Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones1,2

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ABSTRACT  The importance of estrogens in homeostatic regulation of many cellular and biochemical events is well illustrated by the pathophysiologic changes that occur with estrogen deficiency. Many of the major diseases of Western populations are hormone dependent and epidemiologic data have shown a strong association between their incidence and diet. In particular, the importance of a plant-based diet is evident from the current dietary recommendations that emphasize an increase in the proportion and amount of fruit and vegetables that should be consumed. Although interpretation of the role of individual components of the diet is difficult from epidemiologic and dietary studies, it is recognized that there are many plant-derived bioactive nonnutrients that can confer significant health benefits. Among these phytochemicals is the broad class of nonsteroidal estrogens called phytoestrogens, and in the past decade there has been considerable interest in the role of isoflavones because of their relatively high concentrations in soy protein. The isoflavones in modest amounts of ingested soy protein are biotransformed by intestinal microflora, are absorbed, undergo enterohepatic recycling, and reach circulating concentrations that exceed by several orders of magnitude the amounts of endogenous estrogens. These phytoestrogens and their metabolites have many potent hormonal and nonhormonal activities that may explain some of the biological effects of diets rich in phytoestrogens. Am J Clin Nutr 1998;68(suppl):1333S–46S.

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INTRODUCTION

Over the years, epidemiology has provided important clues to possible etiologic factors in disease states. Largely as a result of the findings from population and case-control studies, it is now generally accepted that many of the common diseases of prosperous nations are diet related (1) and could be avoided with significant modification of the diet. Identifying the individual or multiple components of the diet that are responsible for the pathogenesis of many of the common diseases is extremely difficult because of the complex interplay between lifestyle factors, genetics, and many confounding variables. Dietary intervention studies, which are often short-term, provide variable results that may not necessarily reflect responses that could be seen in long-term studies; however, the latter often suffer from lack of compliance, especially if radical changes to the diet are required. Despite these limitations, research into human nutrition has led to an awareness of the health benefits that dietary modification can offer. Specifically, it has been recognized that the human diet contains, in addition to essential micro- and macronutrients, a complex array of naturally occurring bioactive nonnutrients called phytochemicals that, if incorporated into the diet either naturally as an integral part of the food or as a food supplement, may confer significant long-term health benefits. It is this concept that has resulted in the development of the relatively new field of functional foods.

Phytochemicals are plant-derived compounds of natural origin (2, 3), and the number of such classes of compounds identified as having biological activity is rapidly expanding as epidemiologic studies continue to indicate possible associations between diet and disease states. Phytoestrogens are a broad group of plant-derived compounds of nonsteroidal structure that can behave as estrogen mimics. The major classes of phytoestrogens of current interest from a nutritional and health perspective are the lignans and isoflavones (4–7). [A wide range of synthetic estrogens may be consumed as a result of their introduction into the food chain from the environment. These environmental estrogens (8) have been the focus of many reviews elsewhere and are therefore not discussed here.]

A conspicuous feature of the chemical structure of phytoestrogens is the presence of a phenolic ring that, with few exceptions, is a prerequisite for binding to the estrogen receptor (Figure 1) (9). For this reason, phytoestrogens can act as estrogen agonists or antagonists (10–14); their actions at the cellular and molecular level are influenced by many factors, including but not limited to concentration dependency, receptor status, presence or absence of endogenous estrogens, and the type of target organ or cell. The recent identification and cloning of a second and novel estrogen receptor, referred to as Erβ (15), with its unique anatomical distribution in tissues such as bone, brain, vascular endothelia, and bladder and its ligand specificity toward phytoe-
DISTRIBUTION AND ORIGINS OF PHYTOESTROGENS

The phytoestrogens are ubiquitous within the plant kingdom, being synthesized in plants from phenylpropanoids and simple phenols (38–41). Flaxseed and soybean are rich sources of lignans (42, 43) and isoflavones (44–51), respectively, and were identified as important dietary sources for urinary enterolactone (42) and equol (19, 20), which are mammalian-derived compounds not found in plants, or daidzein and genistein, the hydrolysis products of various isoflavonoid glycosides of plants. The extent of intestinal bacterial metabolism will therefore have an effect on the bioavailability of dietary estrogens and consequently be expected to influence the potential for physiologic effects.

Dietary estrogens are weakly estrogenic (10⁻²- to 10⁻³-fold, depending on the system examined) when compared with estradiol or estrone, the principal circulating estrogens of most mammals (10, 21–29). The preferential binding of nonsteroidal estrogens to the Erβ receptor suggests that they may exert their actions through distinct and separate pathways from those of classical steroidal estrogens. Additionally, the lower affinity of several phytoestrogens for serum proteins would be expected to influence the potential for physiologic effects.

