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Phytoestrogens*

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ABSTRACT

We have reviewed the literature regarding the food sources, potency, population intakes, and known biological effects of phytoestrogens in humans using MEDLINE data base from the years 1975–1996. Over 600 articles pertinent to the metabolism of phytoestrogens, including female reproduction (in particular menstruation and menopause), cardiovascular disease, osteoporosis, and cancer were assessed including relevant case control or cohort studies, as well as randomized trials and review articles. Epidemiological studies regarding human data were included, as well as human cell line and animal studies when there were no relevant human data available. We conclude that phytoestrogens exhibit physiological effects in humans. Mild estrogenic changes occur in postmenopausal women. Benefits are seen regarding hypercholesterolaemia. Epidemiological, animal, and *in vitro* data encourage further assessment of the role of phytoestrogens in cancer prevention. (*J Clin Endocrinol Metab* **83:** 297–303, 1998)

PHYTOESTROGENS are plant compounds with estrogen-like biological activity. The use of certain plants in traditional medicine and folklore may be ascribed to their estrogenic properties. For example, the pomegranate is associated with fertility (1), the Thai vine, *Pueraria Mirifica*, is used as a rejuvenant and aphrodisiac (2), and hops were believed by the German clergy in the Middle Ages to lower libido (3).

Following publication of the Allen Doisy bioassay for estrogens in 1923 (4), plant extracts were first reported to exhibit estrogenic activity in 1926 (5). By 1975, several hundred plants had been found to exhibit estrogenic activity on bioassay or to contain estrogenically active compounds (6). Phytoestrogens assumed biological and economic importance in the 1940s, with the outbreak of infertility in sheep grazing on pastures rich in subterranean clover in Western Australia, later known as "Clover Disease" (7).

Phytoestrogens were identified in the urine of nonhuman primates in 1979, (8) and in humans in 1982 (9, 10). Epidemiological studies suggest that consumption of a phytoestrogen-rich diet, as seen in traditional Asiatic societies, is associated with a lower risk of the so-called "Western" diseases such as breast and prostate cancer and cardiovascular disease (11).

Estrogenic activity has been reported amongst compounds produced by animals, plants, and microorganisms, as well as in industrially manufactured chemicals and their breakdown products (12). Pesticides and insecticides, including DDT, also contain estrogen-like compounds and are now classified as xenoestrogens (13). Little is known of the long-term effects of xenoestrogens, however there is substantial concern regarding their potentially deleterious effects. Further discussion of xenoestrogens is beyond the scope of this review.

Classification and Metabolism of the Major Phytoestrogens

There are three main classes of phytoestrogens: isoflavones, coumestans, and lignans, which occur in either plants or their seeds. Resorcylic acid lactones exhibit estrogenic activity and are produced by molds that commonly contaminate cereal crops and hence are better termed mycoestrogens (Fig. 1). A single plant often contains more than one class of phytoestrogen. For example, the soy bean is rich in isoflavones, whereas the soy sprout is a potent source of coumestrol, the major coumestan (1). The major isoflavones, genistein and daidzein, commonly exist as inactive glucosides (14).They are also derived from precursors, biochanin A and formononetin, which are converted to genistein and daidzein respectively, after breakdown by intestinal glucosidases (15). Daidzein is further partially metabolized to equol and O desmethylangiolensin (O-DMA).

The estrogenically active lignans, enterodiol and enterolactone, are derived from the compounds secoisolariciresinol and matairesinol found in plants. (11, 15). These lignan precursors occur in the aleuronic layer of the grain close to the fiber layer.

