Phytoestrogens and Human Health

A review of the scientific literature

Some fifty years ago, researchers became aware that phytoestrogens in alfalfa and clovers could affect the fertility of livestock. More recently, multiple epidemiological studies have found a relationship between high dietary intake of isoflavones and lignans and lower rates of certain cancers, cardiovascular problems, and menopausal symptoms.[1,2,3] As far back as 1985, it was known that phytoestrogens could compete with estradiol for binding to intercellular estrogen receptors.[4] Although still inconclusive, scientific evidence is accumulating to suggest that phytoestrogens may have a role in preventing chronic disease.[5] An especially strong body of evidence suggests that they may be effective in preventing and treating prostate cancer, due to their antiandrogenic properties.[6,7]

Natural SERMs

Current research suggests that phytoestrogens may be natural 'Selective Estrogen Receptor Modulators' (SERMs),[8] which means that they can bind to certain estrogen receptors in some tissues, either activating or down-regulating cellular responses (see the National Cancer Institute's 'Science Behind the News' pages for a good explanation of this process). Depending on concentrations of endogenous estrogens, as well as on which receptor complexes are activated or down-regulated, SERMs can have either estrogenic or anti-estrogenic effects.

Research indicates that phytoestrogens may be beneficial to the skeletal[9,10] and cardiovascular systems.* Through preferential binding to beta-type estrogen receptors ('ER beta'), they activate cardioprotective and bone-stabilizing metabolic processes.[11] Simultaneously, the phytoestrogens appear to down-regulate the activity of the alpha-type estrogen receptors ('ER alpha') prominent in breast and uterine tissue. This is one possible mechanism behind their proposed anticancer effects.[12]

In addition, accumulating evidence suggests that phytoestrogens can favorably affect the balance of estrogen metabolites in the body. 'Bad' metabolites (16 alpha-hydroxyestrone, 4-hydroxyestrone and 4-hydroxyestradiol) are genotoxic and mutagenic.[13,14] The ratio of 'good' (2-hydroxyestrone) to 'bad' metabolites is increasingly being used as a marker to assess cancer risk.[15,16,17] Non-ER mediated effects on growth regulation in human breast cancer cells have also been documented for genistein.[18]

Relative binding affinity (RBA) of various estrogens for human estrogen receptors[12] and rat uterine estrogen receptors[19]
(these numbers vary considerably based on the assays and cell types used)

Molecule	Human ER-alpha	Human ER-beta	Rat uterine
Estradiol	100	100	100
Genistein	1.6	100	0.45
Coumestrol	12	34	0.82
Daidzein	0.2	1.8	0.023
Glycitein	A recent study found the binding affinity of glycitein to be ~ 1/20 that of genistein in a mouse uterine assay; curiously, glycitein increased mouse uterine weight by 150% as compared to 50% for genistein.[84]		
Formononetin Biochanin A	Both compounds show very little affinity for estrogen receptors; however, biochanin A is converted to genistein and formononetin to daidzein in the human body.		

*Several studies have demonstrated that intact soy protein (including the phytoestrogens) has cardiovascular benefits. This is not the case, however, for soy protein from which the isoflavone fraction has been removed, or for the isolated isoflavones. This synergistic effect is not fully understood by science.[83]

How Strong are Phytoestrogens?

The scientific literature contains apparently conflicting reports regarding the relative 'estrogenicities' of the phytoestrogens vs. the endogenous estrogens. Many *in vitro* studies have indicated that phytoestrogens have some 1/100 to 1/1000 the binding affinity of estradiol for cellular estrogen receptors.[19] This has led to the interpretation that phytoestrogens are 100 to 1000 times weaker than estradiol. But the situation is not so simple! Some of the reports on binding affinity did not distinguish between affinities for the 'alpha' and the (more recently discovered) 'beta' type estrogen receptors. There is also evidence that other kinds of receptors, beyond just ER-beta and ER-alpha, may be activated by estrogenic chemicals; this could be the case for phytoestrogens as well.[22]

Once bound to the known estrogen receptors, endogenous estrogens and phytoestrogens may have different activating or down-regulating effects on the 'ER-ERE' (Estrogen Receptor - Estrogen Response Element) complex. This complex ultimately tells the cell which genes to switch on and off, leading to significantly different physiological states.[12]

For example, when genistein binds to ER-beta, the receptor changes shape in a way similar to the change that occurs with the binding of the drug raloxifene (raloxifene is a SERM prescribed for osteoporosis). When estradiol binds to this same receptor, it induces a different shape change.[23,24] This suggests that the ER-genistein pair could have some of the same beneficial effects as the ER-raloxifene combination.

