et al.* 2000).

***In vivo* studies**

The effects of phytoestrogens on mammary tumors *in vivo* have been investigated in chemically induced

(7,12-dimethylbenz(a)anthracene (DMBA) or *N*-methyl-*N*-nitrosomethylurea (NMU)) models of mammary carcinogenesis, nude mice xenografted with breast cancer cell lines and more recently in mouse mammary tumour virus (MMTV)-wt-erbB-2/*neu* transgenic mice. Genistein has been the most widely investigated phytoestrogen, presumably because of the use of soy extracts as an alternative to conventional HRT and because of the comparatively low incidence of breast cancer in Eastern countries, such as Japan and China, where high concentrations of dietary soy products are consumed.

Both genistein and soy protein have been shown to dose-dependently stimulate growth of implanted MCF-7 xenografts (Hsieh *et al.* 1998, Allred *et al.* 2001), whilst a more recent study reported that a soy extract did not stimulate tumor growth in either MCF-7 or MDA-MB-231 xenografts and, when used in association with 17 β -oestradiol, it displayed anti-oestrogenic activity (Gallo *et al.* 2006). In contrast, Shao *et al.* (1998) reported that genistein inhibited growth of these ER-positive and ER-negative xenografts and stimulated apoptosis. Similar anti-tumor effects have also been seen in chemically induced mammary carcinogenesis (Barnes *et al.* 1990, Lamartiniere *et al.* 1998, Constantinou *et al.* 2001) and nude mice (Constantinou *et al.* 1998), although Santell *et al.* (2000) found no growth-inhibitory effects of genistein *in vivo*, even when relatively high circulating plasma concentrations were achieved. In MMTV/*neu* transgenic mice, there were no significant differences in the tumor latency between low- and high-dose isoflavone diets nor in the percentage of disease-free mice at 60 weeks. As anticipated, tamoxifen inhibited tumor growth but tamoxifen in conjunction with a low (but not high) dose isoflavone diet significantly reduced the preventative effect of this oestrogen antagonist (Liu *et al.* 2005). In reviewing the evidence of genistein on breast cancer growth *in vivo*, De Lemos (2001) concluded that this phytoestrogen stimulated growth at low concentrations, but at high concentrations inhibited tumor growth. Few *in vivo* studies have been carried out on other phytoestrogens although anti-tumor effects of apigenin, quercetin, and enterolactone have been reported (Caltagirone *et al.* 2000, Chen *et al.* 2004, Saarinen *et al.* 2005).

Animal models have also been used to look at the cancer chemoprotective effects of resveratrol. Resveratrol increased tumor latency and reduced the number of tumors in an NMU-induced mammary cancer model (Bhat *et al.* 2001), and more recently long-term administration of resveratrol (since birth) was shown to increase the differentiation of lobular structures in the mammary gland and reduce proliferative activity,

whilst reducing numbers and increasing the latency of DMBA-induced tumors (Whitsett *et al.* 2006). Along the same lines, resveratrol reduced tumor growth and angiogenesis but increased apoptosis in ER α negative/ER β positive MDA-MB-231 xenografts *in vivo* (Garvin *et al.* 2006).

Human studies

Several studies have investigated the effects of soy consumption on cell proliferation or biomarkers of cell proliferation in breast tissue. Hargreaves *et al.* (1999) investigated the effect of 60 g/day soy (48 mg total isoflavones) administered for 14 days on breast tissue of premenopausal women. Whilst there was no effect on oestrogen receptor status, proliferation, apoptosis, or mitosis in breast epithelial cells, the levels of apolipoprotein D were significantly lowered and expression of the oestrogen-responsive gene pS2 was increased in nipple aspirate ($P \leq 0.002$), suggesting a weak oestrogenic effect.

In another study, a dietary supplement of 45 mg total isoflavones/day for 14 days significantly increased the thymidine-labeling index and immunocytochemical staining of Ki67 (both biomarkers of cell proliferation) in biopsies of normal breast tissue obtained from women previously diagnosed with either benign or malignant breast disease (McMichael-Phillips *et al.* 1998). These data suggest that short-term dietary soy supplementation can induce proliferation in breast tissue of premenopausal women with breast disease. In contrast, soy consumption has been associated with a reduction of mammographic density (Atkinson & Bingham 2002, Jakes *et al.* 2002), whilst Maskarinec & Meng (2001) reported a positive correlation between self-reported soy food intakes and percentage breast density in women living in Hawaii. Increased mammographic density has been associated with a four- to sixfold increased risk of breast cancer (Atkinson *et al.* 1999). Recent studies on cynomolgus monkeys showed that 12-month dietary soy supplements (equivalent to 129 mg/day) had no effect on the premenopausal breast (Wood *et al.* 2006a), whilst high doses of soy isoflavones (240 mg/day) selectively antagonized oestrogen-induced changes in the postmenopausal primate breast (Wood *et al.* 2006b). Taken together, the overall evidence shows no consistent effects of dietary phytoestrogens on indicators of cell proliferation in normal human breast tissue, although phytoestrogens may increase proliferation in existing breast cancer.

