

# Isoflavones And Other Soy Constituents In Human Health And Disease

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## Abstract

In the past several years, soy and its constituents have garnered considerable attention, from both researchers and health practitioners. Epidemiological data which indicated people from Asian cultures have lower rates of certain cancers, including cancer of the breast, prostate and colon, sparked an interest in soy as a contributing factor. While soy constituents, including saponins, lignans, phytosterols, protease inhibitors, and phytates, have come under investigation, the constituents which seem to hold the most promise from a therapeutic standpoint are the two isoflavones, genistein and daidzein. Numerous epidemiological, human, animal, and *in vitro* studies have demonstrated that soy isoflavones are effective chemopreventive agents for certain types of cancer. Mechanisms involved include antiangiogenesis, estrogen receptor binding, modulation of sex hormone binding globulin (SHBG), anti-inflammatory and antioxidant effects, and inhibition of the enzymes protein tyrosine kinase (PTK) and 5 alpha-reductase. Interaction with many other enzymes has been suggested. Evidence also points to the beneficial effects of soy, particularly the isoflavones, in prevention of cardiovascular disease. Isoflavones appear to inhibit platelet activating factor and thrombin formation. They also increase HDL cholesterol and decrease triglycerides, LDL, VLDL, and total cholesterol. Other potential health benefits of soy include prevention of osteoporosis, via the phytoestrogen effects of isoflavones, and prevention of neovascularization in ocular conditions, via inhibition of angiogenesis.

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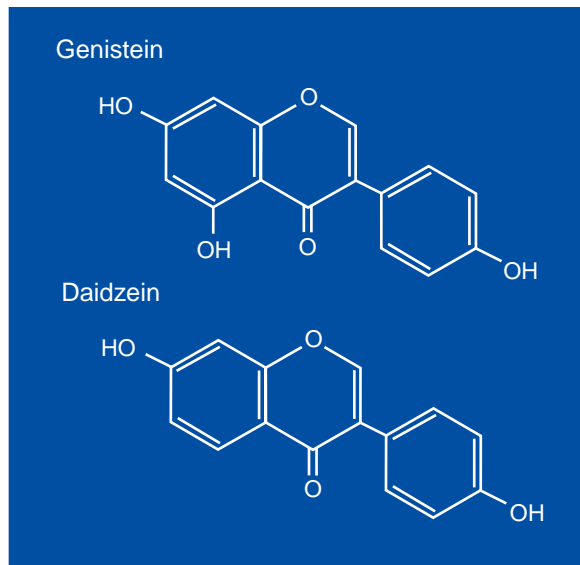
## Introduction

Recent interest in the constituents of soybeans, particularly the isoflavones, has catapulted soy to the status of a promising nutraceutical with potentially significant health benefits. The principle isoflavones in soy are genistein (4',5,7-trihydroxyisoflavone), daidzein (4',7-dihydroxyisoflavone) (see Figure 1), and their metabolites. In addition, soy products are a source of lignans, coumestans, saponins, plant sterols, phytates (inositol hexaphosphate), and protease inhibitors, all of which are also garnering attention for their health-promoting benefits.<sup>1</sup> Soy constituents have been shown to have estrogenic, anti-estrogenic, antiviral,<sup>2</sup> anticarcinogenic,<sup>3-5</sup> bacteriocidal, and antifungal<sup>6</sup> effects. Isoflavones also have antimutagenic,<sup>4</sup> antioxidant,<sup>7,8</sup> mild anti-inflammatory,<sup>9</sup> antihypertensive,<sup>9</sup> and antiproliferative effects.<sup>3,10</sup> This article will focus

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Figure 1. Structure of Genistein and Daidzein



primarily on isoflavones as these are the constituents found in greatest quantity in soy products. Brief reference will be made to other beneficial constituents.

### Classification of Isoflavones

Flavonoids are a subgroup of the larger group of plant constituents, the polyphenols. Flavonoids are further differentiated into isoflavonoids, with isoflavones a subcategory of isoflavonoids. See Figure 2. Isoflavonoids differ from other classes of flavonoids by their greater structural variability, their frequent presence in plants in their free form, rather than as a glycoside, and by the greater frequency of isoprenoid substitution. They are not as ubiquitous in nature as some of the other flavonoids such as flavones and flavonols, being found primarily in one subfamily of Leguminosae, the Papilionoideae.<sup>11</sup> Approximately 600 isoflavonoids have been identified. They are divided into subclasses depending on the oxidation level of the central pyran ring. Isoflavones are the most abundant of the subclasses of isoflavonoids. Genistein and daidzein are two important isoflavones. As can be seen in Figure 1, genistein has a hydroxy group in the 5 position, giving it three hydroxy groups, while daidzein has just two. Due to

