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Potential Health Benefits of Dietary Phytoestrogens: A Review of the Clinical, Epidemiological, and Mechanistic Evidence*

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ABSTRACT

Phytoestrogens represent a family of plant compounds that have been shown to have both estrogenic and antiestrogenic properties. A variety of these plant compounds and their mammalian metabolic products have been identified in various human body fluids and fall under two main categories: isoflavones and lignans. A wide range of commonly consumed foods contain appreciable amounts of these different phytoestrogens. For example, soy and flax products are particularly good sources of isoflavones and lignans, respectively. Accumulating evidence from molecular and cellular biology experiments, animal studies, and, to a limited extent, human clinical trials suggests

that phytoestrogens may potentially confer health benefits related to cardiovascular diseases, cancer, osteoporosis, and menopausal symptoms. These potential health benefits are consistent with the epidemiological evidence that rates of heart disease, various cancers, osteoporotic fractures, and menopausal symptoms are more favorable among populations that consume plant-based diets, particularly among cultures with diets that are traditionally high in soy products. The evidence reviewed here will facilitate the identification of what is known in this area, the gaps that exist, and the future research that holds the most potential and promise. (*J Clin Endocrinol Metab* 83: 2223–2235, 1998)

THE INTAKE of diets low in fat and high in complex carbohydrates from grains, fruits, and vegetables is associated with a lower risk of chronic diseases (1). Although this has been suggested to be due to the adverse effect of fat and the potential health benefits of dietary fiber, other constituents associated with high fiber foods may also be responsible in part for the health benefit of such diets. This review discusses the potential role of phytoestrogens, a dietary component found in some 300 plants, in the reduction of chronic diseases, including coronary heart disease, atherosclerosis, hypercholesterolemia, cancer, and osteoporosis, as well as the reduction of menopausal symptoms.

Phytoestrogens have been identified in bile, urine, semen, blood, and feces in man and animals. Phytoestrogens have 2-phenylnaphthalene-type chemical structures similar to those of estrogens and have been found to bind to estrogen receptors (2). The rapidly growing body of literature in this area indicates that these plant-derived estrogens may exert both estrogenic and antiestrogenic effects on metabolism, depending on several factors, including their concentration,

the concentrations of endogenous estrogens, and individual characteristics, such as gender and menopausal status (3, 4). Phytoestrogens exhibit weak estrogenic activity on the order of 10^{-2} – 10^{-3} that of 17β -estradiol (2, 5, 6), but may be present in the body in concentrations 100-fold higher than endogenous estrogens (7–9). The antiestrogenic activity of phytoestrogens may be partially explained by their competition with endogenous 17β -estradiol for estrogen receptors. This partial estrogenic/antiestrogenic behavior is a common feature of many weak estrogens (10, 11).

Many of the potential health benefits of phytoestrogens may be attributable to metabolic properties that do not involve estrogen receptors, such as their influence on enzymes, protein synthesis, cell proliferation, angiogenesis, calcium transport, Na^+/K^+ adenosine triphosphatase, growth factor action, vascular smooth muscle cells, lipid oxidation, and cell differentiation (3, 4). In view of the current epidemiological, clinical trial, and mechanistic data in this area, phytoestrogens have been generally accepted to have a beneficial, rather than a deleterious, effect in humans. However, at this time it would be premature to recommend specific amounts of dietary phytoestrogen to prevent specific chronic diseases. This review will attempt to illustrate the broad range of actions of these estrogen-like molecules that occur in commonly consumed plant-based foods and to suggest future research directions in this area.

Isoflavones and lignans

The majority of phytoestrogens found in typical human diets can be categorized into two primary classes: isoflavones

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and lignans. Phytoestrogens in the diet may have a role in modulating hormone-related diseases based on their structural similarity to the estrogens 17β -estradiol and diethylstilbestrol. In Fig. 1, note the similar distance between the two -OH groups on equol, enterolactone, and enterodiol and that of 17β -estradiol, which is a factor essential for strong

binding to the estrogen receptor. The structure of phytoestrogens is also similar to the weak estrogen/antiestrogen tamoxifen, which is used for the treatment and prevention of breast cancer. Also, structurally, the presence and position of the -OH groups on the phytoestrogen compounds, estradiol and diethylstilbestrol, are considered one of the prerequisites

