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A SYSTEMIC REVIEW ON EFFECTS OF PHYTOESTROGEN ON BREAST CANCER

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A SYSTEMIC REVIEW ON EFFECTS OF PHYTOESTROGEN ON BREAST CANCER

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Abbreviation

ER	Estrogen Receptor
ERE	Estrogen Response Element
RBA	Relative Binding Affinity
DNA	Deoxy Nucleic Acid
ER+	Estrogen Receptor positive
ER-	Estrogen Receptor negative
PR+	Progesterone receptor positive
PR-	progesterone receptor negative
mERα	membrane Estrogen Receptor alpha
ERK	Estrogen Receptor kinase
SERM	Selective Estrogen Receptor modulators
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
TGF	Transforming growth factor
IGF-1	Insulin like growth factor-1
SHBG	Sex hormone binding globulin
HSD	Hydroxy steroid dehydrogenase
BC	Breast cancer
OR	Odd ratio
RR	Relative risk
CI	Confidence interval
FFQ	Food frequency questioner
PMH	Postmenopausal Hormone
EM	Estrogen metabolite
DMBA	Dimethyl Benz (a) Anthracene
NMU	*N*-Methyl-*N*-Nitrosomethylurea
HRT	Hormone replacement therapy
µM	micro-Molar
DES	Diethylstilbestrol
IC_{50}	50% Inhibitory concentration

Abstract

Background: Interest in the physiological role of bioactive compounds present in plants has increased dramatically over the last decade. Of particular interest in relation to human health are the classes of compounds known as the phytoestrogens, which include several groups of non-steroidal estrogens including isoflavones, lignans and coumestans that are widely distributed within the plant kingdom. Epidemiological studies suggest that diets rich in phytoestrogens, particularly soy and unrefined grain products, may be associated with low risk of breast cancer.

Objective: - This review presents the studies published so far by exploring a link between dietary phytoestrogens and breast cancer.

Methods: A Medline, PubMed and Google internet search was conducted using the keywords breast cancer, phytoestrogens, soybeans, isoflavones, estrogen, estrogen receptor, coumestrole and lignans. Further articles were obtained by cross-matching references of relevant articles.

Results: From the published data we may conclude that overall information about phytoestrogen consumption and breast cancer risk is still scarce: From all prospective studies and the case control studies in Asian populations we get the impression that consumption at young ages (adolescence or earlier) and consumption of high amounts may protect both against pre- and postmenopausal breast cancer.

Conclusion: There is no clear evidence that phytoestrogens intake influences the risk of developing breast cancer.

Keywords: -Breast cancer, phytoestrogens, soybeans, isoflavones, and lignans.

1. Introduction

Phytoestrogens are estrogenic compounds found in plants. The classical definition of phytoestrogens refers to compounds that exert estrogenic effects on the central nervous system, induce estrus, and stimulate growth of the genital tract of female animals (Mindy K *et al.*, 1997).

Some naturally occurring compounds present in plants have been found to possess estrogenic properties. These chemicals have been named “phytoestrogens”. Phytoestrogens display estrogen-like activity since they are structurally similar to human estrogens and therefore they can bind to the estrogen receptor. In this way they are able to mimic or block the action of the human hormone estrogen, although they are much less potent. These compounds enter the human body via the consumption of plants, especially grains, beans, nuts and seeds (Bakker I. 2004).

Phytoestrogens represent a heterogeneous group of herbal substances, which structure is similar to that of 17-β-estradiol. Structures of common phytoestrogens, Selective Estrogen Receptor Modulators (SERMs) and 17-β estradiol are shown in Figure 1.1. They are called estrogen-like molecules or non-steroidal estrogens. In spite of the structural similarity with estradiol, phytoestrogens are diphenolic yet non-steroidal compounds. The group of phytoestrogens includes more than 100 molecules, divided according to their chemical structure (Grace B *et al.*, 2004).

Phytoestrogens were first observed in 1926 but it was unknown if they could have any effect in human or animal metabolism. In the 1940s, it was noticed for the first time that red clover (a phytoestrogens-rich plant) pastures had effects on the fertility of grazing sheep (Thomas J. 2004).

The key structural elements crucial for the estradiol-like effects are: - The phenolic ring that is indispensable for binding to estrogen receptors (ERs), the ring of isoflavones mimicking a ring of estrogens at the receptors binding site, distance between two hydroxyl groups at the isoflavones nucleus similar to that occurring in estradiol and optimal hydroxylation pattern (John K. 2010).

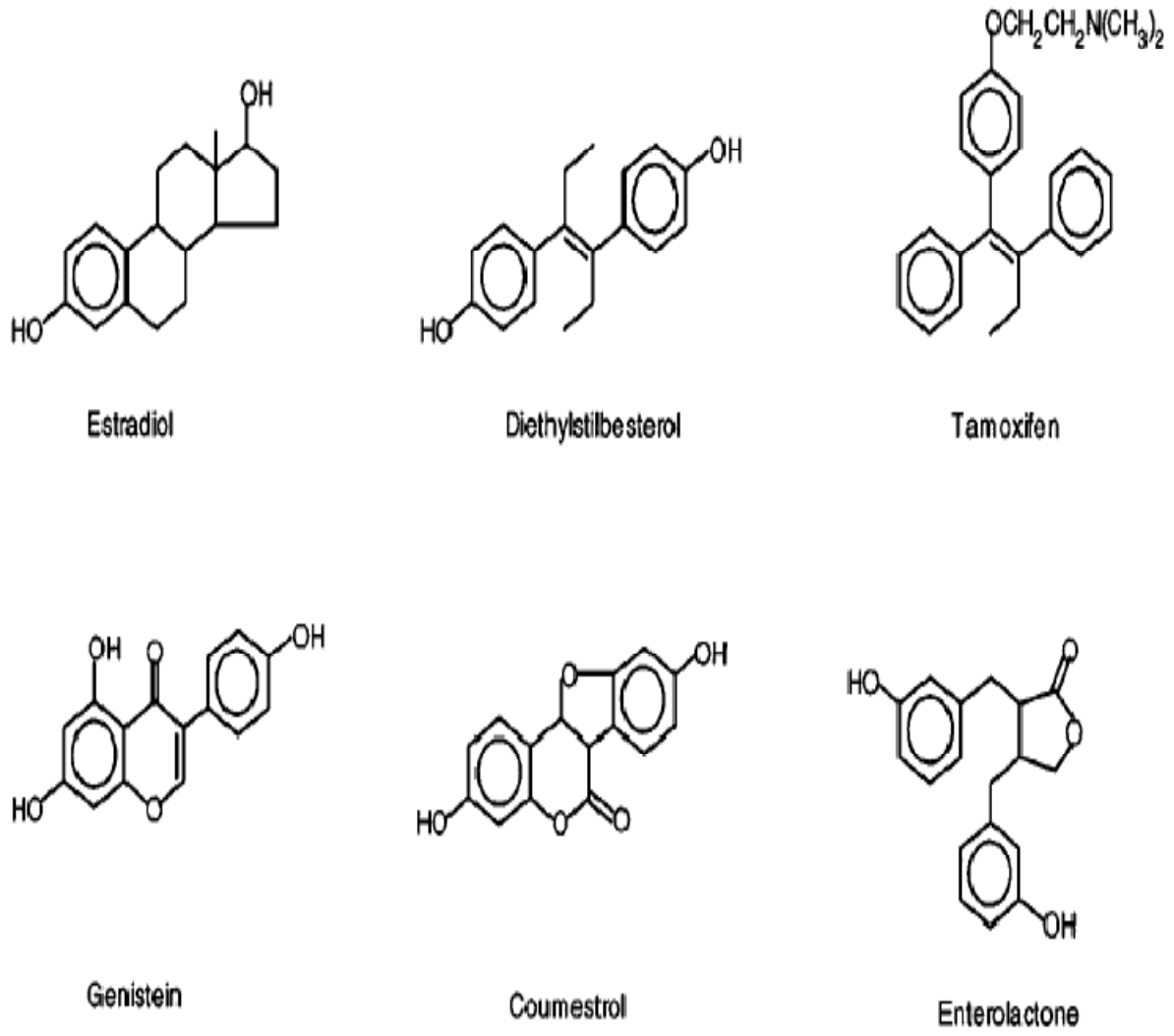


Figure 1.1 Structures of the phytoestrogens genistein (isoflavone), coumestrol (coumestan), and enterolactone (lignan) for comparison with estradiol (natural estrogen), diethylstilbestrol (synthetic estrogen), and tamoxifen (synthetic antiestrogen) (Mindy K *et al.*, 1997).

1.1. Biochemical Classification of Phytoestrogens

The majority of phytoestrogens belong to a large group of substituted phenolic compounds known as flavonoids. Three classes of flavonoids: - the isoflavones, coumestans and prenylated flavonoids are phytoestrogens that possess the most potent estrogenic activity. A class of non-flavonoid phytoestrogens, the lignans has also been identified (Figure 1.2).

The scheme in Figure 1.2 may not be an exclusive list as other phytoestrogens may be identified as constituents of food in the future (Bakker I. 2004).

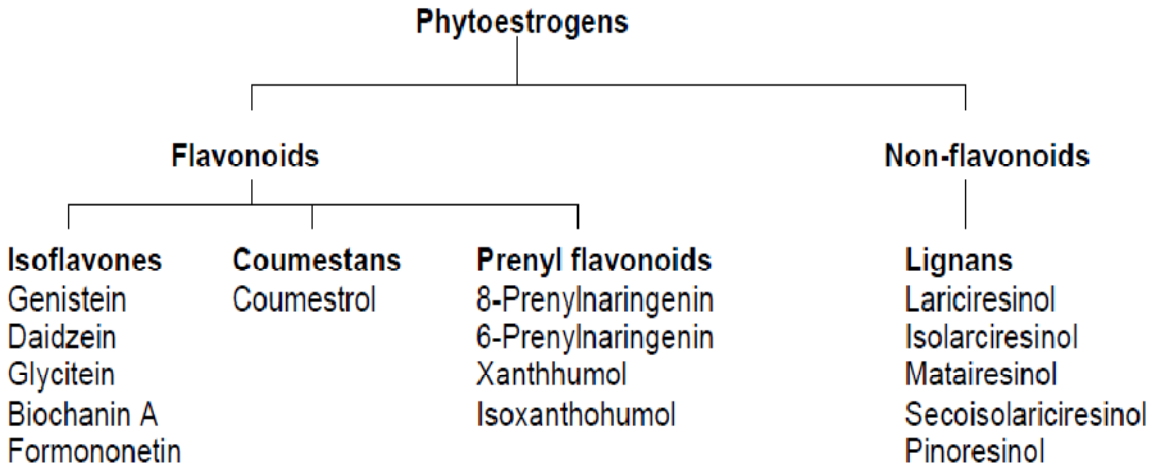


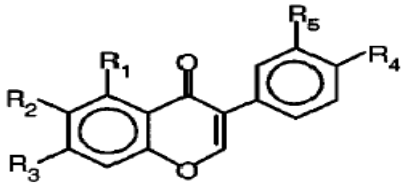
Figure 1.2 various groups of phytoestrogens and members of this group (Bakker I. 2004).

