Abstract

Steroid-hormone dependent cancers, including those of the breast, prostate and colon, are leading causes of morbidity and mortality in western countries. In rural Asian areas, these diseases are relatively uncommon. Dietary factors, including low consumption of fruit, vegetables and soy in the west have been shown in various epidemiologic studies as reasons for these differences. This review discusses flavonoids, one component of these plant foods that is being investigated for their role in chemoprevention. Epidemiological, in vitro, animal and human studies shall be explored to look at mechanisms involved, including steroid hormone activity, effects on cell growth, antioxidant activities, inhibition of chemical carcinogenesis and influences on modulators of cancer risk. Although the in vitro and animal models point to several pathways by which flavonoids may reduce incidence of these cancers, the clinical data are still relatively lacking. More research is needed to determine how best to use foods containing these compounds to reduce steroid hormone-dependent cancer risk.

Keywords: Reviews; Flavonoids; Steroids

1. Introduction

Steroid-hormone dependent cancers, including those of the breast, prostate and colon, are leading causes of morbidity and mortality in western coun-
tries. In Asian countries, particularly rural areas, these diseases are relatively uncommon. Epidemiological studies contribute these differences in incidence to environmental causes, particularly those of diet and lifestyle. Dietary factors include low consumption of fruit, vegetables and legumes, particularly soy, in the west. Flavonoids are one component of these plant foods being investigated for their role in chemoprevention.

Flavonoids are phenolic compounds characterized by their diaryl nucleus (Fig. 1). They are structurally similar to steroid hormones (Fig. 2), particularly estrogens, and therefore have been studied over the past several years for their potential roles in prevention of hormone-dependent cancers. Over 4000 flavonoids have been characterized in edible plant foods, and are consumed in highest amounts in soy, apples, red wine, tea and onions (Table 1) [1]. Human consumption of all flavonoids has been estimated to be a few hundred milligrams to 1 g per day [2].

This review shall present some of the current knowledge and research available in the field of flavonoids and steroid hormone-dependent cancers. Much work is still needed to firmly establish the chemopreventive applications of flavonoids, and the foods in which they are contained.

2. Epidemiological evidence

The original associations between flavonoids and steroid hormone-dependent cancers were made through several ecological studies looking at the low incidences of breast, colon and prostate cancers in Asian countries compared to western ones. Soy consumption is very high in Asia compared to North America and Europe, and it was found that this food could be negatively correlated with cancer incidence [3–5]. Since these initial studies, many other plant foods have been studied for their associations with these cancers.

Most epidemiological studies have concentrated on whole foods, not components present within them. Through these studies, many interesting associations have been made between vegetable consumption and various cancers. Messina et al. have extensively reviewed the in vivo and in vitro data of soy, showing protective effects in both animal models and epidemiologic studies [6]. Steinmetz and Potter [7] have reviewed over 200 case-control and cohort studies, finding that over the majority of those investigating cancers of the breast, colon, cervix, endometrium and ovary have shown statistically significant decreases in incidence with vegetable and/or fruit consumption [8–60]. The only site for
which such a relationship was not found was the prostate [60–64]. However, a Seventh-Day Adventist study, involving over 34,000 vegetarians and omnivores, found that vegetarians had significantly reduced risks of colon and prostate cancer rates.

Epidemiological studies looking specifically at certain flavonoids are becoming more important, as databases and techniques for quantifying consumption of these compounds are improving [61–66]. Flavonoids present in highest amounts in the human diet include the soy isoflavones genistein, daidzein and biochanin A, flavonols quercetin, myricetin and kaempferol, and the flavones luteolin and apigenin [2,67]. One case-control study used this new database [68] to look at 83 prostate cancer patients and 107 age-matched controls. They found that after adjustments for total calories, greater consumption of most phytoestrogens, including isoflavones and other flavonoids, had a slightly protective effect on prostate cancer risk. Moreover, genistein, daidzein, and coumestrol (a structurally-related compound) showed the strongest protective associations. However, the overall intake of the isoflavones was very low compared to Japanese men (1.2 mg versus 39.2
mg/day). Some studies have reported weak protective effects of flavonoids from fruits and vegetables and prostate and colon cancers [67,69]. Others have shown no associations between flavonoid intake and cancer mortality or incidence [70,71]. At present, relationships between whole foods, i.e., vegetables and soy, show much stronger chemopreventive associations than the flavonoids that they contain.

