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DIETARY PHYTOESTROGENS

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ABSTRACT

Broadly defined, phytoestrogens include isoflavones, coumestans, and lignans. A number of these compounds have been identified in fruits, vegetables, and whole grains commonly consumed by humans. Soybeans, clover and alfalfa sprouts, and oilseeds (such as flaxseed) are the most significant dietary sources of isoflavones, coumestans, and lignans, respectively. Studies in humans, animals, and cell culture systems suggest that dietary phytoestrogens play an important role in prevention of menopausal symptoms, osteoporosis, cancer, and heart disease. Proposed mechanisms include estrogenic and antiestrogenic effects, induction of cancer cell differentiation, inhibition of tyrosine kinase and DNA topoisomerase activities, suppression of angiogenesis, and antioxidant effects. Although there currently are no dietary recommendations for individual phytoestrogens, there may be great benefit in increased consumption of plant foods.

CONTENTS

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BACKGROUND

Definition of Phytoestrogens

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 (85). In this review, the term phytoestrogen is used in a broader sense, referring also to chemicals that show effects suggestive of estrogenicity, such as binding to the estrogen receptor (ER), induction of specific estrogen-responsive gene products, and stimulation of ER-positive breast cancer cell growth.

Defined broadly, phytoestrogens can be divided into three main classes: isoflavones, coumestans, and lignans. All are diphenolic compounds with structural similarities to natural and synthetic estrogens and antiestrogens (Figure 1). Although resorcylic acid lactones such as zearalenone have also shown estrogenic effects, they are not intrinsic components of food plants but are secondary mold metabolites of fungal species (mainly *Fusarium*). They therefore are not discussed in this review.

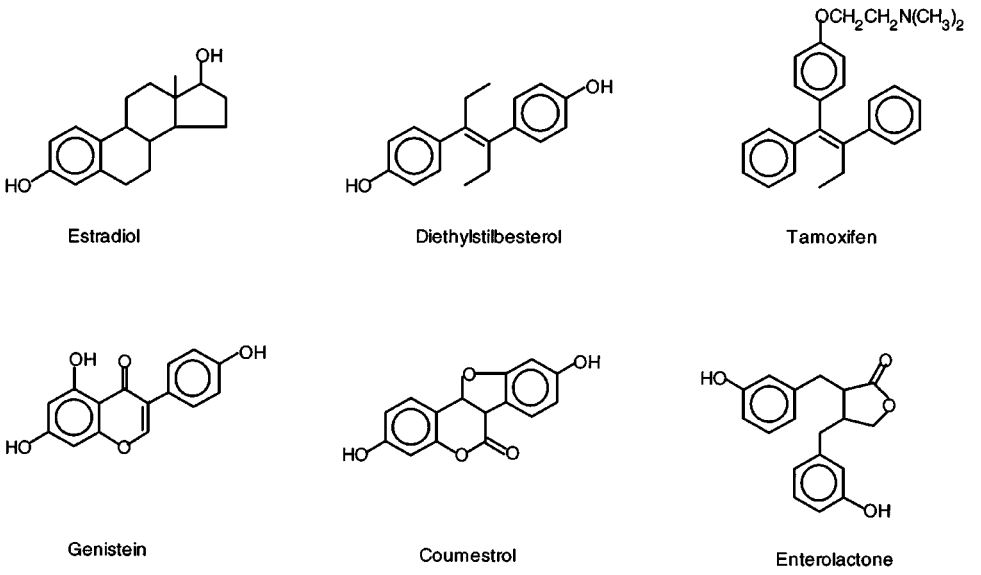
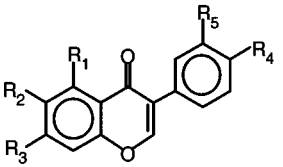


Figure 1 Structures of the phytoestrogens genistein (isoflavone), coumestrol (coumestan), and enterolactone (lignan) for comparison with estradiol (natural estrogen), diethylstilbestrol (synthetic estrogen), and tamoxifen (synthetic antiestrogen).



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 2 Chemical structures of the isoflavones found in soybeans. Daidzein, genistein, and glycitein are also present as acetylglucosides (6″-O-acetyldaidzin, 6″-O-acetylgenistin, 6″-O-acetylglycitin) and malonylglucosides (6″-O-malonyldaidzin, 6″-O-malonylgenistin, 6″-O-malonylglycitin).

Dietary Sources and Chemical Forms

In 1954, Bradbury & White (30) listed 53 plants that possessed sufficient estrogenic activity to initiate estrus in animals. The list was later expanded to over 300 by Farnsworth et al (52). Isoflavones and coumestans have been identified as the most common estrogenic compounds in these plants (113).

Soybeans and soy foods are the most significant dietary sources of isoflavones (45, 101, 137). After normalization for differences in isoflavone molecular weights, they contain approximately 0.2–1.6 mg of isoflavones/g dry weight. Chick peas and other legumes, as well as clover, toothed medic, and bluegrass, have also been identified as isoflavone sources (113).

Soybeans contain three main isoflavones and each is found in four chemical forms (Figure 2). The unconjugated forms, or aglycones, are daidzein,

genistein, and glycitein. Each of these isoflavones is also found as a glucoside (daidzin, genistin, and glycitin), acetylglucoside and malonylglucoside (79). Formononetin and biochanin A, the 4′-methyl ethers of daidzein and genistein, respectively (Figure 2), are found in clover (30).

Wang & Murphy (137) characterized the concentrations and distribution of all 12 isoflavone isomers in 29 commercial soybean foods. Unprocessed soybeans contain 1.2– 4.2 mg of isoflavones/g dry weight, while high-protein soy ingredients such as soy flour and texturized vegetable protein (TVP) contain 1.1–1.4 mg/g dry weight. Soy concentrate, produced by a water or alcohol wash of soy flakes to remove soluble carbohydrates and improve functionality, shows an extremely low isoflavone concentration. Second-generation soy foods such as tofu yogurt and tempeh burger contain 6–20% of the isoflavones found in whole soybeans (137), since most of the matrices in these foods are nonsoybean constituents.

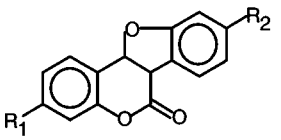
Processing is known to influence the forms of isoflavones found in soy foods. Minimally processed soy flour contains 6″-O-malonyldaidzin and 6″-O-malonylgenistin as the major isomers. In contrast, TVP contains appreciable amounts of 6″-O-acetyldaidzin and 6″-O-acetylgenistin, as a result of the transformation of the malonyl isoflavones to their acetyl forms by heat treatment during extrusion processing. Nonfermented soy foods (e.g. tofu) contain greater levels of glucosides, while fermented soy foods (e.g. tempeh) contain greater levels of aglycones as a result of enzymatic hydrolysis during fermentation (137).

Although a large number of coumestans have been isolated from plants (145), only a few have been shown to possess uterotropic activity. Coumestrol and 4′-methoxycoumestrol (Figure 3) are the estrogenic coumestans found in alfalfa, ladino clover, and other fodder corps (129).

The most significant sources of coumestans in foods are sprouts of clover and alfalfa (56), with coumestrol contents of 5.6 and 0.7 mg/g dry weight, respectively. Split peas, kala chana seeds, pinto bean seeds, lima bean seeds, and soybean sprouts also contain small amounts of coumestrol (15–80 μg/g dry weight) (56, 77).

Although they have not been shown to induce estrus, lignans are considered by some researchers to be phytoestrogens as a result of other estrogen-like actions (see below). Lignans are present in plant foods as well as in human biological fluids. The conversion of plant lignans to mammalian lignans occurs in the gastrointestinal tract as a result of bacterial action (see below). The plant lignans, secoisolariciresinol and matairesinol, are the dietary precursors of the mammalian lignans, enterodiol and enterolactone (Figure 4).

Oilseeds, such as flaxseed, are the richest plant sources of lignans. Flaxseed contains about 0.8 mg of secoisolariciresinol/g dry weight (17). Although the



Coumestan	R_1	R_2
Coumestrol	OH	OH
4'-methoxycoumestrol	OH	OCH_3

Figure 3 The structures of coumestrol and 4′-methoxycoumestrol, two estrogenic coumestans found in alfalfa.

lignan content of foods has not been fully characterized, Thompson et al (131) used in vitro fermentation with human fecal flora to determine mammalian lignan production from a variety of plant foods. Production ranged from 0.02–67.5 mg of lignans/100 g (wet weight) of plant. The highest production was found in oilseeds, including flaxseed and unhulled soybeans (20.5 mg/100 g), with lesser amounts found in dried seaweeds (0.9 mg/100 g), whole legumes (0.6 mg/100 g), cereal brans (0.5 mg/100 g), legume hulls (0.4 mg/100 g), whole grain cereals (0.3 mg/100 g), vegetables (0.14 mg/100 g), and fruits (0.08 mg/100 g).

METABOLISM AND DISPOSITION

Isoflavones and lignans show similar patterns of metabolism and disposition in animals and humans, while the metabolism of coumestans has not been characterized. Both isoflavones and lignans undergo significant metabolism by bacteria in the gastrointestinal tract (119).

The metabolic pathways of daidzein and genistein catabolism in humans were originally proposed by Setchell & Adlercreutz (119) and have been expanded recently by Joannou et al (70), based on the isoflavone metabolites found in human urine (Figure 5). Daidzein is metabolized to dihydrodaidzein, which is further metabolized to both equol and O-desmethylangolensin (O-DMA). Genistein is transformed to dihydrogenistein and is further metabolized to 6′-hydroxy-O-DMA. Human urinary excretion of these metabolites is variable

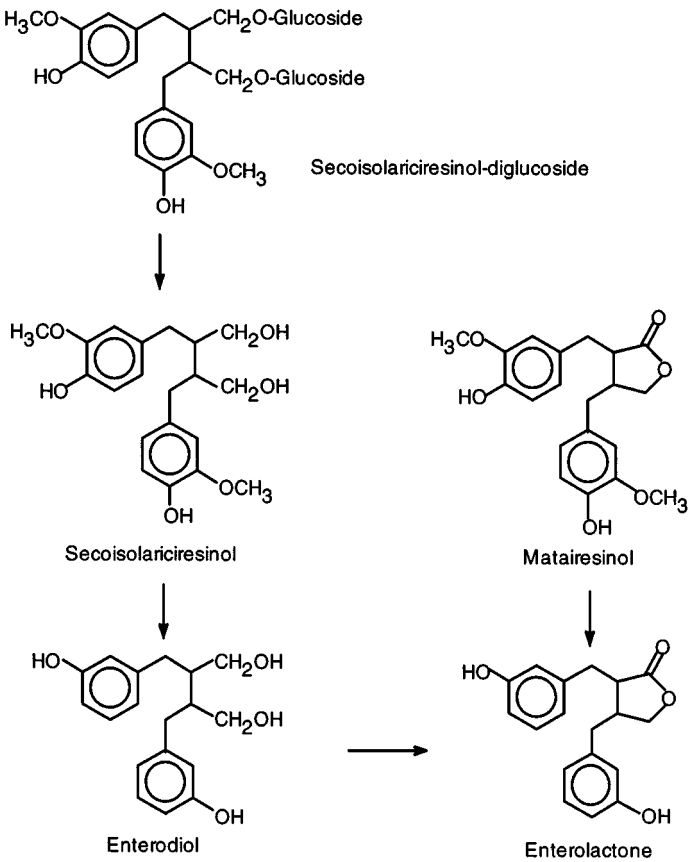


Figure 4 Formation of enterolactone and enterodiol by human fecal flora (modified from 28). Secoisolariciresinol diglucoside is metabolized to enterodiol through hydrolysis of the sugar moiety, dehydroxylation, and demethylation. Enterodiol can then be further oxidized to enterolactone. Matairesinol is converted to enterolactone by gut bacteria through dehydroxylation and demethylation.