1.2. Dietary sources of phytoestrogens

The main classes of phytoestrogens and their common dietary sources are shown in Table 1.1, which suggest only the isoflavones and the lignans are commonly found in a Western diet. It is possible that other phytoestrogen compounds are present in foods, which have not been detected. Until recently, most of the available information on concentrations of phytoestrogens in foods is related to isoflavone aglucones. This is due to the limitations in the analytical methods used. Data on the concentrations of isoflavone glucosides or glucones (i.e. bound to glucose), prenylated flavonoids, coumestans and lignans are more limited (Rishi K. 2002).

Table 1.1 Phytoestrogens and common dietary sources (Bakker I. 2004)

Phytoestrogen Class	Example of dietary source
Isoflavones	Legumes, lentils, chickpeas, soybean
Coumestans	Young sprouting legumes
Lignans	Most cereals, linseed, fruit and vegetable
Prenylated flavonoids	Some beers(hops)



Isoflavone	R_1	R_2	R_3	R_4	R_5
Daidzein	H	H	OH	OH	H
Genistein	OH	H	OH	OH	H
Glycitein	H	OCH_3	OH	OH	H
Daidzin	H	H	O-glucoside	OH	H
Genistin	OH	H	O-glucoside	OH	H
Glycitin	H	OCH_3	O-glucoside	OH	H
Formononetin	H	H	OH	OCH_3	H
Biochanin A	OH	H	OH	OCH_3	H

Figure 1.3 Chemical structures of the isoflavones found in soybeans. Daidzein, genistein, and glycitein are also present as acetylglucosides (Mindy K *et al.*, 1997).

1.3. Metabolism of the Main Phytoestrogens

Phytoestrogens are mainly absorbed as precursor metabolites (Lignans: Matairesinol, Secoisolariciresinol, Isoflavones: Formononetine, Daidzein, Biochanin A) and metabolized by intestinal bacteria's to actual active compounds (figure 1.4 & 1.5). These effective substances will be either further metabolized or excreted without biochemical alteration in the form of urine or feces.

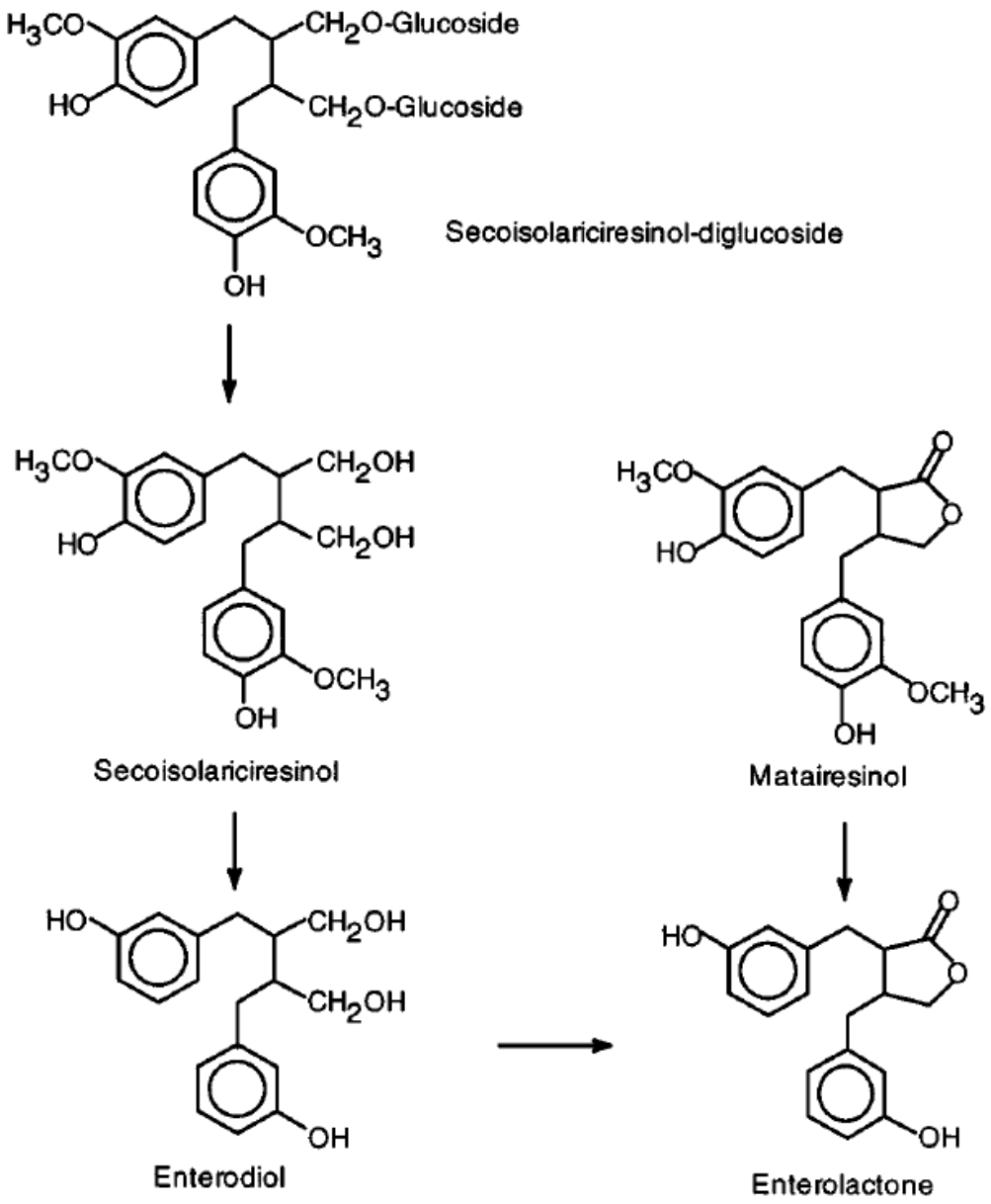


Figure 1.4 Formation of enterolactone and enterodiol by human fecal flora. Secoisolariciresinol diglucoside is metabolized to enterodiol through hydrolysis of the sugar moiety, dehydroxylation, and demethylation. Enterodiol can then be further oxidized to enterolactone. Matairesinol is converted to enterolactone by gut bacteria through dehydroxylation and demethylation (Mindy K *et al.*, 1997).

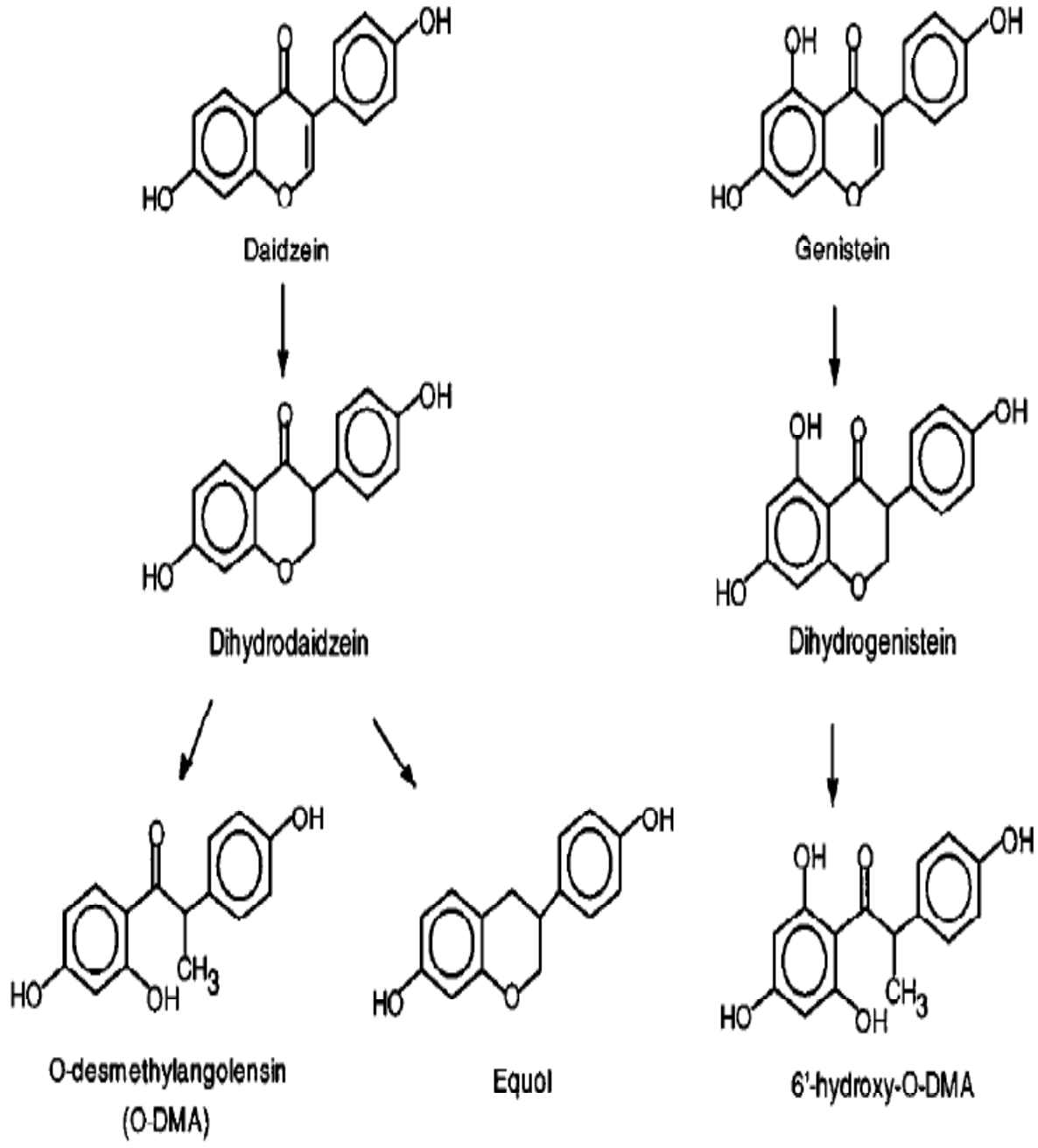


Figure 1.5 Proposed metabolic pathways for the catabolism of daidzein and genistein by human gut bacteria. The formation of O-DMA from daidzein and 6-hydroxy-ODMA from genistein likely involves several reduction reactions. The formation of equol from dihydrodaidzein may go through reduction, dehydration, and further reduction reactions (Mindy K *et al.*, 1997).