3. In vitro and animal models

Over the last few years, various mechanisms by which flavonoids may prevent, or even treat steroid hormone-dependent cancers, have been investigated. These include activities at the tissue, cellular, and even sub-cellular levels.

3.1. Estrogenic activity

Estrogenic effects of flavonoids were first discovered through determining the cause of infertility in Australian sheep eating red clover. The leaves contain up to 5% by dry mass of the soy isoflavones biochanin A and formononetin [72,73]. Of the flavonoids, the soy isoflavone genistein has remained the model “phytoestrogen”. Genistein is structurally similar to estradiol, other steroid hormones (testosterone, progesterone), and synthetic inhibitors including tamoxifen. This isoflavone possesses 1/1000 the estrogenic activity of estradiol [74–76]. Circulating concentrations in individuals consuming moderate amounts of soybeans are nearly 1000-fold higher than peak levels of endogenous estradiol in premenopausal women [77–79]. Genistein and other soy isoflavones have been shown to compete with radiolabeled estradiol for binding to the estrogen receptor [80]. This may be because of the structural similarity between the steroid nucleus of 17β-estradiol and the polyphenolic ring structure of isoflavones. Since other plant-derived flavonoids also possess this structure, they too have become relatively well-studied for estrogenic and anti-estrogenic activities. In contrast, androgenic activities of flavonoids have only recently started being investigated. The data obtained from these studies support important applications for these compounds and the

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<tr>
<th>Flavonoid</th>
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<td>Biochanin</td>
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<td>Genistein</td>
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<td>Quercetin</td>
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<td>Quercetin</td>
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foods in which they are found, in prostate cancer prevention.

Many assays have been utilized to determine steroid hormone activity. These include radiolabeled binding assays, measuring steroid-regulated proteins or reporter proteins and Southern and Northern blotting techniques. Zava and Duwe have determined estrogenic and anti-estrogenic activities of several flavonoids, including genistein, equol, kaempferol and hesperetin using two methods in estrogen receptor (ER)(+) and ER(−) breast cancer cell lines [81]. One measured [125I]estradiol binding to ER in the nucleus in the absence or presence of increasing concentration of flavonoid, from 100 pM to 10 μM. The second technique involved measuring the estrogen-regulated, secreted protein, pS2, after incubating the cells with flavonoid at the above concentrations for 72 h. In both assays, genistein and equol, the metabolite of the soy isoflavone daidzein, were found to have the highest binding affinities, 0.1 that of estradiol. Next came kaempferol (0.012), and finally quercetin (0.001). Hesperetin and β-naphthoflavone did not have blocking activity. All of the compounds found to bind to the ER in the previous study stimulated pS2 production, in the same order as their binding activity. Blocking of pS2 production by tamoxifen further confirmed that binding to the ER was the mechanism observed. Similar results for genistein and other flavonoids were seen through measuring pS2 and/or cathepsin-D transcription [80,82–84]. Our group [85] has evaluated estrogenic activity of over 70 flavonoids and structurally-related compounds using pS2 concentrations in the supernatant as the endpoint. Structure–function relationships were discussed in this study, with the diphenolic structure, and positions of hydroxyl groups residing in the C-7 or 4′ positions (Fig. 1) being most important for estrogenic activity. Flavonoids with hydroxyl groups at the C-3 position, such as quercetin, hinders binding, as is reflected by the low estrogenic/binding affinity in both studies. At least two other studies, using different systems, concur with these results. Miksicek assessed estrogenicity of flavonoids (at 1 μM) using HeLa cells transfected with wild-type, recombinant estrogen cDNA expressed from the plasmid pER-18, and an estrogen-responsive reporter plasmid system measuring production of CAT (pERE-TK-CAT). Genistein displayed highest activity, with significant activities also being seen with kaempferol, apigenin, biochanin A and daidzein. The importance of hydroxyl groups at carbon positions 4′, 7 and/or 6 was shown here, as all compounds determined to have estrogenic activity possessed hydroxyl groups at one or more of these positions [86]. Binding to the ER was confirmed through competition assays with [125I]estradiol. Le Bail et al. used a stably-transfected MCF-7 cell line with a luciferase reporter gene and ERE to determine estrogen agonist and antagonist activities. Significant agonist activities were seen for compounds containing 7 and/or 4′ hydroxyl groups at concentrations as low as 1 μM. Moreover, they determined that phenolic hydroxyl groups in positions 4′ and 7 could be considered to be equivalent to the position 3 and 17-hydroxyl groups of estradiol [87]. These results were also confirmed using a recombinant yeast strain stably transfected with the human ER gene, with upregulation of this gene being the measured endpoint [88]. Finally, resveratrol, a red wine polyphenol, not a flavonoid, but possessing a similar structure, has also been shown to bind to ER in the [125I]estradiol competition assay, and to induce luciferase production in both MCF-7 and T47-D cell lines transfected with ERE-tk109-luc or ERE2-tk109-luc plasmids. Resveratrol also increased progesterone receptor mRNA expression and pS2 expression in MCF-7 cells at 10 μM concentrations [89].