These conjugates can vary considerably among different soybeans or soy-protein products (47, 49, 63) and that this is a function of species differences (51), geographic and environmental conditions (64), and extent of industrial processing of the soybeans (47, 49, 65–68).

All soybean proteins and foods currently available for human consumption contain significant amounts of the isoflavones daidzein and genistein, either as the aglycone (unconjugated form) or as different types of glycoside conjugates (the chemical structures of these conjugates are depicted in Figure 2). These include 6’-O-malonylglucosides, 6’-O-acetylglucosides, and the β-glucosides of daidzein and genistein (69, 70), all of which can be separated by reversed-phase HPLC. Smaller amounts of glycine conjugates are often found in soy proteins (71), whereas very high concentrations of conjugates of glycine are found in the hypocotyledon or germ (70). The malonyl and acetyl glucosides are susceptible to heat and readily convert to the more stable β-glucoside (67); therefore, depending on the extent of processing of the soybean, the relative proportions of these conjugates can vary considerably among different soyfoods (49, 50).

Little is known about the biological activity of the individual glycosidic conjugates of isoflavones. They are readily hydrolyzed by intestinal bacteria (53), which suggests that it would be difficult to directly assess their activity in vivo. The role of conjugation may be important in influencing the bioavail-
were measured by HPLC. Concentrations of conjugated (glycosides) and unconjugated isoflavones in flour and miso. Both soy proteins were boiled for 30 min and the total percentages are highly variable between types and brands of tofu has significant amounts of isoflavones (0.2–0.5 mg/g), the consumers continue to associate soy protein with tofu. Although the conjugation profile can be influenced by heat, the total isoflavone concentration in the soy-protein product remains constant. In studies examining the stability of soy flour and miso to boiling for 30 min, there was no significant change in the amounts of aglycones or β-glycosides, indicating that when present in a food matrix, isoflavones are stable at the usual temperatures encountered in the preparation of soyfoods (Figure 3). Irrespective of the relative proportions of the individual isoflavone conjugates in different soy products, the aglycones are ultimately released either as a result of the conditions used in the processing and preparation of the soyfood or as a result of intestinal bacterial metabolism.

Comprehensive lists of the isoflavone composition of many soy-protein foods of Western and Asian origin were published (49, 50, 68), and several additional studies have confirmed these general findings (51, 63, 69). The large range of total isoflavone concentrations in soy proteins or soyfoods is shown in Figure 4. For most of the soy flours and concentrates, isoflavone concentrations are relatively high (0.5–3.0 mg/g), whereas the soymilks and soy infant formulas have relatively low concentrations of isoflavones. However, the volume intake of these latter products is sufficient to account for a significantly high dietary intake of isoflavones from these foods. The finding of isoflavones in soy infant formulas (66, 68, 72) and the demonstration that they are readily absorbed and excreted by infants fed soymilk formulas (68, 73) has led to questions regarding the potential long-term effects of these bioactive phytoestrogens (74–76).

The great diversity in soy-protein foods that can be incorporated into the average diet is generally underappreciated, and consumers continue to associate soy protein with tofu. Although tofu has significant amounts of isoflavones (0.2–0.5 mg/g), the concentrations are highly variable between types and brands of tofu (63). Many other soyfoods can be incorporated into the human diet to provide greater amounts of isoflavones (49, 50). Products such as soy oils have only traces of isoflavones (46, 49), which is explained by the highly polar nature of the glycosidic conjugates in the soybean and their inability to partition into the lipophilic oil. This is evident from the fact that the efficient extraction of isoflavones from the soybean or soy proteins requires highly polar organic-aqueous solvent mixtures (52, 67, 69). Soy sauce also has a low isoflavone content (47, 49).

Striking compositional differences are found between the types of soy proteins commonly used in China, Japan, and Indonesia and those usually incorporated into foods in Western countries (Figure 5). Many of the soyfoods consumed in the Far East are highly fermented soybean products; many of the bacteria used in their preparation are capable of hydrolyzing the glycosidic conjugates and modifying the composition (77). Analysis of these foods reveals a predominance of the aglycones (49, 50). These compositional differences may be important with regard to metabolism and bioavailability because there are marked differences in the polarity of conjugated and unconjugated isoflavones.

**PHYSIOLOGIC BEHAVIOR OF SOY ISOFLAVONES**

A large amount of work performed almost 2 decades ago established that lignans and isoflavones of dietary origin have common physiologic behavior. Much of this initial work focused on the origins and fate of the lignans enterolactone and enterodiol (43), but several unidentified diphenolic compounds were also recognized. One diphenolic compound, then referred to as compound 382/196 [named from the masses of the molecular ion and base peak in the electron ionization mass spectrum of the trimethylsilyl ether derivative (78, 79)] was subsequently identified as the isoflavone equol (20) only after it was observed that addition of soy protein to the diet of rats and humans caused an impressive increase in urinary excretion.