1.4. Mechanism action of phytoestrogen

1.4.1. Phytoestrogens are modulators of Estrogen Receptor alpha (ERα) and Estrogen Receptor beta (ERβ) mediated activity

Due to positioning of both hydroxyl groups in a special dimensional order phytoestrogens have a high sterical identity to 17β-estradiol and are therefore able to bind at the estradiol receptor (figure 1.6).

Phytoestrogens bind with different Relative Binding Affinity (RBA) to the ER with special preference of the ER-β. Opposite to the RBA of 17β- estradiol of 100, the RBA of genistein is 5 on ER-α and 36 on ER-β. Other phytoestrogens, for example coumestane (coumestrole), will bind even with a significantly higher affinity to the ER-protein (Table 1.2).

Table 1.2 Relative Binding Affinity (RBA) of different hormones and phytoestrogen to the estradiol receptor α and β in mice (Wolf S. 200l)

	RBA	
	ERα	ERβ
17β-Estradiol	100	100
Estron	60	37
17α-Estradiol	58	11
Estriol	14	21
Tamoxifene	7	6
Coumestrol	94	185
Genistein	5	36
B-Zearalanol	16	14

A large body of evidence demonstrates various features of phytoestrogens: estrogen-like action, estrogen receptor binding, ER-transactivation, estrogen dependent target gene expression or cellular growth effects. Different studies, however, provide variable results regarding the estrogen-like potencies of the phytoestrogens. This may be due to the fact that the potencies of several phytoestrogens have been tested in various cell systems using different techniques (i.e. radioligand binding assays, transactivation assays, target gene expression) under different conditions (such as different dosages, in presence or in absence of estradiol), so that results are not comparable (Wolf S. 2001).

Human endometrial Ishikawa cell line that stably expresses human ERα or ERβ are used to determine the potencies of the best known phytoestrogens for both ERα and ERβ activity (Mueller O *et al.,* 2004). They found that the soy derived genistein, coumestrol and equol displayed a preference for transactivation of Estrogen Receptor β-Estrogen Response Element (ERβ-ERE) responses compared to ERα-ERE responses and were 10- to 100-fold less potent than diethylstilbestrol. Resveratrol, enterolactone and its human metabolite 6-OH enterolactone and human metabolites of daidzein were weak agonists to both ERα and ERβ. Interestingly, they showed that phytoestrogens affect the transcriptional activity of ERα and ERβ in an ERE sequence-dependent manner, thus implying a degree of promoter dependency. Their ligand binding measurements revealed that genistein, coumestrole and equol had high binding affinity to ERα and ERβ and with a distinct preference for ERβ. Quercetin, a common phytoestrogen, has been shown to induce ERE-dependent transactivation in MCF-7 cells through both ERα and ERβ but with a higher capacity, like genistein, to stimulate ERβ responses as compared to the stimulation ERα responses (Moutsatsou P. 2007).

Isoflavones, genistein, daidzein, its metabolite equol, and coumestrol were able to modulate the binding of both ERα and ERβ to EREs. Of note, genistein and daidzein preferentially activated the binding of ERβ to ERE, whereas coumestrol and equol showed only a slight preference in the binding of ERβ to ERE compared to the binding of ERα to ERE. Such data lead to the conclusion that phytoestrogens differ not only in their ERE-transactivation potencies and their ER-binding affinities, but also in their ability to increase the ER binding onto DNA-response elements (EREs) (Kostelac D *et al.,* 2003).

The ability of phytoestrogens to modulate cellular proliferation is also dose-dependent and estrogenic status-dependent. Resveratrol, genistein and quercetin have shown biphasic modulation with regard to proliferation of ER positive breast cancer cell lines. They stimulate proliferation at low concentration (physiologically relevant), the ER possibly involved in adverse cell proliferative effects. However, they inhibit proliferation at concentrations higher than 50-60μM, the inhibitory cellular growth effects considered to be exerted by way of ER-independent pathways (Thomas J. 2004).

Genistein anti-proliferative effects are more pronounced in the presence of estradiol, implying that the “good estrogen” action of genistein is relevant to chemoprotection (Shao M et al., 2000).

Phytoestrogens, similar to estrogens, have been shown to act via several membrane-initiated signaling mechanisms in cell lines expressing the membrane version of ERα (mERα), resulting in the activation of Extracellular Receptor Kinase (ERK). Phytoestrogens have been shown not only to exert direct effects on ER activity but also to affect the formation of endogenous 17β-estradiol, thus regulating indirectly estrogen signaling. Indeed, some inhibit aromatase, an enzyme that catalyzes the conversion of testosterone to estradiol, thus protecting against breast cancer via ER-independent mechanisms (Moutsatsou P. 2007). Moreover, lignans and isoflavones possess a distinct antioxidative capacity through the prevalence of two phenyl groups (radical scavengers).

ERα predominates in endometrium, ovarian stroma and breast cells, whereas ERβ predominate in bone, endothelium and brain. Because of the different tissue distribution of ERs, isoflavonoids possess organ-specific estrogenic and antiestrogenic effects. Thus, they fulfill the criteria of SERMs (Oseni T *et al.*, 2008).

The final effect of isoflavonoids seems to depend, in addition to receptor binding, on the circulating levels of phytoestrogens and endogenous estrogen, so that a “certain amount” of estrogen may appear mandatory for isoflavonoid action (Oseni T *et al*., 2008).

The binding capacities of isoflavonoids to ERα and ERβ may not totally reflect their clinically significant effects, because they can be present in the body at 100-fold higher concentrations than endogenous estrogens and they may have direct nongenomic effects (Nikander E. 2004).

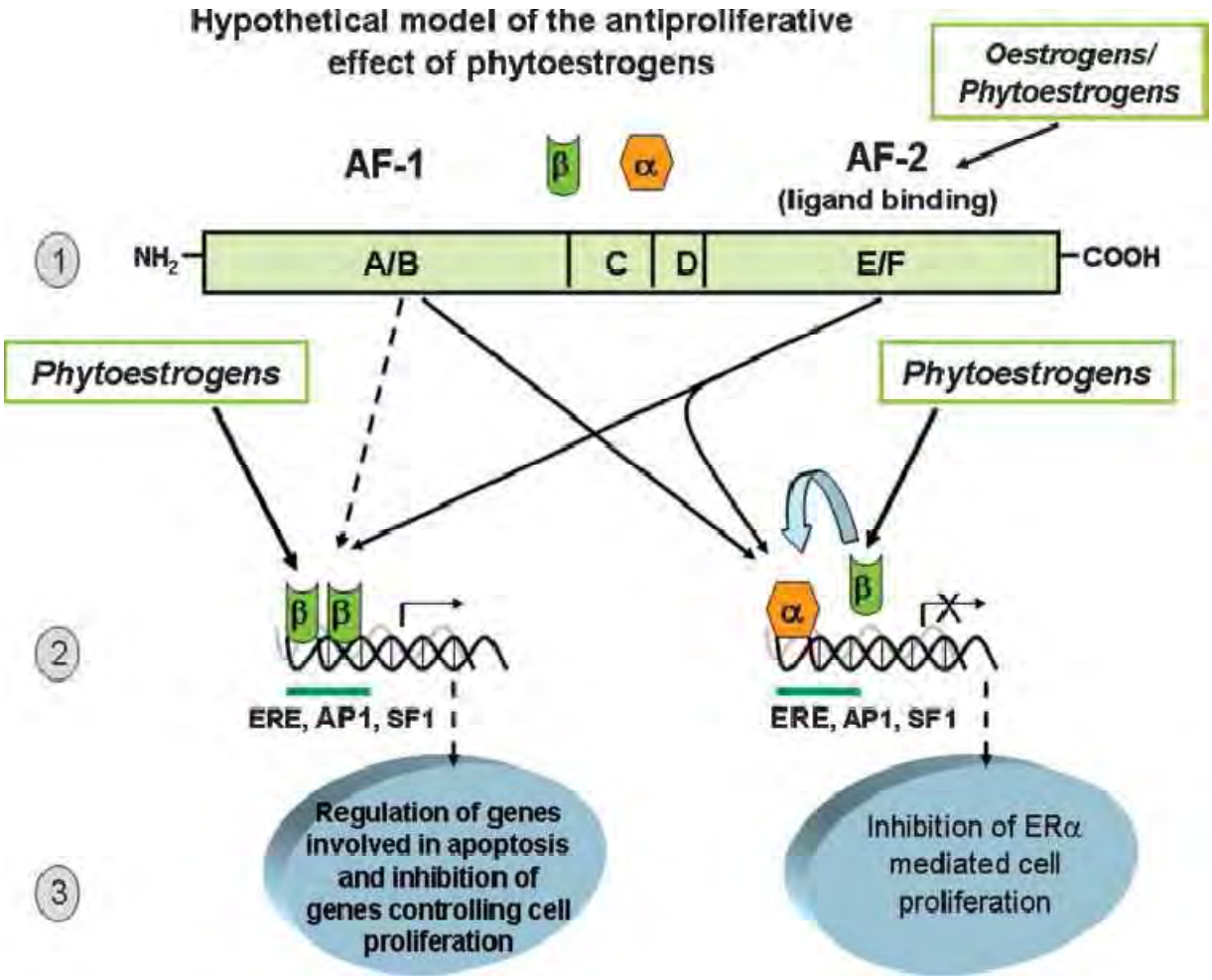


Figure1.6. (1) Functional domains of the ERα and ERβ. Activation function-1 (AF-1) can recruit co-regulators for gene transcription independent of ligand binding to the E/F domain of the receptors. The AF-1 has only weak activity in ERβ receptors compared with α receptors. The ability of AF-2 to recruit co-regulators is dependent on ligand binding and is equally active in both ERα and ERβ. (2) Homodimers of ERβ have greater transcriptional activity at response elements of the DNA (e.g. AP-1) other than the specific estrogen-response element (ERE). This contrasts the binding of ERα homodimers to the ERE. Dimerization of ERβ with ERα is thought to silence ERα. (3) Many phytoestrogens bind preferentially to ERβ, dimers of which may bind to consensus sites, such as AP-1 and activate/inhibit genes that regulate tumor growth. Binding to ERβ may also induce heterodimerization with ERα and hence silence its activation of genes stimulating cell proliferation (Rice S *et a*l., 2006).