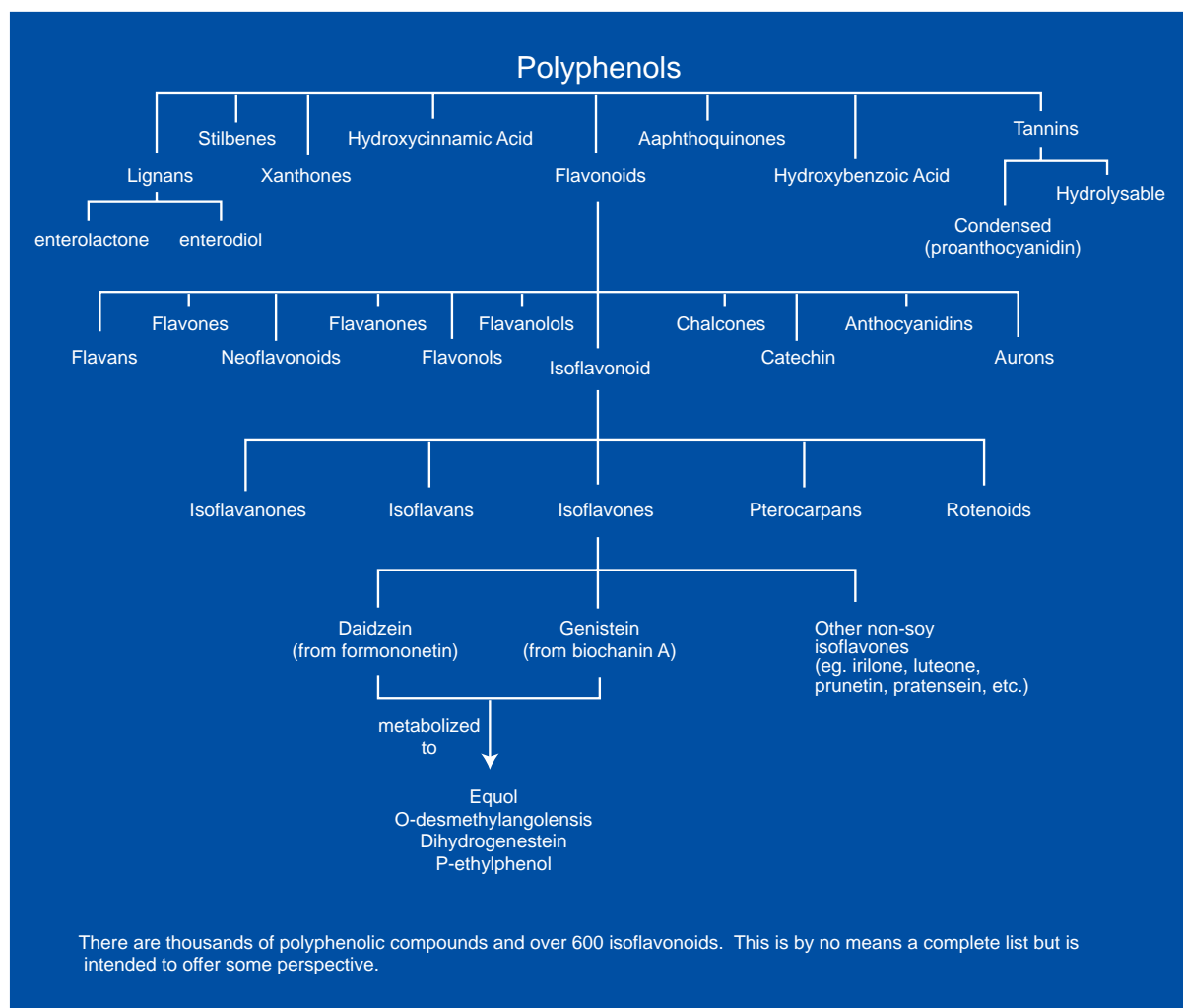
the fact that the 5 hydroxy group on the genistein binds to the 4 ketonic oxygen, genistein is a more hydrophobic molecule than daidzein. This affords genistein some of its unique therapeutic effects.

### Absorption, Metabolism and Excretion of Soy Isoflavones

Isoflavones undergo extensive metabolism in the intestinal tract prior to absorption. Genistein is formed from biochanin A, and daidzein from formononetin.<sup>1</sup> Genistein and daidzein also occur in soy products in the form of their glycosides, genistin and daidzin. In the case of the glycosides, intestinal bacterial glucosidases cleave the sugar moieties, releasing the biologically active isoflavones, genistein and daidzein. In adults, these are further transformed by bacteria to specific metabolites: equol, O-desmethylangolensin, dihydrogenistein, and p-ethylphenol. Due to soy intake by livestock, isoflavone metabolites are also consumed directly in a diet high in dairy products and meat.<sup>12</sup> In at least one study, genistein was well-absorbed in the small intestines by human subjects fed a soy beverage.<sup>13</sup> After absorption, the isoflavones are transported to the liver where they are removed from the portal blood. However, a percentage of the isoflavones in the portal blood can escape uptake by the liver and enter the peripheral circulation. The effectiveness of this hepatic first-pass clearance influences the amount which reaches peripheral tissues.<sup>13</sup> The isoflavones are then eliminated, primarily via the kidneys, similar to endogenous estrogens.<sup>14</sup>

After examining plasma, fecal and urinary concentrations of isoflavones in healthy volunteers, Xu et al concluded the bioavailability of soy isoflavones is influenced by an intact, healthy gut, with microflora capable of converting these isoflavones to their active forms.<sup>15</sup> Wheat fiber appears to decrease the bioavailability of genistein. A small crossover study of seven healthy women found a

Figure 2. Polyphenol classification



more fiber-rich diet resulted in 55 percent less plasma genistein 24 hours after soy intake and a 20 percent reduction in total urinary genistein. The researchers postulated the fairly insoluble wheat fiber reduced the absorption of genistein by its bulking effect and hydrophobic binding.<sup>16</sup> Karr et al found urinary excretion of isoflavones to be reflective of the type and amount of soy ingested.<sup>17</sup> A study conducted on healthy male subjects between the ages of 20 and 40 found urinary excretion of genistein and daidzein was greater after consumption of 112 grams of tempeh, a fermented soy product, than after 125 grams of unfermented soy pieces.<sup>18</sup> This finding seems to indicate fermentation of soy products

increases bioavailability of the isoflavones. Plasma levels of soy isoflavones were also increased after consumption of soy flour and clover sprouts.<sup>19</sup>

### Isoflavone Content of Soy Products

Fukutake et al analyzed soy products for genistein and genistin (the glycoside of genistein) content. The results are outlined in Table 1. In general, they found fermented soy products contain more genistein than soybeans, soy milk and tofu.<sup>20</sup> Alcohol extraction, a process used in the production of many soy protein concentrates and isolates (used in soy protein powders), results in the removal of up to 90 percent of the isoflavones.<sup>21</sup>

Table 1. Levels of Genistein and its glycoside, Genistin in soy foods (analyzed by high performance liquid chromatography)<sup>20</sup>