Equol was first identified as a minor constituent of the urine of pregnant mares (19) and later was found in the urine of many other animal species (22, 80–84), but it had not been identified previously in human urine. Daidzein 7[3-hydroxy]β-glucoside was isolated from soy flour, identified by mass spectrometry, and shown to be converted to equol-glucuronide (52). Our studies showed that the biotransformation of the ingested phytoestrogens occurred by the action of intestinal microflora on the dietary lignan and isoflavone glycosides (53, 79), that these unique dietary estrogens were not excreted in urine or found in

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**FIGURE 3.** Stability to heating of isoflavones in 2 soy proteins: soy flour and miso. Both soy proteins were boiled for 30 min and the total concentrations of conjugated (glycosides) and unconjugated isoflavones were measured by HPLC.

**FIGURE 4.** Total isoflavone concentrations (mg/g) measured in a range of soy-protein products of Western and Asian origin. Data adapted from references 63–68.
URINARY EXCRETION OF ISOFLAVONES

Most studies of the metabolism of isoflavones have focused on urinary excretion. This is partly because of the high concentrations found in urine after soy intake and the methodologic difficulties encountered in measuring the lower concentrations in other biological fluids. Early studies reported urinary equol excretion after a soy challenge (52, 53) and excretion values in several units of expression in subjects consuming omnivorous or vegetarian diets (106–109). Equol excretion was 11–25 mmol/d (3–7 mg/d) after an intake of 40 g textured vegetable protein, which is several orders of magnitude greater than endogenous estrogen excretion. Many investigators have confirmed the high variability in urinary excretion of isoflavones among subjects consuming soy-protein foods (72, 110–117). The reason for this is unclear, but it was found that about one-third of healthy subjects were unable to bio-transform daidzein to equol (53, 86), and much higher concentrations of the precursors therefore appear in the urine of such subjects. An inverse relation exists between equol and daidzein excretion (112) and O-desmethylangolensin (114), an additional metabolite of daidzein (118). More recent studies have indicated that the extent of metabolic transformation of dietary isoflavones is more complex than originally thought (110, 119). A detailed qualitative analysis of human urine revealed many other diphenolic metabolites that represent intermediates in the biotransformation of daidzein and genistein, many of which can only be successfully quantified by mass spectrometry.

Irrespective of the factors governing isoflavone metabolism, measurements of urinary daidzein, genistein, or their metabolites provide a useful indicator of compliance with a diet containing soyfoods. Concentrations of these dietary estrogens are relatively low in subjects consuming an omnivorous diet without soyfoods, whereas in vegetarians there is a tendency toward high urinary excretion of lignans as well as isoflavones (106–109). Several studies examined urinary excretion in response to dietary intervention with plant-based foods (72, 117, 120). Few studies have been performed to establish whether there are dose-response relations between intake of isoflavones and urinary isoflavone excretion, and in them a small number of subjects and different soyfoods (87, 114, 115) were used. Nevertheless, these studies indicate that urinary excretion of isoflavones increases with increasing intake of soy isoflavones, but that absorption, as reflected by urinary excretion, may be saturable at high doses.

Japanese subjects consuming a traditional diet excrete high concentrations of isoflavones (121, 122). Typical HPLC analysis illustrated in Figure 6 shows the urinary excretion of daidzein, genistein, and equol in 6 Japanese adults consuming a traditional diet containing a variety of soy proteins (66). Concentrations of these isoflavones are so high when soy is ingested that relatively

**FIGURE 5.** Relative distribution of conjugated and unconjugated isoflavones in different soy proteins of Western and Asian origin, illustrating the higher proportion of aglycones found in fermented soy proteins.

the blood and bile of germ-free animals (79), and that administration of antibiotics abolished their formation (54). These observations were confirmed by several other groups (85–90). Incubation of textured vegetable protein with cultured human fecal flora resulted in the formation of equol (53), and the absence of equol from the urine of 4-mo-old infants fed soy infant formulas (73) further highlights the essential role that intestinal bacteria play in its formation.

Thus far, there have been no attempts to determine the types of bacteria involved in the metabolism of isoflavones, and it is assumed that similarities exist with the bacterial metabolism of lignans (91). The finding of equol in high concentrations in portal venous blood of rats and in bile established an enterohepatic circulation for isoflavones (79, 85, 90, 92). Recent studies of the pharmacokinetic behavior of genistein in rats also confirmed that in common with endogenous estrogens (93, 94), isoflavones undergo biliary secretion (85, 90, 92); when infused, genistein rapidly appears in bile.