Several researchers have investigated the androgenic and anti-androgenic activities of flavonoids. None have found these compounds to have agonist androgenic activities, but most have observed anti-androgenic [90] and/or inhibition of androgen-mediated activities. Our group evaluated inhibition of prostate-specific antigen (PSA) production in an ER (+), AR (+) breast cancer cell line (BT-474) and prostate cancer cell transfected with the human AR [PC-3(AR)+]. Eighteen of the 72 flavonoids and related compounds tested demonstrated such inhibition of PSA production at concentrations of 10 μM. Several compounds, including genistein and biochanin A, had blocking effects on PSA production at concentrations as low as 0.1 μM [91,92]. Davis et al. examined modulation of PSA expression in the LNCaP (androgen-dependent) VCaP (androgen-independent) prostate cancer cell lines by genistein.
This isoflavone had differential effects on PSA expression in the two cell lines. PSA mRNA and protein expression and secretion were suppressed in the LNCaP cell line at all concentrations, while only high concentrations of genistein inhibited PSA expression in VeCaP. Inhibition of cell proliferation in VeCaP was independent of PSA signaling pathways, leading the authors to conclude that the anti-proliferative effects of genistein were irrespective of androgen responsiveness in this androgen-refractory cell line [93].

Resveratrol, in concentrations of 50, 100 and 150 μM, was given to LNCaP cells in the presence or absence of 1 nM mibolerone, a non-metabolized, synthetic androgen, to evaluate expression of two androgen-regulated proteins, PSA and human glandular kallikrein 2 (hK2), as well as AR. The cells were transfected with either a PSA promoter-luc, hK2 ARE/minimal thymidine kinase promoter/CAT or an AR promoter-luc construct. Resveratrol treatment completely abolished androgen-induction of the PSA promoter, hence inhibiting production of any PSA. Transfection with triple ARE constructs demonstrated similar results. Western blotting of AR protein levels indicated a dose- and time-dependent inhibition of AR with resveratrol, and ARA70, a co-activator, was found through Northern blotting to decrease, with a maximum reduction using 100 μM resveratrol. These results indicate that AR-mediated gene expression and cell processes are affected by resveratrol, and, moreover, repression of expression of ARA70 may further enhance inhibitory effects of this red wine polyphenol on androgen action [94]. Green tea polyphenols have been shown to have similar repressive effects on androgen action in mice [95].

3.2. Cell growth

Many studies have investigated proliferative effects of flavonoids, and mechanisms by which stimulation or inhibition of cell growth may occur. Several experiments have examined the biphasic effects of genistein and other isoflavones, which in ER(+) MCF-7 and T47-D cells stimulate cell growth at concentrations of 10 nM–10 μM, and inhibit proliferation at concentrations of 50–100 μM [81,83,87,96–98].

Over 30 flavonoids have been tested for effects on cell proliferation and potential cytotoxicity, in a variety of cell lines, including colon cancer cell lines Caco-2 and HT-29, and the breast cancer cell line MCF-7, for their influence on proliferation and apoptosis, measured through caspase-3. EC₅₀ values ranged between 40 and 200 μM. In almost all cases, inhibition by flavonoids occurred in the absence of cytotoxicity. Only baicalein, myricetin and flavone were able to induce apoptosis in Caco-2 and HT-29 cells. No alterations of cell growth have been seen for taxifolin, hesperitin or catechin in the HT-29 cell line [99,100]. In the breast cancer cell lines tested, 7-hydroxyflavone, 7,8-dihydroxyflavone and kaempferol did not alter proliferation either [87].