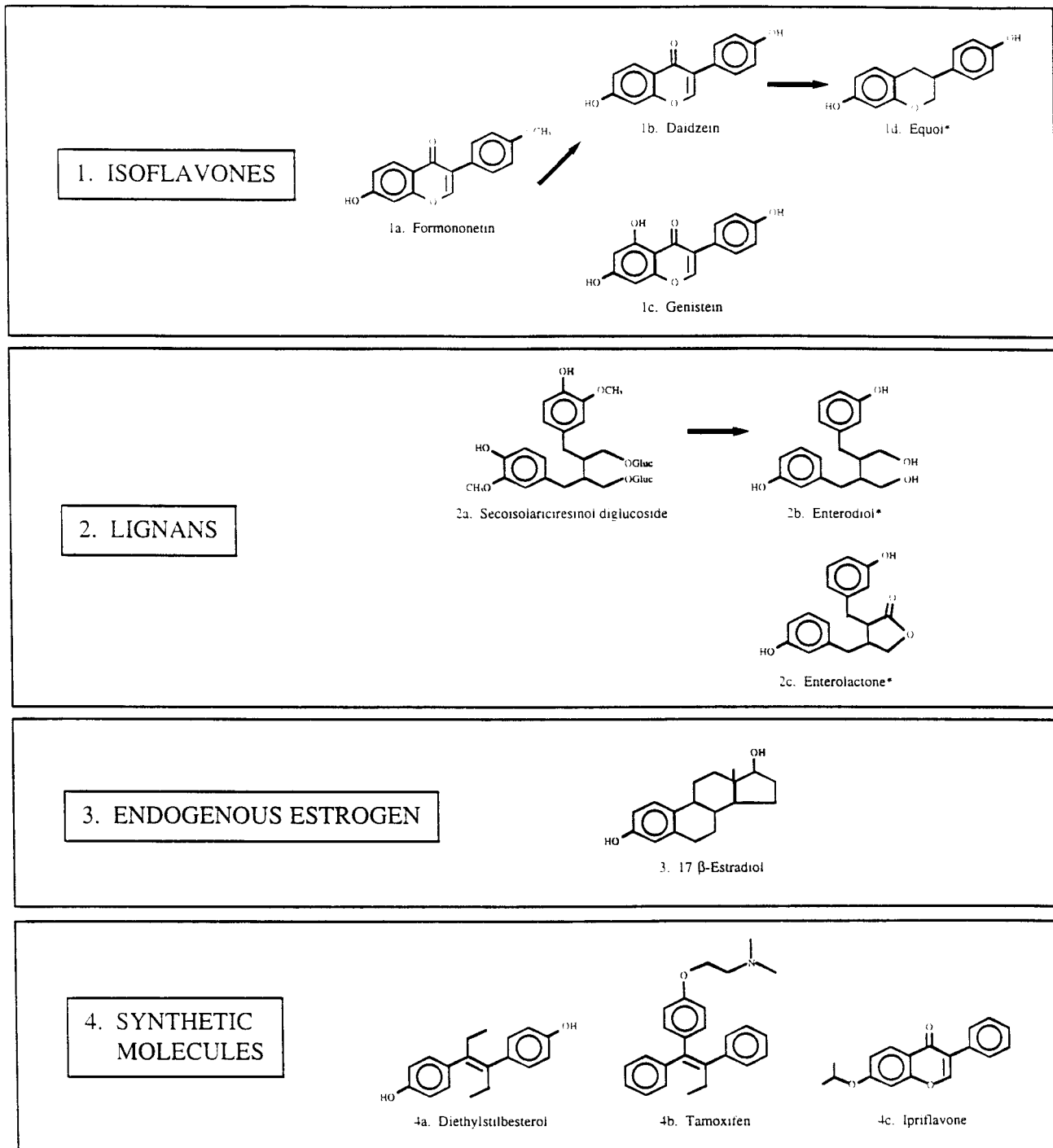


FIG. 1. A comparison of the chemical structures of 1) isoflavones: a) formononetin, b) daidzein, c) genistein, and d) equol*; 2) lignans: a) secoisolariciresinol diglucoside, b) enterodiol*, and c) enterolactone*; 3) endogenous estrogen: a) 17β -estradiol; 4) synthetic molecules: a) the potent synthetic estrogen diethylstilbestrol, b) the potent synthetic antiestrogen tamoxifen, and c) the potent isoflavone derivative ipriflavone. (*, These are the mammalian phytoestrogens found in tissues and biological fluids that are derived from plant phytoestrogens.)

for estrogenic activity (12). The antioxidant potencies of isoflavones are structurally related and closely associated with the presence of hydroxyl groups at positions 4' and 5' and the position of the aromatic ring. The estrogen receptor is capable of binding several structurally diverse compounds that include the natural estrogens and isoflavones, such as genistein (2, 13, 14).

Isoflavones make up the most common form of phytoestrogens. They have a common diphenolic structure that resembles the structure of the potent synthetic estrogens diethylstilbesterol and hexestrol. Two of the major isoflavones found in humans are genistein and daidzein. Genistein and daidzein are parent compounds, which are metabolized from their plant precursors, biochanin A and formononetin, respectively. In plants, isoflavones are inactive when present in the bound form as glycosides, but when the sugar residue is removed, these compounds become activated. These plant compounds undergo fermentation by intestinal microflora, with both metabolites and unfermented parent (aglycone) compounds being liable to absorption. In the body, the parent compounds are re-conjugated to glucuronides, but otherwise do not undergo any further metabolism in the body and are excreted in the urine (15). In the colonic microflora, daidzein may be metabolized to equol or to O-demethylangolensin (O-Dma) and genistein may be metabolized to p-ethyl phenol. Daidzein, genistein, equol, and O-Dma are

the major phytoestrogens detected in the blood and urine of humans and animals.

Isoflavones are found in a variety of plants, including fruits and vegetables, but they are predominantly found in leguminous plants and are especially abundant in soy (Table 1). Reinli and Block have recently compiled reference data on the levels of isoflavones found in a variety of food items (16). The isoflavone contents of different varieties, crops, and harvest years of soybeans vary substantially (17). In addition, the contents of isoflavones in different soy products (e.g. tofu and soy protein concentrates) vary substantially, which can be attributed to various processing steps. For example, processed soy products, such as soy hot dogs and tofu yogurt, may contain only 1/10th the isoflavone content of whole soy beans (0.2–0.3 vs. 2–4 mg isoflavone/g) (17, 18). Yet, even these processed soy products contain larger amounts of isoflavones than do most other legumes or nonleguminous plant foods, such as the 0.002 mg isoflavone/g raw green beans (19).

Once ingested, several factors influence the bioavailability of isoflavones. For example, Hutchins *et al.* (21) assessed urinary isoflavone recovery in postmenopausal women consuming either tempeh, a fermented soybean product, or a comparable quantity of soybean pieces from an unfermented soybean product. Urinary isoflavone recovery was greater for the tempeh. The presence of fiber in the diet has been

TABLE 1. Food sources of isoflavones (micrograms per g)

Food/food group	Total isoflavones	Daidzein	Genistein	Glycetin
Modified from Wang and Murphy, 1994 (17, 18) ^a				
Soybean 1	1,176	365	640	171
Soybean 2	4,215	1,355	2,676	184
Roasted soybeans	2,661	941	1,426	294
Soy flour	2,014	412	1,453	149
Soy granule	2,404	917	1,225	262
Textured vegetable protein 1	2,295	799	1,175	321
Textured vegetable protein 2	2,261	831	1,185	245
Protein isolate 1	621	89	373	159
Protein isolate 2	987	191	640	156
Protein concentrate (alcohol extraction)	73	0	19	54
Tofu	532	238	245	49
Tempeh	865	405	422	38
Miso 1	647	272	281	94
Miso 2	389	107	227	55
Soy hot dog	236	55	129	52
Soy bacon	144	26	83	35
Tempeh burger	386	95	255	36
Tofu yogurt	282	103	162	17
Soy parmesan	88	26	6	56
Cheddar cheese 1	43	0	4	39
Cheddar cheese 2	197	83	62	52
Mozarella cheese	123	24	56	43
Flat noodle	127	15	56	56
Modified from Dwyer <i>et al.</i> , 1994 (20) ^b				
Tofu, brand 1	289	76	213	
Tofu, brand 2	260	73	187	
Tofu, brand 3	313	97	216	
Tofu, brand 4	292	86	206	
Soy drink	28	7	21	
Soy-based specialty formula 1	3	0	3	
Soy-based specialty formula 2	5	1	4	

^a Glucoside, malonyl, acetyl, and aglycone forms of isoflavones combined.