	Genistein	Genistin
Soybeans, Soy nuts and Soy powder	4.6 - 18.2 mcg/g*	200.6 - 968.1 mcg/g
Soy milk and Tofu	1.9 - 13.9 mcg/g	94.8 - 137.7 mcg/g
Fermented Soy** Miso and Natto	38.5 - 229.1 mcg/g	71.7 - 492.8 mcg/g
Calculated daily dietary intake by the Japanese	1.5 - 4.1 mg/day/person	6.3 - 8.3 mg/day/person

\* mcg/gram of food  
 \*\* soy sauce was found to contain both isoflavones but at lower levels than miso or natto (fermented soy beans)

The isoflavone content of soybeans varies considerably depending on the variety of soybean (there are over 10,000 varieties of soybeans), the year harvested, geographic location, and the plant part in question.<sup>22</sup> Non-soy legumes, such as lentils and other beans, do not contain appreciable amounts of isoflavones.<sup>23</sup>

### Other Soy Constituents

**Protease Inhibitors:** Researchers have looked with interest at protease inhibitors (PI) and their potential anti-cancer and anti-inflammatory effects. Two prominent protease inhibitors from soybeans are Bowman-Birk inhibitor (BBI) and Kunitz-Trypsin inhibitor (KTI). BBI is a 71-residue inhibitor which has two independent inhibitory sites involving binding with the proteases, trypsin and chymotrypsin.<sup>24</sup> BBI has been found to inhibit expression of certain oncogenes in irradiated animal models,<sup>25</sup> as well as inhibiting chemically induced carcinogenesis.<sup>26,27</sup> Both *in vitro* and *in vivo* animal models have demonstrated that BBI appears to exert its effects directly on the target organ rather than

by a non-specific effect on metabolism.<sup>28,29</sup> Some researchers have theorized the dietary intake of exogenous PIs indirectly increases endogenous PI formation.<sup>30</sup> Other researchers have questioned the concept that PIs contribute significantly to the anti-cancer effects of soy. This is due, in part, to the fact that both raw and cooked soy products are equally effective in reducing cancer incidence, even though heating virtually destroys all protease activity.<sup>31</sup> Another reason for skepticism is that ingested PIs (such as purified BBI) are very poorly absorbed from the digestive tract.<sup>31</sup> Some researchers have postulated the formation of these protease:protease inhibitor complexes might interfere with protein absorption, offering some cancer protection (epidemiological studies indicate high fat and high protein diets increase cancer risk).<sup>32</sup> Protease inhibitors in soy products have been implicated in pancreatic hypertrophy and hyperplasia in animal models.<sup>33</sup> Whether it is the protease inhibitors and whether this hyperplasia contributes to increased rates of pancreatic cancer in these animals is still a subject of debate.

**Lignans:** Lignans are capable of exerting a phytoestrogenic effect in humans. In addition, they exhibit anti-tumor and antiviral activity.<sup>11</sup> The most prevalent lignans in mammals are enterolactone and enterodiol, formed by gut bacteria, from the plant precursor lignans, matairesinol and secoisolariciresinol, respectively.<sup>12</sup> Oil seeds, such as flaxseed, contain about 100 times the lignan content of other plants. Other sources of lignans in descending order of importance are dried seaweed, whole legumes (including soy), cereal bran, legume hulls, whole grain cereals, vegetables, and fruit.<sup>1</sup> Gender differences in urinary lignan excretion have been observed, with men excreting more enterolactone and less enterodiol than women. The researchers felt this implied a difference in colonic bacterial metabolism of lignans between the genders.<sup>34</sup> Administration of antibiotics nearly completely eliminates the formation of these mammalian lignans from their precursors.<sup>12</sup>

**Phytosterols:** Phytosterols, such as  $\beta$ -sitosterol, are found in high concentrations in soy products. Although poorly absorbed, they bind cholesterol in the gut.<sup>31</sup> Dietary content of phytosterols differs widely among populations. The typical Western diet contains about 80 mg/day, while the traditional Japanese diet contains approximately 400 mg/day.<sup>35,36</sup>

**Coumestans:** The phytoestrogen, coumesterol, as well as other coumestan isoflavonoids, have been found by some researchers in significant quantities in soy foods of all types, including soybeans, soy flour, soy flakes, isolated soy protein, tofu, soy drinks, and soy sprouts.<sup>37</sup> On the other hand, Adlercreutz reports its presence only in soy sprouts.<sup>12</sup> The most abundant source is mung bean sprouts. It is also found in significant quantities in other members of the Leguminosiae family, including *Trifolium* and *Medicago* spp.<sup>11</sup>

**Saponins:** Saponins are distributed widely in the plant kingdom, including in

soybeans. They appear to have anti-cancer properties by virtue of their antioxidant and anti-mutagenic properties.<sup>38</sup> They also bind cholesterol and bile acids in the gut.<sup>31</sup> An *in vitro* study demonstrated saponins isolated from soybeans exhibited potent antiviral effects on the HIV virus. Saponin B1 completely inhibited HIV-induced cytopathic changes and virus-specific antigen expression within six days after infection. Saponin B2 exhibited similar, although less potent, effects.<sup>39</sup>

**Phytates:** Although phytic acid (inositol hexaphosphate) has been implicated in blocking the absorption of minerals, the phytate content of plants, including soy, seems to be responsible for some of the anti-cancer properties of vegetable-based foods. Phytic acid is a highly charged antioxidant, capable of scavenging hydroxyl radicals and chelating metal ions such as the pro-oxidant, iron. Graf and Eaton reported the iron-chelating ability of phytate to be more important than the fiber in dietary colon cancer prevention.<sup>40</sup> Vucenik et al reported anti-tumor effects of phytic acid both *in vitro* and in animal models.<sup>41</sup> Phytates also appear to enhance natural killer cell activity.<sup>42</sup> Rao et al found these potent antioxidant effects to protect against cardiac ischemia and reperfusion injury in animal models.<sup>43</sup> For a summary of soy constituents and their functions, please see Table 2.