In addition to binding affinity, another factor to consider is the influence of high plasma levels of phytoestrogens which can be present at some 100 to 1,000 times the concentration of endogenous estrogens[20] (and even higher in soy-formula-fed infants). In addition, phytoestrogens may have different bioavailabilities than endogenous estrogens, due to the fact that they bind less tightly to steroid hormone serum transport proteins.[21]

Complicating the picture is the fact that many phytoestrogens are converted by human colon bacteria into other compounds (including enterodiol, enterolactone, and equol). Some of these metabolites are more potent than their precursors, while others are less so. Different individuals, depending on factors such as their particular gut flora and/or genetic makeup, produce different concentrations and proportions of these metabolites. There is also evidence that phytoestrogen activity is modulated by the levels of a person's endogenous estrogens.

Furthermore, the estrogenic effect of any particular compound is not the same in different types of cells and tissues. Nor is it identical in different species, so it is not possible to directly apply the results of *in vitro* and animal research to humans. Finally, the different sexes (in both animals and man) can have different responses to phytoestrogens. Receptor-binding affinity, then, is only one factor amongst many that determines the actual hormonal effects of any particular phytochemical.

Overall, the situation is far more complex than we once realized. Many biochemical factors are involved; all phytoestrogens are not the same; all tissues do not respond identically; some people respond differently than others. Considering this, we must remember to focus on information from traditional usage, epidemiological studies, and clinical trials when trying to understand the true effects of phytoestrogens on human health. It is important not to draw premature conclusions (as is often done in the popular press) from animal and *in vitro* research.

Areas of Concern

Isoflavones in infant formulas

Estimates of isoflavone intake in the traditional Japanese diet range from 15 - 200 mg/day. However, scientific data on human exposure to higher doses is difficult to find. Nonetheless, approximately one million American infants ingest large doses of phytoestrogens in soy-based formula every year. These children sustain plasma phytoestrogen concentrations of up to 7,000 nm/L (compared to an average of 744 nm/L in adult Japanese women).[25] A recent study in the *Lancet* noted that the average daily exposure to phytoestrogens from baby formula was 6 - 11 times higher than a hormonally active dose in adults, and plasma concentrations of isoflavones were some 13,000 - 22,000 times higher than endogenous estrogen concentrations in the infants studied.[26]

Should this extreme level of dosage have obvious short-term adverse health effects, one would expect them to show up in the extensive literature. However, the only conclusive reports of negative reactions to soy formulas have been due to allergies (an estimated 3 - 4% of infants are allergic to soy).[27] Studies following children through adolescence have not reported any obvious adverse reproductive effects.[28] A retrospective cohort study published in JAMA (2001) examined 811 subjects in their twenties and thirties, and found no statistically significant differences between those who had soy formula vs. those who had cow's milk as infants.[29] Whether or not early exposure to high doses of isoflavones has any positive or negative effects on cancer rates or cognitive and neurological parameters in later life is not yet known.

For a commentary on the toxicology and pharmacology of isoflavones, including information on soy formula, see the IFT's Toxicology and Safety Division newsletter, Spring 2002. For a more cautious evaluation, see this page from Cornell. One published study noted a statistically significant correlation between soy infant formula and premature thelarche in a few children under (but not over) the age of two.[30] There is some speculation that soy formula could be contributing to the increase in premature puberty among American girls, but scientific data is lacking. The bibliography from Cornell has a section on hormones in food and premature puberty.