Other actions of phytoestrogens

Whilst this review has focused on the direct action of phytoestrogens on oestrogen synthesis and

oestrogen receptors in relation to breast cancer, other actions have been ascribed to these compounds that may act independently of the ER or indirectly impinge on ER signaling. Phytoestrogens have been shown to increase the levels of human sex hormone binding globulin that will consequently reduce the concentration of circulating free 'active' hormones (Brezinski *et al.* 1997, Adlercreutz *et al.* 1998, Berrino *et al.* 2001). Such an effect could be significant in the progression of breast cancer although local synthesis is considered more important than circulating concentrations of hormones. Highly reactive oxygen have been shown to play a role in the development of cancer and several studies have shown that phytoestrogens can act as anti-oxidants, although the concentrations at which anti-oxidant activity is observed are unlikely to be reached through dietary means (Wei *et al.* 1995, Arora *et al.* 1998, Harper *et al.* 1999, Wilson *et al.* 2002).

Some phytoestrogens are inhibitors of cell-signaling pathways. For example, genistein is an inhibitor of protein tyrosine kinase (Akiyama *et al.* 1987), whilst apigenin and quercetin are inhibitors of the phosphatidylinositol kinase (PI3K) pathway (Nguyen *et al.* 2004). Resveratrol has been reported to inhibit Src tyrosine kinase and blocks Stat 3 activation in malignant cells (Kotha *et al.* 2006). Both MEK/ERK 1/2 and PI3K/AKT pathways can be activated by growth factors and thus phytoestrogens may modulate such control of breast tumor growth (Limer & Speirs 2004, Weldon *et al.* 2005). In addition, activated oestrogen receptors have also been shown to interact with these cell-signaling pathways (Moggs & Orphanides 2001, Bardin *et al.* 2004), and a recent study has shown that resveratrol modulated the PI3K pathway through an ER α -dependent mechanism (Pozo-Guisado *et al.* 2004). Therefore, the cellular actions of phytoestrogens may be complex. Although some kinetic studies show that phytoestrogens may bind competitively with steroid substrates to steroidogenic enzymes, other evidence shows they can alter enzyme expression (Whitehead & Rice 2006). Indeed, recent real-time RT-PCR studies in our laboratory have shown that certain phytoestrogens and low-dose mixtures of phytoestrogens are potent inhibitors of aromatase expression (Rice *et al.* 2006). Therefore, further experiments are required to elucidate the actions of phytoestrogens on cell-signaling pathways rather than their ability to bind to oestrogen receptors.

Epidemiology of breast cancer in relation to dietary phytoestrogens

In the early 1980s, a possible link between isoflavones and lignans and the prevention of breast cancer was noted and this led to numerous studies to evaluate this hypothesis.

The epidemiology of breast cancer in relation to dietary intake of phytoestrogens has been adequately reviewed (Adlercreutz 2003, Ziegler 2004) and the current opinion, mainly based on studies of immigrant populations, suggests that early exposure to relatively high concentrations of soy phytoestrogens may have a protective effect on breast cancer in later life (Adlercreutz 2003). Animal studies support this proposal (Lamartiniere 2000) as does a recent study in Japanese women (Shu *et al.* 2001). However, results from epidemiologic studies are mixed, even from studies in Asian populations (Yamamoto *et al.* 2003).

Evidence relating to a high dietary intake of lignans, particularly in vegetarian diets, and the incidence of breast cancer is more controversial (Adlercreutz 2003). Some studies have shown a modest reduction in breast cancer in women with a high intake of lignans (McCann *et al.* 2004, Ziegler 2004), whilst another study showed that higher enterolactone excretion was associated with a non-significant increase in breast cancer risk (den Tonkelaar *et al.* 2001). In contrast, it was reported that both low (below the 12.5th percentile) and high (above the 87.5th percentile) plasma enterolactone concentrations were associated with an increased incidence of breast cancer (Hulten *et al.* 2002). It should be noted that the metabolism of plant lignans to mammalian lignans in the gut is dependent on diet. An increase in dietary fat decreased urinary excretion of lignans whilst whole grain fiber increased the production of enterolactone (see Adlercreutz 2003).