### Soy Isoflavones in Infant Formulas and Breast Milk: Benefit or Risk?

Recent advances in understanding the phytoestrogen content of soy foods have led researchers to examine the isoflavone content in commonly consumed infant formulas. A recent study published in the July 5, 1997 issue of *The Lancet* examined the isoflavone content of 25 randomly selected samples from five major brands of soy-based infant formulas. There were significant levels of isoflavones, particularly in the form of the glycosides of

Table 2. Soy constituents and their functions

Constituents	Function
Protease Inhibitors (Bowman-Birk inhibitor; Kunitz-Trypsin inhibitor)	<ul style="list-style-type: none"> <li>• inhibit oncogene expression</li> <li>• inhibit chemically induced carcinogenesis</li> <li>• implicated in pancreatic hypertrophy (animal studies)</li> </ul>
Lignans (enterolactone; enterdiol)	<ul style="list-style-type: none"> <li>• phytoestrogenic (agonistic/antagonistic effects on estrogen)</li> <li>• antitumor</li> <li>• antiviral</li> </ul>
phytosterols ( $\beta$ -sitosterol)	<ul style="list-style-type: none"> <li>• binds cholesterol in the gut</li> </ul>
Coumestans (coumesterol)	<ul style="list-style-type: none"> <li>• phytoestrogen (with agonistic and antagonistic effects on estrogen)</li> </ul>
Saponins	<ul style="list-style-type: none"> <li>• antioxidant</li> <li>• binds cholesterol &amp; bile acids in the gut</li> <li>• antiviral (HIV)</li> </ul>
Phytates	<ul style="list-style-type: none"> <li>• antioxidant</li> <li>• chelate metalons (such as iron)</li> <li>• enhances natural killer cell activity</li> </ul>
Isoflavones (Genestein; Daidzein and their metabolites)	<ul style="list-style-type: none"> <li>• phytoestrogen (agonistic/antagonistic effects on estrogen)</li> <li>• anti-mutagenic</li> <li>• antioxidant</li> <li>• anti-inflammatory</li> <li>• antiproliferative</li> <li>• antihypertensive</li> <li>• angiogenesis inhibition</li> </ul>

tored into the equation, than the typical dose necessary to exert hormone-like effects in adults. They found negligible concentrations of isoflavones in breast milk and cow's milk.<sup>14</sup> There was some evidence of the daidzein metabolite, equol, in the infants who were fed cow's milk, confirming previous observations that cow's milk also contains some isoflavones. In this study, the plasma concentration of isoflavones in breast-fed babies was 1/200th the level in soy-formula fed babies. Franke and Custer, on the other hand, reported in 1996 that human breast milk from mothers consuming soy foods provided significant levels of isoflavones.<sup>44</sup> Some researchers have concluded that the isoflavone content of human breast milk appears to be a reflection of the mother's diet.

These findings have raised some interesting questions and stimulated lively debate. Adverse effects of

genistein and daidzein, in all samples tested. The plasma concentrations of genistein and daidzein were compared in four-month-old infants fed exclusively soy formula, cow's milk formula, and breast milk. A four-month-old infant was estimated to be ingesting between 4.5 and 8.0 mg/kg body weight per day of total isoflavones. This is a proportionately greater concentration per body weight than that found in adults consuming soy foods.

Additionally, the researchers estimated the daily exposure of infants to soy isoflavones was 6-11 times higher, with bodyweight fac-

phytoestrogens on development and reproductive capacity of livestock, wildlife and experimental animals have been reported.<sup>45</sup> There has been very little clinical experience with human infants and phytoestrogens, however. In animal models, phytoestrogens have had effects similar to other estrogens, included interfering with normal reproductive system development.<sup>46</sup> On the other hand, phytoestrogenic isoflavones have been found to possess some important anti-cancer properties. Genistein, given in only three doses to

newborn mice, decreased breast cancer incidence and tumor numbers significantly.<sup>44</sup> Although millions of Asians have consumed large quantities of soy foods for hundreds of years without any apparent health risk and seemingly with health benefits, long-term studies are needed to clarify the safety of using soy-based infant formulas and to assess the potential beneficial or adverse effects of consuming phytoestrogens in the form of soy isoflavones early in life.

### The Hormonal Effects of Phytoestrogens in Adults

Plant lignan and isoflavonoid glycosides are converted by gut bacteria in the intestines to compounds with molecular weights and structures similar to steroid hormones (see Figure 3). The pattern of isoflavonoid and lignan excretion in the urine is similar to endogenous estrogens.<sup>47</sup> Studies of the effects of phytoestrogens on hormone levels are conflicting. Lu et al found the consumption of soy products by premenopausal women resulted in decreased circulating ovarian steroids and adrenal androgens, as well as increased length of the menstrual cycle.<sup>48</sup> Six healthy females, age 22-29, were given 12 oz soy milk three times daily with meals for one month. Daily isoflavone intake was approximately 100 mg each of daidzein and genistein (in the form of their glycosides, daidzin and genistin). The estradiol levels decreased by 31 percent on days 5-7 of the cycle, 81 percent on days 12-14, and 49 percent on days 20-22. Luteal phase progesterone levels decreased by 35 percent, and DHEA sulfate levels decreased progressively during the month by 14-30 percent. The length of the menstrual cycle increased during the soy feeding month from 28.3 +/- 1.9 days to 31.8 +/- 5.1 days.