In humans, after consumption of plant lignans and isoflavones, complex enzymatic metabolic conversions occur in the gastrointestinal tract, resulting in the formation of heterocyclic phenols with a close similarity in structure to estrogens (10) (Fig. 2). Absorbed phytoestrogen metabolites undergo enterohepatic circulation and may be excreted in the bile (16) deconjugated by intestinal flora, reabsorbed, reconjugated by the liver, and excreted in the

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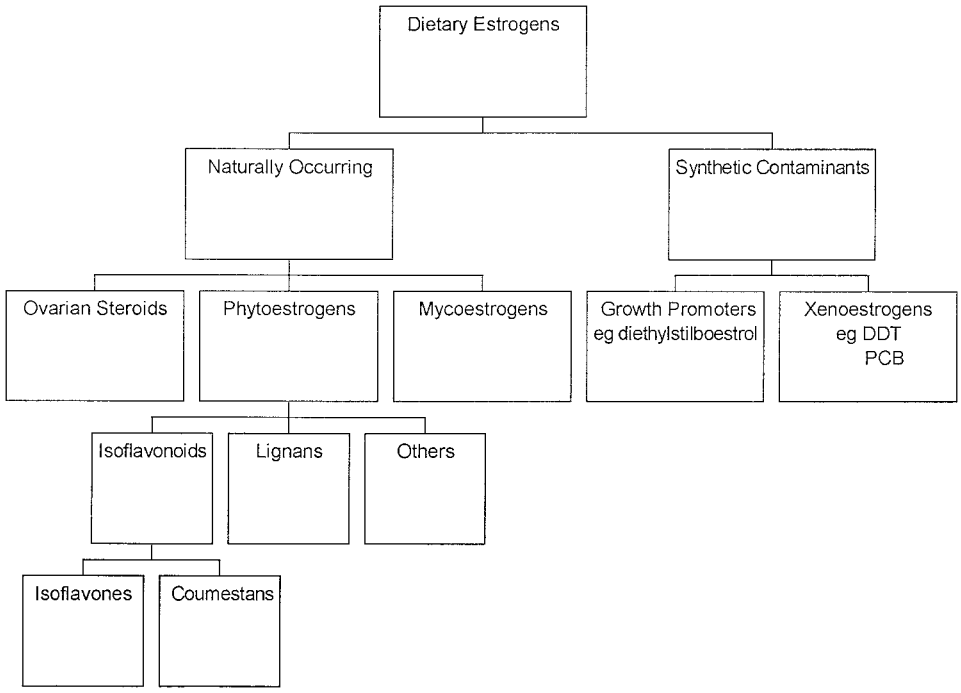

FIG. 1. Sources and classification of dietary estrogens.

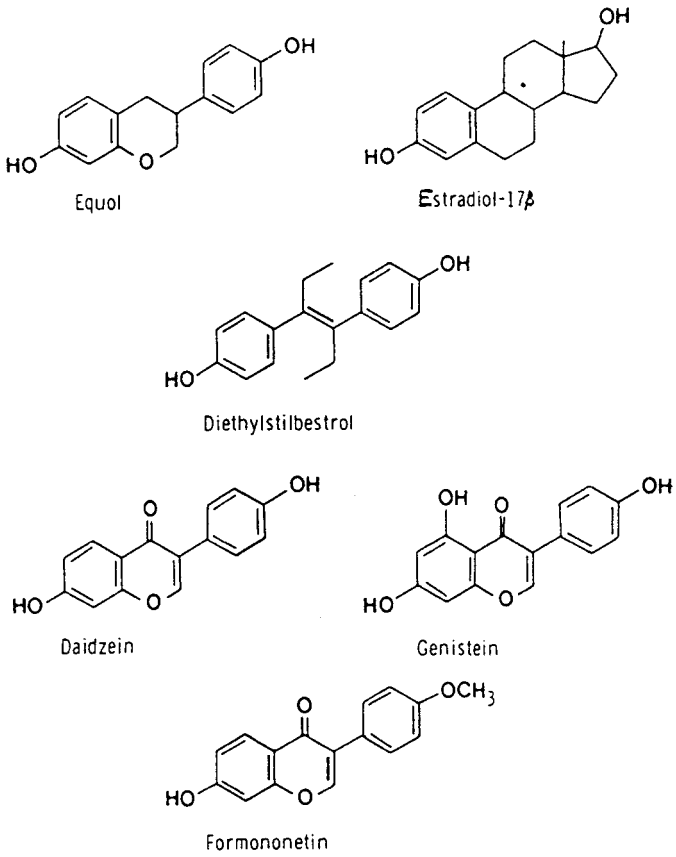

FIG. 2. A comparison of the chemical structure of the phytoestrogen equol, formed in the gastrointestinal tract of man and animals, estradiol-17b, diethylstilboestrol, and several phytoestrogens of plant origin. Permission granted by the author Setchell KDR, Ref. 10.