1.5. Biological Activity of Phytoestrogens

Lignans and Isoflavones are weak estrogenic and partially antiestrogenic agents. They furthermore possess antimicrobial, anticarcinogenic and anti-inflammatory potentials. The following effects of phytoestrogens are known:-

Increase of SHBG with consecutive decreases of free steroid hormone concentrations. Primarily this is valid for androgens, but also for estradiol. Blocking of the estradiol receptor as a consequence of high concentrations of phytoestrogens. Despite the weak binding affinity, estradiol will be replaced from the receptor due to a significant mass of substrate. The estradiol receptor will be blocked like a proliferation inhibitor (Wolf S. 2001).

The most essential effect of the phytoestrogens is intracellular enzyme inhibition: Genistein is able to inactivate the signal chain of all growth factors of the tyrosine kinase family, for example Insulin-like Growth Factor (IGF-1), Insulin, Epidermal Growth Factor (EGF), Transforming Growth Factor (TGFβ) and Fibroblast Growth Factor (FGF) by blocking the tyrosine kinase as represented in figure 1.7. Furthermore, an inhibitory effect takes place on the aromatase, 17-β-OH-dehydrogenase and 5α-reductase as depicted on figure 1.8 (Rice S *et a*l., 2006).

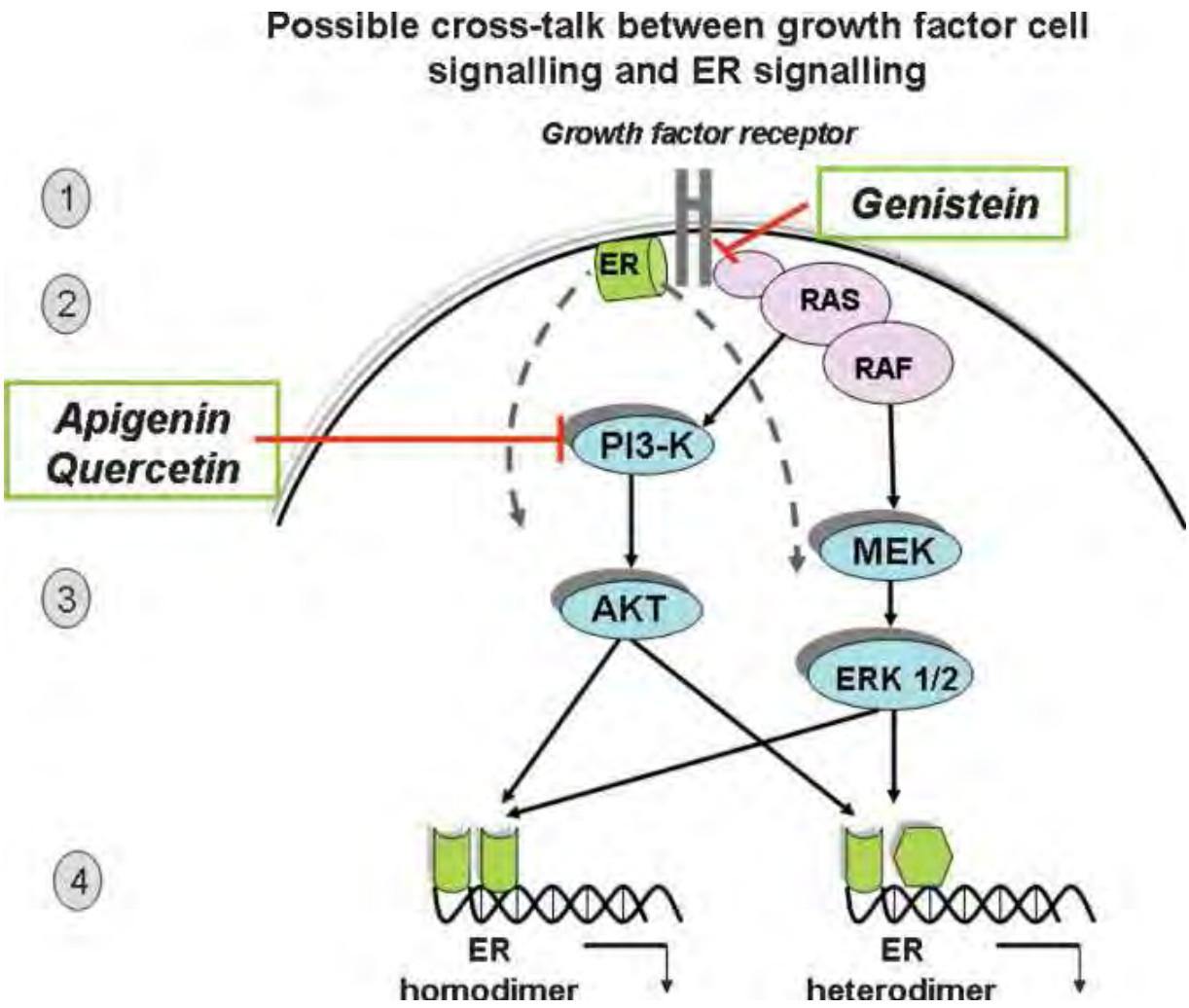


Figure 1.7 Activation and interaction of ER with cell-signaling pathways and inhibition by phytoestrogens.

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 estrogen 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 (Rice S *et a*l., 2006).

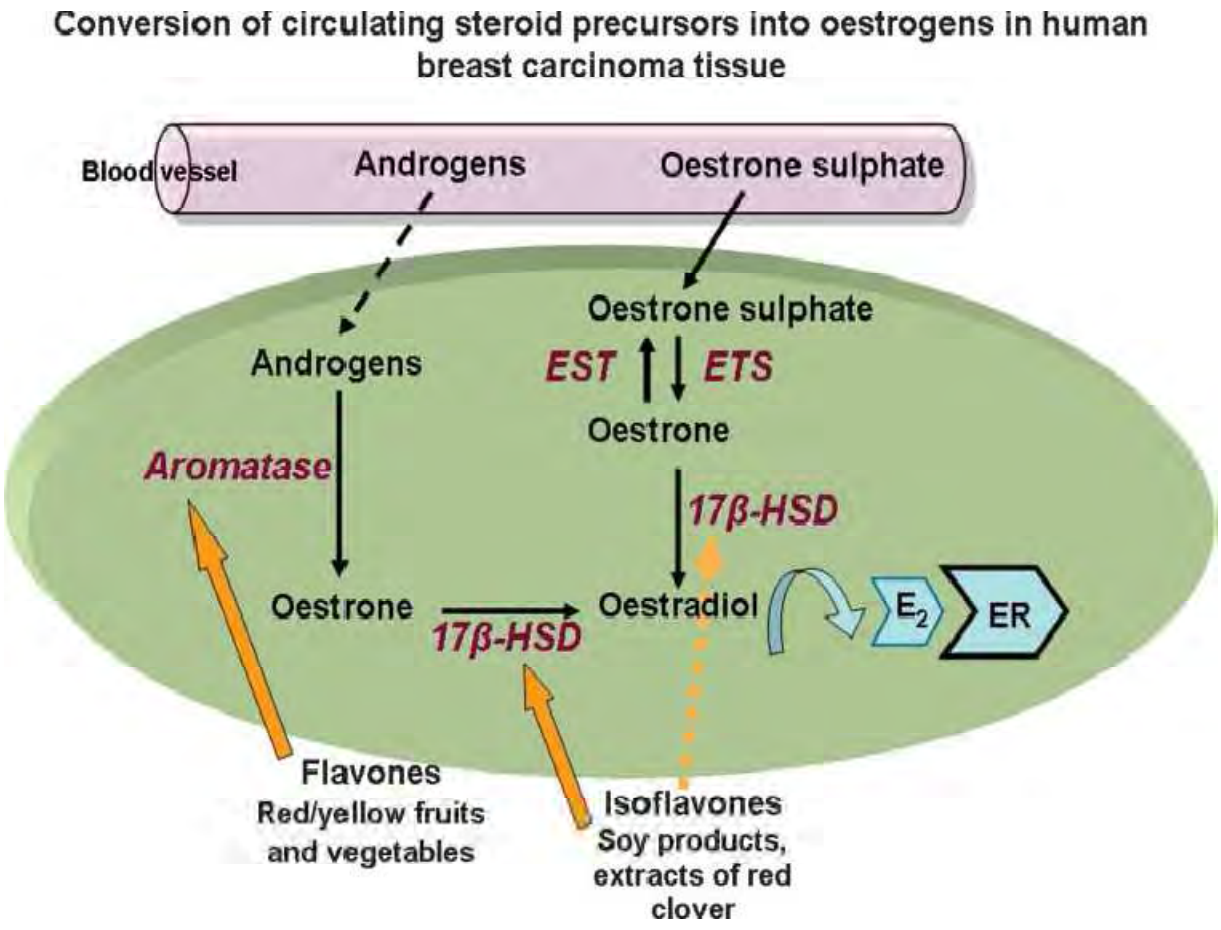


Figure 1.8 Steroid synthesis in intratumoral stromal and carcinoma cells and the potential sites at which flavones and isoflavones may inhibit the production of biologically active estrogens. Androgens taken up by carcinoma cells include androstenedione and DHEA, the latter of which is converted to androstenedione by 3β-HSD1. Androstenedione is converted to estrone by aromatase. Estrone sulphate is converted to estradiol by ETS and 17β-HSD.ETS, estrogen sulfatase; EST, estrogen sulfotransferase/SULT1E1; HSD, hydroxysteroid dehydrogenase (Rice S *et al*., 2006).