Along with steroid-hormone metabolism (95–97), the liver probably plays a key role in the further metabolism of isoflavones by conjugating the aglycone with glucuronic acid and, to a lesser extent, sulfuric acid. Hepatic conjugation of the lignan enterolactone by UDP-glucuronosyltransferase from rabbit liver microsomal preparations was shown (98), and plasma and urinary isoflavones are predominantly conjugated with glucuronic acid and to a lesser extent sulfuric acid (78, 86, 99, 100). The efficiency of conjugation of isoflavones is high and consequently the proportion of circulating free isoflavones is small. Interestingly, the finding that enterolactone, enterodiol, and equol were predominantly conjugated to glucuronic acid in portal venous blood (78) is difficult to explain and suggests that conjugation of isoflavones may occur in the intestinal wall during absorption from the gut. This has been subsequently confirmed in rats by using everted intestinal sac preparations (90). Similarly, our studies (KDR Setchell, L Zimmer-Nechemias, JE Heubi, unpublished observations, 1996) showed that when daidzein and genistein were administered orally as a single bolus dose to healthy premenopausal women and to men, only small proportions of these unconjugated isoflavones appeared in plasma.

The metabolism of isoflavones has been studied extensively in agricultural animals (101, 102) because of the infamous role of isoflavones as causative agents in the infertility syndrome in sheep known as clover disease (103). Studies with hepatic micro-
simple HPLC methods based on reversed-phase chromatography can be used for their detection and to evaluate compliance (66, 114). Urinary isoflavone excretion was found to vary among individuals, but these excretion data were similar to values found after acute administration of soyfoods containing 50–100 mg isoflavones. These observations led to the suggestion that the low incidence of many hormone-dependent diseases in countries in which soy is a staple may be related to the exposure to these dietary estrogens (53, 55).

Claims have been made for sex differences in the biotransformation and excretion of soy isoflavones (72), but sample size was insufficient for the statistical power necessary to determine differences based on the known, large, interindividual variability in metabolic handling. No sex differences were apparent in other studies (53, 117). In acute studies of dietary challenge, maximal urinary excretion of isoflavones occurs within 24 h and there is general consensus that no more than 30% of the ingested dose of isoflavone can be accounted for by the concentrations in urine and plasma of omnivores averaged 240 nmol/d (60 mg/d) with large variability, and although concentrations were higher in the feces of vegetarians, overall amounts were relatively low (88, 124). These findings imply that a large fraction of the ingested dose of isoflavones is now unaccounted for by the various isoflavone metabolites being examined, which is consistent with our observations when we examined the pharmacokinetics of daidzein and genistein and the β-glycosides in humans.

Human intestinal flora may degrade the isoflavone nucleus to more simple phenols. In sheep, for example, p-ethylphenol was shown to be a quantitatively important metabolite of genistein (23, 125). In recent studies using HPLC with electrochemical detection (Figure 7), p-ethylphenol was identified in the urine and plasma after administration of pure isoflavones. However, the quantitative importance of this metabolite is unknown (PH Gamache, unpublished communications, 1996). The paucity of data on the metabolic fate of isoflavones is likely to be addressed by the synthesis of chemically inert stable-labeled tracers incorporating isotopes of 13C rather than deuterium (126), for which uncertainty over the chemical and metabolic stability of the isotope may limit interpretation of data. At present, stable-labeled tracers are not commercially available and are expensive and difficult to synthesize.

PLASMA CONCENTRATIONS OF ISOFLAVONES
Few studies have measured circulating concentrations of isoflavones; this reflects the greater difficulty of measurement in plasma compared with urine. Mass spectrometry continues to be the most appropriate method for accurately measuring plasma concentrations of isoflavones over a wide dynamic range (86, 99, 100, 127). With improvements in sensitivity of HPLC detection techniques, particularly with electrochemical detection (PH Gamache et al, unpublished communications, 1996) or the development of immunoassays (128), more convenient and versatile methods will undoubtedly become available in the future. Plasma concentrations of daidzein and genistein have been measured by gas chromatography–mass spectrometry with selected monitoring of specific ions characteristic of the trimethylsilyl ether derivatives (86, 99) or tert-butyldimethylsilyl ethers (127) of daidzein, genistein, and equol. These techniques are based on the methods commonly used for steroid hormone analysis and are highly specific and sensitive.