(74), and only about 30–40% of subjects excrete significant quantities of equol after isoflavone consumption (120).

The plant lignans secoisolariciresinol and matairesinol are converted by human gut bacteria to the mammalian lignans, enterolactone and enterodiol (28) (Figure 4). Secoisolariciresinol diglucoside is transformed to enterodiol through reactions involving hydrolysis of the sugar moiety, dehydroxylation, and demethylation. Enterodiol can be further oxidized to enterolactone.

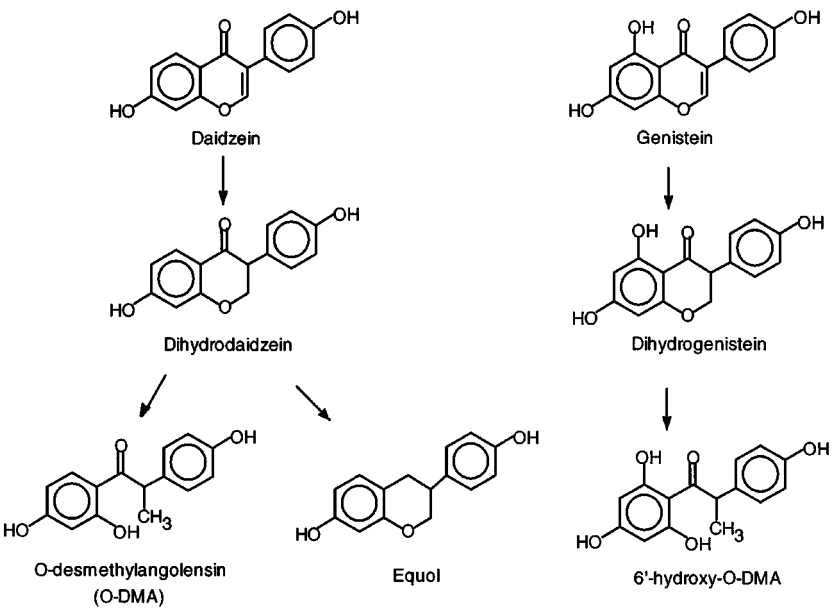


Figure 5 Proposed metabolic pathways for the catabolism of daidzein and genistein by human gut bacteria (modified from 70). The formation of O-DMA from daidzein and 6′-hydroxy-O-DMA from genistein likely involves several reduction reactions. The formation of equol from dihydrodaidzein may go through reduction, dehydration, and further reduction reactions.

Matairesinol is converted to enterolactone through dehydroxylation and demethylation.

Isoflavone and lignan absorption and utilization require a series of deconjugation and conjugation steps. Absorption is facilitated by hydrolysis of the sugar moiety by human gut bacterial β-glucosidases, gastric hydrochloric acid, and β-glucosidases in foods (74). After absorption in the small intestine, isoflavones and lignans are conjugated with glucuronic acid and sulfate by hepatic phase II enzymes (UDP-glucuronosyltransferases and sulfotransferases). Lignan and isoflavone conjugate profiles in human urine suggest that glucuronic acid is the primary moiety (8). Like endogenous estrogens, these conjugates are excreted through both urine and bile and undergo enterohepatic circulation. After excretion into bile, conjugated isoflavones and lignans can be deconjugated once again by gut bacteria. Deconjugation may promote reabsorption, further metabolism, and degradation in the lower intestine (119, 146).

Human metabolism and excretion of isoflavones after soy consumption show considerable individual variation (69, 73, 146). In a study reported by Xu et al

(147), 12 adult women received a single dose of 0.7, 1.3, or 2.0 mg of isoflavones/kg of body weight in soy milk (ratio of daidzein:genistein = 56:44). Before soy milk consumption, isoflavone levels in plasma, urine, and feces were extremely low, increasing several hundred times after consumption. At 6.5 h after soy consumption, average plasma concentrations of daidzein and genistein increased from negligible levels to 0.79 and 0.74 μM, respectively, after a dose of 0.7 mg of isoflavones/kg; to 1.22 and 1.07 μM, respectively, after a dose of 1.3 mg/kg; and to 2.24 and 2.15 μM, respectively, after a dose of 2.0 mg/kg (147). The average 24-h urinary recoveries of daidzein and genistein were approximately $21 \pm 8\%$ and $9 \pm 5\%$ of consumption, respectively. The average 24-h fecal excretion of daidzein and genistein was 1–2% of the ingested amounts, although one subject showed a fecal isoflavone recovery that was 10 times greater than that of the other subjects. This low recovery may be due to metabolism to compounds that were not monitored or to broad tissue distribution that has not been characterized.

In a recent study reported by Coward et al (46), four volunteers consumed beverages containing 20 g of isolated soy protein twice daily for 14 days. The total intake of genistein and daidzein with each serving was 21.0 and 13.6 mg, respectively, a molar ratio of 1.46. Plasma concentrations of genistein and daidzein from samples taken at 6.5 h after soy consumption on days 1, 7, and 14 ranged from 496–644 nM and 289–424 nM, respectively. The genistein/(daidzein + metabolites) molar ratio varied from 1.30 to 1.54.

Soy food processing appears to influence isoflavone bioavailability. Urinary isoflavone excretion was similar in 17 male subjects consuming either 112 g of fermented soy tempeh or 125 g of unfermented soybean pieces for nine days each (69). Urinary recovery of daidzein and genistein, however, was higher when subjects consumed the tempeh diet ($9.7 \pm 0.6\%$ and $1.9 \pm 0.1\%$, respectively) than when they consumed the unfermented soy diet ($5.7 \pm 0.6\%$ and $1.3 \pm 0.1\%$, respectively). These data suggest that the isoflavone aglycones in fermented food may be more bioavailable than their glucosides.

Animal studies, on the other hand, suggest that the extent of isoflavone absorption is similar for the aglycone and conjugated forms, although the initial absorption rate of aglycone forms is greater than that of the conjugates. In the study by King et al (76), rats were given a single dose of genistein (20 mg/kg of body weight) or an equivalent dose of its conjugated forms as an isoflavone-rich soy extract. Plasma genistein concentrations at 2 h after dosing were 11.0 ± 2.3 μM in the genistein-treated rats compared with 4.9 ± 0.2 μM in the soy extract-treated rats, but there were no significant differences at 8 h and later. The mean urinary excretion rate during the first 2 h after dosing was more than 10 times higher in the genistein group than in the soy extract group, but the percentage of dose recovered in urine over 48 h was not different ($19.9 \pm 2.4\%$

and $17.5 \pm 1.1\%$ in the genistein and soy extract groups, respectively). There were no significant differences in the recovery of genistein in feces ($21.9 \pm 2.8\%$ and $21.1 \pm 2.5\%$ of dose for the genistein and soy extract groups, respectively). These results suggest that the extent of absorption of genistein is similar for the aglycone and conjugated forms. Although higher initial plasma concentrations may be achieved with the aglycone, by 8 h the concentrations are similar.

Few studies have been performed in which urinary and fecal lignan excretion have been measured after consumption of controlled quantities of dietary lignans. In 19 premenopausal women consuming 10 g of ground flaxseed per day for three menstrual cycles, both urinary and fecal lignan excretion increased significantly and varied greatly among subjects (3- to 285-fold increase) (80, 81). Urinary excretion of enterodiol increased from 1.09 ± 1.08 to 19.48 ± 1.10 μmol/day, and excretion of enterolactone increased from 3.16 ± 1.47 to 27.79 ± 1.50 μmol/day (81). Excretion was not altered by the phase of the menstrual cycle nor by the duration of flaxseed consumption. Fecal lignan excretion also increased significantly with flax consumption, from 80.0 ± 80.0 to 2560 ± 3100, 640 ± 480 to $10{,}300 \pm 7580$, and 7.33 ± 10.0 to 11.9 ± 8.06 nmol/day for enterodiol, enterolactone, and matairesinol, respectively (80).

To the best of our knowledge, plasma lignan concentrations after known amounts of plant lignan feeding have not been reported in either human or animal studies. Neither have coumestan concentrations been reported in any biological fluids after known amounts of coumestan feeding.

Few data have been published on tissue distribution of phytoestrogens. Yueh & Chu (151) reported the tissue distribution of daidzein in rats 15 min after intravenous injection of 40 mg of daidzein/kg of body weight. Daidzein concentration was found to be high in plasma, liver, lung, and kidney at about 30 μg/g wet weight; to be moderate in skeletal muscle, spleen, and heart at about 15–20 μg/g wet weight; and to be low in brain and testis at about 2–5 μg/g wet weight. To the best of our knowledge, no other studies of phytoestrogen tissue distribution have been reported.

PHYSIOLOGICAL LEVELS IN HUMANS

Phytoestrogens have been identified in many physiological fluids in humans consuming ordinary diets. Those identified in urine include enterolactone (122, 128), enterodiol (122), equol (16), daidzein, O-DMA, matairesinol (19, 20), genistein (2), and glycitein (74). Daidzein, genistein, O-DMA, equol, enterolactone, enterodiol, and matairesinol have been identified in both human plasma and feces (3, 4).