Other flavonoids that are inhibitory at high concentrations in both ER(+) and ER(−) breast cancer cell lines include quercetin, luteolin, biochanin A, coumestrol, daidzein, kaempferol and apigenin [84,97,101]. None were shown to work through cytotoxic mechanisms. However, when MCF-7 cells were exposed to 50 or 100 μM genistein continuously for up to 10 days, DNA synthesis was strongly inhibited from days 1–4, after which time cytotoxicity occurred [97]. In T47-D breast cancer cells, 20 μM genistein, quercetin and kaempferol markedly inhibited growth. Only quercetin was inhibitory over the entire concentration range of 100 nM–20 μM. At the highest concentrations, extensive chromatin fragmentation was observed, suggesting apoptosis [96]. This is similar to short-term effects of flavone, which has been observed, through semi-quantitative reverse transcription polymerase chain reaction (RT-PCR), to inhibit cell proliferation through apoptosis. Flavone was highly selective towards transformed cells only, and thus may be important for chemotherapeutic uses in colon cancer [100].

No obvious structure–function relationships have been observed for effects on proliferation, either for type of flavonoid (isoflavone, flavone, flavanone) nor hydroxylation pattern [87,99].

At concentrations greater than 25 μM, the soy isoflavones genistein, biochanin A, equol, and to some extent daidzein have been shown to modulate the proliferative activity of environmental carcinogens, including o,p′-DDT, 4-nonylphenol and 5-ctylyphenol. Genistein was the most potent, with an IC₅₀ of 25–33 μM [102]. Therefore, diet-derived
flavonoids may be protective against environmental xenoestrogens.

Inhibition of cell proliferation by genistein and other flavonoids, have been shown to be associated with specific arrest of the cell cycle. Genistein treatment of MCF-7 cells (10 \( \mu M \)) causes reversible arrest at the G2/M phase of the cell cycle [103]. Quercetin arrests the cell cycle at G1 and S-phase boundary (Table 2). Moreover, the two flavonoids together have been shown to synergistically inhibit growth in OVCAR-5 ovarian cancer cells [104]. One mechanism of cell cycle arrest and apoptosis by apigenin, luteolin and quercetin has been shown to be through wild type p53 accumulation [105]. These results may be of interest in clinical treatment of breast and ovarian cancers.

3.3. Antioxidant activities

Flavonoids have been shown to have both antioxidant and pro-oxidant activities in vitro, and in animal models. Activities and structural requirements for these activities have been determined by several investigators. However, conflicting potencies have been reported, dependent on the assay and methods used. Using an oxygen radical absorbance capacity assay, and three different reactive species—a peroxyl radical generator, a hydroxyl radical generator, and Cu\(^{2+}\), a transition metal—Cao et al. determined both antioxidant and pro-oxidant activities of various flavonoids, and structure–function relationships. They found that both antioxidant and Cu\(^{2+}\) pro-oxidant activities were dependent on the number of hydroxyl substitutions, with compounds that contained multiple hydroxyl groups showing anti-peroxyl radical activities several times stronger than Trolox, an \( \alpha \)-tocopherol analogue. Dihydroxyl substitutions at C-3’ and 4’ were particularly important to peroxyl radical absorbing activity. O-Methylation of hydroxyl groups inactivated both antioxidant and pro-oxidant activities [106]. Flavonoids including naringenin, naringin, hesperetin and apigenin were also found to form pro-oxidant metabolites that oxidized NADH and glutathione upon oxidation by peroxidase/hydrogen peroxide. The implications of pro-oxidant activities of flavonoids is uncertain [107,108].

The importance of the 4’ hydroxyl group and 5’,7 dihydroxy was determined through examination of antioxidant activity in the aqueous phase through ABTS+ total antioxidant activity assay (Fig. 1). The order of reactivity for isoflavones in radical scavenging was genistein>daidzein, genistin, biochanin A, daidzin. No activity was seen for formononetin or ononin [109]. Examining other flavonoids, quercetin, cyanidine, which contain 3’,4’-dihydroxy substituents and conjugation between A and B rings have antioxidant activities fourfold greater than that of Trolox. Kaempferol, which does not have the \( ortho \)-dihydroxy substitution, and catechin and epicatechin, which do not possess the 2,3 double bond, have greater than 50% reduction of antioxidant activity. Free radical scavenging was highest with EGCG, then quercetin (50% less effective). Genistein, daidzein, hesperetin and naringenin were not effective [110].