urine (17). Lignans and isoflavones can be measured in urine, plasma, faeces, semen, bile, saliva, and breast milk (18). Concentrations of the different phytoestrogen metabolites vary widely between individuals even when a controlled quantity of an isoflavone or lignan supplement is administered. As dietary phytoestrogen metabolism is predominantly determined by the gastrointestinal flora, antibiotic use, or bowel disease and gender will modify metabolism (10, 19–21).

Concurrent dietary intake, in particular high dietary fiber, vegetable and fruit intake and duration of exposure, exert a major influence on lignan and isoflavone metabolism (22–24). There is need for caution in relating dietary components to disease states due to the complex interactions between ingested foods, metabolism and absorption of specific dietary constituents as, similarly dietary fat, fiber, protein, alcohol, and micronutrients may all independently effect endogenous estrogen metabolism (25–29).

Food Sources

The isoflavones displaying estrogenic activity occur almost exclusively in legumes and beans (1, 14, 30) and in products that contain most or all of the bean (31, 32).

Second generation soy foods are made by adding soy ingredients to a wide variety of manufactured foods, hence the isoflavone content is diminished, for example tofu yoghurt and soy noodle (33–35). Soy sauce contains few isoflavones.

Lignans are found widely in cereals, fruit, and vegetables. They occur in high concentration in flaxseed also known as linseed (15, 30, 36) and in lesser concentration in whole grain cereals, vegetables, fruit and seeds (1) Table 1. Estimation of

TABLE 1. Classification and food sources of phytoestrogens

Isoflavones		Lignans			Coumestans	
Legumes	Soybean products	Whole grain cereal	Fruits, Veges, Seeds	Alcoholic sources	Bean sprouts	Fodder crops
Soybeans	Soy meal	Wheat	Cherries	Beer from hops	Alfalfa	Clover
Lentils	Soy grits	Wheat germ	Apples	Bourbon from corn	Soybean sprouts	
Beans	Soy flour	Barley	Pears			
haricot	Tofu	Hops	Stone fruits			
broad	Soy milk	Rye	Linseed			
kidney		Rice	Sunflower seeds			
lima		Brans	Carrots			
Chick peas		Oats	Fennel			
			Onion			
			Garlic			
			Vegetable oils including olive oil			

the concentration of lignans in foods has been limited by assay technique (36).

Coumestans occur predominantly with germination for example beans sprouting and also in fodder crops (1).

Resorcylic acid lactones, including zearalenone and zearalenol, are mycotoxins, and are produced by Fusarium species of mold growing on crops stored in damp conditions. These compounds are most concentrated in the seed hull which is commonly removed in preparation of foodstuffs (1).

Phytoestrogen levels in various populations

Current information about the amount of phytoestrogens in human diets is limited. Variations in phytoestrogen content occur because of genetic differences in plants such as soy varieties, location, season, infection with fungal diseases, processing, and as part of the plant's defence response (37).

Macrobiotics and other vegetarians such as Seventh Day Adventists have the highest excretion values of lignans (38). Asian populations, such as those in Japan, Taiwan and Korea, are estimated to consume 20–150 mg/day of isoflavones, with a mean of about 40 mg from tofu and miso (38).

Potency and biological effects

The biological potencies of phytoestrogens vary. The majority of these compounds are nonsteroidal in structure and vastly less potent than the synthetic estrogens (10^{-3} to 10^{-5}), and they vary between species, routes of administration, and end points used (1). The relative potencies, determined by human cell culture bioassays (compared with estradiol, to which we give an arbitrary value of 100) are: coumestrol 0.202, genistein 0.084, equol 0.061, daidzein 0.013, and formononetin 0.0006 (11, 39).