Adlercreutz et al (5) summarized their data accumulated over many years regarding the urinary excretion of lignans and isoflavones in various populations

(Finnish women, American women, Asian immigrant women in Hawaii, breast cancer patients, and Japanese women and men) and dietary groups (omnivores, vegetarians, lacto-ovovegetarians, adherents of macrobiotics, and those who consume the traditional Japanese diet). American adherents of macrobiotics, lacto-ovovegetarians, and Japanese men excreted the greatest quantities of urinary isoflavones, with levels at 3412–8770, 885–2188, and 1820–3630 nmol/day, respectively. Japanese women showed the greatest variation (347–6610 nmol/day), and Finnish breast cancer patients showed the lowest urinary isoflavone excretion (67.5–324 nmol/day). American adherents of macrobiotics had the greatest urinary lignan excretion (15,228–35,363 nmol/day), while Finnish breast cancer patients showed a low urinary lignan excretion of 1302–2835 nmol/day. Despite their high urinary isoflavone excretion, Japanese men and women had very low urinary lignan excretion (840–1792 nmol/day).

Adlercreutz et al (3) reported fecal excretion of isoflavones and lignans in 10 omnivore and 10 vegetarian women. For the omnivore women, mean values of total daidzein, genistein, equol, O-DMA, enterolactone, enterodiol, and matairesinol were 45.4, 11.6, 14.9, 5.67, 1510, 147.7, and 22.3 nmol/day, respectively. For the vegetarian women, mean values of total daidzein, genistein, equol, O-DMA, enterolactone, enterodiol, and matairesinol were 259.1, 189.6, 267.8, 114.7, 3280, 479.2, and 89.3 nmol/day, respectively.

Characterization of the concentrations of isoflavones and lignans in human plasma was reported by Adlercreutz et al (4). In this study, two fractions (free + sulfate and glucuronide) of isoflavones and lignans were measured. Data were collected from 28 Finnish women (14 omnivores and 14 vegetarians) and six Japanese men who consumed a traditional Japanese diet. Among the Finnish women, except for both fractions (free + sulfate and glucuronide) of matairesinol and equol and the glucuronide fraction of genistein, all the plasma isoflavones and lignans were significantly greater in the vegetarians. For the female Finnish vegetarian subjects, mean values of total daidzein, genistein, equol, O-DMA, enterolactone, enterodiol, and matairesinol were 18.5, 17.1, 0.7, 0.8, 89.1, 5.4, and 0.06 nM, respectively. For the female Finnish omnivore subjects, mean values of total daidzein, genistein, equol, O-DMA, enterolactone, enterodiol, and matairesinol were 4.2, 4.9, 0.8, 0.07, 28.5, 1.4, and 0.02 nM, respectively. For the six men consuming a traditional Japanese diet, plasma concentrations of total daidzein, genistein, equol, O-DMA, enterolactone, enterodiol, and matairesinol varied from 60–924, 90–1204, 0.54–24.6, 0.98–223, 2.87–17.8, 0.68–1.61, and 0.21–3.5 nM, respectively.

To the best of our knowledge, usual physiological levels of coumestans have not been reported in human and animal studies, although the analytical methods (56, 89) for monitoring plasma and urinary coumestrol are available.

PHYSIOLOGICAL EFFECTS

Hormonal Effects

FERTILITY The estrogenic activity of isoflavones was first recognized in relation to a syndrome known as clover disease in sheep (24, 31, 98, 124). After grazing on pastures of subterranean and red clover, Australian ewes suffered from a severe reproductive disorder that resulted in permanent infertility. In searching for the cause of the infertility, researchers identified the isoflavones genistein and daidzein and their precursors biochanin A and formononetin as components of the clover. Millington et al (95) reported that the formononetin content, not the biochanin A or genistein content, was positively correlated with the estrogenicity of the clover. Further research led to the identification of equol in the urine of sheep given an intraruminal dose of formononetin (31) and in the plasma of sheep consuming red or subterranean clover (124). Shutt & Cox (125) reported that equol possessed more estrogenic activity than did daidzein or O-DMA and concluded that equol was responsible for the estrogenic effects observed in sheep.

There appears to be great species variability in the effects of phytoestrogens on fertility. Doses of equol in concentrations similar to those seen in clover disease do not cause damage in mares (97), and high daily consumption of phytoestrogens has not been associated with permanent infertility in cattle (32). The differences in sensitivity to phytoestrogens between species are not fully understood, although differences at the level of the ER have been suspected (88).

The estrogenic effects of isoflavones are also believed to have played a major role in the reproductive failure and liver disease that threatened the existence of the captive cheetah population. Setchell et al (121) suggested that the high concentrations of daidzein and genistein present in the cheetahs' diet may have been among the major factors in the decline of fertility and the presence of liver disease in this species.

In rodents, isoflavones have been shown to stimulate uterine growth. In the mouse uterine growth assay, genistein and daidzein are roughly 100,000 times less effective than estradiol and diethylstilbestrol (DES) (26), while glycitein and its conjugates are found to be nonestrogenic (105). The relative potencies of DES, genistein, genistin, and daidzin administered through stomach intubation have been reported to be 100,000, 1.00, 0.66, and 0.26, respectively, in the B6D2F1 mouse (49). However, not all mouse strains are susceptible to isoflavone estrogenicity. Although effects of uterine hypertrophy have been seen in B6D2F1 and B6C3F1 strains, the Swiss albino CD-1 mouse does not respond, and the ICR mouse shows only a slight response (50, 51). It is possible that nonsusceptible strains rapidly inactivate isoflavones, or they may lack the

capability to form estrogenic metabolites. There may also be strain differences in target tissue sensitivity to isoflavones.

Effects of neonatal exposure to coumestrol and equol on the development of the rat reproductive tract have been examined (93). When Sprague-Dawley pups were injected subcutaneously with 100 μg of coumestrol on postnatal days 1–5, premature uterine gland development and increased uterine weight were observed. At later ages, uterine weight was significantly lowered, and there was a severe suppression in ER levels. When 100 μg of equol was given, it lowered uterine weight at the later ages but did not affect ER levels. When given on postnatal days 10–14, both coumestrol and equol caused a dose-dependent inhibition of uterine gland growth, although not as severe as with either DES or tamoxifen. Coumestrol was estimated to be about 1000 times more potent than equol and behaved much like DES with respect to its effects on uterine weight, glands, and ER levels.

SEXUAL DIFFERENTIATION Prenatal exposure to genistein has been shown to influence sexual differentiation. The dosage and timing of exposure during development appear to be important. In a study reported by Levy et al (84), markers of sexual differentiation included birth weight, anogenital distance at birth, gonadotropin releasing hormone–stimulated luteinizing hormone (LH) secretion, volume of the sexually dimorphic nucleus in the preoptic area of hypothalamus (SDN-POA), puberty onset, and vaginal cyclicity. Pregnant Charles River CD rats were subcutaneously injected daily on gestation days 16–20, with either 25,000 μg of genistein, 5000 μg of genistein, 5 μg of DES, 50 μg of estradiol, or corn oil. Compared with the corn oil control, DES- and estradiol-treated animals showed significantly enlarged SDN-POA volumes. There was an apparent but nonsignificant decrease in SDN-POA volume in the females treated with 5000 μg but not 25,000 μg of genistein, and no significant differences were found among any males. Females in the 25,000-μg genistein group and both sexes in the DES and estradiol groups had significantly smaller birth weights. Both sexes in the 5000-μg genistein group and the estradiol group, as well as males in the DES group, had significantly shorter anogenital distance at birth. Females in the 5000-μg genistein group had significantly later onset of vaginal opening than those in the corn oil control group.

Effects of neonatal exposure to genistein on sexual differentiation were also studied in rats through neonatal subcutaneous injections of either corn oil or of 1, 10, 100, 200, 400, 500, or 1000 μg of genistein (48). Although only the 10-μg dose of genistein was associated with increased pituitary response to gonadotropin releasing hormone, increasing genistein was associated with decreasing LH secretion on postnatal days 1–10. The volume of the SDN-POA was increased by the 500- and 1000-μg doses. These results suggest that low

doses of genistein have nonandrogenizing, pituitary-sensitizing effects, while higher doses mimic the more typical effects of estrogens in masculinizing the brain and decreasing pituitary response.

Coumestrol has also been shown to influence sexual differentiation in rats. Weanling 21-day-old female Sprague-Dawley rats were fed a diet containing 0.01% coumestrol on days 21–24 or 22–60. Compared with control subjects, the coumestrol-fed animals showed progestin receptor induction in the uterus, pituitary, and SDN-POA, earlier vaginal opening, lower body weight, and irregular vaginal cycles (143). When female rat pups consumed coumestrol through lactation from rat dams fed 0.01% coumestrol in their diet, 83% of the pups showed the cornified smears of a persistent estrous state by 132 days of age (91% of control subjects were cycling regularly at the same age) and failed to show an LH elevation after estradiol priming followed by progesterone (142).

BINDING TO THE ESTROGEN RECEPTOR AND EFFECTS ON GROWTH OF ESTROGEN-DEPENDENT CELLS Phytoestrogens have been shown to bind the ER, although weakly in comparison to estradiol. Coumestrol, daidzein, genistein, equol, and O-DMA have been reported to bind the ER in cytosol preparations of sheep uteri with relative binding affinities of 5, 0.1, 0.9, 0.4, and 0.05% of estradiol, respectively, (125). Coumestrol and genistein also have demonstrated binding to cytoplasmic ER present in the pituitary gland and the hypothalamus of the ewe (96). These data suggest that in the absence of endogenous estrogens, these phytoestrogens may be able to exert weakly estrogenic effects.

Estrogenic effects of phytoestrogens are suggested by their abilities to stimulate growth of estrogen-dependent MCF-7 human breast cancer cells (92). Coumestrol (90, 92, 141), genistein (90, 92, 141), biochanin A (90, 141), daidzein (141), and enterolactone (99) have been shown to enhance cell proliferation at concentrations below 1–10 μM. The concentrations for half maximal growth have been reported to be 0.0018, 0.0025, 0.04, and 0.3 μM for coumestrol, genistein, biochanin A, and daidzein, respectively (141). Genistein has been shown to stimulate expression of estrogen-responsive pS2 mRNA at concentrations as low as 0.01 μM and to induce proliferation of ER-positive MCF-7 cells but not ER-negative MDA-MB-231 cells at concentrations between 0.01 and 1.0 μM (138).