^b Values shown are averages of two lots, one purchased in June and the other in December of 1992. Biochanin A, coumestrol, and formononetin were assayed, but found in only negligible amounts.

shown to correlate positively with urinary excretion of phytoestrogens (22). Several investigators have reported that individual variability in colonic microflora plays an important role in determining the preferred pathways of isoflavone metabolism and the bioavailability of isoflavones. For example, equol is notably present in the blood and urine in some individuals and is absent in others (21, 23, 24). Lu *et al.* provided 12 oz. soymilk with each meal for 1 month to healthy women (25) and men (26) and reported that the urinary recovery of ingested isoflavones ranged, on the average, from 15–66% for the individual isoflavones. Over the 1-month study period, the percentage of urinary recovery decreased by approximately one third among the women, but remained stable among the men. Related findings from another study of urinary recovery indicated that, on the average, 10–37% of daidzein and genistein from a single day's intake of soy milk was recovered in 48-h urine collections (23). A notable distinction in this latter study was that for five of the seven healthy women participants who had less than 1% fecal recovery of isoflavones, the amount of urinary recovery was 10–16%. For the remaining two women with relatively greater fecal recovery of at least 5%, the amount of urinary recovery was not inversely associated but, rather, was directly associated with a urinary recovery of 27–32%, suggesting that both absorption and intestinal degradation are important factors in determining bioavailability. These data support the observation that there is substantial individual variability in isoflavone metabolism. Among these and other studies, it has been consistently observed that virtually all of the urinary recovery of genistein and daidzein, from a single time point of intake, is complete within 24 h, although Kelly *et al.* (27) reported that postchallenge urinary recovery of equol and O-Dma continued to be substantial for at least 3 days.

Lignans are compounds possessing a 2,3-dibenzylbutane structure and exist as minor constituents of many plants, where they form the building blocks for the formation of lignin (as distinguished from lignan) found in the plant cell wall. They are constituents of higher plants (gymnosperms and angiosperms), such as whole grains, legumes, vegetables, and seeds, with exceptionally high concentrations of lignans found in flaxseed (Table 2). Although previously thought to be present only in higher plants, mammalian lignans have been detected in the biological fluids of man and animals.

As with the isoflavones, the chemical structure of plant lignans differs somewhat from that of mammalian lignans (Fig. 1). Most of the structural changes occur in the colon, liver, and small intestine during enterohepatic circulation. Mammalian lignans differ in structure from plant lignans in that they have phenolic hydroxyl groups in the meta position only in their aromatic rings. Once in the colon, they are absorbed and then are conjugated with glucuronic acid or sulfate in the liver, reexcreted through the bile duct, deconjugated by the bacteria, and reabsorbed. Some reach the kidney and are excreted in the urine (21). Lignans are excreted in bile and urine as conjugated glucuronides and in feces in the unconjugated form. Originally, mammalian lignans were observed in urinary steroid profiles and thought

TABLE 2. Food sources of lignans (micrograms per g)

Food/food group	Total lignans	Enterodiol	Enterolactone
Modified from Thompson <i>et al.</i> , 1991 (28)			
Flaxseed meal	675.4	85.2	590.2
Flaxseed flour	526.8	118.2	408.6
Cereals			
Triticale	9.2	5.2	4.0
Wheat	4.9	4.1	0.8
Oats	3.4	2.5	0.9
Brown rice	3.0	1.7	1.3
Corn	2.3	2.0	0.3
Rye	1.6	0.7	0.9
Barley	1.1	0.4	0.7
Cereal brans			
Oat bran	6.5	2.6	3.9
Wheat bran	5.7	2.7	3.0
Rice bran	1.8	1.3	0.5
Oilseeds			
Soybean	8.6	6.9	1.7
Sunflower seeds	4.0	2.0	2.0
Peanuts	1.6	1.0	0.6
Legumes, dried whole			
Lentil	17.9	7.9	10.0
Kidney	5.6	3.3	2.3
Navy bean	4.6	3.5	1.1
Pinto bean	2.0	1.5	0.5
Vegetables			
Garlic	4.1	0.8	3.3
Asparagus	3.7	1.4	2.4
Carrot	3.5	2.8	0.6
Sweet potato	3.0	2.4	0.6
Broccoli	2.3	1.6	0.7
Mushroom	0.6	0.4	0.1
Celery	0.3	0.2	0.1
Cucumber	0.3	0.2	0.1
Tomato	0.2	0.1	0.1
Fruits			
Pear	1.8	1.1	0.7
Plum	1.5	0.5	1.0
Strawberry	0.8	0.4	0.4
Banana	0.7	0.6	0.1
Orange	0.4	0.3	0.1
Cantaloupe	0.4	0.2	0.2
Apple	0.3	0.3	0.0

Values are expressed as mammalian lignan production by fecal flora (micrograms) from foods (per g).

to be new endogenous hormones. The first mammalian urinary lignan identified was given the trivial name enterolactone, and a second mammalian lignan is referred to as enterodiol. Enterolactone and enterodiol are metabolites of the plant lignans matierresinol and secoisolariciresinol, respectively. The higher the dietary intake of precursors, the higher the mammalian lignan production in the colon and the higher the excretion rate in the urine. Few data have been published on lignans from dietary sources that are metabolized to mammalian lignans, but clinical trials performed by Kirkman *et al.* (29) demonstrated that the excretion of the lignans, enterodiol and enterolactone, was higher during a carotenoid (carrot and spinach) and cruciferous (broccoli and cauliflower) vegetable diet, than during a vegetable-free diet, suggesting that these vegetables may provide a source of mammalian lignan precursors. In this study, men excreted more enterolactone and less enterodiol than women, implying a gender difference in colonic bacterial metabolism of lignans.

Sources and typical intake levels of phytoestrogens

The major dietary source of phytoestrogens in most populations is soy. Soybeans and their products are consumed by humans in many forms, including whole soybeans, tofu, tempeh, and soy milk. Soy protein, primarily in the form of nontosted defatted soybean flakes, can be isolated from the whole bean for consumption through processing. Isolated soy protein, which contains up to 50% of the phytoestrogens found in unprocessed soybeans (Table 1), can be consumed through a variety of products, such as baked goods, confections, meat products, texturized foods, and nutritional supplements (30–33). The majority of these products are quite palatable. The concentration of genistein in most soy food materials ranges from 1–2 mg/g protein. Flax is also a concentrated source of phytoestrogens, particularly the lignans (Table 2). Other plant foods contain trivial amounts of phytoestrogens compared to soy and flax. However, small amounts from a large number of relatively poor sources can add up to appreciable amounts. In a small study of macrobiotics, lactovegetarians, and omnivores, it was reported that the macrobiotics excreted more than 4-fold the amount of urinary phytoestrogen as did the lactovegetarians, who, in turn, excreted double the amount of the omnivores (9). Many Asian populations that have low rates of breast and prostate cancer consume 20–80 mg/day genistein, almost entirely derived from soy, whereas the dietary intake of genistein in the United States has been estimated at 1–3 mg/day (34).