Estrogenic and antiestrogenic activity

Phytoestrogens are of biological interest because they exhibit both *in vitro* and *in vivo* weak estrogenic and antiestrogenic actions (1).

The estrogenic effect of phytoestrogens was first observed as reproductive disturbances in sheep (7). Isoflavones stimulate uterine hypertrophy in laboratory animals exhibiting estrogenic action (1, 40). When administered with estradiol, genistein functions as an antiestrogen, decreasing uterine estradiol uptake in animal models (32). Isoflavones exhibit anticarcinogenic activity *in vivo.* Laboratory animals fed soy-fortified diets, have predominantly less breast tumor proliferation after stimulation with direct acting [N-methyl-N′-nitrosourea (NMU)] and indirect acting [dimethylbenz[a]anthracene (DMBA)] tumor-inducing agents (32).

Genistein has been the phytoestrogen of greatest interest at present and, *in vitro*, has been shown to exert both proliferative (estrogenic) and antiproliferative (antiestrogenic) effects in human cell lines (41, 42). In the human estrogen receptor (ER)-positive MCF-7 breast cancer cell line, these effects are biphasic and concentration dependent, with stimulation of cell growth occurring at low concentrations of genistein [10^{-5}–10^{-8} M] and inhibition at higher concentrations [10^{-4}–10^{-5} M](41). At low concentrations genistein competes with estradiol for binding to the ER with a 50% inhibition concentration of 5×10^{-7} M and stimulates the expression of pS2 mRNA, a specific marker of ER-mediated estrogen like activity(41). Sathyamoorthy (42)and others have shown a similar stimulatory effect with daidzein, equol, and enterolactone at 10^{-6} M concentrations.

The antiproliferative effects of genistein described above occurred in both ER-positive and ER-negative cell lines and thus appear not to be mediated by the ER (41). It has been proposed that genistein, and perhaps other phytoestrogens, inhibit tumor cell growth by interfering with the tyrosine kinase activity of activated growth factor receptors and cytoplasmic tyrosine kinases, which are essential for the transduction of mitogenic signals. Akiyama and others (43) reported that genistein inhibits epidermal growth factor-receptor (EGF-R) tyrosine autophosphorylation, whereas Peterson and Barnes (44) were unable to demonstrate this effect and concluded that the isoflavones exert their inhibitory effects either downstream from EGF-R tyrosine autophosphorylation or by some other mechanism (44).

Extrapolation of cell culture studies to humans is questionable, especially in terms of tumor growth inhibition. A high soy diet results in an approximate plasma level of 0.5–4 mmol/l of genistein (45, 46), whereas the studies describing an antiproliferative effect indicate a minimum cell culture concentration of 10–100 mmol/l of genistein (39, 32).

Other reported biologic properties

Isoflavones exhibit additional anticarcinogenic activity *in vitro* and have been reported to inhibit angiogenesis (47) and

cell cycle progression (48). Other effects include aromatase enzyme inhibition (49, 50), stimulation of sex hormone binding globulin (SHBG) synthesis (51, 52), antioxidant properties (53), and digitalis-like activity (54).

Effects in humans

Premenopausal women

A cyclic pattern of lignan excretion has been observed during the menstrual cycle in humans and in Vervet monkeys (8). It is probable that the precursors of these lignans are of dietary origin, however there is also a hormonal influence on the metabolism and absorption of these compounds (55). Dietary intervention with 60 g soy protein daily has been reported to increase follicular phase length (56). This effect was associated with suppression of the midcycle surges of FSH and LH (56). Twelve ounces of soy milk three times daily decreases serum 17 beta-estradiol and luteal phase serum progesterone in 22–29-yr-old females (57). In contrast, daily 10 g flax seed supplementation increases luteal phase duration, but with no difference in follicular phase length in normally cycling women (58). An increase in SHBG was not observed in these studies (56, 58) in contrast to earlier *in vitro* reports (11, 51). McMichael-Phillips (59) has reported that 14 days of 60 g soy supplementation increases the proliferation rate of premenopausal breast epithelium.