There is still no conclusive evidence that a high dietary intake of phytoestrogens and the reduced incidence of breast cancer are directly related or whether phytoestrogens are simply biomarkers of a healthy diet and life-style. Despite the evidence that some phytoestrogens at low doses can promote the growth of breast cancer cell lines and increase biomarkers of cellular proliferation in human breast cells after a short-term diet rich in phytoestrogens, current evidence would suggest that a high dietary intake of phytoestrogens does not increase the risk of breast cancer.

Efficacy of phytoestrogens as alternatives to HRT

Many botanical preparations are sold to alleviate menopausal symptoms, but the most widely used are extracts of soy or red clover that contain isoflavones or black cohosh (BC; *Actaea racemosa*; Wuttke *et al.* 2003, Beck *et al.* 2005). The latter, containing triterpene glycosides and aromatic acids, is not strictly a phytoestrogen, and whilst the ‘active’ components of extracts of BC have not yet been identified, total extracts of BC do not bind to oestrogen receptors or show direct classical oestrogenic effects (Viereck *et al.* 2005).

Hot flushes/flushes and night sweats are the most frequent symptoms of the menopause and the most common reason for women to seek symptomatic relief. Therefore, most studies investigating the efficacy of phytoestrogens as alternatives to HRT have specifically focused on the incidence, frequency, and intensity of hot flushes. Unfortunately, most studies to date have been on a small scale and of short-term duration and the majority was not randomized, double-blind or even placebo-controlled trials (Low Dog 2005, Speeroff 2005). The topic has been adequately reviewed and overall evidence shows that phytoestrogen extracts of soy and red clover have little or no effect; at best only 10% reduction of symptoms beyond that of the placebo effect (Kurzer 2003, Geller & Studee 2005). Some studies have shown that black cohosh reduced symptoms, primarily hot flushes and mood disorders (Geller & Studee 2005, Viereck *et al.* 2005), but recent studies have shown no significant effects of this extract on hot flushes (Newton *et al.* 2005, Verhoeven *et al.* 2005).

Conclusion

Equating epidemiological evidence with experimental evidence is fraught with problems. Epidemiological evidence suggests that exposure to relatively high concentrations of phytoestrogens during development or early life may be important in programming an individual’s risk of developing cancer in late life. This could explain why the reduced risk of certain cancers observed among migrants increases with subsequent generations. Alternatively, this may simply reflect changing life-styles and dietary habits unrelated to phytoestrogen intake.

The question of dietary intake versus experimental doses at which phytoestrogens may exert effects is an important consideration. Studies have shown that the average daily intake of phytoestrogens in some Eastern populations is around 30 mg/day compared with

Western societies in which the dietary intake is <10 mg/day or much lower. Reported circulating concentrations of various phytoestrogens range from nanomolar to low micromolar concentrations and yet, with the exception of *in vitro* studies on the growth of ER-positive breast cancer cell lines, most experiments have shown that relatively high concentrations of phytoestrogens, in the micromolar range, are required to exert any pharmacological effects, i.e. at concentrations higher than those likely to be achieved as a result of dietary exposure. It is not known, however, whether or not phytoestrogens accumulate in tissues and whether they accumulate in their conjugated forms, as they mainly exist in the circulation, or in their free active forms.

Short-term dietary supplementation has been shown to have proliferative effects on breast tissue in premenopausal women with breast tumors (not in women without breast disease) and animal studies have provided conflicting data as to whether phytoestrogens stimulate or inhibit chemically induced tumors or tumor implants. Generally, high concentrations of phytoestrogens are required to inhibit specific steroidogenic enzymes and hence local production of oestrogens that could be important in oestrogen-dependent breast cancers. Therefore, there is experimental evidence for both a promotional and a protective effect of phytoestrogens on breast cancer, but at the present time it is impossible to reconcile dietary/supplement exposure with epidemiological and experimental studies. Of major concern is that phytoestrogen supplements are over-the-counter drugs and women who do not find relief of menopausal symptoms with recommended dosages may simply up the dose of such ‘natural’ alternatives and achieve circulating concentrations of these compounds that may have deleterious effects on their health. Further work is required to determine the cellular actions of phytoestrogens beyond the oestrogen receptor and the effects of combinations of different phytoestrogens during long-term exposure.

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