Potential health benefits of phytoestrogens

Phytoestrogens may have favorable estrogenic effects on the risk of cardiovascular disease and are thought to be hypocholesterolemic, anticarcinogenic, antiproliferative, antiosteoporotic, and hormone altering (3, 4, 35). These health benefits may be attributable to a variety of potential mechanisms that are both estrogen receptor dependent and independent and have been mentioned briefly in the introduction. With consideration to these possible mechanisms in mind, we will attempt to look at specific diseases and conditions and how phytoestrogens may effect them.

Cardiovascular disease

Several lines of evidence, including epidemiological, clinical trial data, and basic science, suggest the plausibility of a causal, inverse relationship between phytoestrogens and cardiovascular disease. The well established low rates of cardiovascular diseases and the high intakes of dietary phytoestrogens in Asian populations relative to those in other industrialized countries are consistent with a potential protective effect of phytoestrogens (36, 37). However, this association is confounded by other concomitant dietary differences (e.g. low saturated fat intake in Asian populations), making it difficult to attribute the observed differences in disease rates to phytoestrogen intake. Estrogen-related studies constitute a separate and nondietary line of epidemiological evidence that suggests a potential health benefit of phytoestrogens. As suggested by many epidemiological studies of hormone replacement therapy in postmenopausal women, there appears to be an associated reduction in the

risks of coronary heart disease and a possible cardioprotective role (38–40). These observational data are complemented by clinical trials and animal and cell studies.

Several human clinical trials with phytoestrogens have reported inconsistent serum lipid effects. One study noted a significant reduction in total cholesterol in premenopausal women when they consumed soy products with 45 mg conjugated isoflavones/day relative to levels during a control period when they were fed isoflavone-free soy products. The treatment group difference was significant despite the small sample size and the selection of healthy, normocholesterolemic women who had limited room for detectable improvements (41). In another study, hypercholesterolemic, postmenopausal women were randomly assigned to 6 months of 40 g protein supplementation/day from a casein nonfat dry milk source, an isolated soy protein source, or a soy protein source with approximately half of the phytoestrogen content removed. Relative to the casein milk protein, both soy products lowered nonhigh density lipoprotein (non-HDL) cholesterol and increased HDL cholesterol significantly, and therefore, both soy groups experienced an improved total cholesterol/low density lipoprotein (LDL) cholesterol ratio. However, the benefit could not be attributed to the phytoestrogen (42). Gooderham *et al.* (43) reported no significant cholesterol effects when 20 healthy men were fed supplements of 60 g/day of either soy protein or casein for 28 days despite the 100- to 150-fold increase in plasma isoflavone concentrations of genistein and daidzein during the soy protein diet. The investigators appropriately point out that there was limited room for favorable cholesterol changes because of the low baseline concentrations. In another study, moderately hypercholesterolemic (mean, 6.0 mmol/L), postmenopausal women were given 45 g/day of either soy flour or wheat flour for 12 weeks (44). After 12 weeks, a modest, but insignificant, decrease in cholesterol was observed in both treatment groups, but there was no significant difference between the soy *vs.* wheat flour groups in cholesterol concentrations despite significantly higher urinary concentrations of phytoestrogens for the women taking the soy flour.

In several randomized trials using nonhuman primates, phytoestrogens were used to explore the possibility of a hypocholesterolemic effect. Anthony *et al.* (45) assessed the effect of soybean protein's alcohol-extractable components (including the isoflavones genistein and daidzein) on plasma lipid and lipoprotein concentrations. In this study, peripubertal male and female rhesus monkeys were fed moderately atherogenic diets containing soy protein with phytoestrogens or soy protein with the phytoestrogens removed by alcohol extraction. Compared with the alcohol-extracted soy protein, the phytoestrogen-intact soy protein had favorable effects on plasma lipids and lipoprotein concentrations, specifically by significantly reducing LDL, very low density lipoprotein, and total plasma cholesterol concentrations in both males and females and significantly increasing HDL cholesterol concentrations in females. An important finding with cancer implications was that the phytoestrogen treatments had no adverse effects on the reproductive system in either sex. An inherent limitation in using an alcohol extraction process to manipulate phytoestrogen content is that

other putative cholesterol-lowering factors besides the phytoestrogens are also removed through this processing, such as saponins and plants sterols, thereby making it difficult to attribute treatment differences specifically to the phytoestrogens (45, 46). Rather than using soy products with and without the alcohol extraction process, another group of investigators gave ovariectomized rats a variety of isolated environmental estrogens, including the isoflavone genistein. Oral doses were given daily in amounts ranging from 0.1–3.0 mg/kg BW. After daily treatment for 4 days or 5 weeks, cholesterol concentrations were significantly lower in the genistein group compared to the control values at all dosage levels (47).

Two proposed mechanisms for the hypocholesterolemic effect of phytoestrogens are the up-regulation of LDL receptors and/or the inhibition of endogenous cholesterol synthesis. Phytoestrogens in soy protein may stimulate the clearance of cholesterol, probably by up-regulating LDL receptors, and thereby increasing LDL receptor activity (48). This may be of particular significance for hyperlipidemics. Lignans may also affect cholesterol homeostasis, as they have been shown to inhibit the activity of cholesterol-7 α -hydroxylase, the rate-limiting enzyme in the formation of primary bile acids from cholesterol (49, 50).

Lipoprotein(a) [Lp(a)] is a cholesterol-carrying particle in the blood that is structurally similar to LDL, with the addition of the apoprotein(a) moiety. Increasing evidence over the past decade has indicated that Lp(a) is an independent risk factor for coronary heart disease (51). Despite its resemblance to the LDL particle, Lp(a) levels in the blood are not responsive to many of the conventional diet, lifestyle, or pharmacological approaches to lowering LDL cholesterol levels. To date, estrogen treatment is one of the few factors that has successfully lowered Lp(a) levels (52, 53). Although the effects of phytoestrogens on Lp(a) in humans have not been studied directly, the primate study cited above (45) and the structural and receptor binding similarities between phytoestrogens and endogenous estrogens suggest a potential benefit of the plant estrogens and warrant further investigation to test this.