Postmenopausal women

Japanese women are reported to have a low frequency of hot flushes compared with postmenopausal western women, in part attributed to their high phytoestrogen consumption (60). Whether this is valid has been recently challenged (61). Evidence from several human studies demonstrates that certain dietary phytoestrogens can produce mild estrogenic effects in the postmenopausal woman, including estrogen-like effects on vaginal cytology (62–64) and reductions in hot flushes (64–7) (Table 2). However, the findings are inconsistent; four weeks' supplementation with textured vegetable protein produced no effect. There are variations in response within studies, and there is no clear correlation in response to estrogenic changes in vaginal cytology and the effect on hot flushes. This illustrates the difficulty in making objective assessments of hot flushes due to the natural resolution of flushes over time. The variations in response may depend on populations studied, soy products used, and study design, particularly with respect to duration of exposure, variability in response, and nonresponse in some postmenopausal women to phytoestrogen supplementation (24). Phytoestrogen supplementation may benefit some women in alleviating menopausal symptoms, however it is difficult to make specific recommendations of formulation and dosage.

Disease Relationships

Cardiovascular disease

There is evidence to support the hypothesis that phytoestrogen consumption contributes to the lower incidence of cardiovascular disease in Asian countries and in vegetarians and that phytoestrogens may be cardioprotective (11).

A study of Rhesus male and female monkeys has shown that isoflavone-intact soy protein supplementation results in a hypocholesterolemic effect when compared with a soy diet depleted of phytoestrogen ($P < .004$) (68). Both male and female animals receiving the soy diet were observed to have low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol values 30–40% lower than controls. High density lipoprotein (HDL) cholesterol ratio increased by 50% in female and 20% in male animals (68).

Another study has investigated the effects of soy protein in male cynomologus macaques. The animals were fed an atherogenic diet and randomized to supplementation with either casein, soy protein with isoflavones intact (soy+), or soy protein with isoflavones extracted (soy-). The soy+ diet had a lower total cholesterol, higher HDL cholesterol, and lower LDL and VLDL cholesterol. Similarly the extent of coronary artery atherosclerosis was negligible in the monkeys fed the soy+ diet compared with the other two groups in whom atheromatous plaque was detected (69). Furthermore the soy+ diet enhanced dilator responses of atherosclerotic coronary arteries to acetylcholine in female rhesus monkeys (69).

Cassidy *et al.* observed a 9% reduction in total cholesterol in a small study of normolipemic premenopausal women given a 60-g soy protein supplement (56). Consumption of 25-g soy protein enriched bread resulted in a decreased total serum cholesterol and increased HDL cholesterol in hypercholesterolemic men (70). A soybean protein diet in subjects with Type II hyperlipoproteinemia may lower cholesterol on average by 20% (71). A meta-analysis of 38 published controlled clinical trials of soy protein consumption that averaged 47 g per day and serum lipid and lipoprotein concentrations found that consumption of soy protein was associated significantly with mean reductions in total cholesterol (9.3% decrease, 95% CI 0.35–0.85 mmol/L), LDL cholesterol (12.9% decrease, 95% CI 0.30–0.82 mmol/L), and triglycerides (10.5% decrease, 95% CI 0.003–0.29 mmol/L) (72). The hypocholesterolemic effect appears to be signifi-

TABLE 2. Effect of phytoestrogens on vaginal cytology and menopause symptoms: summary of studies