Hydrogen donating ability of a range of phytoestrogens were assessed using electron spin resonance spectroscopy, the ferric-reducing ability of plasma assay and Trolox equivalent antioxidant capacity. The ability of compounds to inhibit lipid peroxidation was also examined in vitamin E-deficient liver microsomes. Only kaempferol was found to have significant antioxidant activity in this study. Genistein had the highest activity of the isoflavones, however, isoflavones as a group were relatively poor hydrogen donors. Equol and coumestrol had higher potencies than isoflavones, but they too were weak [111,112]. Inhibition of microsomal lipid peroxidation by isoflavones and metabolites were found to have the relative activities of isoflavane>isoflavonone> isoformononin >isoformoninone [113]. In 12-\( \beta \)-tetradeoxy canoyl phorbol 13-acetate (TPA)-activated HL-60 cells, genistein had potent activity against hydrogen peroxide. Daidzein had moderate antioxidant effects,
and apigenin and biochanin A had no effect. In contrast, genistein, apigenin and prunectin were equally potent in inhibiting superoxide anion generation by xanthine/xanthine oxidase. For both systems, hydroxyl substitution at the 4’ position was important [114].

3.4. Carcinogenesis

Flavonoids have been demonstrated to reduce carcinogenesis in animal models and to modulate enzymes implicated in the carcinogenic process. Their effects on initiation and promotion stages of the carcinogenic process have been evaluated, and several mechanisms have been proposed, including influences on development and hormonal activities.

The initial animal work was performed by Troll et al., who demonstrated a significant reduction of mammary cancer by X-irradiation in Sprague–Dawley (SD) rats fed a raw soybean diet, compared to those fed casein (44% developed tumors versus 74%). Although this protection was attributed to protease inhibitors present in soy, other compounds, including isoflavones could have been at least partially responsible [115]. Messina et al. have conducted many animal studies using soy and isoflavones in chemical carcinogenesis models of mammary and other tumors. The majority of these show protective effects [6].

The soy isoflavones genistein, daidzein and biochanin A, as well as soy protein and soy milk have all been used to determine effects on mammary carcinogenesis. When female SD rats were fed a rat soybean diet containing 10% fermented soy milk or 0.02% or 0.04% isoflavone mixture during and after 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) exposure, mammary tumors were significantly smaller, and tumor multiplicity significantly lower than controls [116]. Similarly, when SD rats were fed a 10% soybean or 10% miso supplemented diet, or were given 10 or 50 mg/kg body mass biochanin A for 18 weeks, tumor multiplicity was significantly decreased. The 50 mg/kg biochanin A dose also significantly reduced tumor incidence compared to control (32 versus 80%), and proliferation cell nuclear antigen labeling index. When MCF-7 cells or MDA-MB-468 cells were treated with 15, 30 or 45 μM genistein, then implanted into a nude mouse xenograft model, the tumorigenic potential of the cells was diminished [117]. Not all studies agree with these results. SD rats fed one of four purified diets containing casein or soy with 0.03, 0.4 or 0.81 mg isoflavones for 2 weeks, then treated with 7,12-dimethylbenz[a]anthracene (DMBA) or peanut oil, showed only a trend (P<0.09) for an inverse relationship between tumor incidence and isoflavone intake [118]. Cohen et al. found no significant inhibition of nitrosomethylurea (NMU)-induced mammary carcinogenesis for rats taking soy protein-supplemented diets [119].

Timing of isoflavone treatment may be important. SD rats were exposed to 0, 25 or 250 mg genistein/kg AIN-76A diet from conception to day 21 post-partum. At day 50 post-partum, all were treated with 80 mg DMBA/kg body mass to induce mammary cancer. Once sacrificed, it was found that genistein feeding resulted in a dose-dependent protection against the development of mammary tumors, with fewer tumors per rat. Moreover, it was found that the mice given genistein had fewer terminal end buds (TEBs), which are mammary structures most susceptible to carcinogenesis [120]. These results were confirmed in a similar study [121]. 500 μg/g body mass genistein given subcutaneously to prepubertal female SD rats prior to DMBA administration at day 50 also resulted in fewer TEBs and more lobules [122]. It was concluded that genistein could suppress the development of chemically-induced mammary cancer without reproductive or endocrinological toxicities [123].

Genistein, daidzein, their glycosylated forms (genistin and daidzin), as well as soy products have also be protective against early stages of 3,2’-dimethyl-4-aminobiphenyl (DMAB)-induced prostate cancer [124], and azoxymethane (AOM)-induced colon cancer in rat models [125]. Therefore, although not all studies show chemopreventive effects, the vast majority indicates that soy isoflavones and food sources may be useful in preventing steroid hormone-dependent cancer development.