Independent of the possible role of soy protein in the reduction of plasma cholesterol concentrations, studies of cultured vascular cells have demonstrated that increased concentrations of isoflavonoids alter cellular processes associated with lesion development (54). In low density cultures of proliferating endothelial cells, genistein induced marked morphological changes. When concentrations were increased up to 25 μ mol/L, genistein induced a highly spread morphology compatible with growth arrest. In contrast, confluent quiescent endothelial cells did not exhibit toxicity signs even at genistein concentrations up to 200 μ mol/L (55). These data suggest that genistein targets only proliferating cells, leaving quiescent, nondividing cells unaffected. This characteristic is important with respect to possible use of the compound in therapeutic applications, as fewer side-effects are expected. However, the physiological relevance of the above findings, using high concentrations of phytoestrogens, are unclear. Based on simple pharmacokinetic calculations involving daily intake of isoflavones and ab-

sorption from the gut, distribution to peripheral tissues, and excretion, it is unlikely that blood isoflavone concentrations, even in high soy consumers, could be greater than 1–5 μ mol/L (56).

Proteolytic degradation of the extracellular matrix by endothelial cells is controlled by angiogenic factors, such as basic fibroblast growth factor (bFGF), that induce the production of urokinase-type plasminogen activator and its physiological inhibitor, plasminogen activator inhibitor-1. Experiments have demonstrated that genistein markedly reduced both bFGF-stimulated and basal levels of both plasminogen activator and plasminogen activator inhibitor-1 activity in bovine microvascular endothelial cells. Moreover, genistein inhibited the bFGF-induced migration of endothelial cells in wounded confluent monolayers of endothelial cells (55). Inhibition of production of proteolytic enzymes and migration of endothelial cells by genistein, therefore, represent a more complex interference of the compound with important early events of angiogenesis other than endothelial cell proliferation.

Various studies have suggested that genistein's role as a protein tyrosine kinase inhibitor is responsible for an anti-thrombotic effect (57). An increase in tyrosine phosphorylation at tyrosine residues of platelet proteins is associated with the platelet's activation, and protein tyrosine phosphorylation is subsequently increased after thrombin stimulation (58). Therefore, it may be feasible that a protein tyrosine inhibitor such as genistein would be able to decrease tyrosine phosphorylation, which would lead to decreased platelet activation and have downstream events, such as a reduction in the deposition and aggregation of platelets and a decrease in the progression of atherosclerosis. Sargeant *et al.* (59, 60) demonstrated that human platelets that were preincubated for 30 min with 100 μ mol/L genistein had reduced thrombin-, ADP-, and thapsigargin-evoked protein tyrosine phosphorylation. In another study conducted by Nakashima *et al.* (61), human platelets that were preincubated for 5 min with 10 μ g/mL genistein were able to completely prevent platelet aggregation induced by collagen and thromboxane A₂ analogs. In contrast to the collagen and thromboxane A₂ analog-induced thrombins and to the studies conducted by Sargeant, Nakashima observed that concentrations of genistein up to 100 μ g/mL had no significant effect on thrombin-induced platelet activation. Nakashima's group also demonstrated that daidzein, which has no inhibitory activity for protein tyrosine kinase, was able to suppress platelet responses elicited by both collagen and thromboxane A₂. These results demonstrated by Nakashima indicate that the ability of genistein and daidzein to block platelet response induced by collagen or thromboxane A₂ is due to their ability to prevent thromboxane A₂ from binding to its receptor *vs.* its ability to inhibit protein tyrosine phosphorylation. In a clinical trial involving 60 g/day of a soy protein isolate beverage powder for 28 days, a dramatic rise in plasma isoflavone concentrations was observed, but no significant effect on platelet aggregation was detected (43). More studies will need to be conducted to clarify the exact mechanisms by which these phytoestrogens affect various platelet responses and their potential have antithrombotic activity.

Cancer

It has been well established that cancer rates differ strikingly in various populations of the world. Hormone-related cancers of the breast, ovary, endometrium, and prostate have been reported to vary by as much as 5- to 20-fold between populations, and migrant studies indicate that the differential is largely attributable to environmental factors rather than genetics (62, 63). The highest rates of these cancers are typically observed in populations with Western lifestyles that include relatively high fat, meat-based, low fiber diets, whereas the lowest rates are typically observed in Asian populations with Eastern lifestyles that include plant-based diets with a high content of phytoestrogens (62, 64). In a case-control study, Ingram *et al.* (65) reported a significant reduction in breast cancer risk among both premenopausal and postmenopausal women who consume phytoestrogens. In a study of Asian-Americans of Chinese, Japanese, and Filipino heritage, it was reported that tofu consumption was significantly and inversely associated with breast cancer (66). Phytoestrogen intake was not determined in this study, but should have paralleled the tofu intake given the high content of phytoestrogens in soy products. Similar findings were reported from a case-control study of women in Singapore in which soy intake was inversely and animal product intake was positively associated with breast cancer, although the findings were significant only among premenopausal, not postmenopausal, women (67). Among the multiethnic population of Hawaii, Goodman *et al.* (68) reported that soy and fiber consumptions were both associated with a decreased risk of endometrial cancer, a finding that was limited to women who had never used unopposed estrogens and had never been pregnant. Dietary studies and urinary analysis of lignans in postmenopausal women have shown that lignan secretion is significantly lower in urine in women with breast cancer than in healthy omnivorous and vegetarian women (8). In a study conducted in Boston and Helsinki, it was demonstrated that the lowest excretion of enterolactone and equol was found in a group of postmenopausal breast cancer patients compared to control omnivorous and vegetarian women (69).

Both endogenous and exogenous sex hormones have been associated with various cancers. High levels of biologically active androgens or estrogens are associated with increased risk of prostate cancer in men and ovarian and breast cancer in women. It has been hypothesized that substances such as xenoestrogens, compounds that affect estrogen production and metabolism, can also increase the risk of breast cancer (70). Diets that lower these androgen or estrogen levels are associated with low risk of breast, prostate, and ovarian cancer (71). The level of circulating estrogens, particularly the portion that is biologically active, is higher in breast cancer patients than in healthy controls (72). Population groups with lower risk of breast cancer have demonstrated lower levels of circulating estrogen than those with higher risk (73, 74). For breast, ovarian, endometrial, and colon cancer, there is a consistent relationship between reproduction, exogenous hormone use, and risk factors. Despite the positive associations of endogenous and exogenous estrogens with these cancers, plant estrogens are inversely associated with cancer.