Studies were performed in which plasma isoflavone concentrations were determined in omnivores and vegetarians and in healthy subjects before and after acute dietary intervention with soy-protein foods (86, 99, 100, 115). Plasma concentrations of daidzein, genistein, equol, and desmethylandroglensin were relatively low and generally <40 nmol/L (10 ng/mL) in humans consuming diets without soy and were considerably higher in vegetarians (99). In the plasma of Japanese men, concentrations of these substances were 7- to 110-fold higher than in a similarly aged group of Finnish men, and in some instances exceeded 2.4 μmol/L (600 ng/mL) (129). When soy was ingested acutely, plasma daidzein, genistein, and equol concentrations increased markedly and values of 0.08–2.4 μmol/L (20–600 ng/mL) were attained, although there was considerable variability in reported values (86, 100, 115).

Early studies of dietary estrogens established that enterolactone, enterodiol, and equol were present mainly as glucuronide conju-
The concentrations of unconjugated isoflavones were relatively low (<0.03 μmol/L, or <8 ng/mL), even after soy intake, reflecting the large capacity for conjugation during their enterohepatic cycling (78, 127). Apparently, no attempts were made to determine the concentrations of isoflavone glycosides in plasma after soy ingestion, presumably because it is generally believed, although not proven, that intestinal hydrolysis of the glycoside is rapid and efficient. Plasma equol was reported to be present in high concentrations in only 4 of 12 subjects challenged with soy flour (86), reaffirming the original observations that about one-third of the general population cannot form equol from a dietary isoflavone challenge (53). Our recent studies indicate that equol is generally not found in the first day after acute studies of soy intake and suggest that residence time in the intestine is an important factor for formation of equol, which appears to be a time-dependent process.

Factors that influence the concentrations of isoflavones in plasma have not been studied to any extent and there are considerable gaps in knowledge of the pharmacokinetics of dietary isoflavones. There have been no age-related differences reported, although limited studies have examined the plasma concentrations in young and old vegetarians and omnivores (99), with no obvious difference in values found in the small sample sizes studied. Most importantly, other factors that might influence bioavailability, such as the effect of the food matrix and the extent of intestinal bacterial fermentation and intestinal transit time, have yet to be examined in any detail.

The chemical composition of the dietary isoflavone may be a key determinant of its bioavailability and extent of biotransformation. Daidzein was reported to be more bioavailable than genistein (115), but the study design used was inappropriate for drawing this conclusion. The absence of isoflavones in plasma 24 h after oral intake of soy, given that the half-life is 7.9 h (127), indicates limitations of the methods used for measurement. It is difficult to determine pharmacokinetics when using a mixed matrix with multiple forms of the isoflavones administered together. The conclusions from this study (115) are at variance with data recently obtained for the pharmacokinetics of the pure compounds daidzein, genistein, daidzin, and genistin (127). Apparent bioavailability determined from the plasma appearance and disappearance curves after single-bolus oral administration of 50 mg of each of the isoflavones was similar for genistein and daidzein, and the glycosidic conjugates were more bioavailable (127). In women, peak concentration (Cmax) range was 300–3200 nmol/L (80–800 ng/mL), but the time to attain Cmax was generally between 6 and 8 h after ingestion (Figure 8). The half-life of plasma disappearance was relatively long at 7.9 h. Similar pharmacokinetics were obtained for adult men. These are impressive concentrations of isoflavones when compared with the endogenous plasma concentrations of estradiol throughout life (Figure 9), and even allowing for the relatively weaker estrogenic activity, it is probable that the concentrations are sufficient to account for the many in vivo and in vitro biological effects that have been attributed to isoflavones.

There have been no reports of the tissue distribution of isoflavones. However, recent studies have shown that when genistein is injected intraperitoneally to rats, it rapidly appears in the brain tissue and in microdialysate fluid from the corpus striata. Shown in Figure 10 are a series of multichannel voltammograms obtained at timed intervals after the intraperitoneal injection of genistein to an adult Sprague-Dawley rat and the concentrations of genistein and its metabolite p-ethylphenol in the microdialysate fluid before and 40 min after administration of this isoflavone (PH Gamache et al, unpublished communications, 1996). These studies indicate that isoflavones can readily cross the blood-brain barrier.

**FIGURE 8.** The plasma appearance and disappearance curves for daidzein (Dz) and genistein (Gs) observed in different women who were given orally 50 mg of the individual isoflavones. Concentrations (ng/mL) are expressed over time in a log versus linear plot to indicate the rate of disappearance. To convert values from ng/mL to nmol/L, multiply by the following: for daidzein, 3.94; for genistein, 3.70.

**FIGURE 9.** A comparison of the range in plasma concentrations (pg/mL) of phytoestrogens (■) after the ingestion of soy protein with the concentrations of circulating estradiol (□) in men and women. Data are shown on a log scale.
growth promoters (134–136) after the long-term deleterious effect of diethylstilbestrol in humans was realized (137, 138). The findings that isoflavone concentrations were several orders of magnitude higher than endogenous estrogens in biological fluids after dietary intake of modest amounts of soy protein raised the question of whether biological effects could also be expected in humans (52, 53).