In another study, also on premenopausal women, Lu et al found 60 grams of soy protein, with 45 mg of isoflavones daily, resulted in suppression of

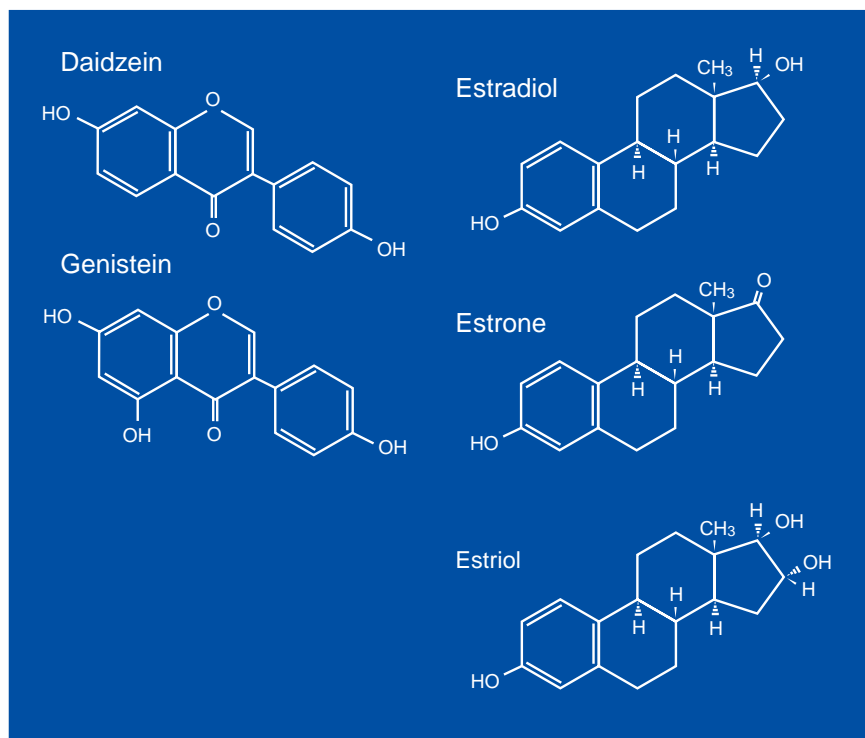
midcycle surges of FSH and LH. Plasma concentrations of estradiol increased during the follicular phase in the soy group, while cholesterol decreased 9.6 percent. The researchers noted a similar effect occurs in women given tamoxifen. There were no significant differences in estradiol levels between the soy and control groups at midcycle or during the luteal phase.<sup>49</sup> At least one study found soy protein isolate to have a stimulatory effect on breast tissue in premenopausal women, characterized by increased breast secretions, epithelial cell hyperplasia, and elevated levels of serum estradiol.<sup>50</sup>

Wang et al found genistein competed with estradiol binding to estrogen receptors, with 50 percent inhibition occurring at  $5 \times 10^{-7}$  M.<sup>51</sup> A study of soy-supplemented postmenopausal women found a slight estrogenic effect on vaginal cytology. However, no difference between soy-supplemented subjects and controls in regard to serum FSH, LH, sex hormone binding globulin, endogenous estradiol or body weight was observed.<sup>52</sup>

Many studies on phytoestrogens focus on the use of coumestrol, as it has more potent phytoestrogenic effects than lignans, genistein and daidzein. *In vitro* studies have found both genistein and coumestrol inhibit the conversion of estrone to 17-beta estradiol. Coumestrol exhibited the strongest inhibition.<sup>53</sup> An *in vitro* study monitoring the expression of the estrogen-responsive protein pS2 in breast cancer cell MCF-7 tissue culture to assess the estrogenic response of various plant substances, found the following substances elicited estrogen-like activity: daidzein, equol, nordihydroguaiaretic acid, enterolactone, and kaempferol. The substances tested which did not appear to have estrogen-like activity were quercetin and enterodiol.<sup>54</sup>

The effects of phytoestrogens vary greatly depending on the species of animal. the particular phytoestrogen compound being

Figure 3. Structure of estrogens compared to isoflavones



tested, the age of the animal, the length of time of ingestion, the presence or absence of exogenous estrogen, the target tissue in question, and the dosage used. The phytoestrogen, coumestrol, was found to have an estrogenic effect, as demonstrated by changes in uterine and brain tissue, when given to prepubescent rats; however, when given to adult female rats, ovarian cycling was inhibited. When given for 10 days to neonatal rats, there was no effect on estrous cycling; but, when given for 21 days, it interfered with normal cycling, once the rats reached adulthood. The females exhibited persistent estrous and lack of an LH surge, while the males demonstrated a decrease in sexual behavior.<sup>55</sup>

Historically, the consumption of soy products in Asian cultures, from a very young age, has not resulted in any apparent negative effects related to hormone imbalances. It is unlikely that animal studies are effective at predicting the effects of phytoestrogens in the human model.<sup>56</sup>

The mechanisms for the anti-estrogenic effect of phytoestrogens are largely unknown but some experts believe it is unlikely to be a direct receptor-mediated effect.<sup>12</sup> One mechanism of action of lignans and isoflavonoids is to stimulate sex hormone-binding globulin (SHBG), also known as sex steroid binding protein (SBP), synthesis in the liver. SHBG binds to cell surface receptors, resulting in regulation of bioavailability and activity of hormones.<sup>12</sup> *In vitro*, in MCF-7 human breast cancer cells, SHBG has been found to down-regulate estradiol.<sup>57</sup>

It appears phytoestrogens exert mild agonistic and antagonistic effects on estrogen, depending on the level of endogenous estrogen present and on the tissue being tested. *In vitro* studies demonstrate an estrogenic effect in the absence of endogenous estrogen, and an anti-estrogenic effect in the presence of estrogen.<sup>12</sup> Much research in this area remains to be done.

Much of the effect of phytoestrogens might be due to enzyme inhibitions. It appears phytoestrogens have an inhibitory effect on many enzymes involved in the biosynthesis and metabolism of steroid hormones. The effect on enzymes is further discussed below.

### Mechanism of Action of Soy Isoflavones

There are many proposed mechanisms for the therapeutic effects of isoflavones. The mechanisms include inhibition of protein tyrosine kinase (PTK), binding of estrogen receptors (although soy's inhibition of cancer cell growth does not seem to be entirely estrogen dependent),<sup>58</sup> inhibition of production



of reactive oxygen species,<sup>59</sup> induction of DNA strand breakage resulting in apoptosis or cell death,<sup>58</sup> inhibition of angiogenesis,<sup>60</sup> modulation of sex steroid binding protein,<sup>61</sup> inhibition of 5 alpha-reductase,<sup>62</sup> inhibition of P-form phenolsulfotransferase (PST) - mediated sulfation,<sup>63</sup> inhibition of thrombin formation and platelet activation,<sup>64</sup> and increased LDL receptor activity.<sup>65</sup> The therapeutic implications of each of these mechanisms is elaborated below.