Phytoestrogens and Breast Cancer

Isoflavones

Based on results from some *in vitro* and animal studies, concern has arisen that the estrogen agonist effects of isoflavones might increase the growth of breast cancer cells. Though there is still some controversy, the majority of scientific opinion seems to be coming down in

favor of using phytoestrogen-containing foods for the prevention and treatment of breast cancer.[31]

Several studies have indicated that countries with the highest phytoestrogen consumption have the lowest rates of breast cancer,[32,33] but other epidemiological studies suggest the lack of a causative relationship. No studies, however, have found an increased risk of breast cancer with increased soy consumption. A recent epidemiological study involving 2,983 women found no association between the average American dietary intake of phytoestrogens (equivalent to less than one serving of tofu/week) and breast cancer risk.[46]

Many *in vitro* experiments detected anti-cancer effects from phytoestrogens at high concentrations (but mild stimulatory effects at lower concentrations).[31] Animal studies have noted both cancer-inhibitory and cancer-promoting effects. Several reports[34,35] have indicated that exposure of young rats (but not adult rats) to genistein results in a large reduction in mammary cancer later in life. One human study found a similar protective pattern for women who ate tofu as teenagers.[36]

Data regarding the effect of phytoestrogens on hormonal patterns has been mixed; however, one group reported that a daily dose of 154 mg isoflavones in ~ 1 liter of soymilk reduced serum progesterone levels in premenopausal women by 45%.[37] Recent information from the well-publicized discontinued HRT study[38] indicates that the combination of estrogen and progestins is more cancer-promoting than estrogen alone. [Full text of this study is available here.]

Evidence in favor of phytoestrogens indicates that isoflavones (and the antiestrogenic drug tamoxifen) can decrease the density of breast tissue in post-menopausal women; increased density is associated with increased cancer risk, and conventional HRT increases density.[31] Concerning the concurrent use of isoflavones and Tamoxifen, *in vitro* data shows mixed results, but one animal study has indicated that soy products may increase the benefits of the drug.[39]

There is also evidence that isoflavones and lignans may exert anti-cancer effects through other mechanisms, independent of their interactions with estrogen receptors.[41] For example, isoflavones at physiological concentrations have been found to inhibit an enzyme which catalyzes the transformation of the weaker estrogen, estrone, into the more cancer-promoting estradiol.[42] Another study found that phytoestrogens inhibit a second enzyme important in steroid biosynthesis.[43] Isoflavones also exhibit some antioxidant activity, which may contribute to cancer prevention.[44,45] Finally, several studies quoted in Messina and Loprinzi[31] report that phytoestrogens have anti-angiogenesis effects, discouraging the growth of new blood vessels that tumors need for survival.

For a thorough and well-balanced review of the phytoestrogen-breast cancer literature, see *Soy for Breast Cancer Survivors: A Critical Review of the Literature*.[31] The authors examine both positive and negative findings and conclude: "the honest response to each of these

diametrically opposed claims [soy is beneficial vs. soy is harmful] is that no convincing data exist to support either claim. In fact, there are strongly conflicting data regarding both. As such, if women (with or without breast cancer) enjoy partaking of soy products, then it seems quite reasonable for them to partake of them...moderation in intake is probably wise." (p 3103S)

Black Cohosh and Red Clover - Positive Effects

Interestingly, two new studies[47,48] investigating alcohol extracts of black cohosh (*Cimicifuga racemosa*) found antiestrogenic activity and no proliferative effects on breast cancer cells *in vitro;* in fact, the extract was shown to inhibit estradiol-induced proliferation of cancer cells. Black cohosh contains both phenolic and triterpenoid phyto (-anti?-) estrogens[49] (but apparently not formononetin as was previously reported).[50] An animal study just published in *Cancer Research* has also found that *Cimicifuga* extract did not stimulate the growth of estrogen-sensitive tumors.[51] For more information on black cohosh, see this monograph from the Institute for Natural Products Research. Please be aware that as it grows in popularity, this Native American herb could easily become endangered in the wild; environmentally sensitive cultivation must be encouraged to ensure a sustainable supply.

Red clover (*Trifolium pratense*) is an herb traditionally used for the prevention and treatment of cancer. Its principal isoflavones are biochanin A and formononetin. *Trifolium* also contains lesser amounts of genistein and daidzein; it is one of the few plants to have all four of these compounds. Biochanin A and formononetin exhibit weak activity in estrogen receptor assays; however, once inside the human body, biochanin A is transformed into genistein and formononetin into daidzein. Curiously, red clover blossoms - the part used in herbal medicine - have a very low isoflavone content compared to the leaves.[52] Evidence is accumulating to support the use of red clover and its isoflavones:

- This animal study found that biochanin A was more effective at reducing tumor multiplicity than either miso or soybeans.[53]
- A 1998 investigation reported that *Trifolium* extracts also bind to progestin receptors, and exhibit an anti-estrogenic effect on breast cancer cells *in vitro*.[54]
- A new study demonstrated that biochanin A inhibited the growth of a particular type of breast cancer cells *in vitro*.[55]
- Recent research from the University of Florida found similar results with prostate cancer cells.[56]
- An *in vitro* study with transformed human endothelial cells found that physiological concentrations of biochanin A inhibited growth.[57]
- Biochanin A and genistein were found to inhibit the production of PSA (a biomarker of cancer activity) in both breast and prostate cancer cell lines.[58]
- Environmentally, red clover is an excellent choice: it can be cultivated in great abundance. Organic farmers use red clover as a cover crop to enrich the soil with nitrogen.