2. Literature Review

2.1. Breast Cancer

Normal breast cells grow and divide in a controlled manner to replace cells that have died because of damage or age. Breast cancer is a disease in which abnormal breast cells increase in size, divide and destroy normal tissue. Breast cancer is the most common malignancy among females affecting approximately one out of ten women. Ageing of population in the industrialized world is the most obvious cause of increased breast cancer occurrence; indeed, the risk of developing breast cancer after 65 years of age is 5.8 times higher than before 65, and 150-fold higher than before 30 years of age. In addition to advanced age, a few dozens of other breast cancer predisposing factors have been identified; however, all these diverse risks can be assigned to either of two major categories: excessive exposure to estrogens and deficiency in maintenance of genomic integrity (Imyanitov N *et al*., 2004).

The development of breast cancer is highly dependent on the hormones associated with ovarian function, as such hormone-related events occurring premenopausally, or even in adolescence, may determine whether breast cancer develops postmenopausally. These hormone-related risk factors include early onset of menarche, late onset of menopause, delayed age of first pregnancy and, in postmenopausal women, an elevated free estradiol concentration (Bingham A et al., 1998). There is also evidence from studies of migrants, that the development of breast cancer can be influenced by environmental factors (Mense M *et al.*, 2010).

Breast cancer is one of the most common cancers among women in the United Kingdom and incidence continues to rise. In Ethiopia, where breast cancer is especially fatal given late presentation, limited resources, low awareness of breast cancer and its symptoms, and strong traditional beliefs that can delay biomedical care. However, where new and effective treatments are increasingly available and accessible to the population, understanding and smoothing breast cancer care navigation for patients throughout the healthcare system could significantly improve timely detection and treatment (Dye D *et al*., 2009)

2.2. Phytoestrogens and Breast Cancer Risk

The importance of estrogens in the etiology of breast cancer is widely recognized. Estrogens have been implicated in the initiation and promotion stages of breast cancer, and lifetime estrogen exposure is a major risk factor for breast cancer development.

Estrogens exert their carcinogenic effects via ER-dependent mechanisms as well as their genotoxic metabolites. Phytoestrogens exhibit a wide array of pharmacologic properties, and recently, interest in the potential benefits of diets high in phytoestrogens has intensified, especially those related to chemoprevention. The link between phytoestrogens and breast cancer prevention has been the subject of numerous studies, and the epidemiology of breast cancer in relation to phytoestrogen consumption has recently been extensively reviewed. Generally, epidemiologic studies have been inconclusive, and the relationship between phytoestrogens and breast cancer prevention remains uncertain. Some studies have revealed the modest protective effects of phytoestrogens; others have detected no association between phytoestrogen intake and breast cancer risk; and a few have reported marked protective effects (Gikas D *et al*., 2011).

2.2.1. Phytoestrogens and markers of breast cancer risk

It is thought that an increased risk of breast cancer may be associated, at least in part, with an individual's lifetime exposure to estrogen. It has been suggested that an increase in menstrual cycle length results in lower exposure to estradiol and, over a lifetime, this could correlate to a lower risk of breast cancer. The finding that menstrual cycles in Asian women, consuming a relatively high-soy high-phytoestrogen diets, are generally longer than in Western women and breast cancer incidence is lower in the former lend strong support to this theory (Bingham A et al., 1998).

Large multicentre study conducted among Asian-Americans (n= 597) report that high intake of tofu (≥ 120 times/year) was associated with a lowered risk of breast cancer (OR = 0.85; 95% CI= 0.74-0.99) compared with low intake (< 13 times/year). This finding was demonstrated in both pre- and postmenopausal women following adjustment for age, geographical location, ethnicity and migration history. However, the association was only significant in women born in Asia and not among women of Asian origin born in the US (Wu H *et al*., 1996).

Although most case-control studies have indicated some protective effect of soy, findings have been inconsistent, and some have failed to show any relationship between phytoestrogen intake and breast cancer development.

There has been some evidence that the menopausal status of a woman may modulate the effects of soy. Case-control studies have generally found more evidence for a protective role in premenopausal women versus postmenopausal. This lends support to a current hypothesis that phytoestrogens' effects are dependent on the hormonal status of the woman, with stimulatory effects in low estrogen environments, while in high-estrogen states, they may block the effects of estrogen (Adlercreutz H. 2002).

Phytoestrogens may exert a protective effect by lowering plasma free estrogen and androgen levels through an increase in SHBG concentration. However, the concentration of SHBG is influenced by many factors, including changes in bodyweight, making cross sectional comparisons difficult. The results of short-term intervention studies following supplementation with either flaxseed or soy failed to show any significant increases in SHBG concentration. In premenopausal women (n= 36) given a soy beverage, containing 38 mg total isoflavones, over two menstrual cycles did not find a significant difference in the urinary 2α- hydroxyoestrone:16α hydroxyestrone ratio (Martini C *et al.,* 1999).

In contrast to the above paragraph, 430 Chinese-American, Japanese-American, and Filipino-American women, ages 20 to 55 years, and living in San Francisco-Oakland (California), Los Angeles (California), or Oahu (Hawaii) were studied by collecting 12-hour urine samples. They were postmenopausal (n = 167) or premenopausal in luteal phase (n = 263). Among all women, 2α- hydroxylated Estrogen Metabolite (EM) and 4α-hydroxylation EM were 16% higher (P-trend = 0.02) and 19% higher (P-trend = 0.03) in the highest versus lowest soy tertiles, respectively. In contrast, 16α- hydroxylated EM were 11% lower (P-trend < 0.01). Results were consistent across ethnic and menopausal groups and after adjustment for westernization measured by birthplace (Asia or United States). Findings suggest that regular soy intake is associated with increased ratios of 2α:16α EM and with higher relative levels of 4α-hydroxylated EM. The observed variations in estrogen metabolism might modify breast cancer risk (Furman J *et al.*, 2009).

The effect of soy protein isolate (10-128 mg isoflavones/day) on the plasma hormone and SHBG concentrations of postmenopausal women (n= 14) who were divided into equol and non-equol excretors were considered. Women who excreted equol generally displayed lower concentrations of estrone, estrone sulfate, androgens and prolactin. The equol excretors also had higher concentrations of SHBG and midluteal progesterone. The authors conclude that the plasma hormone profile of equol excretors is associated with a reduced risk of breast cancer (Duncan M *et al.*, 2000).

A study in Australian women (n= 144) showed that high excretion of both equol (OR= 0.27; 95% CI= 0.10-0.69) and enterolactone (OR= 0.36; 95% CI = 0.15–0.86) were associated with a lowering of breast-cancer risk. This effect was particularly strong for equol, which was associated with a 4-fold reduction in risk. Enterolactone was associated with a 3-fold reduction in risk. There were no associations with the parent phytoestrogens daidzein and matairesinol, suggesting that metabolism of these compounds by the gut microflora may be an important factor in reducing the risk of breast cancer (Ingram D *et al*., 1997).

Urinary excretion of lignans and isoflavones (which is assumed to reflect intake) and the risk of breast cancer have suggested a protective role for soy. In a sub-study of a population based case-control study (n= 60) in China, women with a total urinary isoflavone excretion in the highest tertile displayed a 50% reduction in breast cancer risk compared with those in the lowest tertile (OR= 0.14; 95% CI= 0.02-0.88) (Zheng W *et al*., 1999)

A case control study reported that postmenopausal women with breast cancer (n=18) had lower urinary daidzein (p= 0.03) and a trend towards lower genistein (p= 0.08) excretion compared with controls. In addition, women with breast cancer were found to have higher levels of testosterone than those in the control group (p= 0.05). There were no differences between any of the other hormone parameters measured (Murkies A *et al.*, 2000).

Urinary excretion of genistein and enterolactone in postmenopausal women with (n= 88) and without (n= 268) breast cancer were studied. The results showed that increased urinary excretion of genistein was weakly, non-significantly associated with a reduced risk of breast cancer (OR for highest tertile compared with the lowest tertile was 0.83; 95% CI= 0.46-1.51).

Whereas increased urinary excretion of enterolactone was weakly, non-significantly associated with an increased risk of breast cancer (OR for highest tertile compared with the lowest tertile was 1.43; 95% CI= 0.79-2.59) (Den-Tonkelaar I *et al*., 2001).

2.3. Effects of phytoestrogen studies on Animal Models

Many studies have explored the role of phytoestrogens in breast cancer using rodent models of breast cancer initiation and growth. Animals genetically bred to develop breast cancer or the use of a chemical carcinogen administered to the animals have both been used to study the effects of phytoestrogens on breast cancer tumorigenesis. Researchers have also used human breast cancer cell lines (mostly MCF-7, which are ER+ breast cancer cells) injected into laboratory animals and then modulated the animal's diet with phytoestrogens (Duffy C *et al.*, 2007).

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. 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 (Rice S *et a*l., 2006).

When newborn female rats are treated with genistein and then exposed to a chemical carcinogen (DMBA), genistein induces an increased latency and a decreased incidence and number of induced mammary tumors compared with animals treated with vehicle alone. This protective effect is much more marked when animals are treated with genistein during the neonatal or prepubertal period; the number of tumors is less markedly decreased when genistein is administered later. These *in vivo* studies suggest an antitumor activity of genistein. Whether genistein acts as a chemopreventive agent or as an agent to stimulate tumor growth will probably depend on the age at which females receive genistein, on the timing of genistein administration (i.e. before or after the induction of the tumor), and on the dose of genistein (This A *et al.*, 2001).