It was initially believed that phytoestrogen action would be predominantly hormonal, but as this field of study has expanded over the past decade, it has become apparent from the diversity of properties of isoflavones (31–33, 139) that no single action can explain many of the in vivo and in vitro effects of isoflavones, and that these molecules almost certainly exert multiple effects in the same organism. This is consistent with steroid action in general. In vivo and in vitro studies have provided conflicting data about the hormonal actions of isoflavones and lignans (13, 14, 25, 29, 140–146). Observed in vitro effects are consistent with estrogenic action, and the antagonistic action of isoflavones is apparent from the competitive inhibition of estradiol for binding to the estrogen receptor (24–26). Note, however, that most of these studies were carried out many years before the discovery of the new estrogen receptor Erβ (15). On the basis of this rather simplistic view of potential physiologic actions for isoflavones, it was proposed that a soy-containing diet would be beneficial in protecting against or treating breast cancer (53).

Animal studies using classic models of chemically-induced breast cancer showed that a diet containing soy protein significantly reduced tumor formation (123, 147, 148) and that this effect was lost when isoflavones where removed from the soy protein (123). DNA-adduct formation induced by 7,12-dimethylbenz[a]anthracene was also found to be reduced by genistein administration (149). Furthermore, administration of genistein to neonatal rats led to a significant reduction in tumor formation when tumors were chemically induced in rats as adults phase of life (150, 151). These latter findings may indicate that early adaptation to phytoestrogen exposure may confer benefit in later life and could partly explain why the incidence of breast cancer is so much lower in Japan and China, where soy is commonly consumed throughout life, than in Western populations (58, 152–154). Tumor inhibition in these animal models occurred at levels of exposure that could be considered within the range of dietary intake in humans consuming manageable amounts of soy-protein foods.

Many in vitro investigations using several different cell lines, including estrogen receptor-independent cells (36), have confirmed the in vivo findings of tumor suppression by isoflavones, but it has become apparent that the mechanism of action may not be exclusively hormonal (12, 155). Genistein is a specific inhibitor of protein tyrosine kinases (34) and DNA topoisomerases I and II (156, 157) and arrests cell growth by interfering with signal transduction pathways (158–160). Additionally, phytoestrogens exhibit many other activities, including but not limited to antioxidant (161–164), antiproliferative (165, 166), and antiangiogenic (167, 168) activities, and they inhibit the actions of cytokines and growth factors (35, 160, 169, 170), all of which may contribute to the effectiveness of isoflavones as potential anticancer agents. Genistein was also found to inhibit cytokine-stimulated nitric oxide formation (171); in view of the role of nitric oxide in inflammation and carcinogenesis, this could explain the observed reduction in the extent of inflammation in a rat model of prostatitis (172).

Nonsteroidal estrogens in vitro inhibit the activity of the enzyme aromatase (P450arom; 173–176) and stimulate the synthesis of sex hormone binding globulin (177–179). A demonstration of these effects in vivo would provide further support for dietary estrogens in cancer prevention. Peripheral synthesis of estrogens from androgens occurs in adipose tissue, and to limit localized estrogen-stimulated tumor cell growth, aromatase inhibitors are being added to the arsenal of chemotherapeutic agents (180) for breast cancer (181). Sex hormone binding globulin regulates the concentration of circulating free estrogen (182). However, although dietary recall data indicated that vegetarians and subjects with a high urinary excretion of phytoestrogens have higher concentrations of sex hormone binding globulin (177, 183–185), prospective short-term dietary intervention studies using diets rich in phytoestrogens showed no effects on serum sex hormone binding globulin concentrations (86, 112, 186–188).

Without the implementation of large, long-term, prospective clinical trials, which are expensive and time consuming, it will be difficult to definitively prove that phytoestrogens have anti-cancer effects in humans, even though there is now much circumstantial evidence to support this contention. What is clear is that modification of the diet on a regular basis by the inclusion of soy-protein foods containing isoflavones leads to a significant alteration of the hormonal characteristics of the menstrual cycle of premenopausal women (111, 112). These effects include a prolongation of the length of the menstrual cycle, specifically the length of the follicular phase, and a marked suppression in the normal midcycle surges in the gonadotropins luteinizing hormone and follicle-stimulating hormone. Because menstrual cycle length is significantly longer in Japanese and Chinese women and is inversely related to breast cancer risk (189, 190), the effect of dietary intervention with soy protein is considered beneficial in terms of disease prevention (112). The underlying mechanism for these effects is suggested to be hormonal.