Antiestrogenic effects of phytoestrogens have also been observed. At concentrations 100–1000 times that of estradiol (the probable levels in human plasma after regular phytoestrogen consumption), it has been proposed that phytoestrogens may be able to compete effectively with endogenous mammalian estrogens, bind the ER, and prevent estrogen-stimulated growth in mammals (5). This may also result in interference with the release of gonadotropins and

interruption of the feedback-regulating system of the hypothalamus-pituitary-gonadal axis. There are some data to support this hypothesis. Genistein and coumestrol have competetively suppressed the binding of [^{3}H]estradiol to ER when added to rat and human mammary tumor tissue (135), and daidzein, equol, and matairesinol have competed effectively with [^{3}H]estradiol for the rat uterine estrogen nuclear type II binding site (bioflavonoid receptor) with a 50% inhibitory concentration (IC_{50}) of 1–10 μM (7). Prolonged exposure of MCF-7 cells to 1.0 μM genistein, a concentration likely to be reached with regular soy consumption, has decreased ER expression and suppressed estradiol-stimulated responses (138), and incubation with physiologic concentrations of estradiol and enterolactone has resulted in reduced growth stimulation compared with either compound alone (99).

High concentrations (greater than 10 μM) of genistein (107, 138) and enterolactone (99) have been shown to significantly inhibit MCF-7 cell growth. Since similar effects have been shown in estrogen-independent MDA cells (107, 138), it is likely that the inhibitory effects are independent of the ER.

EFFECTS ON ESTROGEN SYNTHESIS AND AVAILABILITY Kellis & Vickery (72) first reported several natural and synthetic flavones to be inhibitors of aromatase enzyme, the rate-limiting enzyme in estrogen synthesis in humans. In an in vitro study, placental aromatase activity was inhibited by daidzein, equol, O-DMA, enterolactone, and enterodiol with IC_{50} values of $<$1 mM, 150 μM, 160 μM, 14 μM, and 30 μM, respectively (1). In a similar study, enterolactone, coumestrol, and biochanin A showed suppression of aromatase activity in a human preadipose cell culture system with K_i values of 14.4, 1.3, and 49 μM, respectively (IC_{50} values of 74, 17, and 113 μM, respectively), although genistein, daidzein, O-DMA, and equol had no effects (35, 136).

Phytoestrogens have been proposed to decrease free estrogen concentrations via stimulation of sex hormone binding globulin (SHBG) synthesis in the liver. Enterolactone (1–10 μM) has been shown to increase SHBG synthesis by HepG2 cells (7), although studies in humans have not shown increased SHBG levels in subjects consuming phytoestrogen-rich diets.

EFFECTS ON HUMAN REPRODUCTIVE HORMONES Phytoestrogens have been shown to affect the menstrual cycle and concentrations of reproductive hormones in premenopausal women. Phipps et al (109) reported a study of 19 premenopausal women studied for two diet periods of three menstrual cycles each in a crossover design. The subjects showed a longer average luteal phase length when consuming 10 g of flaxseed powder/day in addition to their habitual diets (12.6 ± 0.4 vs 11.4 ± 0.4 days) (109). There were no significant differences between flax and control cycles for concentrations of either estradiol or estrone during the early follicular, midfollicular, or luteal phases. Although

flaxseed ingestion had no significant effect on luteal phase progesterone concentration, the luteal phase progestrone/estradiol ratio was significantly higher during the flax seed cycles.

A number of studies have been reported on the effects of soy consumption on reproductive hormones in premenopausal women. Cassidy et al (38) reported that six premenopausal women receiving 60 g of soy protein/day (containing 45 mg of isoflavones) for one menstrual cycle in a metabolic ward had significantly increased follicular phase length and/or delayed menstruation. Mid-cycle surges of LH and follicle stimulating hormone (FSH) were significantly suppressed, and follicular phase estradiol was increased by the soy feeding (38). In another study by the same group (39), subjects consumed 23–45 mg of isoflavones/day as TVP ($n = 6$) or miso ($n = 3$), or they consumed an isoflavone-free control diet ($n = 5$) for one menstrual cycle. Follicular phase length was significantly increased, peak progestrone concentrations were delayed, and mid-cycle peaks of LH and FSH were suppressed while the subjects consumed the soy. No effects were observed when subjects consumed the isoflavone-free soy protein diet, which suggests that the observed effects were due to the isoflavones. In a study reported by Lu et al (87), six premenopausal women were given 12 oz of soymilk with each of three meals (total isoflavone intake of 200 mg/day) in a metabolic ward for one month. Serum estradiol and luteal phase progesterone concentrations were decreased during feeding, and menstrual cycle length was increased. Although these studies are suggestive of hormonal effects, studies of one menstrual cycle are insufficient for evaluation of phase and cycle effects. Longer studies (at least two menstrual cycles per diet) with a larger number of subjecs are needed to confirm these results in humans.

Phytoestrogen consumption has shown modest estrogenic effects in postmenopausal women. In a study reported by Baird et al (18), 97 postmenopausal women were randomly assigned to a group that was provided with soy foods for four weeks or a control group that was instructed to eat as usual. Daily intake of soy consisted of 38 g of dry TVP or 114 g of whole soybean. In addition, 25 g of soy splits were consumed daily as a snack. Daily intake of isoflavones was 165 mg/day. The percentage of vaginal superficial cells was increased for 19% ($P = 0.06$) of those eating soy compared with 8% of control subjects, although FSH, LH, and SHBG were unaffected. Phytoestrogen supplementation with soy flour, flaxseed, or red clover sprouts was shown to increase vaginal cell maturation in postmenopausal women (144), although in a recent study with soy and wheat flour supplementation, no effects on vaginal cell maturation were observed (100).

Tumor Cell Differentiation and Mitogenesis

It is widely accepted that agents that promote terminal differentiation of human tumor cells inhibit cancer cell proliferation (115). The therapeutic use of

all-*trans* retinoic acid in the treatment of acute promyelocytic leukemia (66) is based on this mechanism. This approach is becoming important not only in chemotherapy but also in cancer chemoprevention (43).

Agents that function as inducers of cell differentiation belong to a variety of chemical classes and modulate numerous cellular processes. Many such inducers are inhibitors of enzymes whose actions promote cell proliferation. Protein tyrosine kinases (PTKs) and DNA topoisomerases belong to this class of enzymes.

PROTEIN TYROSINE KINASES PTKs are oncogene products that catalyze phosphorylation of their own tyrosine residues as well as those in other proteins, such as growth factors, involved in tumor cell signal transduction and proliferation (68). They are thought to induce cell proliferation in part because of effects on growth factor–stimulated signal transduction. Isoflavones, especially genistein, have been shown to inhibit cellular PTKs (29, 34, 41). In vitro experiments with the epidermal growth factor receptor from the plasma membrane of human epidermoid carcinoma A-431 cells showed genistein to inhibit receptor tyrosine autophosphorylation and tyrosine phosphorylation of both natural and artificial compounds, with IC_{50} values of 2.6–37 μM (10).

PTK inhibition has been proposed to explain the growth-inhibiting effects of genistein in a number of different human cancer cells. Genistein has been shown to induce terminal differentiation and inhibit proliferation in a dose-dependent manner in human leukemia and melanoma cells (43, 44). It reduced cell growth and induced differentiation of melanoma cells at concentrations of 45 μM and caused early changes in their chromatin structure, detectable as protein-linked DNA strand breaks (75). Genistein has also been shown to inhibit growth and induce differentiation in human leukemia cells at concentrations of 37 and 74 μM (44) and to inhibit growth of human prostate cancer (108), stomach, esophageal, and colon cancer cells (149). Both biochanin A and genistein have shown cytotoxic effects on stomach, esophageal, and colon cancer cell growth at concentrations greater than 148 and 74 μM, respectively (149). No data regarding the effects of lignans and coumestans on tumor cell differentiation or PTK inhibition have been reported.

DNA TOPOISOMERASES DNA topoisomerases are enzymes that catalyze topologic changes in DNA and are required for DNA replication (40, 57). Several studies have shown that genistein inhibits both topoisomerase I and II activity (44, 75, 91, 106). Genistein has been shown to inhibit the relaxation of pBR322 (supercoiled) DNA catalyzed by topoisomerase II at concentrations greater than 7.4 μM (106). Decatenation activity of purified calf thymus DNA topoisomerase II was initially inhibited at genistein concentrations of 20 μM

and was completely inhibited at 80 μM (91). Genistein has also been shown to induce topoisomerase II–mediated double-stranded breaks, which suggests that genistein may stabilize the topoisomerase II-DNA complex, allowing the breaks to occur but preventing them from being resealed (44).

Angiogenesis

Phytoestrogens have been shown to suppress angiogenesis (55). Genistein, when added with basic fibroblast growth factor, delayed the proliferation of endothelial cells derived from bovine brain capillaries. Retardation of endothelial cell growth by genistein may occur (*a*) as a consequence of the competitive inhibition of ATP binding to the catalytic domain of tyrosine kinase; (*b*) as a consequence of the attenuation of activity of S6 kinase, an enzyme that is also activated by basic fibroblast growth factor; or (*c*) through the modulation of topoisomerases I and II (96).

Antioxidant Effects

A number of studies have shown isoflavones to exert antioxidant effects in vitro and in vivo. Naim et al (104) reported that isoflavones inhibited lipoxygenase action and prevented peroxidative hemolysis of sheep erythrocytes in vitro. Phenolic compounds in soybeans, defatted soy flour, soy protein concentrates, and soy isolates have been shown to exert appreciable antioxidant activity as detected by the rate of β-carotene bleaching in a lipid-aqueous system (112). In a study reported by Wei et al (140), genistein was found to be the most potent inhibitor of hydrogen peroxide production in 12-O-tetradecanonylphorbol-13-acetate–activated HL-60 cells ($IC_{50} = 25\,\mu$M). Daidzein showed weaker effects ($IC_{50} = 150\,\mu$M), and biochanin A showed none. In addition, genistein was a potent inhibitor of superoxide anion generation by xanthine/xanthine oxidase ($IC_{50} = 1$–$2.5\,\mu$M), with daidzein showing a moderate inhibitory effect ($IC_{50} = 5\,\mu$M) and biochanin A exhibiting no effect.

Genistein has also been shown to increase activities of antioxidant enzymes. Feeding 250 ppm of genistein to 6- to 7-week-old female CD-1 mice for 30 days significantly enhanced the activities of catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase by 10–30% in skin and small intestine (140). Rat hepatic cumene hydroperoxidase activity was increased significantly after the feeding of a soybean isoflavone extract (240 mg of isoflavones/kg of diet) for one week (62). Soy isoflavones, thus, can act as antioxidants directly or indirectly through the enhancement of antioxidant enzyme activities.

To the best of our knowledge, no data regarding the antioxidant effects of lignans and coumestans have been reported.