### Therapeutic Applications

**Cancer:** The first clues soy diets might provide protection from cancer came from epidemiological studies, in which people from Asian cultures eating a diet high in soy foods, such as tofu, demonstrated lower rates of several types of cancers, including types not typically considered to be hormone- or diet-related. Messina et al reviewed 21 epidemiological studies which evaluated the effect of soy diets on 26 different cancer sites. An evaluation of the effect of non-fermented soy products in these studies found that 10 showed decreased risks for rectal, stomach, breast, prostate, colon, and lung cancers, while 15 showed no significant effect. Only one, in which fried bean curd was evaluated, showed an increased risk for esophageal cancer. On the other hand, the effects of fermented soy products – miso soup and soybean paste – were much less consistent. Twenty-one studies, involving 25 cancer sites, evaluating fermented soy products, found an increased cancer risk in four studies, mixed results in four, no significant effects in 14, and a decreased risk in three. The increased risks of cancer from consumption of fermented soy products appear to involve primarily the gastrointestinal tract – esophageal, stomach, colorectal, and pancreatic cancers.<sup>66</sup> Please see Table 3 for a summary of these studies.

To put the epidemiological studies in some perspective, Adlercruetz et al found high

urinary excretion of the soy isoflavones, equol, daidzein, and O-desmethylangolensin in both men and women living in rural Japan.<sup>67</sup> Most soy foods contain about 1-2 mg/g of genistein. Asian cultures tend to consume 20-80 mg/day. The usual dietary intake of genistein in Western cultures is 2-3 mg/day. Messina et al examined 26 animal studies and reported that 17 (65%) of them demonstrated a protective effect of soy from experimental carcinogenesis.<sup>66</sup>

There are many proposed mechanisms for the anti-cancer benefits of soy-based foods. Inhibition of PTK activity has been proposed as a major mechanism in the prevention of carcinogenesis. While synthetic PTK inhibitors have been proposed for the treatment of cancer, expected toxicity has restricted their development. In 1987, it was discovered genistein is a natural PTK inhibitor.<sup>58</sup> Tyrosine kinase inhibition results in the inhibition of leukotriene production, (products of inflammation which have been implicated in the stimulation of tumor growth). *In vitro* studies found pretreatment of cancer cell lines with genistein completely inhibited leukotriene production.<sup>68</sup>

Influence on a number of other enzymes has been suggested as a possible mechanism for the anti-cancer properties of isoflavones. Some of these enzymes include DNA topoisomerases,<sup>69,70</sup> ribosomal S6 kinase activity,<sup>71</sup> phospholipase C-gamma,<sup>72</sup> phosphatidylinositol kinases,<sup>73</sup> and mitogen-activated protein kinase.<sup>74</sup> In addition, genistein demonstrated *in vitro* inhibition of phenolsulfotransferase, an enzyme involved in sulfation-induced carcinogenesis.<sup>63</sup>

*In vitro* studies have found genistein to be a very potent inhibitor of neovascularization or angiogenesis, one of the proposed mechanisms for cancer growth inhibition.<sup>60</sup> Isoflavone effects on hormone regulation, expression and metabolism have been elaborated above and are discussed further below in the sections on breast and prostate cancer.

Table 3. Summary of epidemiological studies of soy and cancer.

Studies	Fermented miso/soybean paste	Unfermented tofu/bean curd	other soy products
Breast Cancer (4 Studies)	1. ↓ risk 2. ↓ risk, premenopause (no effect postmenopause) 3. ↓ risk 4. no significant effect (type of soy examined not noted)		
Prostate Cancer (3 Studies)	1. no significant effect 2. no significant effect 3. no significant effect	3. ↓ risk	
Colorectal Cancer (6 Studies)	1. no significant association (type of soy not delineated) 2. ↑ risk (colon) 3. ↑ risk (rectal) 5. no association (colorectal)	3. no significant association 4. ↓ risk (not statistically significant) 6. ↓ risk rectal (↓ risk colon, not statistically significant)	3. no significant association 5. ↓ risk rectal (not colon)
Lung Cancer (4 Studies)	1. no association (bean curd or soybean paste)	2. ↓ risk 3. ↓ risk	4. ↓ risk (soy in general)
Stomach Cancer (16 Studies)	1. ↑ risk (miso) 1. no effect (bean paste) 2. ↓ risk (not significant) 3. no significant association 4. ↓ risk (soybean paste) 5. ↑ risk (miso) 6. ↑ risk 7. ↑ risk 8. no significant association 9. no significant association 10. ↓ risk	1. ↓ risk 2. ↓ risk 3. ↓ risk (not significant) 12. ↓ risk 14. no significant association	1. no effect soybean, fried bean curd 11. ↓ risk (soybeans) 13. ↓ risk (soy milk) 15. no significant association (non-miso soy products) 16. ↓ risk (soy milk & other soy products)
Esophageal Cancer (2 Studies)	1. ↑ risk for males (no effect for females)	1. no significant association	2. ↑ risk (fried bean curd)
Gall Bladder/Bileduct Cancer (2 Studies)	1. no significant effect from any soy product 2. ↓ risk bile duct (not significant) no effect gall bladder		
Liver Cancer (1 Study)	1. no significant association		
Pancreatic Cancer	1. ↑ risk		

\* Numbers denote studies

For more specific information on these and references for these studies please see Messina M, Persky V, Setchell KDR, Barnes S. Soy intake and Cancer Risk: A Review on the *In Vitro* and *In Vivo* Data. *Nutrition and Cancer* 1994;21:113-131.