Mice received test diets containing either fermented soy bean extract (100, 200, 400 mg total isoflavones/kg diet), genistein (200 mg/kg diet), daidzein (200 mg/kg diet) or genistein and daidzein (100 + 100 mg/kg diet) for 21 days. All diets, with the exception of that containing a combination of genistein and daidzein, reduced the ratio of 16α-hydroxyoestrone: 2α-hydroxyestrone in urine. These findings suggest the test diets may exert a cancer protective effect by shifting the metabolism of estradiol toward inactive rather than genotoxic metabolites (Kishida T *et al.*, 2000).

Dietary exposure to genistein (1000 mg/kg diet) did not protect against development of DMBA-induced mammary cancer in ERα wild-type mice. Mammary adenocarcinoma was observed in 56% of these animals. However, tumour development was not observed in ERα knockout mice. The authors conclude induction of DMBA-induced mammary tumours is ERα-dependent (Day K *et al.*, 2001).

2.4. Effects of phytoestrogen studies in vitro

Elevated levels of estrogen in plasma can stimulate the growth of mammary cancer cells. The cell line MCF-7, derived from a human breast tumour, is commonly used to assess the estrogenicity of compounds in vitro (Matsumura A *et al.*, 2005). These cells express ERα and exhibit a biphasic growth response to estrogens. Over recent years, many in vitro studies on mammary cells have identified the effects of the main phytoestrogens, especially genistein and daidzein. Studies of the effects of increasing doses of genistein on induction of an estrogen-dependent protein (pS2) and on the quantity of DNA, reflecting proliferation, in cultures of MCF7 cancer cells (expressing the ERα), demonstrated a biphasic effect of genistein on mammary cells, depending on the concentrations in the culture medium (This A *et al.*, 2001).

At 'physiological' doses (i.e. at doses corresponding to plasma concentrations achieved with a high soy intake (100 nM/L to 1 μM/L), genistein stimulates cellular proliferation and this effect is dependent on ER. At physiological doses, in the presence of physiological doses of estradiol, genistein behaves like a competitive inhibitor for the binding site of E_2 to ER and slightly inhibits cellular proliferation, since it has a lower activity than E_2.

At pharmacological doses (>10 μM/L), it markedly inhibits cellular proliferation. This effect is not ER-dependent and is probably related to inhibition of the tyrosine kinase activity of growth factor receptors.

On clearly defined systems, the key determinants of the activity of genistein therefore appear to be the genistein and estradiol concentrations in the culture medium (This A *et al.*, 2001).

3. Objective

General Objective

- To review the effects of phytoestrogen on breast cancer

Specific Objective

- To provide a basic overview of phytoestrogen, its classification, source and metabolism
- To explain the mechanism action of phytoestrogens
- To explain the association between phytoestrogen use and the risk of breast cancer
- To summarize current evidence regarding the most pressing clinical questions patients and providers may have about phytoestrogens and breast cancer.

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4. Methods

A PubMed, MEDLINE data based and Google internet search was conducted using the keywords breast cancer, soybeans, phytoestrogens, isoflavonoids, and lignans. Each term also was searched alone without the breast cancer term. Further articles were obtained by cross-matching references of relevant articles. In addition, each reference that was obtained was reviewed for citations to articles that may have been missed in the search of the publication databases. Abstracts and full texts were independently screened to establish the suitability for inclusion to this review.

Inclusion criteria

Criteria for the selection of articles included English language and human subjects. Studies had to be of case–control and cohort design, evaluating the risk of invasive breast cancer in relation to phytoestrogen exposure and reporting odds ratios (ORs) or relative risks, as well as 95% confidence intervals (95% CIs). In order to be included in this review, studies had to have data on individual level (original data) and to be published after 2000.

Data extraction

Information extracted from each study included geographic region and design, sample size, dietary assessment method, the number of cancer cases and risk estimates with CIs.

5. Results

Many case-control and cohort studies have been conducted by exploring the role of phytoestrogens in breast cancer risk. By using inclusion criteria, 18 articles were considered as relevant. Out of these, 10 studies conducted on human, four on animal models and four in vitro studies. In this senior paper, 10 articles on human studies have been reviewed on the basis of the inclusion criteria, as described above.

Research on the relation between phytoestrogens and breast cancer risk has been limited in scope. Most epidemiologic studies have involved Asian women and have examined the effects of traditional soy foods (e.g., tofu), soy protein, or urinary excretion of phytoestrogens. Horn-Ross L and his colleague's research examine the effects of phytoestrogenic compounds on breast cancer risk in non-Asian US women. African- American, Latina, and White women aged 35–79 years, who were diagnosed with breast cancer between 1995 and 1998, were compared with women selected from the general population via random digit dialing. Interviews were conducted with 1,326 cases and 1,657 controls. Usual intake of specific phytoestrogenic compounds was assessed via a food frequency questionnaire and a newly developed nutrient database. The average phytoestrogen consumption was 3,174 μg per day for cases and 3,326 μg per day for controls; this difference was not statistically significant (p =0.46). Phytoestrogen intake was not associated with breast cancer risk (odds ratio = 1.0, 95% confidence interval: 0.80, 1.3 for the highest vs. lowest quartile). (Horn-Ross L *et al*., 2001).

The Shanghai breast cancer study, a population based case control study, examined the association between soy food intake and breast cancer risk among women in Shanghai. The study included 1459 breast cancer cases and 1556 age-matched controls. A non-significant reduction in risk ($p< 0.10$) was observed amongst women who reported eating soy foods at least once per week (OR= 0.78; 95% CI= 0.52-1.16). Following adjustments for confounding factors, women in the highest decile compared to the lowest decile intake group were shown to have a 30% lower risk of breast cancer (OR= 0.66; 95% CI= 0.46-0.95). The reduction in risk was also greater for women with estrogen and progesterone receptor positive (ER+/PR+) breast cancer (OR= 0.44; 95% CI= 0.25-0.78) than those with any other ER/PR status (Dai Q *et al*., 2001).

The Shanghai study also obtained information on adolescent (13-15 years) dietary soy food intakes and reported a significant inverse association between intake at this age and risk of breast cancer ($p< 0.001$) in later life. The inverse association was reported for both pre- and postmenopausal women. Details of adolescent soy food intakes were also obtained from the mothers of participant's aged < 45 years. After adjustment for a variety of other risk factors, adolescent soyfood intake was inversely associated with risk, with ORs of 1.0 (reference), 0.75 (95% CI, 0.60–0.93), 0.69 (95% CI, 0.55– 0.87), 0.69 (95% CI, 0.55– 0.86), and 0.51 (95% CI, 0.40–0.65), respectively, for the lowest to highest quintiles of total soyfood intake (trend test, $P < 0.001$). Adolescent soyfood intakes reported by participants' mothers were also inversely associated with breast cancer risk (P for trend < 0.001), with an OR of 0.35 (95% CI, 0.21– 0.60) for women in the highest soyfood intake group. (Shu X *et al*., 2001).

Prospective cohort study in Japan, frequent miso soup and isoflavone consumption was associated with a reduced risk of breast cancer. In January 1990, 21,852 Japanese female residents (aged 40–59 years) from four public health center areas completed a self-administered questionnaire, which included items about the frequency of soy consumption. Through December 1999, 209,354 person follow-up and 179 women were diagnosed with breast cancer. Cox proportional hazards regression was used to estimate the relative risks (RRs) and 95% confidence intervals (CIs) for breast cancer in relation to consumption of miso soup, soyfoods, and estimated isoflavones. All statistical tests were two-sided. Consumption of miso soup and isoflavones, but not of soyfoods, was inversely associated with the risk of breast cancer. The associations did not change substantially after adjustment for potential confounders, including reproductive history, family history, smoking, and other dietary factors. Compared with those in the lowest quartile of isoflavone intake, the adjusted RRs for breast cancer for women in the second, third, and highest quartiles were 0.76 (95% CI = 0.47 to 1.2), 0.90 (95% CI = 0.56 to 1.5), and 0.46 (95% CI = 0.25 to 0.84), respectively (P-trend = .043). The inverse association was stronger in postmenopausal women (P-trend = .006) (Yamamoto S *et al.*, 2003).

Population- based case-control study of breast cancer by age 50 in southern Germany to evaluate the association between dietary intake of different phytoestrogens and premenopausal breast cancer risk. Dietary information was collected from 278 premenopausal cases and 666 age-matched controls, using a validated FFQ. Using multivariate logistic regression, the highest *vs.* lowest intake quartiles of daidzein and genistein yielded significantly reduced ORs (95% CI) for breast cancer risk of 0.62 (0.40–0.95) and 0.47 (0.29–0.74), respectively. The protective effects of daidzein and genistein were found only for hormone receptor-positive tumors. High intake of other isoflavonoids, e.g., formononetin and biochanin A, as well as the sum of isoflavonoids were not associated with a decrease in risk. Breast cancer risk significantly decreased with a high intake of the plant lignan matairesinol (OR =0.58, 95% CI 0.37–0.94) but not secoisolariciresinol or the sum of plant lignans. However, both estimated mammalian lignans, enterodiol and enterolactone, were inversely associated with breast cancer risk, with ORs of 0.61 (95% CI 0.39–0.98) and 0.57 (95% CI 0.35– 0.92), respectively. There results suggest an important role of dietary intake of daidzein and genistein, despite low levels, as well as of matairesinol and mammalian lignans to reduce premenopausal breast cancer risk in this study population (Linseisen J *et al.*, 2004).

Association between phytoestrogen intake and breast cancer risk in a large prospective study in a Dutch population with a habitually low phytoestrogen intake were studied by Keinan-Boker L and his colleagues. The study population consisted of 15,555 women aged 49–70 year who constituted a Dutch cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC; 1993–1997). Data concerning habitual dietary intake in the preceding year were obtained by using a validated food-frequency questionnaire. A total of 280 women were newly diagnosed with breast cancer during follow-up. The median daily intakes of isoflavones and lignans were 0.4 (interquartile range: 0.3–0.5) and 0.7 (0.5–0.8) mg/d, respectively. Relative to the respective lowest intake quartiles, the hazard ratios for the highest intake quartiles for isoflavones and lignans were 1.0 (95% CI: 0.7, 1.5) and 0.7 (0.5-1.1), respectively. Tests for trend were nonsignificant (Keinan-Boker L *et al.*, 2004).