Investigator (yr)	Phytoestrogen	Numbers	Vaginal cytology	Hot flushes
Wilcox (1990)	45 g SF	25	$\uparrow P < 0.05$	
Murkies (1995)	45 g SF	58	NS	$\downarrow P < 0.001$
Baird (1995)	TVP ⅓ substitute	94	NS	
Dalais (1996)	45 g SG	52	$\uparrow P < 0.03$	NS
	45 g linseed		NS	$\downarrow P < 0.02$
Harding (1996)	80 mg SP drink	20		$\downarrow P < 0.03$
Brezezinski (1996)	80 g tofu, miso, 10 g linseed	165	$\uparrow P < 0.005$	$\downarrow P < 0.004$

Phytoestrogen SF, soy flour; SP, soy protein; SG, soy grit enriched bread; TVP, textured vegetable protein; NS, not significant.

cantly related to pretreatment plasma cholesterol (72). A recent study of normolipemic postmenopausal women supplemented with a 40-mg phytoestrogen pill demonstrated a 22% increase in HDL cholesterol and no significant change in other parameters (73). Dietary soy phytoestrogens may provide cardioprotective benefits via a direct effect on lipids. Other reported properties, such as inhibition of platelet aggregation and antioxidant effects, may also be important (1, 74).

Osteoporosis

Osteoporosis is related to multiple factors including aging, hormone deficiency, and diet. There is a paucity of data regarding the possible role of phytoestrogens in bone metabolism and the incidence of osteoporosis. Dietary soybean prevents significant bone loss in ovarectomized rats ($P < 0.001$) (75). Ipriflavone (7 isopropoxy-isoflavone), a synthetic flavonoid, inhibits osteoclast recruitment and function, and 600 mg/day has prevented bone loss at the distal radius in osteoporotic postmenopausal women (76). Postmenopausal women randomized to receive casein, soy protein with either 1.39 mg total isoflavones/g protein (ISP), or 2.25 mg total isoflavones/g protein (ISP+) for six months demonstrated increased bone mineral content and density with ISP+ compared with controls ($P < 0.005$) (77). Postmenopausal women increased bone mineral content when fed 45 g soy enriched bread compared with controls fed wheat kibble bread ($P < 0.03$) (64). At present there is little published data to support a specific role for phytoestrogens in the prevention of osteoporosis, and further studies of longer duration are needed.

Cancer

The incidence of hormone dependent tumors is lower in Asia and Eastern Europe than western countries (11, 78) and amongst vegetarians (79, 80). Breast, ovarian, prostate, and colon cancer show a negative correlation with cereal and phytoestrogen intake when comparing cancer mortality rates and food availability data between countries (78).

Breast cancer

Japanese immigrants to North America have a higher incidence rate of breast cancer than their counterparts in Japan (81). Breast cancer rates of North American-born Japanese and "early" Japanese immigrants are almost identical to those of caucasian North Americans, whereas "late" immigrants have an incidence rate intermediate between the former groups and that of Japanese residing in Japan (81). Such findings support the role for environmental conditions in the etiology of breast cancer. Japanese women, in their homeland, who have breast cancer have a higher number of *in situ* tumors with fewer nodal metastases, and those with metastases have less nodal spread, than women with breast cancer in the United States or Britain (82). Hirayama (83) reported a significant graded inverse association in Japanese women between risk of breast cancer and consumption of miso (soybean paste soup). A diet high in soy products conferred a low risk of breast cancer in premenopausal women in Singapore, with no effect observed in postmenopausal women (84).

Baghurst and Rohan (79) investigated the relationship between dietary fiber and breast cancer in a case controlled study and reported a progressive reduction in breast cancer relative risk with each quintile of increasing fiber intake for women. Those in the highest quintile of nonstarch polysaccharide intake had a relative risk of 0.46. Whether this relates to interference with the enterohepatic circulation of both endogenous estrogens and phytoestrogens and hence an overall reduction in total circulating estrogens, to the phytoestrogen content of the dietary fiber, the exclusion of other carcinogenic foods or the concurrent ingestion of other influencing macronutrients is yet to be ascertained. Low urinary lignan values, a measure of low fiber intake, have been reported in breast cancer patients (11).