At issue in the study of soy isoflavones in the treatment of cancer is whether the concentration achieved by dietary consumption of soy products is enough to influence tumor growth. Studies on human volunteers consuming soy beverages, which provided 42 mg genistein and 27 mg daidzein daily, resulted in peripheral blood concentrations of 0.5-1.0 microM,<sup>13</sup> a concentration much lower than that necessary to inhibit growth of cultured cancer cells.<sup>75</sup> However, these same researchers found non-transformed mammary epithelial cell cultures to be much more sensitive to genistein, with inhibition of growth stimulation occurring in the range of 1-2 microM. This suggests a role of isoflavones as chemopreventive rather than chemotherapeutic agents.

*Breast Cancer:* Case-controlled, epidemiological, *in vitro*, and animal studies point to effectiveness of soy isoflavones in the prevention of breast cancer. A case-controlled study, published in the October 4, 1997 issue of *The Lancet*, examined the effect of phytoestrogens on breast cancer risk. One hundred forty-four women with early diagnosed breast cancer were paired with age- and area-of-residency-matched controls. Prior to treatment, a questionnaire, and 72-hour urine and blood tests were administered. Urine was assayed for the isoflavones daidzein, genistein, and equol, and the lignans enterodiol, enterolactone, and matairesinol. Adjustments were made for age at menarche, parity, and alcohol and total fat intake. Increased excretion of daidzein, equol, and enterolactone was associated with a reduction in the risk for development of breast cancer. The most significant correlation was between the levels of the soy isoflavone, equol, and the risk of breast cancer, with those in the highest quartile of equol excretion exhibiting only one-fourth the risk of those in the lowest quartile – a four-fold reduction in risk. The lignan, enterolactone, and the isoflavone, daidzein,

were associated with a three-fold reduction in risk. The daidzein results were insignificant, after correcting for confounding variables. Similar trends were noted for both pre- and postmenopausal groups. Unfortunately, no reliable data for genistein was available due to instability of the derivative of genistein being tested and interference by an unknown compound.<sup>76</sup> Two case-controlled studies, one in Singapore<sup>77</sup> and one in Japan,<sup>78</sup> found significant protection from soy intake for pre- but not postmenopausal women.

Epidemiological studies demonstrate an inverse relationship between soy intake and incidence of breast cancer (see Table 3). Americans have two to three times the breast cancer rate of Asians eating a traditional diet.<sup>79</sup> An epidemiological study of Asian-American women found tofu intake to correlate inversely with breast cancer incidence, after adjustment for other dietary, menstrual and reproductive factors.<sup>80</sup> This effect was observed both in pre- and postmenopausal women. In summary, all four of the human studies examined seemed to indicate a protective effect of soy against breast cancer in premenopausal women. The effect on post-menopausal women was significant in two of the four studies.

*In vitro* experiments with human breast cancer cells confirm genistein to be a potent inhibitor of cell growth, regardless of estrogen receptor status. Other isoflavones, daidzein and biochanin A, demonstrated weaker growth inhibition.<sup>81-83</sup> Pagliacci et al reported the *in vitro* inhibition of MCF-7 human breast cancer cells occurred through blocks at critical points in cell cycle control as well as via induction of apoptosis.<sup>84</sup> Wang et al found genistein produced a concentration-dependent effect on breast cancer cell cultures. At lower concentrations ( $10^{-8}$  to  $10^{-6}$  M) genistein stimulated growth, while higher concentrations ( $>10^{-5}$ ) inhibited growth. They concluded the effect of genistein at the lower concentrations appeared to be estrogen receptor mediated,

Table 4. Results of a meta-analysis of the effects of soy protein intake on serum lipids.<sup>92</sup>

Soy Diet compared to control (meat protein) diet		
	mg/dl	%
Total Cholesterol	23.2 mg/dl decrease	9.3 % decrease
LDL Cholesterol	21.7 mg/dl decrease	12.9 % decrease
Triglycerides	13.3 mg/dl decrease	10.5% decrease
HDL Cholesterol	1.2 mg/dl increase (not statistically significant)	2.4% increase

while effects at higher concentrations were independent of estrogen receptors.<sup>51</sup>

Genistein, when administered to neonatal<sup>85</sup> or prepubescent rats,<sup>86</sup> suppressed the development of chemically induced mammary tumors without causing toxicity to the development of the endocrine or reproductive systems. Barnes et al found soy in the form of raw soybeans as well as soy protein isolate inhibited mammary tumors in experimental models.<sup>87</sup>

**Prostate Cancer:** Epidemiological evidence points to the benefits of soy constituents in the prevention of prostate cancer. Japanese men who consume a low-fat, high soy diet have low mortality rates from prostate cancer. Isoflavones in the plasma of Japanese men were between 7 and 110 times higher than in Finnish men, with genistein present in the highest concentrations.<sup>88</sup> Mechanisms suggested include genistein-induced prostate cancer cell adhesion, direct growth inhibition, and induction of apoptosis. Growth inhibition appears to be independent of genistein's estrogenic effects.<sup>89</sup> An *in vitro* study indicated the isoflavones genistein, biochanin A, and equol were potent inhibitors

of 5 alpha-reductase,<sup>62</sup> the enzyme necessary for the conversion of testosterone to dihydrotestosterone (implicated in prostate cancer).

Studies have found that animals fed soy isolates high in the isoflavones, genistein and daidzein, demonstrated a reduced incidence of prostate cancer and a 27 percent longer disease-free period after exposure to chemical carcinogens than animals fed a soy isolate low in isoflavones.<sup>90</sup> This not only points to the potential chemoprotective effects of soy, but seems to point to the importance of the isoflavones over other soy constituents. Peterson and Barnes found the isoflavones, genistein and biochanin A, but not daidzein, to inhibit several human prostate cancer cell lines.<sup>91</sup>

**NIH Recommendations:** The committee of the National Institutes of Health (NIH) studying chemoprevention from soy products made the following recommendations:

“1. Future dietary studies involving soybeans should be carried out using soy products rather than isolated compounds, since soybeans appear to contain several potential anticarcinogens.

“2. Standardized and improved analytical methods are needed so that the contents of all soy-based materials employed in soybean research, whether soybean fractions or soy products, can be accurately described.