EFFECTS ON HUMAN HEALTH

Symptoms of Menopause

The onset of menopause (cessation of menses) is caused by the failure of the ovaries to respond to gonadotropins, as a result of the depletion of available follicles for growth (58). It is widely believed that the reduction of gonadal estrogens causes a number of physiological changes that result in symptoms such as hot flashes, atrophic vaginitis, and vaginal dryness and that contribute to the development of osteoporosis and increased risk of coronary heart disease. These changes can be reversed by exogenous estrogens given locally or systemically.

The major symptoms of menopause have been assumed to occur universally. However, Lock (86) suggested that some menopausal experiences (e.g. hot flashes) vary from culture to culture. He reported that fewer postmenopausal Japanese women than postmenopausal Canadian women complain of hot flashes during menopause. At the same time, only about 4% of postmenopausal Japanese women use estrogen replacement therapy, compared with about 30% of postmenopausal women in the United States (42). Although the difference in symptoms could be due to many factors, Adlercreutz et al (6) have suggested that the weakly estrogenic actions of a phytoestrogen-rich diet may explain the reduced frequency of menopausal symptoms in Japanese women.

Murkies et al (100) reported a study in which 58 postmenopausal women with at least 14 hot flashes per week received 45 g of soy flour or wheat flour (control) per day as a supplement to their regular diet over 12 weeks in a randomized, double blind design. Hot flashes significantly decreased in both the soy and wheat flour groups (40% and 25% reductions, respectively) with a rapid response in the soy flour group in six weeks. Menopausal symptom scores also decreased significantly in both groups (100). The lack of difference between the two groups may be due to either a strong placebo effect or a time-dependent decline in symptoms.

Osteoporosis

Like menopausal symptoms, osteoporosis has been related to decreased gonadal steroid production. Common sites of fracture among postmenopausal women are the vertebrae, forearm, and hip, while such fractures occur much less frequently in men of a similar age (58). Estrogen replacement therapy has been shown to reduce the risk of osteoporosis in postmenopausal women (47). Although the mechanisms are not entirely clear, it has been proposed that estrogen (*a*) reduces sensitivity of bone tissue to the resorptive effects of parathyroid hormone; (*b*) blocks the release of interleukin-1, a potent bone resorption agent; or (*c*) directly modulates osteoblast activity (15).

Estrogenic effects of phytoestrogens have been proposed to prevent bone resorption and promote increased bone density. Coumestrol has been shown

to inhibit bone resorption and stimulate bone mineralization in vitro (133). Genistein has been found to have effects similar to Premarin, an estrogen with bone-retaining properties, in maintaining trabecular bone tissue in rats (11). Kalu et al (71) found significantly less bone loss and a delayed onset of age-related bone loss in rats consuming soy protein compared with those consuming casein. Dietary soybean protein has also been shown to prevent bone loss caused by ovarian hormone deficiency in an ovariectomized rat with osteoporosis (14). The markers of bone formation (serum alkaline phosphatase) and of bone resorption (serum tartrate–resistent acid phosphatase) were both significantly greater in the ovariectomized + soybean group compared with the control animals that had surgery but no ovariectomy. Despite the higher rate of bone turnover in the ovariectomized + soybean group, the vertebral and femoral bone densities in this group were significantly greater than in the ovariectomized group. In addition, the fourth lumbar vertebra calcium and phosphorus contents in the ovariectomized + soybean group were greater than those in the ovariectomized group, although the difference was not statistically significant. These data suggest that bone formation promoted by soy protein exceeds the resorption caused by ovariectomy (14).

Ipriflavone, a synthetic isoflavone with daidzein as its major metabolite, has been shown to prevent bone loss in animals and patients with osteoporosis (9, 148). Although not entirely clear, the mechanism may be through direct effects on the osteoblast (25) or through effects on secretion of calcitonin, the hormone-suppressing bone resorption (148).

Soy intake has also been suggested to protect against bone loss by mechanisms independent of its estrogenic effects. First, soy foods are a good source of calcium. Miso and tempeh contain 92 and 77 mg of calcium per 0.5 cup serving, respectively. Tofu coagulated from soymilk by calcium salt has about 406 mg calcium per quarter block (61). Second, a high soy-protein diet may prevent the urinary calcium loss seen with a high animal-protein diet. Breslau et al (33) compared calcium loss during consumption of soy protein versus animal protein and found 50% more calcium loss in subjects who consumed animal protein than in those who consumed soy protein.

Cancer

A great deal of evidence supports the hypothesis that adequate dietary lignan and isoflavone intakes reduce cancer risk (5, 119). Several papers have reviewed the potential roles of phytoestrogens in preventing breast, colon, and prostate cancer (5, 21, 94).

A number of epidemiologic studies have examined the relationship between soy food consumption and cancer risk. Tofu consumption has been associated with a reduced risk of prostate cancer in Japanese men (123). Japanese men who consumed tofu more than five times a week had half the risk of prostate cancer

compared with Japanese men who consumed tofu less than once a week. Tofu consumption has also been negatively correlated with gastric cancer in Japanese men and women (59, 102) and with lung cancer in Chinese men (130). Daily intake of miso, a fermented soybean, has been associated with a reduced risk of gastric cancer in a 12-year study of over 265,000 Japanese adults (64). Chinese men and women who consume more than 5 kg of soybeans each year have been shown to have a 40% reduction in risk of stomach cancer (150), and Chinese women who consume soy foods less than once a week have been shown to have 3.5 times the risk of lung cancer and twice the risk of breast cancer compared with women who consumed soy foods daily (78, 82). Chinese men who consume more than 9 kg of soybean sprouts, tofu, or dried tofu each year have been shown to have one third the risk of rectal cancer compared with men who consumed less than 2 kg each year (65).

Animal studies have shown reduced cancer development with soybean consumption. Feeding a 30% soybean diet to mice protects the liver from cancer development induced by the nitrosamine precursors, dibutylamine and nitrite (53). Rats that consumed a soy-based diet developed fewer mammary tumors following administration of carcinogens than did rats on soy-free isonitrogenous and isocaloric diets (22). In female F344/N rats with hepatocarcinogenesis initiated by diethylnitrosamine and promoted by phenobarbital, consumption of soybean isoflavone extract at 240 or 480 mg/kg of diet normalized the activity of hepatic glutathione peroxidase, an antioxidant enzyme suppressed by phenobarbital (83). In that study, phenobarbital promotion was inhibited as reflected in the volume of altered hepatic foci for γ-glutamyltransferase and placental glutathione transferase positive after three months of feeding.

Serraino & Thompson (117) reported that supplementation of a high-fat diet with lignan-rich flaxseed flour or defatted flaxseed meal (5% or 10%) reduced epithelial cell proliferation by 38.8–55.4% and nuclear aberrations by 58.8–65.9% in the female rat mammary gland, with optimum effects seen at 5% flaxseed flour. In another study reported by the same group, five groups of male Sprague-Dawley rats were fed a high-fat (20% corn oil) basal diet with or without supplementation with 5% or 10% flaxseed meal or flaxseed flour for four weeks, following a single injection of azoxymethane (15 mg/kg of body weight) (118). In the descending colon of supplemented groups, the total number of aberrant crypts and foci were significantly reduced by 41–53% and 48–57%, respectively.

In vitro studies have revealed numerous mechanisms by which isoflavones may be cancer preventive. Genistein has been shown to inhibit the growth of both estrogen receptor-negative and -positive human breast cancer cell lines (IC_{50} = 24–44 μM) (107). Genistein and daidzein (IC_{50} of 2.2 μM and 8 μM, respectively) inhibit production of inositol phosphates, key intracellular signals

of proliferation stimulated by aluminum tetrafluoride in 3T3 cells (63). Genistein inhibits the autophosphorylation of the epidermal growth factor receptor in A431 human epidermoid carcinoma cells ($IC_{50} = 2.6\ \mu M$) (10) and DNA topoisomerase II in human leukemic MOLT-4 and HL-60 cells ($IC_{50} = 31.5$ and 48 μM, respectively) but not in normal human proliferating lymphocytes (132). Genistein also blocks endothelial cell proliferation and in vitro angiogenesis (IC_{50} of 5 and 150 μM, respectively) (55). It is important to remember that the IC_{50} for some of these effects is very high relative to the physiological concentrations of the phytoestrogens.

On the other hand, some phytoestrogens at low concentrations (less than 10 μM) have shown proliferative effects through ER-mediated pathways in vitro (89, 138, 141). In vivo, formononetin stimulated mammary tissue proliferation in castrated female BALB/c mice after daily subcutaneous injection of 40 mg/kg for five days (139). Proliferation was increased 3.3-fold over saline-treated control subjects.

Heart Disease

Increasing risk of ischemic heart disease among postmenopausal women is associated with a deficiency of gonadal steroid production. In general, postmenopausal women experience decreased plasma concentrations of high density lipoprotein (HDL) cholesterol and increased plasma concentrations of low density lipoprotein (LDL) cholesterol as a result of the decline in estrogen (27). In fact, estrogen replacement therapy has been shown to decrease LDL cholesterol and increase HDL cholesterol and to prevent heart disease in postmenopausal women. Higher estrogen levels are thought to contribute to the reduced heart disease observed in premenopausal women as compared to postmenopausal women and men.

It has been proposed that phytoestrogens may act as estrogen agonists, producing effects on lipoproteins similar to those caused by estrogen. This is supported by epidemiologic data showing that the incidence of coronary heart disease and hypercholesterolemia is greater in populations that consume diets rich in animal protein than in those that consume diets rich in vegetable protein (127). Numerous studies have reported that soy protein substituted for animal protein in the diet reduces the concentration of blood total cholesterol and LDL cholesterol in humans (36, 126). The decrease in blood cholesterol has generally been greater in hypercholesterolemic than in normocholesterolemic subjects (37). A significant decrease of total and LDL cholesterol and apolipoprotein B concentrations was found in mildly hypercholesterolemic men when they consumed 50 g of isolated soy protein daily in addition to a low-fat, low-cholesterol diet (111). A recent meta-analysis of 38 controlled clinical trials has concluded that consumption of soy protein rather than animal protein significantly decreases

blood cholesterol, LDL cholesterol, and triglycerides (12). It also suggested that phytoestrogens account for 60–70% of the effects seen.