Equol

Equol is a metabolite of daidzin, the glucoside form of daidzein.[59] It is produced by some 30 to 40% of people who ingest the isoflavone, the richest sources of which are soy, kudzu root, and red clover leaf. In estrogen receptor assays, equol exhibits roughly the same binding affinity as genistein; however, it tends to stay in circulation longer, presumably increasing exposure of tissues to its effects. The ability to produce equol seems to be genetic and not influenced by diet.[60] One study reports that people who produce equol have hormonal profiles associated with a lower risk of breast cancer: lower concentrations of androstenedione, dehydroepiandrosterone (DHEA), estrone, cortisol, and testosterone; and higher concentrations of sex hormone binding globulin (SHBG).[61]

Lignans

The lignans, represented in the diet mainly by secoisolariciresinol and matairesinol, have demonstrated beneficial effects with breast,[62,63,64] prostate,[65,66,67] and colon[68,85] cancer as well as with hypercholesterolemic atherosclerosis[69] and chronic kidney disease.[70,71] In the colon, bacteria convert the botanical lignans into the mammalian lignans enterodiol and enterolactone. Evidence suggests that a healthy colon flora population may be necessary for humans to derive significant benefit from lignans.

In vitro, lignans have been demonstrated to bind to sex hormone binding globulin (SHBG), displacing estradiol and testosterone.[72] Several animal studies have found that lignans have significant anticarcinogenic effects. A human clinical trial with postmenopausal women found that flaxseed (rich in lignans) supplementation favorably altered the balance of 'good' vs 'bad' estrogen metabolites in a dose-dependent manner.[73] Another study reported that flax significantly lowered estradiol and estrone levels in postmenopausal women.[74] The latest research indicates that high levels of lignans are associated with lower breast cancer risk and one recent study[75] noted that the risk reduction was considerably greater in women possessing at least one A2 allele of the CYP17 gene associated with increased risk of breast cancer. (This gene codes for an enzyme important in the synthesis of androstenedione, a precursor of estradiol). The researchers suggested that "there may be a threshold effect in which lignans provide the greatest protective effect among women who have higher endogenous hormone levels, and presumably, higher breast cancer risk." (p 3040)

Numerous epidemiological studies have shown an inverse correlation between cancer incidence and fruit and vegetable consumption; lignans are among the many compounds likely to be responsible for this effect.[76] It has also been demonstrated that women with breast cancer have lower plasma levels of lignans than women without breast cancer.[86]

For more information on the role of isoflavones and lignans in breast and prostate cancer, see the new review Flavonoids and steroid hormone-dependent cancers.[76] For another informative review see Dietary agents in cancer prevention: flavonoids and isoflavonoids.[87]

Phytoestrogens and the Brain

A NIH-funded investigation examined 3,734 Japanese men in Hawaii who had been tracked since 1965 for a cardiovascular longitudinal study.[77] Cognitive function was assessed according to standard parameters in the living participants and their wives (aged 71 - 93 years). NMR imaging and later autopsies looked for changes in brain tissue. It was found that those who had consumed the greatest quantity of tofu in midlife had lower cognitive test performance and lower brain weight than those who had consumed the least tofu. The authors noted that the degree of impairment in the highest consumption vs. the lowest consumption group was "roughly of the magnitude as would be caused by a four year difference in age or a three year difference in education." (p 252)

They postulated that the observed effect might be due to isoflavones inhibiting key enzymes in estrogen synthesis pathways. Estrogen is known to be involved in repair of neural structures that degenerate over time, and it has been observed that higher levels of estrogen are associated with lower incidence of Alzheimer's disease in women. In answering subsequent criticism of this study, the authors commented that dietary "recommendations on the basis of our findings alone would be premature. Nonetheless, should our findings be supported by other research, the public health implications would be enormous." [78]

For an alternative interpretation of the Tofu and Brain Aging study, see this article from the director of the Functional Foods for Health program at the University of Illinois (home of the NAPRALERT database).