“3. Basic research in the absorption, metabolism, and physiology of potential anticarcinogens in humans should be conducted.”

**Cardiovascular Disease:** A large meta-analysis of 38 controlled studies of the effects of soy diets, with animal protein diets serving as the controls, found a statistically significant decrease in serum lipids in the soy group. The changes were most significant in hypercholesterolemic subjects<sup>92</sup> (see Table 4). The intake of energy, fat, saturated fat, and cholesterol was similar between the two groups. Gooderham et al reported no effect on platelet aggregation or serum lipid levels in healthy, normocholesterolemic men fed soy protein compared to casein.<sup>93</sup>

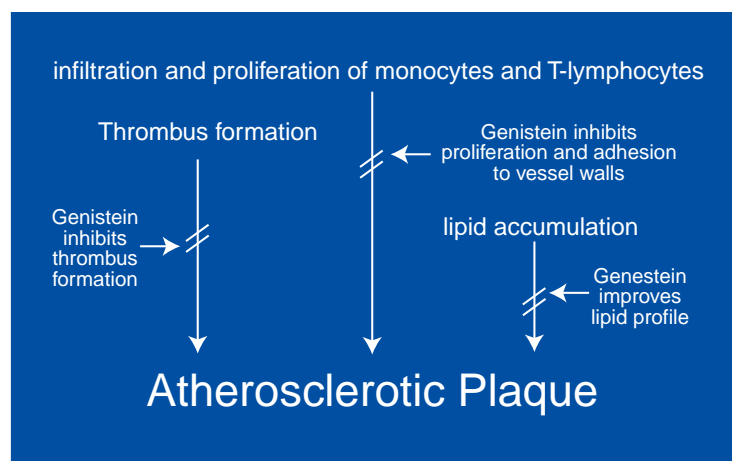
One of the proposed mechanisms for the hypolipidemic effect involves an increase in LDL receptor activity in both humans and animals.<sup>65</sup> Other metabolic changes which have been noted in animals and humans on soy diets include increased cholesterol and bile acid synthesis, increased apolipoprotein B and E receptor activity, and decreased hepatic secretion of lipoproteins (associated with increased clearance of cholesterol from the bloodstream).<sup>94</sup> Proposals for the specific constituents involved include the amino acid profile, saponins, phytic acid, fiber, as well as the effects of isoflavones discussed below.<sup>94</sup>

Arterial thrombus formation is generally initiated by an injury to the endothelial cells lining the blood vessels. One of the first events after an injury is thrombin formation. This leads to a cascade of events including platelet activation, resulting in thrombus formation. Genistein has been found to inhibit thrombin formation and platelet activation.<sup>64</sup> The pathogenesis of atherosclerotic plaque formation also involves, in addition to lipid accumulation, the infiltration of monocytes and T-lymphocytes into the artery wall, contributing to the thickening of the wall and occlusion of the vessel. Monocytes and lymphocytes are permitted to adhere to the endothelial cell

surfaces via the expression of certain “adhesion molecules.” The infiltration and proliferation appear to be controlled by peptide growth factors. Increased levels of isoflavones, genistein in particular, appear to alter the growth factor activity, and inhibit cell adhesion and proliferation, all activities necessary for lesion formation in the intima of the blood vessels<sup>95</sup> (see Figure 4).

Animal studies with monkeys have confirmed the cardioprotective effects of soy. Soy protein diets, when compared to casein diets, resulted in significant improvements in

Figure 4. Genistein inhibits plaque formation.



lipid profiles, insulin sensitivity, and a decrease in arterial lipid peroxidation.<sup>96</sup> Furthermore, animal studies also indicate the isoflavone content of the soy is an important factor. Monkeys were fed soy isolates high in isoflavones and compared in a cross-over trial with a soy isolate in which the isoflavones had been removed via alcohol extraction. LDL, VLDL, and total cholesterol:HDL ratios were significantly lowered, while HDL was significantly elevated in the group on the isoflavone-rich diet.<sup>21</sup> No lipid lowering effect occurred in the group on the casein diet.

**Other Potential Therapeutic Benefits:** While research on the health benefits of soy constituents has focused primarily on the chemopreventive effects for cancer and cardiovascular disease, there are a few other conditions which might benefit from the addition of soy isoflavones to the diet.

*Osteoporosis:* Animal studies indicate soy isolates enhance bone density. Ovariectomized rats fed a high soy diet demonstrated enhanced bone density of the vertebral bodies and femoral bone compared to the group fed a casein diet. While there was considerable bone turnover in the soy-fed group, bone densities suggest formation exceeded resorption.<sup>97</sup> Further studies on the use of soy isoflavones for the prevention and treatment of osteoporosis are warranted.

*Eye disorders:* Neovascularization complicates many eye disorders such as proliferative diabetic retinopathy, and is responsible for corneal transplant rejection. Substances which exhibit the capacity to inhibit angiogenesis could play an important role in preventing this vascularization. An animal study demonstrated that genistein, when injected subconjunctivally, inhibited corneal neovascularization. While this was not a human study with the use of oral doses, this study has opened the door for future investigation.

## Conclusion

Research indicates soy and its individual constituents have several potential health benefits. The primary isoflavones, genistein and daidzein, as well as their metabolites, exert a wide array of effects which appear to offer protection against cancer, cardiovascular disease, osteoporosis, and ocular neovascularization. Many of the studies to date have been either epidemiological, animal, or *in vitro*. Further controlled human trials are needed to confirm the preliminary findings reported in these studies. Until the isoflavone content of individual soy products is routinely

listed on the packaging, it might be difficult to assure adequate consumption of therapeutic dose of isoflavones by dietary changes alone. Soy constituents, particularly the isoflavones, have come under scrutiny, due to their phytoestrogen effects. Because in some cases they act as estrogen agonists and at other times, antagonists, the use of these isoflavones in cancer patients as well as in infant formulas is controversial. Further study to determine whether their use in these situations is harmful or beneficial is indicated.

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