The hypothesized mechanisms for the cholesterol-lowering effects of soy protein have been reviewed, although the exact mechanisms and responsible soy components have not been fully identified (110). The metabolic changes observed in a variety of animals, and in some cases humans, fed soy protein include increased cholesterol synthesis, increased bile acid synthesis (or fecal bile acid excretion), increased apolipoprotein B or E receptor activity, and decreased hepatic lipoprotein secretion and cholesterol content, which are associated with increased clearance of cholesterol from the blood. Some researchers (67, 103, 134) suggest that dietary soy protein impairs cholesterol absorption and/or bile acid reabsorption. This is observed in some animal species, such as rabbits and rats, but not in humans, nor is it observed when amino acids replace intact soy protein. Other scientists (23, 54, 60, 114, 116) propose that changes in endocrine status, such as alterations in insulin:glucagon ratio and thyroid hormone concentrations, are responsible. One hypothesis (114) suggests that the amino acid composition of soy causes changes in cholesterol metabolism (possibly via the endocrine system).

Although it is possible that nonprotein components associated with soy protein (such as saponins, fiber, phytic acid, minerals, and isoflavones) affect cholesterol metabolism (110), a recent study with rhesus monkeys supports the significance of soy isoflavones (13). Compared with an alcohol-extracted soy protein, phytoestrogen-intact soy protein significantly reduced LDL + VLDL cholesterol concentrations in both males and females (about 30–40% lower), significantly increased HDL cholesterol concentrations in females (about 15% higher), and significantly decreased total cholesterol/HDL cholesterol ratios (approximately 20% lower for males and 50% lower for females). These data suggest that soy isoflavones contribute to the hypolipidemic effects of soy consumption.

CONCLUSIONS

Phytoestrogens are present in the human diet in substantial amounts. They have been shown to exert many biological effects in cell culture systems, animals, and humans. Many of these effects are considered to be health protective, although a few potentially harmful effects have been shown as well. The nature of the effects may vary as a result of a number of factors, including age at exposure, exposure dose, differences among compounds, presence of other dietary components, and other yet unknown factors.

A recent conference (the Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, Brussels, September 15–18, 1996;

proceedings to be published in the *American Journal of Clinical Nutrition*) demonstrated the large amount of research currently underway in the area of soy. In the next few years, as this work is published, there will be a tremendous increase in publications in the areas of isoflavone pharmacokinetics; usual exposure in humans, particularly infants; tissue distribution; physiological effects in humans; and the mechanisms of effects on bone, kidney, heart disease, and cancer.

Before recommendations are made regarding consumption of specific phytoestrogens in humans, it will be important to further characterize their physiological effects and margins of safety. Further research is needed to evaluate effects at physiological and pharmacological levels; to determine the effective doses for beneficial as well as harmful effects; and to evaluate the interactions of phytoestrogens with each other and with other dietary components. Although current data are not sufficient to support dietary recommendations for individual phytoestrogens, there may be great benefit in increased consumption of plant foods.

Literature Cited

1. Adlercreutz H, Bannwart C, Wähälä K, Mäkelä T, Brunow G, et al. 1993. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J. Steroid Biochem. Molec. Biol.* 44:147–53
2. Adlercreutz H, Fotsis T, Bannwart C, Wähälä K, Brunow G, et al. 1991. Isotope dilution gas chromatographic-mass spectrometric method for the determination of lignans and isoflavonoids in human urine, including identification of genistein. *Clin. Chim. Acta* 199:263–78
3. Adlercreutz H, Fotsis T, Kurzer MS, Wähälä K, Mäkelä T, et al. 1995. Isotope dilution gas chromatographic-mass spectrometric method for the determination of unconjugated lignans and isoflavonoids in human feces, with preliminary results in omnivorous and vegetarian women. *Anal. Biochem.* 225:101–8
4. Adlercreutz H, Fotsis T, Watanabe S, Lampe J, Wähälä K, et al. 1994. Determination of lignans and isoflavonoids in plasma by isotope dilution gas chromatography-mass spectrometry. *Cancer Detect. Prev.* 18(4):259–71
5. Adlercreutz H, Goldin BR, Gorbach SL, Höckerstedt KAV, Watanabe S, et al. 1995. Soybean phytoestrogen intake and cancer risk. *J. Nutr.* 125:757–70S
6. Adlercreutz H, Gorbach S, Goldin B. 1992. Dietary phyto-oestrogens and the menopause in Japan. *Lancet* 339:1233
7. Adlercreutz H, Mousavi Y, Clark J, Höckersted K, Hämäläinen EK, et al. 1992. Dietary phytoestrogens and cancer: in vitro and in vivo studies. *J. Steroid Biochem. Molec. Biol.* 41:331–37
8. Adlercreutz H, Van der Wildtz J, Kinzel J, Attalla H, Wähälä K, et al. 1995. Lignan and isoflavonoid conjugates in human urine. *J. Steroid Biochem. Molec. Biol.* 52:97–103
9. Agnusdei D, Zacchei F, Bigazzi S, Cepollaro C, Nardi P, et al. 1989. Metabolic and clinical effects of ipriflavone in established post-menopausal osterporosis. *Drugs Exp. Clin. Res.* 15(2):97–104
10. Akiyama T, Ishida J, Nakagawa S, Ogaware H, Watanable S, et al. 1987. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.* 262:5592–95
11. Anderson JJ, Ambrose WW, Garner SC. 1995. Orally dosed genistein from soy

and prevention of cancellous bone loss in two ovariectomized rat models. *J. Nutr.* 125:799S
12. Anderson JW, Johnstone BM, Cook-Newell ME. 1995. Meta-analysis of the effects of soy protein intake on serum lipids. *N. Engl. J. Med.* 333:276–82
13. Anthony MS, Clarkson TB, Hughes CL Jr, Morgan TM, Burke GL. 1996. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. *J. Nutr.* 126:43–50
14. Arjmandi BH, Alekel L, Hollis BW, Amin D, Stacewicz-Sapuntzakis M, et al. 1996. Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. *J. Nutr.* 126:161–67
15. Aurbach GD, Marx SJ, Spiegel AM. 1992. Metabolic bone disease. In *Williams Textbook of Endocrinology,* ed. JD Wilson, DW Foster, 28:1477–517. Philadelphia: Saunders. 8th ed.
16. Axelson M, Kirk DN, Farrant RD, Cooley G, Lawson A, et al. 1982. The identification of the weak oestrogen equol [7-hydroxy-3(4′-hydroxyphenyl)chroman] in human urine. *Biochem. J.* 201:353–57
17. Axelson M, Sjovall J, Gustafsson BE, Setchell KDR. 1982. Origin of lignans in mammals and identification of a precursor from plants. *Nature* 298:659–60
18. Baird DD, Umbach DM, Lansdell L, Hughes CL, Setchell KDR, et al. 1995. Dietary intervention study to assess estrogenicity of dietary soy among postmenopausal women. *J. Clin. Endocrinol. Metab.* 80:1685–90
19. Bannwart C, Adlercreutz H, Fotsis T, Wähälä K, Hase T, et al. 1984. Identification of *O*-desmethylangolensin, a metabolite of daidzein, and of matairesinol, one likely plant precursor of the animal lignan enterolactone, in human urine. *Finn. Chem. Lett.* 4–5:120–125
20. Bannwart C, Fotsis T, Heikkinen R, Adlercreutz H. 1984. Identification of the isoflavonic phytoestrogen daidzein in human urine. *Clin. Chim. Acta* 136:165–72
21. Barnes S. 1995. Effect of genistein on in vitro and in vivo models of cancer. *J. Nutr.* 125:777–83S
22. Barnes S, Grubbs C, Setchell KD, Carlson J. 1990. Soybeans inhibit mammary tumors in models of breast cancer. *Clin. Biol. Res.* 347:239–53
23. Barth CA, Scholz-Ahrens KE, Pfeuffer M, Hotze A. 1990. Response of hormones and lipid metabolism to different dietary proteins. *Monogr. Atheroscler. No. 16.* Basel, Switzerland: Karger
24. Bennetts HW, Underwood EJ, Shier FL. 1946. A specific breeding problem of sheep on subterranean clover pastures in western Australia. *Aust. Vet. J.* 22:2–12
25. Benvenuti S, Tanini A, Frediani U, Bianchi S, Masi L, et al. 1991. Effects of ipriflavone and its metabolites on clonal osteoblastic cell line. *J. Bone Miner. Res.* 6:987–96
26. Bickoff EM, Livingston AL, Hendrickson AP, Booth AN. 1962. Relative potencies of several estrogen-like compounds found in forages. *J. Agric. Food Chem.* 10:410–12
27. Bierman EL, Glomset JA. 1992. Disorders of lipid metabolism. In *Williams Textbook of Endocrinology,* ed. JD Wilson, DW Foster, 26:1367–95. Philadelphia: Saunders. 8th ed.
28. Borriello SP, Setchell KDR, Axelson M, Lawson AM. 1985. Production and metabolism of lignans by the human faecal flora. *J. Appl. Bacteriol.* 58:37–43
29. Boutin JA. 1994. Minireview—Tyrosine protein kinase inhibition and cancer. *Int. J. Biochem.* 26:1203–26
30. Bradbury RB, White DE. 1954. Oestrogens and related substances in plants. *Vitam. Horm.* 12:207–33
31. Braden AWH, Hart NK, Lamberton JA. 1967. The oestrogenic activity and metabolism of certain isoflavones in sheep. *Aust. J. Agric. Res.* 18:335–48
32. Braden AWH, Thain RI, Shutt DA. 1971. Comparison of plasma phyto-oestrogen levels in sheep and cattle after feeding on fresh clover. *Aust. J. Agric. Res.* 22:663–670
33. Breslau NA, Brinkley L, Hill KD, Pak CYC. 1988. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. *J. Clin. Endocrinol. Metab.* 66:140–46
34. Burke TR. 1992. Protein-tyrosine kinase inhibitors. *Drugs Future* 17:119–31
35. Campbell DR, Kurzer MS. 1993. Flavonoid inhibition of aromatase enzyme activity in human preadipocytes. *J. Steroid Biochem Mol. Biol.* 46:381–88
36. Carroll KK. 1991. Review of clinical studies on cholesterol-lowering response to soy protein. *J. Am. Diet. Assoc.* 91:820–27
37. Carroll KK, Kurowska EM. 1995. Soy consumption and cholesterol reduction: review of animal and human studies. *J. Nutr.* 125:594–97S
38. Cassidy A, Bingham S, Setchell KDR. 1994. Biological effects of a diet of soy protein rich in isoflavones on