The lack of effect of a soy protein devoid of isoflavones (113), similarly observed in earlier animal models of breast cancer (123), supports a role for dietary estrogens in regulating menstrual-cycle characteristics (113). Interestingly, these biological responses to dietary change are similar to the effects of tamoxifen (191, 192), an antiestrogen now in use as a prophylactic for...
the prevention of breast cancer in high-risk women (193). Soy was suggested as an effective nonpharmacologic alternative for disease prevention in such women (111).

There has been considerable interest in the effects of dietary estrogens on prostatic cancer. In vitro studies showed that genistein inhibits the growth of cultured prostate cells (37), and several isoﬂavones and lignans were found to inhibit the activity of the steroid-metabolizing enzyme 5α-reductase in human genital skin ﬁbroblasts and prostatic tissue (194). The activity of this enzyme is important in androgen metabolism and is lower in Japanese men, who have a low incidence of prostate cancer (195). Animal studies conﬁrmed a protective effect of soy against prostatic dysplasia in a mouse model (196), suggesting a possible association with phytoestrogen exposure.

Although the above-mentioned studies in premenopausal women were the ﬁrst to show a hormonal effect of soy protein, the hypocholesterolemic effect of soy was ﬁrst recognized > 30 y ago (reviewed in 197, 198) and seemingly rediscovered recently. A meta-analysis of 38 controlled clinical trials of soy proteins showed signiﬁcant reductions in serum total cholesterol and LDL cholesterol (199). In 34 of the 38 studies evaluated, serum cholesterol concentrations were reduced with an average intake of 47 g soy protein/d. Decreases in serum total cholesterol averaged 0.59 mmol/L (23 mg/dL), equivalent to a mean reduction from baseline of 9.3%. As expected, the extent of reduction is highly dependent on the initial serum cholesterol concentration. Most studies have evaluated the effects of soy protein in patients with hypercholesterolemia, but a mean reduction of 9.6% in serum total cholesterol occurred in premenopausal women with normal cholesterol concentrations after 1 mo of 60 g textured vegetable protein/d (112).

Interestingly, similar reductions in serum cholesterol were observed with tamoxifen therapy (200, 201), highlighting the weak estrogenic action of this potent antiestrogen (202).

Cholesterol homeostasis is exquisitely sensitive to estrogen. This is evident in estrogen-deﬁcient states associated with increases in serum cholesterol concentrations that are correctable by oral estrogen therapy (203). The mechanism of action of soy protein in lowering serum cholesterol has been the subject of extensive investigation over many decades with no clear-cut answers. It is probable that there are numerous explanations for its effects (199, 204), including the lack of cholesterol in soy, which plays a key role in the regulation of cholesterol synthesis (73). Many of the components, some yet to be identiﬁed in soy, have been implicated in its hypocholesterolemic effect. Phytoestrogens may play a role in lowering serum cholesterol (6). In rhesus monkeys, studies using diets made from soy isolates (20%) with and without isoﬂavones showed that phytoestrogens probably contribute to the hypocholesterolemic effect. These studies showed relatively large reductions in total, LDL, and VLDL cholesterol in both male and female animals without any overt effects on the reproductive tract (204–207). These data are compelling but do not conﬁrm conclusively that the effect is solely due to isoﬂavones. Nevertheless, there are other properties of isoﬂavones that are of beneﬁt in reducing risk for cardiovascular disease. Isoﬂavones may slow the development of plaque formation by inhibiting cell adhesion and altering the activity of speciﬁc growth factors, such as platelet-derived growth factor and cytokines, which inﬂuence lesion formation (208). These effects are likely mediated by inhibition of tyrosine kinases. Because oxidative modiﬁcation of LDL is an important mechanism in atherosclerosis, the antioxi-

With cardiovascular disease being a leading cause of death in postmenopausal women, the hypocholesterolemic effect of a soy-protein diet should be of beneﬁt in counteracting the typical increase in serum cholesterol associated with estrogen deﬁciency. A recent study in postmenopausal women showed signiﬁcant reductions in luteinizing hormone, follicle-stimulating hormone, and serum total and LDL cholesterol with a diet that included textured vegetable protein (211). Furthermore, the potential of using dietary estrogens as an alternative to hormone replacement therapy in the postmenopausal period to limit some of the symptoms and effects of estrogen loss is an area under active investigation. It is suggested that the frequency and severity of hot ﬂushes is lower in Japanese than in Western women (212) and that this could be due to their exposure to dietary estrogens from soy protein (121). Several studies examined the estrogenic effects of soy isoﬂavones on vaginal epithelia and on the frequency of hot ﬂushes in postmenopausal women (188, 213, 214). A beneﬁcial effect in reducing the frequency of hot ﬂushes was reported after 6 and 12 wk of consuming a mixed diet containing soy protein (214), but estrogenic effects on vaginal epithelia were less apparent and inconsistent (188, 213, 214). Studies in macaques failed to show any effect of soy isoﬂavones on vaginal cytology when compared with conjugated equine estrogens (215), but this may be because of species differences in the metabolic handling of dietary estrogens or because nonhuman primates are typically exposed to phytoestrogen-rich diets. It is difﬁcult to evaluate the relatively short-term human studies that have been reported thus far, and there is a need for long-term studies to more critically evaluate and compare the potential beneﬁt of dietary sources of estrogen with conventional hormone replacement therapy.