Case-control study within the 383 women (87 pre- or perimenopausal women [mean age, 52 years] and 296 postmenopausal women [mean age, 59 years]) who developed breast cancer were selected as case subjects and were matched to 383 controls, on date of blood sampling. Plasma levels of isoflavones (daidzein, genistein, glycitein, O-desmethylangolensin, and equol) and lignans (enterodiol and enterolactone) was measured. Breast cancer odds ratios were calculated for tertiles of phytoestrogen plasma levels using conditional logistic regression analysis. Higher levels of all isoflavones were associated with lower breast cancer risk. For genistein, the strongest decrease was seen and the 32% reduction in the upper tertile compared with the lowest tertile was statistically significant (OR, 0.68; 95% CI, 0.47 to 0.98). Women with detectable equol levels were shown to have decreased breast cancer risk, compared with women with nondetectable levels (OR, 0.87; 95%CI, 0.63 to 1.21), and when women with detectable levels above the median were compared with women with nondetectable levels, the protection was somewhat stronger (OR, 0.77; 95%CI, 0.49 to 1.21) (Verheus M *et al.*, 2007).

According to Suzuki R and his colleague's, large population-based prospective cohort of postmenopausal women, they observed a significant inverse association between lignan intake and overall breast cancer risk, especially among Postmenopausal Hormone (PMH) user. Among 51,823 women with an average 8.3-year follow-up, 1,284 invasive breast cancer cases were diagnosed, with details of ER/PR status available for 1,188 cases. Of these, 716 were ER+ PR+, 279 ER+ PR-, 50 ER- PR+, and 143 ER- PR- tumours. Compared to women in the lowest quartile (<712 µg/ day), the multivariable adjusted relative risks (RR) for the highest quartile (≥1036 µg/ day) were 0.83 (95% confidence interval=0.70–0.97; P-trend=0.042) for overall, 0.86 (0.69–1.08) for ER_+PR_+, 0.77 (0.54–1.09) for ER_+PR_-, 0.92(0.56–1.52) for ER-PR- (Suzuki R *et al.*, 2008).

One of the first large population-based epidemiologic studies in Asian American women was designed specifically to investigate the role of dietary factors and breast cancer risk. This study was one of the first to examine dietary patterns in relation to breast cancer risk in Asian American women a group experiencing rapid increases in breast cancer incidence. Mediterranean diet was inversely associated with risk; the odds ratio (OR) was 0.65 (95% CI: 0.44, 0.95) in women with the highest scores (≥ 8; most adherent) compared with those with the lowest scores (0–3; P for trend = 0.009), after adjustment for key covariates.

They also used factor analysis and identified 3 dietary patterns (Western-meat/starch, ethnic-meat/ starch, and vegetables/soy). In a combined index of the 3 patterns, women who were high consumers of Western and ethnic meat/ starch and low consumers of the vegetables/soy diets showed the highest risk (OR: 2.19; 95% CI: 1.40- 3.42; P for trend = 0.0005). SHBG concentrations were 23% lower in women with a high intake of the meat/starch pattern and a low intake of the vegetables/soy pattern than in those with a low intake of the meat/starch pattern and a high intake of the vegetables/soy pattern (P for trend =0.069). Thus, changes in dietary patterns may have contributed, in part, to the increasing trend of breast cancer incidence in Asian Americans and in Asia (Wu H *et al.*, 2009).

Table 3 Case-control and cohort Studies Examining Phytoestrogen Intake and Breast Cancer Risk on human

Authors, Year, and Country of Study	**Design**	**Diet assessment Method**	**Sample size Case/control**	**Results**
Horn-Ross L *et al.*, 2001,USA	Population based case control	FFQ	1,326/ 1,657	No association; no change with analysis by menopausal status, individual phytoestrogens, or ethnic groups (odds ratio = 1.0, 95% confidence interval: 0.80, 1.3 for the highest vs. lowest quartile).
Dai Q *et al.*, 2001, shanghai	Population based case control	FFQ	1,459 / 1,556	Reduced breast cancer risk for women in highest decile total soy intake versus lowest decile (OR, 0.66 [0.46 to 0.95]; *P* for trend = 0.02
Shu X *et al.*, 2001, shanghai	Population based case control	FFQ	1,459 / 1,556	Reduced risk of breast cancer in upper quartile of soy intake during adolescent compared with lowest quartile (OR, 0.75 [0.57 to 0.93]) in premenopausal and postmenopausal women
Keinan-Boker L *et al.*, 2004, Netherland	Prospective Cohort study	FFQ	280/ 15,555	No relationship between isoflavone and lignans with breast cancer risk. Relative to the lowest intake quartiles, the hazard ratios for the highest intake quartiles for isoflavones and lignans were 1.0 (95% CI: 0.7, 1.5) and 0.7 (0.5, 1.1), respectively. P-trend was nonsignificant.
Yamamoto S *et al.*, 2003, Japan	Prospective cohort study	FFQ	179/21,852	Decreased risk of breast cancer, RRs for breast cancer for women in the second, third, and highest quartiles were 0.76 (95% CI = 0.47 to 1.2), 0.90 (95% CI = 0.56 to 1.5), and 0.46 (95% CI = 0.25 to 0.84), respectively (P-trend = .043).

Table 3 Case-control and cohort Studies Examining Phytoestrogen Intake and Breast Cancer Risk on human (Continued)

Linseisen J *et al.*, 2004, Germany	Population based case control	FFQ	278/ 666	Reduced risk of breast cancer in highest versus lowest quartiles of daidzein and genistein (OR, 0.63 [95% CI 0.40 to 0.95]; OR, 0.47 [95% CI 0.29 to 0.74, respectively]); intake of enterodiol and enterolactone were also inversely associated with breast cancer risk (OR, 0.61 [95% CI 0.39 to 0.98] and 0.57 [95% CI 0.35 to 0.92], respectively)
Grace B *et al.*, 2004, UK	Prospective cohort study	Urinary daidzein, genistein, glycitein, equol, enterodiol, and enterolactone	114/ 13,070	Urinary and serum isoflavone levels were associated with increased risk of breast cancer, statistically significant for equol and daidzein; for a doubling of level, (OR, 1.34 [95% CI 1.06 to 1.70]) for urine equol; (1.46 [95% CI 1.05 to 2.02]) serum equol; and (1.22 [95% CI 1.01 to 1.48]) for serum daidzein
Verheus M *et al.*, 2007, Netherlands	Nested case control	FFQ	383/ 383	Higher levels of all isoflavones were associated with lower breast cancer risk. For genistein, the risk estimate for the highest versus the lowest tertile was 0.68 (95% CI, 0.47 to 0.98).
Suzuki R *et al.*, 2008, Sweden	Prospective Cohort study	FFQ	1284/ 51 823	Significant inverse association between lignan intake and overall breast cancer risk. RR for the highest quartile (≥1036 mg /day) were 0.83 (95% confidence interval =0.70–0.97; P-trend=0.042)
Wu H *et al.*, 2009, USA	Case control	FFQ	1248/ 1148	Decrease breast risk OR was 0.65 (95% CI: 0.44, 0.95) in women with the highest scores compared with those with the lowest (P for trend =0.009)

6. Discussion

Interest in the physiological role of bioactive compounds present in plants has increased dramatically over the last decade. Of particular interest in relation to human health are the classes of compounds known as phytoestrogens, which embody several groups of non-steroidal estrogens including isoflavones and lignans that are widely distributed within the plant kingdom. These compounds have a wide range of hormonal and non-hormonal activities in animal and in vitro models, and these suggest plausible mechanisms for potential physiological effects of diets rich in these compounds in humans. Experimental and epidemiological data are available to support the concept that phytoestrogen-rich diets exert physiological effects and preliminary human studies suggest a potential role for dietary phytoestrogens in hormone-dependent disease (Gikas D *et al.*, 2011).

The biological action of these compounds is complex and their ultimate cellular actions are determined by many factors including the phytoestrogen compound studied, cell line used species (including genetic polymorphisms) and tissue under examination. The principal postulated anti-carcinogenic mechanism involves their weak estrogen like activity. Phytoestrogens bind to estrogen receptors, initiate only a modest response and at the same time, they block the binding of more potent estrogens. There is structural similarity to the potent synthetic anti-estrogen tamoxifen (Peeters M *et al.,* 2003). Furthermore, numerous other biological activities independent of the ER have been ascribed to phytoestrogens such as antioxidant, anti-proliferative, anti-angiogenic and pro-apoptotic effects. Recent studies suggest that human gut bacterial metabolism of dietary phytoestrogens can alter their biological activities. As such, inter-individual differences in the ability to harbor certain intestinal bacteria might be associated with inter-individual differences in metabolism of phytoestrogens and hence variations in health and/or disease susceptibility (only 30%-50% of the human population can metabolize daidzein to equol) (Atkinson C *et al.*, 2005).

The anti-carcinogenic potential of phytoestrogen on breast cancer risk was evaluated in 10 studies that measured dietary soy (product) (or isoflavones) intake in relation to breast cancer in human in this review.

Four of these studies were prospective cohort studies and one out of these four studies did not found any statistically significant protective effect (Keinan-Boker L *et al*., 2004). Keinan-Boker L and his colleagues studies fail to show any statistical significant on breast cancer protective effects. This is may be due to insufficient consumption of phytoestrogen (i.e. Dietary intake of isoflavones was low). The results of several studies suggest that isoflavone consumption should be high at certain ages (e.g. prepuberty) to yield protective effects. This may explain why they observed no beneficial effects of isoflavone intake in their study: overall consumption was very low, and they obtained data regarding recent consumption only, but not consumption at a young age.