Protective effects of isoflavones including soy, measured by tumor number, incidence, metastases, and latency (85–7), are seen in animal models with experimentally induced breast cancer. Prepubertal genistein-treated rats developed fewer mammary gland terminal-end buds, with significantly less cells in the S-phase of the cell cycle, and more lobules than controls at 50-days-old (88).

Breast cell lines saturated with predominantly genistein exhibited receptor stimulation at low concentrations, (42–3, 89) and dose dependent inhibition at increased concentrations (40, 89–91).

The epidemiological, animal, and cell-line data suggest that phytoestrogens may play a role in breast cancer. Prepubertal phytoestrogens may cause precocious maturation of breast terminal end buds to more differentiated lobules and subsequent breast cancer protection. In contrast, increased post-pubertal exposure, without breast maturation from significant phytoestrogen ingestion in childhood, or another stimulus for breast maturation such as full-term pregnancy, could potentially increase breast cancer risks via an agonistic estrogenic action.

Prostate cancer

Mills (92) reported that increased consumption of beans, lentils and peas, tomatoes, and dried fruits was associated with significantly decreased prostate cancer risk in 14,000 Seventh Day Adventist men (92). Hirayama (83) showed the protective effects from prostate cancer with consumption of green leafy vegetables, relative risk 0.5 ($P < 0.03$), but found no association with soybean paste (83). A prospective study of men of Japanese ancestry living in Hawaii has shown a decreased prostate cancer risk in those who consume rice and tofu (93), and a study of Japanese immigrants to North America reported an increase in the incidence rate of prostate cancer with younger age at immigration, supporting the hypothesis that environmental changes, including diet, can impact on cancer risk even in later life (79).

In human prostate cancer cell lines, high concentrations of genistein and biochanin A inhibit the growth of androgen-dependent and independent cells. However fairly high concentrations of genistein were required to achieve this effect (44).

Overall, the epidemiological data suggest that phytoestro-

gens may play a protective role against the development prostate cancer (51); however, the physiological significance of the inhibitory effects of genistein on prostate cancer cell lines *in vitro* at this stage is difficult to evaluate.

Conclusion

Phytoestrogens appear to have physiological effects in humans, with the most supportive data being related to the effects of soy protein supplements on lipids and lipoproteins and on vascular function. Mild estrogenic effects have been seen in postmenopausal women; however, larger and longer term studies are needed to more thoroughly document clinical effects and to examine the target effects on estrogen responsive tissue such as breast and endometrium. Phytoestrogens are now available in tablet form, and this will enhance future study designs.

Cancer data is still in its infancy. Extrapolating from human cell line and animal studies has limitations. More human studies are needed to establish the role of phytoestrogens as estrogen agonists and antagonists and of their actions on tyrosine kinases and other growth factor inhibitors in the cancer population. Although epidemiological and animal data suggest phytoestrogens may play a protective role against both breast and prostate cancer (50), the antiproliferative effects observed in breast and prostate cancer cell lines must be interpreted with caution because of the difficulty in correlating the phytoestrogen exposure to the cells *in vitro* to *in vivo* tissue levels in humans. Studies of the inhibitory effects of genistein on the growth of untransformed, normal cells are needed if an increased phytoestrogen dietary intake is to be widely recommended. Furthermore the temporal relationships between dietary phytoestrogen exposure in humans and carcinogenesis needs to be investigated. Data from the animal models described indicate prepubertal exposure may be essential for the breast cancer protective effects to be manifest. Western women, particularly postmenopausal women who have the greatest breast cancer risk, are being encouraged to suddenly increase their phytoestrogen intake without consideration of this potentially significant and possibly adverse temporal effect.

In conclusion, it is naive to try to extract a single component of a total life style from a community such as Japan, where several other significant lifestyle factors are operative, and expect to see a distinct correlation with disease. It may be that phytoestrogen ingestion needs to be life-long and combined with other low-risk dietary constituents and behaviors for the protective effects to be significantly manifest. To date, major dietary studies relating to cancer prevention have been mostly limited to the effects of soy; however, other foods and their interactions also warrant investigation.

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