The incidence of osteoporosis and the risk of hip fracture is signiﬁcantly lower in postmenopausal Japanese women than in postmenopausal Western women (216, 217). The discovery of estrogen receptors in osteoblast cells (218, 219) and the importance of estrogen in down-regulating the activity of osteoclasts, thereby limiting bone resorption, is well recognized even though the exact mechanism of action is unclear. Phytoestrogens have the potential for minimizing bone loss in the menopausal period as evidenced by the clinical use of ipriflavone (220), an approved alternative to estrogen replacement for treating acute ovarian-deﬁcient states. This synthetic isoﬂavone interestingly undergoes extensive intestinal bacterial biotransformation to many metabolites, including daidzein (221). Animal studies showed positive effects of isoﬂavones on bone. Genistein was found to have modest bone-conserving properties when administered in low doses to ovariectomized, lactating rats, and although this animal model may not be ideal for humans, interestingly, the effects of genistein were similar to the responses obtained with Premarin (Ayerst Laboratories, New York; 222). Similarly, the lower bone density observed 1 mo after ovariectomy of 95-d-old rats was not seen in animals that were maintained on either estradiol or soybean protein, suggesting that soy protein has the potential to protect against the bone loss associated with ovarian estrogen deﬁciency (223).

Although most studies investigating the health beneﬁts of phytoestrogens have focused on hormone-dependent diseases,
the potential to exploit the nonhormonal actions of isoflavones has also been examined. Several isolated studies showed that isoflavones, and generally genistein, have other potentially useful applications. The antioxidant activity of genistein inhibits oxidative DNA damage induced by ultraviolet light in calf thymuses (164), which might have implications for photocarcinogenesis, and genistein has been found to prevent hepatic injury caused by the release of inflammatory cytokines during ischemia and reperfusion (170). The liver is an initial target site after phytoestrogens are absorbed from the intestinal tract; high concentrations of dietary estrogens are found in portal venous blood (79). In a series of papers, daidzin and daidzein were isolated from the root of the kudzu plant (Radix puerariae), which is an herb used in Chinese medicine to treat alcohol addiction, and found to be potent inhibitors of human mitochondrial aldehyde dehydrogenase (ALDH-2) and alcohol dehydrogenase (γ-ADH) (224–226). It was proposed that these isoflavones might be useful in the treatment of alcohol abuse after studies showed that intraperitoneal daidzin in ethanol-prefering Golden Syrian hamsters suppressed the desire for alcohol (225). Subsequent studies in rats showed that daidzin administration lowered and delayed peak blood alcohol concentrations compared with control animals, but there was no effect on the activities of hepatic alcohol dehydrogenase or aldehyde dehydrogenase activities (226). It is difficult to reconcile the effects of this isoflavone-glycoside, but it was proposed that the effect on gastric emptying was due to the antioxidant activity because similar responses were observed with vitamin E. Although these studies suggest a further potential clinical application for isoflavones, they also highlight differences in response with different routes of administration of isoflavones.

CONCLUSION

Interest in the field of dietary estrogens has exploded in the past 5 y. The evidence showing that these nonsteroidal estrogens have an array of potent biological activities is indisputable, and animal and clinical studies are providing convincing evidence for potentially beneficial effects of a diet containing these compounds. The threshold intake of dietary estrogens necessary to achieve a biological effect in humans appears to be 30–50 mg/d, which is readily attainable by the inclusion of modest amounts of soy protein in the average Western diet. As has happened with many other bioactive substances of plant origin, isoflavones and lignans are now being extracted to provide the consumer with commercial phytoestrogen supplements as an alternative to a soy-protein diet. Although it may be difficult for adults to consume sufficiently large enough quantities of isoflavones from normal dietary sources to cause the type of deleterious effects previously experienced by several animals species (103, 130, 131), there is a distinct possibility of risk associated with the use of these compounds as uncontrolled over-the-counter pharmacologic agents, because estrogens exhibit biphasic responses that are highly dose dependent.

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