Findings from *in vitro* and animal studies

A recent study[79] compared the neurotrophic effects of six different isoflavones to the effect of estradiol in order to determine if the isoflavones had estrogen agonist properties in cultured human hippocampal cells. Estradiol protected neuronal mitochondria from damage and promoted neuron process outgrowth (a cellular correlate of memory). The phytoestrogens had no effect on these parameters. They did, however, demonstrate a modest protective effect on the cell membranes, which the researchers suspected was due to their antioxidant properties.

Numerous studies have demonstrated that isoflavones can affect the brain metabolism and neurological performance of mice and rats (references in 77). A recent review[80] of neurobehavioral effects reports the following results of treatment with high levels of dietary phytoestrogens:

- brain aromatase (an enzyme which converts androgens to estrogens) levels were not significantly affected in perinatal, maternal, or adult rats
- volumes of the sexually dimorphic nucleus (in the preoptic area of the hypothalamus, it is dependent on testosterone for its development) were smaller in adult male rats

fed a phytoestrogen-free diet than in those fed a phytoestrogen-rich diet

- adult female rats performed better on visual-spatial memory tasks when fed a phytoestrogen-rich diet
- adult male rats performed worse on the same tests when fed a phytoestrogen-rich diet
- both male and female adult rats on the phytoestrogen-rich diet were less anxious when performing tasks than those on the phytoestrogen-free diet

The implications of these neurological findings for humans are still uncertain.

Phytoestrogens and the Thyroid

Soy has long been known to have effects on the thyroid. Isoflavones in soy (and flavonoids from other sources as well) inhibit the enzyme thyroid peroxidase, which is involved in thyroid hormone synthesis. This study explored the inhibitory effects of genistein and daidzein, which were completely reversed with the addition of sufficient iodine.[81] Clinical problems from ingesting high levels of phytoestrogens, such as aggravated hypothyroidism or goiter, can occur in iodine-deficient or hypothyroid individuals.

A recent review from investigators at the National Center for Toxicological Research reaffirms that iodine deficiency increases the anti-thyroid effects of soy, while iodine supplementation reverses them. In rat studies, genistein-fortified diets decreased thyroid peroxidase activity in a dose-dependent manner; however, other parameters of thyroid function were unaffected (including serum levels of the hormones triiodothyronine, thyroxine, and thyroid stimulating hormone).[82]

This article from phytoestrogen researcher Mark Messina, Ph.D., gives a balanced overview of the situation. For another review of the soy-thyroid connection, see the Soy and Thyroid Review (2001) from the University of Illinois.

For information on pharmacokinetics, see the Intermediate page.

For more information

- Many questions remain regarding the benefits of the various phytoestrogens for human health; a great deal of research is underway - according to one source, more than 600 studies are being published every year!
- For an actively updated bibliography of studies relating to phytoestrogens, hormonal development, and breast cancer, see this page from Cornell University.
- PubMed has indexed more than 25 reviews of the phytoestrogen research for the year

2002 alone.

- A special supplement to the Journal of Nutrition (2002 Mar; 132(3):S) is dedicated to phytoestrogen studies. Other issues of this journal contain numerous relevant studies as well.
- The Journal of the American College of Nutrition has multiple articles concerning phytoestrogens.
- An extensive thesis on phytoestrogens from Witold Mazur, one of the leading experts in the field, can be found here. (Have patience downloading: it's a big PDF file).
- Also see the extensive (slow download!) report on Phytoestrogens in the Human Diet from the Institute for Environment and Health (UK).
- An excellent review of human clinical and epidemiological findings has recently been published: Albertazzi, P. and D. Purdie. 2002. The nature and utility of the phytoestrogens: a review of the evidence. *Maturitas, the European Menopause Journal*. 42: 173 - 185.
- A beautiful professional monograph on black cohosh is available from the American Herbal Pharmacopoeia.
- A detailed analysis of the phytoestrogen content of 33 commercially available supplements can be found in this study.[52]

- An interesting interview with isoflavone/breast cancer researcher David Zava, Ph.D., and author John Lee, M.D., ('What Your Doctor May Not Tell You About Breast Cancer') can be found here.

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