The remaining two of these studies were inversely related with breast cancer risk (Yamamoto S *et al*., 2003; Suzuki R *et al*., 2008). According to Yamamoto S and his colleagues studies, among premenopausal and postmenopausal Japanese women aged 40 to 59 years that specifically asked about miso soup, soybeans, tofu, and natto did suggest a protective effect of breast cancer. Compared with those in the lowest quartile of isoflavone intake the adjusted RRs for breast cancer for women in the second, third, and highest quartiles were 0.76 (95% CI = 0.47 to 1.2), 0.90 (95% CI = 0.56 to 1.5), and 0.46 (95% CI = 0.25 to 0.84), respectively (P-trend = .043). The inverse association was stronger in postmenopausal women (P-trend = .006), this is may be due to inhibition of estradiol biosynthesis. According to this study, highest quartile of isoflavone intake is protective in comparison to lowest quartile isoflavone intake (Yamamoto S *et al*., 2003). Suzuki R and his colleagues also observed a significant inverse association between lignan intake and overall breast cancer risk, especially among PMH user (Suzuki R *et al*., 2008). The possible biological mechanism is not clear, but in vitro studies also showed that lignan ENL in the presence of estrogens suppressed the estrogen-induced proliferation in MCF-7 breast cancer cell and stimulated the synthesis of sex hormone-binding globulin in liver cells (Rice S *et a*l., 2006).

One out of these four-cohort study shows that isoflavone consumption associated with increasing breast cancer (Grace B *et al*., 2004). According to Grace B and his colleagues, urinary and serum isoflavone levels were associated with increased risk of breast cancer, statistically significant for equol and daidzein. For a doubling of level (OR, 1.34 [95% CI 1.06 to 1.70]) for urine equol; (1.46 [95% CI 1.05 to 2.02]) serum equol; and (1.22 [95% CI 1.01 to 1.48]) for serum daidzein.

From the remaining six case-control studies, one out of these six case control studies did not found any statistically protective effect (Horn-Ross L *et al*., 2001). Horn-Ross L and his colleague's analyses showed no association between phytoestrogen exposure and breast cancer risk in there population. These findings were similar for breast cancer in both pre- and postmenopausal women and for specific phytoestrogenic compounds, classes of compounds, and total exposure. Note that the highest quartile of consumption in this population was about only 3 mg/day, a level equivalent to less than one serving of tofu per week. In contrast, the average intake of phytoestrogens in Asian countries has been estimated to range from about 15 to 30 mg/day. Therefore, one of the reasons why these studies fail to show protective effect is due to minimum phytoestrogen consumption. Thus, their findings do not preclude the possibility of a threshold effect with a reduction in risk limited to higher levels of exposure (such as those for Asian and Asian-American women).

However, five of these six case control studies show significant decrease of breast cancer risk (Dai Q *et al*., 2001; Shu X *et al*., 2001; Linseisen J *et al*., 2004; Verheus M *et al*., 2007; Wu H *et al*., 2009). All of these studies found that high level of soy consumption reduces breast cancer risk. According to Dai Q and his colleagues and Shu X and his colleagues, women in the highest decile compared to the lowest decile intake group were shown to have a 30% lower risk of breast cancer (OR= 0.66; 95% CI= 0.46-0.95). The reduction in risk was also greater for women with estrogen and progesterone receptor positive (ER+/PR+) breast cancer (OR= 0.44; 95% CI= 0.25-0.78) than those with any other ER/PR status (Dai Q *et al*., 2001; Shu X *et al*., 2001). Linseisen J and his colleagues study also shows that highest *vs.* lowest intake quartiles of daidzein and genistein yielded significantly reduced ORs (95% CI) for breast cancer risk of 0.62 (0.40–0.95) and 0.47 (0.29–0.74), respectively. The protective effects of daidzein and genistein were found only for hormone receptor-positive tumors (Linseisen J *et al*., 2004).

Similar to the above paragraph Verheus M and his colleagues also shows higher levels of all isoflavones were associated with lower breast cancer risk. For genistein, the strongest decrease was seen and the 32% reduction in the upper tertile compared with the lowest tertile was statistically significant (OR, 0.68; 95% CI, 0.47 to 0.98).

Women with detectable equol levels were shown to have decreased breast cancer risk, compared with women with nondetectable levels (OR, 0.87; 95%CI, 0.63 to 1.21), and when women with detectable levels above the median were compared with women with nondetectable levels, the protection was somewhat stronger (OR, 0.77; 95%CI, 0.49-1.21) (Verheus M *et al*., 2007).

Case-control studies have generally found more evidence for a protective role in premenopausal women versus postmenopausal. This lends support to a current hypothesis that phytoestrogens' effects are dependent on the hormonal status of the woman, with stimulatory effects in low estrogen environments, while in high-estrogen states, they may block the effects of estrogen.

We need however to underline the fact that the majority of these studies were done in Asian populations were the consumption of soy is homogeneously high. Relations with breast cancer may thus be difficult to detect if consumption for the total population is above a threshold level.

Soy would need to exhibit strong anticancer effects at these Western intake levels that correspond with low intake levels in Asian countries. It is difficult to evaluate the likelihood of such a low-dose effect. Plasma isoflavone levels in Western women are much less than 1μ mol/L. This level has not been adequately explored in experimental studies, which have used levels orders of magnitude higher. Thus, for the results in Western women to represent a true association between soy intake and breast cancer risk, they would involve anticarcinogenic effects that have not yet been demonstrated. A possible explanation for similar reductions in breast cancer risk associated with different soy intake levels in Western and Asian women relates to the timing of exposure (Messina J *et al.*, 2001).

Asian women are likely to have been exposed to soy during early life and shows reduced breast cancer risk. This observation is consistent with associations between childhood soy exposure and reduced breast cancer risk in Asian-American studies. An association between soy intake and reduced breast cancer risk among Asian-Americans observed only in the subgroup not born in the United States (Shu X *et al.,* 2001; Wu H *et al*., 2009).

If early life is the critical period for soy exposure, then studies based on adult exposure may not capture the association with breast cancer risk and could underestimate the association in Asian women. Conversely, because most Western women would not have experienced early-life soy food exposures, their exposures in adulthood may be relatively more important in affecting risk. However, this would still require anticarcinogenic effects at fairly low exposure levels during adulthood (Shu X *et al*., 2001).

Systemic pharmacokinetic and dose-response studies are required to determine which dietary compounds can be absorbed in efficiently enough, and to estimate the amounts in diet sufficient to exert the biological effects. Because soy additives in the diet are difficult to quantify with most food frequency instruments and tofu intake is unlikely to correspond with total soy intake in most Western diets, the potential for misclassification may be larger in studies of Western women than Asian women. Use of a single spot urine sample to measure isoflavone levels could be another source of misclassification. These levels may be highly variable owing to time of day and timing with respect to meals. Absorption of isoflavones can differ by ethnicity or other factors, leading to variations in excreted levels despite similar levels of soy protein intake (Horn-Ross L *et al*., 2001*)*.

A recent meta-analysis of cohort and case-control studies examining soy intake and breast cancer risk found that high versus low soy intake was associated with a small reduced breast cancer risk (odds ratio [OR] 95%, confidence interval [CI] 0.75 to 0.99). In this Meta analysis, the protective effect of soy consumption on breast cancer risk appeared to be stronger among premenopausal women. However, the researchers noted a high degree of heterogeneity among studies and lack of a dose-response relationship between soy and breast cancer risk. In addition, the methods of measuring and categorizing soy were different among the studies. The classification of high versus low soy intake in the meta-analysis was based on the cut points chosen by the authors of each study and, hence, was not standardized. In addition, the populations studied were different and, hence, food sources of phytoestrogen differed. Such methodological differences among studies make it difficult to pool results and interpret findings (Duffy C *et al.*, 2007).

Both in vitro studies with breast cancer cell lines and in vivo animal studies suggest that timing of exposure to phytoestrogens and dose of phytoestrogen may be a key component in determining its effects (This A *et al.,* 2001). Overall, studies have shown that low doses (≤10 µM) of genistein, biochanin A, and daidzein stimulate in vitro growth of MCF-7 and T-47D cells, but not 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. Coumestrol, like genistein, has a bi-phasic effect on cell growth with increased proliferation at low doses and anti-proliferative activity at high doses (≥10 µM) (Rice S *et a*l., 2006).

Lignans have also been investigated for their effects on cell growth. At low doses enterolactone stimulated but at concentrations above 10 µM, it inhibited proliferation of MCF-7 cells (Rice S *et al*., 2006).

We can easily see from the data above that almost all studies so far showed no evidence for an increased risk of breast cancer with increased phytoestrogen intake. An absence of risk is important since the putative physiological effects of phytoestrogens have created a market that has been utilized by the nutritional industry. Soy supplements are on the market now and soy flour is increasingly used in the nutritional industry due to its Food and Drug Administration (FDA) approved claim of reducing lipid levels and preventing cardiovascular diseases (Peeters M *et al.,* 2003).

We should not forget that soya has been consumed for thousands of years in Asian countries and over many generations of exposure, there is significant evidence for safe use. Overall, prospective epidemiological studies on phytoestrogens and breast cancer are scarce, limiting our knowledge about the exposure disease relation.

7. Conclusion

The global movement for consuming phytoestrogens rich diet is increasing and tabletized concentrated isoflavone extracts are being promoted heavily. This is because epidemiological data and animal, human and *in vitro* studies support the role of phytoestrogens in lowering the risk of various types of cancers (especially breast and prostate cancer) and cardiovascular disease.

Research suggests that the relationship between phytoestrogens and breast cancer is not straightforward. There is evidence for both a protective role and a stimulatory role in breast cancer cell growth. Many phytoestrogens could show biphasic effects on cell growth, stimulatory at low concentrations and inhibitory at higher concentrations. Since the inhibitory responses at concentrations above 10^{-6}M are seen also in the presence of estradiol, they must be distinguished from any estrogen antagonist responses, but it remains to be resolved as to whether they result from specific non-ER mediated mechanisms or from general toxicity.

The nature of the relationship between phytoestrogens and breast cancer likely depends on a number of factors, including the timing of the phytoestrogen exposure, individual differences in metabolism, hormonal milieu, whether phytoestrogens are consumed as food or as supplement, and the growing conditions and processing practices for the plants that contain phytoestrogens.

8. Recommendation

- Although current data are not sufficient to support dietary recommendations for individual phytoestrogens, there may be great benefit in increased consumption of plant foods.

- Further research is required to evaluate its effects at physiological and pharmacological levels in order to determine the effective doses for beneficial as well as harmful effects, to evaluate the interactions of phytoestrogens with each other and with other dietary components.

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