the menstrual cycle of premenopausal women. *Am. J. Clin. Nutr.* 60:333–40
39. Cassidy A, Bingham S, Setchell KDR. 1995. Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. *Br. J. Nutr.* 74:587–601
40. Champe PC, Harvey RA. 1987. DNA synthesis. In *Lippincott's Illustrated Reviews: Biochemistry*, ed. PC Champe, RA Harvey, pp. 357–68. Philadelphia, PA: Lippincott
41. Chang CJ, Geahlen RL. 1992. Protein-tyrosine kinase inhibition: mechanism-based discovery of anti-tumor agents. *J. Nat. Prod.* 55:1529–60
42. Colditz GA, Stampfer MJ, Willett WC, Hunter DJ, Manson JE, et al. 1992. Type of postmenopausal hormone use and risk of breast cancer: 12-year follow-up from the Nurses' Health Study. *Cancer Causes Control* 3(5):433–39
43. Constantinou A, Huberman E. 1995. Genistein as an inducer of tumor cell differentiation: possible mechanisms of action. *Proc. Soc. Exp. Biol. Med.* 208(1): 109–15
44. Constantinou A, Kiguchi K, Huberman E. 1990. Induction of differentiation and DNA strand breakage in human HL-60 and K-562 leukemia cells by genistein. *Cancer Res.* 50:2618–24
45. Coward L, Barnes NC, Setchell KDR, Barnes S. 1993. Genistein, daidzein, and their β-glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. *J. Agric. Food Chem.* 41:1961–67
46. Coward L, Kirk M, Albin N, Barbes S. 1996. Analysis of plasma isoflavones by reversed-phase HPLC-multiple reaction ion monitoring-mass spectrometry. *Clin. Chim. Acta* 247:121–42
47. Ettinger B. 1988. Prevention of osteoporosis: treatment of estradiol deficiency. *Obstet. Gynecol.* 72:12–17S
48. Faber KA, Hughes CL Jr. 1993. Dose-response characteristics of neonatal exposure to genistein on pituitary responsiveness to gonadotropin releasing hormone and volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in postpubertal castrated female rats. *Repro. Toxicol.* 7(1):35–39
49. Farmakalidis E, Hathcock JN, Murphy PA. 1985. Oestrogenic potency of genistin and dadzin in mice. *Food Chem. Toxicol.* 23:741–45
50. Farmakalidis E, Murphy PA. 1984. Oestrogenic response of the CD-1 mouse to the soya-bean isoflavones genistein, genistin and daidzin. *Food Chem. Toxicol.* 22:237–39
51. Farmakalidis E, Murphy PA. 1984. Different oestrogenic responses of ICR, B6D2F, and B6C3F1 mice given diethylstilboestrol orally. *Food Chem. Toxicol.* 22:681
52. Farnsworth NR, Bingel AS, Cordell GA, Crane FA, Fond HHS. 1975. Potential value of plants as sources of new antifertility agents. *J. Pharmacol. Sci.* 64:717–54
53. Fitzsimmons JTR, Orson NV, El-Aaaser AA. 1989. Effects of soybean and ascorbic acid on experimental carcinogenesis. *Comp. Biochem. Physiol.* 93A:285–90
54. Forsythe WA III. 1986. Comparison of dietary casein or soy protein effects on plasma lipids and hormone concentrations in the gerbil. *J. Nutr.* 116:1165–71
55. Fotsis T, Pepper M, Adlercreutz H, Flerischmann G, Hase T, et al. 1993. Genistein, a dietary-derived inhibitor of in vitro angiogenesis. *Proc. Natl. Acad. Sci. USA* 90:2690–94
56. Franke AA, Custer LJ, Cerna CM, Narala KN. 1995. Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. *Proc. Soc. Exp. Biol. Med.* 208(1):18–26
57. Granner DK. 1990. DNA organization and replication. In *Harper's Biochemistry*, ed. RK Murray, Granner DK, PA Mayes, VW Rodwell, pp. 366–382. East Norwalk, CT: Appleton & Lange
58. Hadley ME. 1993. Endocrinology of pregnancy, parturition, and lactation. In *Endocrinology*, ed. ME Hadley, pp. 505–29. Englewood Cliffs, NJ: Prentice Hall. 3rd ed.
59. Haenszel W, Kurihara M, Segi M, Lee RKC. 1972. Stomach cancer among Japanese in Hawaii. *J. Natl. Cancer Inst.* 49: 969–88
60. Ham JO, Chapman KM, Essex-Sorlie D, Bakhit RM, Prabhudesai M, et al. 1993. Endocrinological response to soy protein and fiber in mildly hypercholesterolemic men. *Nutr. Res.* 13:873–84
61. Haytowitz DB, Matthews RH. 1986. Composition of foods: legumes and legume products. In *Agriculture Handbook*, No. 8 series. Washington DC: Hum. Nutr. Inform. Serv., USDA
62. Hendrich S, Lee KW, Xu X, Wang HJ, Murphy PA. 1994. Defining food components as new nutrients. *J. Nutr.* 124:1789–92S
63. Higashi K, Ogawara H. 1992. Effects of isoflavone compounds on the activation of phospholipase C. *Chem. Pharm. Bull.* 40:157–60

64. Hirayama T. 1982. Relationship of soybean paste soup intake to gastric cancer risk. *Nutr. Cancer.* 3:223–33
65. Hu J, Liu Y, Yu Y, Zao T, Liu S, et al. 1991. Diet and cancer of the colon and rectum: a case-control study in China. *Int. J. Epidemiol.* 20:362–67
66. Huang ME, Ye YC, Chen SR, Chai JR, Lu JX, et al. 1988. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* 172:567–72
67. Huff MW, Carroll KK. 1980. Effects of dietary protein on turnover, oxidation, and absorption of cholesterol, and on steroid excretion in rabbits. *J. Lipid Res.* 21:546–58
68. Hunter T, Cooper JA. 1985. Protein-tyrosine kinases. *Annu. Rev. Biochem.* 54:897–930
69. Hutchins AM, Slavin JL, Lampe JW. 1995. Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented and unfermented soy products. *J. Am. Diet. Assoc.* 95:545–51
70. Joannou GE, Kelly GE, Reeder AY, Waring M, Nelson C. 1995. A urinary profile study of dietary phytoestrogens. The identification and mode of metabolism of new isoflavonoids. *J. Steroid Biochem. Molec. Biol.* 54(3/4):167–84
71. Kalu DN, Masoro EJ, Yu BP, Hardin RR, Hollic BW. 1988. Modulation of age-related hyperparathyroidism and senile bone loss in Fischer rats by soy protein and food restriction. *Endocrinology* 122:1847–54
72. Kellis JT Jr, Vickery LE. 1984. Inhibition of human estrogen synthetase (aromatase) by flavones. *Science* 225:1032–34
73. Kelly GE, Joannou GE, Reeder AY, Nelson C, Waring MA. 1995. The variable metabolic response to dietary isoflavones in humans. *Proc. Soc. Exp. Biol. Med.* 208(1):40–43
74. Kelly GE, Nelson C, Waring MA, Joannou GE, Reeder AY. 1993. Metabolites of dietary (soya) isoflavones in human urine. *Clin. Chim. Acta* 223:9–22
75. Kiguchi K, Constantinou AL, Huberman E. 1990. Genistein-induced cell differentiation and protein-linked DNA strand breakage in human melanoma cells. *Cancer Commun.* 2:271–78
76. King RA, Broadbent JL, Head RJ. 1996. Absorption and excretion of the soy isoflavone genistein in rats. *J. Nutr.* 126:176–82
77. Knuckles BE, De Fremery D, Kohler GO. 1976. Coumestrol content of fractions obtained from wet-processing of alfafa. *J. Agric. Food Chem.* 24:1177–80
78. Koo LC. 1988. Dietary habits and lung cancer risk among Chinese females in Hong Kong who never smoked. *Nutr. Cancer.* 11:155–72
79. Kudou S, Fleury Y, Welti D, Magnolato D, Uchida T, et al. 1991. Malonyl isoflavone glycosides in soybean seeds (Glycine max MERRILL). *Agric. Biol. Chem.* 55:2227–33
80. Kurzer MS, Lampe JW, Martini MC, Adlercreutz H. 1995. Fecal lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. *Cancer Epidemiol. Biomarkers Prev.* 4:353–58
81. Lampe JW, Martini MC, Kurzer MS, Adlercreutz H, Slavin JL. 1994. Urinary lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. *Am. J. Clin. Nutr.* 60:122–28
82. Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, et al. 1991. Dietary effects on breast-cancer risk in Singapore. *Lancet* 337:1197–200
83. Lee KW, Wang HJ, Murphy PA, Hendrich S. 1995. Soybean isoflavone extract suppresses early but not later promotion of hepatocarcinogenesis by phenobarbital in female rat liver. *Nutr. Cancer.* 24(3):267–78
84. Levy JR, Faber KA, Ayyash L, Hughes CL Jr. 1995. The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. *Proc. Soc. Exp. Biol. Med.* 208(1):60–66
85. Lieberman S. 1996. Are the differences between estradiol and other estrogens, naturally occurring or synthetic, merely semantical? *J. Clin. Endocrinol. Metab.* 81:850
86. Lock M. 1991. Contested meanings of the menopause. *Lancet* 337:1270–72
87. Lu LJW, Anderson KE, Grady JJ, Nagamani M. 1995. Effects of soya consumption for one month on steroid hormones in premenopausal women: implications for breast cancer risk reduction. *Cancer Epidemiol. Biomarkers Prev.* 5:63–70
88. Lundh T. 1995. Metabolism of estrogenic isoflavones in domestic animals. *Proc. Soc. Exp. Biol. Med.* 208:33–39
89. Lundh TJO, Pettersson H, Kiessling KH. 1988. Liquid chromatographic determination of the estrogens daidzein, formononetin, coumestrol, and equol in bovine blood plasma and urine. *J. Assoc. Off. Anal. Chem.* 71:938–41
90. Mäkelä S, Davis VL, Tallly WC, Korkman J, Salo L, et al. 1994. Dietary estrogens act through estrogen receptor-mediated processes and show no