The association of phytoestrogens with decreased cancer incidence implies either a lack of estrogenicity or estrogen antagonism at these sites in conjunction with other possible mechanisms.

Several animal studies suggest that phytoestrogens retard cancer development. Newborn female rats were treated with either genistein or dimethylsulfoxide (vehicle). Subsequently, they were exposed to a carcinogen, dimethylbenz(a)anthracene, that is known to induce mammary tumors. Animals treated neonatally with genistein had increased latency and decreased incidence and multiplicity of mammary tumors compared with vehicle-treated animals (75, 76). These findings support the view that these compounds may have protective effects with regard to estrogen-dependent cancer.

Androgens are implicated in the development of prostate cancer (77). The conversion of testosterone to the more potent metabolite dihydrotestosterone by prostate-specific steroid 5α -reductase is a key mechanism in the action of androgens in the prostate and is important in the promotion and progression of prostate diseases (78). Clinical prostate cancers often respond to androgen deprivation therapy. Thus, a reduction in androgen levels should affect carcinogenesis processes. The administration of 5α -reductase inhibitors results in a substantial decrease in prostatic sections of the normal gland and a substantial increase in cell death in normal and transformed prostatic cells (79).

It has been suggested that for men, phytoestrogens may confer some level of protection against prostate cancer (3, 34). Epidemiological data have consistently reported a relatively low incidence of prostate cancer in Asian populations whose diet is rich in phytoestrogens, especially the isoflavonoids in soy and other legumes (80, 81). The highest incidence has been reported in North American black males (82) in whom the age-adjusted incidence is 125 times greater than that in men in Shanghai, China (83). This geographic variation is a major feature of prostate cancer, with Asian men generally being much less susceptible to this disease than Europeans and North Americans. However, Japanese men who migrate to America adopt the prostate cancer incidence of the indigenous population within one or two generations (84). These epidemiological data support the concept that diet may inhibit the promotion and progression of prostatic cancer in Asian men. It has also been reported that lower levels of 5α -reductase activity have been found in Japanese men (85). These men also have a higher urinary excretion and higher plasma levels of phytoestrogens than their Western counterparts (81, 86).

Recent molecular biology studies have shown that substrates of lignans and isoflavonoid phytoestrogens inhibit the conversion of testosterone to the more biologically active dihydrotestosterone. Genistein, biochanin A, and equol were able to inhibit 5α -reductase activity by 80% when used at a concentration of 100 $\mu\text{mol/L}$ (87). In one particular clinical case, a 66-yr-old man took 160 mg phytoestrogens daily for 1 week before a radical prostatectomy. The prostatectomy specimens revealed mild patchy microvacuolations and prominent apoptosis, whereas no changes were seen in normal prostate cells (88). These degenerative changes in the prostatectomy specimen, especially the apoptosis, were in-

dicative of androgen deprivation and typical of a response to estrogen therapy (89). In one animal study, rats maintained on a soy-free diet for 11 weeks developed severe inflammation of the lateral prostate, whereas rats maintained on a soy-containing diet or commercial rat chow did not develop any signs of prostatitis. This finding suggests that soy of dietary source may play a protective role against the pathogenesis of prostatitis. One possible explanation could be that as soybeans contain a number of phytoestrogens that are weak estrogens, the soy-free diet might disturb the androgen-estrogen ratio (90). Several investigators have also reported that phytoestrogens inhibit the growth of cultured prostate cancer cells (91, 92). Thus, a diet rich in phytoestrogens may prevent prostate cancer by a variety of mechanisms, including reducing circulating androgen levels, increasing concentrations of sex hormone-binding globulin (SHBG), competitive binding to cellular hormone receptors, and apparent reduction in the production of dihydrotestosterone.

The primary lignans and isoflavones have been shown to reduce the proliferation of cells, including those in estrogen-sensitive breast cancer cell lines, other tumors, and uterus (93). The effects of phytoestrogens on the proliferation of cancer cell lines have been studied under a variety of experimental conditions. Current data indicate that these effects are largely dependent on the specific conditions, such as the concentration of phytoestrogen, the presence or absence of other endogenous estrogens, and the particular cell line (*e.g.* estrogen receptor dependent or independent). Although the majority of the studies in this area have reported an inhibitory effect of phytoestrogens (2, 94–98), it has been noted that in many cases the concentration range of phytoestrogen has been supraphysiological (2, 56, 95–98). Zava *et al.* (2) investigated the effects of a broad range of genistein concentrations on estrogen receptor (ER) binding, induction of the estradiol-regulated antigen pS2, and cell proliferation rate in ER-positive and ER-negative human breast cancer cells grown *in vitro*. They reported that genistein exhibited antiestrogenic effects and inhibited cell proliferation, but only at concentrations higher than those achievable under normal physiological conditions ($>10 \mu\text{mol/L}$). At concentrations of genistein within the physiological range (1 nmol/L to $10 \mu\text{mol/L}$), genistein stimulated the growth of MCF-7 cells. This observation is not consistent with the epidemiological or animal data for reasons that may be explained by additional factors involved in the experimental conditions.

Adlercreutz *et al.* (3) noted that despite the apparent positive effects of phytoestrogens or the intake of soy or linseed on breast cancer risk, it must be kept in mind that phytoestrogens are weak estrogens and, under certain experimental conditions, will always stimulate cell proliferation and estrogen-dependent gene expression. Mousavi and Adlercreutz (98) examined the *in vitro* effect of enterolactone and estradiol on MCF-7 cell proliferation. At concentrations between $0.5\text{--}2.0 \mu\text{mol/L}$, enterolactone alone stimulated cell proliferation, but at the same concentrations, it also inhibited the proliferation of estradiol-stimulated growth. Similarly, Panno *et al.* (94) reported that genistein, not alone but in the presence of estradiol, inhibited the mitogenic activity of the MCF-7 cell line. As circulating levels of estradiol can be

found in both men and women at all stages of the life cycle, the effects of phytoestrogens in cell cultures that contain estrogens should be more relevant than the effects observed in the absence of estrogen.

Another important experimental condition is the type of cell line used in these studies of phytoestrogens and cancer. Some, for example, are estrogen receptor dependent, and some are not. In one study, the inhibition of estrogen receptor-positive MCF-7 breast cancer cells was reversed by the addition of excess competing estrogen. The investigators concluded that the antiproliferative effect of genistein was exerted via an estrogen receptor-dependent pathway (95). However, this conclusion is not supported by two separate studies that each used a variety of cell lines, some that were estrogen receptor dependent and some that were estrogen receptor-independent (2, 96). Peterson *et al.* (96) concluded from their studies that the mechanism of genistein growth inhibition in human breast cancer cells did not depend on the presence of functional estrogen receptor signaling.

The antioxidant properties of genistein may be partially responsible for its anticarcinogenic effects. Research has demonstrated that genistein strongly inhibits tumor promoter-induced H_2O_2 formation both *in vivo* and *in vitro*. Wei *et al.* reported that genistein suppressed H_2O_2 production by 12-*O*-tetradecanoylphorbol-13-acetate-stimulated human polymorphonuclear leukocytes and HL-60 cells in a dose-dependent manner (99). In addition, genistein moderately inhibited superoxide anion formation by HL-60 cells and scavenged exogenously added H_2O_2 under the same conditions as in cell culture. The fact that genistein potently inhibits oxidant formation and protooncogene expression suggests that the antioxidant properties and antiproliferative effects of genistein may at least in part be responsible for the anticarcinogenic mechanism(s) (100).

Some phytoestrogens have been shown to inhibit enzymes that are associated with cell proliferation (*e.g.* ornithine decarboxylase, protein tyrosine kinase, and DNA topoisomerase) and enzymes involved in the production of estrone from the androgens (*e.g.* aromatase), thus denying the tumor a source of endogenous estrogen (101). Pharmacologists have realized that tyrosine kinase inhibitors (TKI) have potential as anticancer agents in both prevention and therapeutic protocols. The risk of synthetic TKI-induced toxicity led to the discovery of the naturally occurring TKI in genistein found in soy. Genistein is a specific inhibitor of tyrosine protein kinases, topoisomerase II, and protein histidine kinase. Protein tyrosine kinase activity is associated with cellular receptors for epidermal growth factor, insulin, insulin-like growth factor I, platelet-derived growth factor, and mononuclear phagocyte growth factor. The tyrosine kinases seem to play an important role in cell proliferation and transformation. These enzymes have been associated with oncogene products of the retroviral *src* gene family and are correlated with the ability of retrovirus to transform cells. Lignans and isoflavonoids as well as foods containing large amounts of these compounds or their precursors seem to inhibit cancer cell growth (102). In addition, genistein inhibits DNA topoisomerase II and ribosomal S6 kinase, both of which may lead to protein-linked DNA strand breaks in cancerous cells, ar-

rest of tumor cell growth, and induction of differentiation of several malignant cell lines into lines that may be benign (99).

An overview of the epidemiological, clinical trial, animal model, and cell culture data suggests that phytoestrogens may confer cancer-protective benefits. However, there are many variable factors to be considered, the literature contains several inconsistencies, and there are many important questions remaining to be answered. Results from a clinical trial by Petrakis *et al.* (103) suggest that caution may still be warranted in promoting the intake of soy products. In their study, the physiological effects of a commercial soy protein isolate on breast secretory activity was examined, using nipple aspirate fluid as one of several outcome measures. In this 1-yr study, soy protein isolate consumption was associated with outcomes that could be considered adverse, including increased secretion of breast fluid, the appearance of hyperplastic epithelial cells, and increased concentrations of estradiol. The study was subject to several limitations, including a substantial rate of drop-out and the lack of a simultaneous control population (to avoid carry-over effects), and the researchers themselves report their results cautiously as pilot study findings. However, this study does point out that not all reported outcomes associated with soy intake have been beneficial.

Osteoporosis

The continual loss of bone mass in the elderly is a natural process of aging. Women have a higher incidence of osteoporotic fractures than men due to their lower peak bone mass, but in addition, the abrupt decrease in estrogen secretion in postmenopausal women accelerates bone loss. Currently, osteoporosis-related fractures are lower in Asia than in most Western communities, possibly due to the phytoestrogen-rich soybeans and vegetables consumed in large quantities in the Asian diet (104). Ho *et al.* (105) investigated the rates of hip fracture in Hong Kong and the U.S. and reported that for men and women 85 yr of age or more, the rates in Hong Kong were roughly one third the rates in the U.S.

Estrogen replacement therapy has been proven effective in the reduction of postmenopausal osteoporosis (106). Human clinical trials examining the relationship between phytoestrogen intake and osteoporosis are sparse. One recent study examined the effect of soy protein and phytoestrogens on bone mineral density in hypercholesterolemic postmenopausal women. The women were randomly assigned to three treatment arms, each with 40 g/day supplemental protein from 1) soy protein with a high concentration of isoflavones, 2) soy protein with a moderate concentration of isoflavones, or 3) a casein nonfat dry milk. For the group taking soy protein with a high concentration of isoflavone, compared to the casein nonfat dry milk group, significant increases were found in both bone mineral density and bone mineral content in the lumbar spine, but not in other skeletal areas. The group receiving the moderate concentration of isoflavone experienced intermediate and nonsignificant changes in the lumbar spine (42).

Indirect evidence for the potential benefits of phytoestrogens with regard to bone metabolism comes from a growing

number of studies of ipriflavone (7-isopropoxyisoflavone), an isoflavone derivative. In daily doses ranging from 200–600 mg/day, this synthetic, nonhormonal drug has been shown to be effective in promoting bone mass and preventing bone loss (107–110). More research is needed to determine whether natural phytoestrogens have a similar effect on bone metabolism as the synthetic ipriflavone. Caution is warranted in extrapolating ipriflavone study results to the effects of phytoestrogens, as the pharmacological doses of ipriflavone used in most investigations are considerably higher than the levels of phytoestrogen intake realistically achievable through diet alone.