antiestrogenicity in cultured breast cancer cells. *Environ. Health Perspect.* 102:572–78
91. Markovits J, Linassier C, Fosse P, Couprie J, Pierre J, et al. 1989. Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. *Cancer Res.* 49:5111–17
92. Martin PM, Horwitz KB, Ryan DS, McGuire WL. 1978. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocrinology* 103:1860–67
93. Medlock KL, Branham WS, Sheehan DM. 1995. Effects of coumestrol and equol on the developing reproductive tract of the rat. *Proc. Soc. Exp. Biol. Med.* 208(1):67–71
94. Messina MJ, Persky V, Setchell KDR, Barnes S. 1994. Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr. Cancer* 21:113–31
95. Millington AJ, Francis CM, McKeown NR. 1964. Wether bioassay of annual pasture legumes. *Aust. J. Agric. Res.* 15:527–36
96. Molteni A, Brizio-Molteni L, Persky V. 1995. In vitro hormonal effects of soybean isoflavones. *J. Nutr.* 125:751–56S
97. Montfort SL, Thomson MA, Czekala NM, Kasman LH, Schakleton CH, et al. 1984. Identification of a non-steroidal estrogen, equol, in the urine of pregnant macaques: correlation with steroidal estrogen excretion. *J. Steroid Biochem.* 20:869–76
98. Moule GR, Braden AWH, Lamond DR. 1963. The significance of oestrogens in pasture plants in relation to animal production. *Animal Breed Abstr* 31:139–57
99. Mousavi Y, Adlercreutz H. 1992. Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. *J. Steroid Biochem. Molec. Biol.* 41:615–19
100. Murkies AL, Lombard C, Strauss BJG, Wilcox G, Burger HG, et al. 1995. Dietary flour supplementation decreases post-menopausal hot flushes: effect of soy and wheat. *Maturitas* 21:189–95
101. Murphy PA. 1982. Phytoestrogen content of processed soybean products. *Food Technol.* 36:62–64
102. Nagai M, Hashimoto T, Yanagawa H, Yokoyama H, Minowa M. 1982. Relationship of diet to the incidence of esophageal and stomach cancer in Japan. *Nutr. Cancer* 3:257–68
103. Nagata Y, Ishiwaki N, Sugano M. 1982. Studies on the mechanism of the antihypercholesterolemia action of soy protein and soy protein-type amino acid mixtures in relation to their casein counterparts in rats. *J. Nutr.* 112:1614–25
104. Naim M, Gestetner B, Bondi A, Birk Y. 1976. Antioxidative and antihemolytic activity of soybean isoflavones. *J. Agric. Food Chem.* 24:1174–77
105. Naim M, Gestetner B, Kirson I, Birk Y, Bondi A. 1973. A new isoflavone from soya beans. *Phytochemistry* 12:169–70
106. Okura A, Arakawa H, Oka H, Yoshinari T, Monden Y. 1988. Effect of genistein on topoisomerase activity and on the growth of [Val 12]Ha-ras-transformed NIH 3T3 cells. *Biochem. Biophys. Res. Commun.* 157:183–89
107. Peterson G, Barnes S. 1991. Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistence gene. *Biochem. Biophys. Res. Commun.* 179:661–67
108. Peterson G, Barnes S. 1993. Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. *Prostate* 22:335–45
109. Phipps WR, Martini MC, Lampe JW, Slavin JL, Kurzer MS. 1993. Effect of flax seed ingestion on the menstrual cycle. *J. Clin. Endocrinol. Metab.* 77:1215–19
110. Potter SM. 1995. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J. Nutr.* 125:606–11S
111. Potter SM, Bakhit RM, Essex-Sorlie DL, Weingartner KE, Chapman KM, et al. 1993. Depression of plasma cholesterol in men by consumption of baked products containing soy protein. *Am. J. Clin. Nutr.* 58:501–6
112. Pratt DE, Birac PM. 1979. Source of antioxidant activity of soybeans and soy products. *J. Food Sci.* 44:1720–22
113. Price KR, Fenwick GR. 1985. Naturally occuring oestrogens in foods—a review. *Food Addit. Contam.* 2:73–106
114. Sanchez A, Hubbard RW. 1991. Plasma amino acids and the insulin/glucagon ratio as an explanation for the dietary protein modulation of atherosclerosis. *Med. Hypotheses* 35:324–29
115. Sartorelli AC. 1985. Malignant cell differentiation as a potential therapeutic approach. *Br. J. Cancer* 52:293–302
116. Scholz-Ahrens KE, Hagemeister H, Unshelm J, Agergaard N, Barth CA. 1990. Response of hormones modulating plasma cholesterol to dietary casein or soy protein in minipigs. *J. Nutr.* 120:1387–92
117. Serraino M, Thompson LU. 1991. The effect of flaxseed supplementation on early

risk markers for mammary carcinogenesis. *Cancer Lett.* 60(2):135–42
118. Serraino M, Thompson LU. 1992. Flaxseed supplementation and early markers of colon carcinogenesis. *Cancer Lett.* 63(2):159–65
119. Setchell KDR, Adlercreutz H. 1988. Mammalian lignans and phytoestrogens. Recent studies on their formation, metabolism and biological role in health and disease. In *Role of the Gut Flora in Toxicity and Cancer,* ed. IR Rowland, pp. 315–45. London: Academic
120. Setchell KDR, Borriello SP, Hulme P, Kirk DN, Axelson M. 1984. Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *Am. J. Clin. Nutr.* 40:569–78
121. Setchell KDR, Gosselin SJ, Welsh MB, Johnston JO, Balistreri WF, et al. 1987. Dietary estrogens—a probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology* 93:225–33
122. Setchell KDR, Lawson AM, Mitchell FL, Adlercreutz H, Kirk DN, et al. 1980. Lignans in man and in animal species. *Nature* 287:740–42
123. Severson RK, Nomura AMY, Grove JS, Stemmermann GN. 1989. A prospective study of demographics, diet, and prostate cancer among men of Japanese ancestry in Hawaii. *Cancer Res.* 49:1857–60
124. Shutt DA, Braden AWH. 1968. The significance of equol in relation to the oestrogenic responses in sheep ingesting clover with a high formononetin content. *Aust. J. Agric. Res.* 19:545–53
125. Shutt DA, Cox RI. 1972. Steroid and phytoestrogen binding to sheep uterine receptors in vitro. *Endocrinology* 52:299–310
126. Sirtori CR, Even R, Lovati MR. 1993. Soybean protein diet and plasma cholesterol: from therapy to molecular mechanisms. *Ann. NY Acad. Sci.* 676:188–201
127. Stamler J. 1979. Population studies. In *Nutrition and Lipids and Coronary Heart Disease,* ed. RI Levy, BM Rifkind, BH Dennis, N Ernst, pp. 25–88. New York: Raven
128. Stitch SR, Toumba JK, Groen MB, Funke CW, Leemhuis J, et al. 1980. Excretion, isolation and structure of a new phenolic constituent of female urine. *Nature* 287:738–40
129. Stob M. 1983. Naturally occurring food toxicants: oestrogens. In *Handbook of Naturally Occurring Food Toxicants,* ed. M Rechcigl Jr, pp. 81–100. Boca Raton, FL: CRC
130. Swanson CA, Mao BL, Li JY, Lubin JH, Yao SX, et al. 1992. Dietary determinants of lung-cancer risk: results from a case-control study in Yunnan Province, China. *Int. J. Cancer* 50:876–80
131. Thompson LU, Robb P, Serraino M, Cheung F. 1991. Mammalian lignan production from various foods. *Nutr. Cancer* 16:43–52
132. Traganos F, Ardelt B, Halko N, Bruno S, Darzynkiewicz Z. 1992. Effects of genistein on the growth and cell cycle progression of normal human lymphocytes and human leukemic MOLT-4 and HL-60 cells. *Cancer Res.* 52:6200–8
133. Tsutsumi N. 1995. Effect of coumestrol on bone metabolism in organ culture. *Biol. Pharm. Bull.* 18(7):1012–15
134. Van der Meer R, de Vries HT, van Tintelen G. 1988. The phosphorylation state of casein and the species-dependency of its hypercholesterolemic effect. *Br. J. Nutr.* 59:467–73
135. Verdeal K, Brown RR, Richardson T, Ryan DS. 1980. Affinity of phytoestrogen for estradiol-binding proteins and effect of coumestrol on growth of 7,12-dimethylbenz(a) anthracene-induced rat mammary tumors. *J. Natl. Cancer Inst.* 64:285–90
136. Wang C, Mäkelä T, Hase T, Adlercreutz H, Kurzer MS. 1994. Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. *J. Steroid Biochem. Molec. Biol.* 50:205–12
137. Wang HJ, Murphy PA. 1994. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem.* 42:1666–73
138. Wang TTY, Sathyamoorthy N, Phang JM. 1996. Molecular effects of genistein on estrogen receptor mediated pathways. *Carcinogenesis* 17(2):271–75
139. Wang W, Tanaka Y, Han Z, Higuchi CM. 1995. Proliferative response of mammary glandular tissue to formononetin. *Nutr. Cancer* 23:131–40
140. Wei H, Bowen R, Cai Q, Barnes S, Wang Y. 1995. Antioxidant and antipromotional effects of the soybean isoflavone genistein. *Proc. Soc. Exp. Biol. Med.* 208(1): 124–30
141. Welshons WV, Rottinghaus GE, Nonneman DJ, Dolan-Timpe M, Ross PF. 1990. A sensitive bioassay for detection of dietary estrogens in animal feeds. *J. Vet. Diagn. Invest.* 2:268–73
142. Whitten PL, Lewis C, Naftolin F. 1993. A phytoestrogen diet induces the premature anovulatory syndrome in lactationally exposed female rats. *Biol. Reprod.* 49:1117–21

143. Whitten PL, Naftolin F. 1992. Effects of a phytoestrogen diet on estrogen-dependent reproductive processes in immature female rats. *Steroids* 57:56–61
144. Wilcox G, Wahlqvist M, Burger H, Medley G. 1990. Oestrogenic effects of plant foods in postmenopausal women. *Br. Med. J.* 301:905–6
145. Wong E. 1975. The isoflavonoids. In *The Flavonoids*, ed. JB Harbourne, TJ Mabry, H Mabry, pp. 780–84. London: Chapman & Hall
146. Xu X, Harris KS, Wang HJ, Murphy PA, Hendrich S. 1995. Bioavailability of soybean isoflavones depends upon gut microflora in women. *J. Nutr.* 125:2307–15
147. Xu X, Wang HJ, Murphy PA, Cook L, Hendrich S. 1994. Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. *J. Nutr.* 124:825–32
148. Yamazaki I, Kinoshita M. 1986. Calcitonin-secreting property of ipriflavone in the presence of estrogen. *Life Sci.* 38:1535–41
149. Yanagihara K, Ito A, Toge T, Numoto M. 1993. Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. *Cancer Res.* 53:5815–21
150. You WC, Blot WJ, Chang YS, Ershow AG, Yang ZT, et al. 1988. Diet and high risk of stomach cancer in Shandong, China. *Cancer Res.* 48:3518–23
151. Yueh TL, Chu HY. 1977. The metabolic fate of daidzein. *Sci. Sin.* 20:513–22