Endogenous hormones, menstrual cycles, and menopausal symptoms

Endogenous hormones. The possibility that phytoestrogen intake could affect levels of endogenous sex hormones has been examined by several investigators, using different study designs. In an observational study of Finnish women, an inverse relationship between urinary excretion of enterolactone and plasma LH has been reported (15). However, the causal link between phytoestrogen intake and circulating levels of endogenous estrogens has not been firmly established. In a large randomized clinical study of soy supplementation among postmenopausal women over 4 weeks, no estrogenic effects were observed despite evidence of high quantities of isoflavone absorption. There were no significant changes in serum FSH, LH, serum hormone-binding globulin (SHBG) levels or in vaginal cytology (111). In one randomized clinical trial, premenopausal women were fed diets consisting of different soybean products with and without isoflavones. Follicular phase length was significantly increased, and peak progesterone, LH, and FSH levels were suppressed in subjects who were fed 69 g texturized vegetable protein/day, which included 45 mg conjugated isoflavones. There were no effects observed in those subjects who were fed isoflavone-free soy protein (41). One study investigated the effect of daily intakes of 100 mg daidzein and 100 mg genistein for 1 month in six premenopausal women. In this small, uncontrolled trial, there was a significant decrease in estradiol and dehydroepiandrosterone sulfate and a nonsignificant increase in menstrual cycle length (25). It remains difficult to draw firm conclusions in this area due to the small sample sizes, the short study durations, the lack of appropriate control, the heterogeneity of the study populations, and the wide range of specific endogenous hormones in these investigations of phytoestrogen intake. Some of the reported biological effects of a diet consisting of soy products containing isoflavones are similar to those induced by the potent synthetic antiestrogen tamoxifen. Tamoxifen, when used therapeutically in breast cancer patients, led to decreases in circulating concentrations of LH and FSH. The decreases in LH and FSH levels were consistently associated with a longer follicular phase (112).

SHBG. Estrogens and androgens are relatively insoluble in aqueous solutions and, therefore, are bound to transport proteins in the circulation, primarily SHBG and albumin. Only a small portion of these steroids (*e.g.* <2%) are trans-

ported in the free form. It is only the free or unbound steroids that are thought to be biologically active and taken up by the tissues. Changes in total hormone concentration result in relatively small changes in the size of the free hormone fraction, whereas changes in SHBG concentration result in relatively large changes in the amount of free and bound hormones. Both lignans and isoflavones have been reported to stimulate the synthesis of SHBG by HepG2 liver cancer cells in culture (113, 114). This is consistent with an observational study of 34 women in whom urinary lignan concentrations were significantly and directly correlated with SHBG concentrations and inversely correlated with the proportion and concentration of free estradiol (15). However, the cell culture data and observational associations between phytoestrogens and SHBG have not been borne out in clinical trials involving phytoestrogen intake. Several short term studies of either flax or soy consumption in men or women for 4–12 weeks reported significant increases in phytoestrogen intake and urinary excretion, but no significant increases in SHBG concentrations (41, 111, 115, 116). With one exception (111), these intervention trials were relatively small, and therefore, their results should be interpreted cautiously. At this time a causal association between realistic intake levels of phytoestrogens and increases in SHBG concentrations remains equivocal or at least weak.

Menstrual cycle length. The associations between phytoestrogen intake and breast cancer and between menstrual cycle length and breast cancer make the link between phytoestrogens and menstrual cycle length an important area of investigation. In an assessment conducted by Olsson *et al.* (117), significantly shorter menstrual cycle length was found for breast cancer patients than for control subjects. It has been suggested that menstrual cycle length is 2–3 days longer in Asian women than in Western women, which could be due in part to the ingestion of substantial amounts of nonsteroidal estrogens present in soy (118, 119). In a 6-month crossover trial with 18 healthy premenopausal women given 10 g/day supplemental flax seed powder for one of the two 3-month study periods, intake of flax seed containing enterodiol and enterolactone resulted in a nonsignificant increase of 0.8 days in menstrual cycle length (116). Lu *et al.* (25) reported an increase of greater than 3 days in cycle length after 1 month of soy milk consumption, but this finding, among only six women and with no appropriate control group, was not statistically significant. The larger, 1-month study of Baird *et al.* (111) reported no significant effect of soy consumption on cycle length. At this time it would appear premature to attribute the longer menstrual cycle lengths of Asian women with typically high levels of soy consumption, specifically to phytoestrogen intake.

Menopausal symptoms. It has been suggested that the consumption of foods containing phytoestrogens contributes to the lower rate of menopausal symptoms among Japanese women compared with women in Western countries. Hot flushes, one unpleasant symptom of menopause, is reportedly lower among women in Japan than those in Canada, which may be due to the high phytoestrogen intake from soy foods in Japan (120). The limited clinical trial data in this area

are equivocal. Murkies *et al.* (44) examined the effect of 45 g/day soy *vs.* wheat flour on hot flushes among women self-reporting at least 14 hot flushes/week. Urinary recovery of phytoestrogens indicated that the women randomized to the soy flour excreted up to 15-fold more of specific phytoestrogens than the women ingesting wheat flour, yet there were no significant differences in hot flushes between the 2 groups. Over the 12-week study, the number of hot flushes decreased significantly from baseline in both groups, but the absence of a difference between the 2 types of flour indicates that the benefit could not be attributed to the soy or its phytoestrogen content. Further research in this area is needed.

Summary

Although large gaps still exist in our understanding of phytoestrogens and their impact on human health, the growing body of literature in this area suggests that they may confer substantial health benefits. Phytoestrogens can be a significant contributor of nonsteroidal estrogens of dietary origin that may have health effects that are especially relevant to women's risk of hormone-associated diseases. Structurally, the plant estrogens share many similarities with endogenous estrogens. Mechanistically, it has been shown that the phytoestrogens can bind to estrogen receptors. Functionally, it appears that the phytoestrogens may exert both estrogenic and antiestrogenic effects depending on circulating levels of endogenous sex hormones. Animal studies and a smaller number of clinical trials with humans have suggested that the health benefits of phytoestrogens may extend into several areas, including lowering levels of blood cholesterol, enhancing endothelial function, inhibiting several stages of cancer initiation and progression, promoting the conservation of bone mass, and favorably influencing menstrual and menopausal symptoms, among others. These findings and associations are consistent with epidemiological observations that high intake levels of soy and soy products, the major source of dietary phytoestrogens, are associated in both men and women with low rates of cardiovascular disease, cancer, and osteoporosis and in postmenopausal women with smaller numbers of hot flushes. Phytoestrogens may be among the dietary factors affording protection against hormone-dependent cancers and diseases in vegetarians and semivegetarians. Prevention of these diseases via increasing intakes of plant-based compounds may provide an important alternative therapy.

Increasing consumption of soy, soy products, and plant-based foods, in general, is consistent with current recommendations to increase fiber and antioxidant intakes while lowering and replacing sources of saturated fat and cholesterol in the diet. Until more is learned, current evidence indicates that there are few risks and many potential benefits from increasing intakes of plant-based foods that are good sources of phytoestrogens. Questions and issues that remain to be resolved include optimal dosages, possible gender differences in response to phytoestrogens, demonstration that observed health benefits can be attributed directly to phytoestrogens rather than to other components of soy and phytoestrogen-rich foods, and the relative impact of the many

specific phytoestrogens that fall into the two broad categories of isoflavones and lignans.

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