Other flavonoids evaluated for anticarcinogenic effects in the colon include quercetin, rutin and hesperidin. Female CF1 mice were treated with 0.1, 0.5 or 2.0%/diet quercetin, or 1.0 or 4.0%/diet rutin for 50 weeks to assess inhibition of AOM-induced colonic neoplasia. No alterations were seen for mice
fed flavonoid-supplemented diets without AOM treatment. However, among AOM-treated mice, both 2.0% quercetin and 4.0% rutin significantly inhibited hyperproliferation and the shift of S-phase cells to middle and upper portions of the crypts. Moreover, significantly fewer focal areas of dysplasia (FADs) were found with both of these treatments, and 2.0% quercetin reduced tumor incidence. 4.0% rutin showed a trend for lower tumor incidence [126]. A second study performed by this group fed quercetin supplementation of 0.5, 2.0 or 5.0%, or 2.0% or 4.0% rutin with 5% or 20% corn oil to female CF1 mice for 9 weeks. 80% of quercetin-fed mice on the high fat diet remained free of FADs, compared to 29% of those on the high fat control diet (P<0.01). For rutin, the difference was bordering significance (P<0.08) [127]. Similar results for AOM-induced mice have been shown by other groups [128]. Quercetin- and rutin-supplementation in normal mice have also been shown to increase numbers of colonic epithelial cells per crypt column, apoptotic index and redistribution of apoptotic cells along the crypt axis. Quercetin alone has also been observed to induce FAD in normal mice fed AIN-76A diets [126].

Hesperidin, the major flavanone in orange juice has been shown to inhibit chemically induced colon carcinogenesis [129], and double strength orange juice slows down DMBA-induced mammary cancer in rats [130]. Based on these studies, Miyagi et al. tested the hypothesis that not-from-concentrate orange juice given to male F344 rats instead of drinking water could inhibit AOM-induced colon cancer. Cancer was initiated by two subsequent injections of AOM at 22 and 29 days of age, and on day 35, half of the rats were put on orange juice-modified diet to equilibrate the macronutrient profiles between the two groups. At 33 weeks of age, the rats were sacrificed and colons analyzed. A 22% reduction in tumor incidence was found for the group fed orange juice compared to control (P<0.05), with a trend towards smaller average tumor burden (P=0.13). The labeling index and proliferation zone were lower, and enhanced cell differentiation was found in the bottom two-thirds of the crypt [131]. Pure hesperidin and diosmin, also found in citrus fruits, were fed for 5 weeks (initiation treatment) or 28 weeks (post-initiation treatment) at levels of 1000 ppm each or in combination (900+100 ppm) to AOM-induced male F344 rats. At the end of 32 weeks, incidence and multiplicity of neoplasms with or followed by flavonoid treatment were significantly smaller than with AOM alone (P<0.001). The combination of the two flavonoids was not better than each alone. Both significantly inhibited development of aberrant crypt foci, and were significantly associated with lower labeling index and other markers of carcinogenesis [129]. These studies indicate that flavonoids, either purified or within foods, are chemopreventive against colon carcinogenesis.

4. Influences on modulators of risk for steroid hormone-dependent cancers

Few clinical trials have been conducted to determine the influences of flavonoids on risk factors for steroid hormone-dependent cancers. Most of those that have been conducted, have looked at potential modulation of breast cancer risk factors by soy isoflavones. The results of these studies are conflicting, and confusing.

Urinary excretion of isoflavones have been assessed in many studies as a marker of isoflavone consumption. To determine whether this method is reliable, several groups have correlated database calculations for isoflavones based on self-reporting and urinary isoflavone excretion. All have shown very significant correlations, along a broad range of intakes [132–135]. Therefore the use of food frequency questionnaires for measurements of soy intake appear to be relatively reliable.

Risk factors that have been associated with decrease in risk of breast cancer include lower plasma concentrations of ovarian hormones, higher levels of sex hormone binding-globulin (SHBG), longer menstrual cycle, particularly the follicular phase, and high urinary isoflavone excretion. Therefore, many of the clinical trials conducted were to determine the effects of soy feeding on these endpoints. In one of the initial soy feeding studies, Cassidy et al. examined the effects of soy feeding on ovarian cycle. A 60-g amount of soy protein, providing 45 mg isoflavones/day significantly (P<0.01) increased follicular phase length and/or delayed menstruation. Moreover, midcycle surges of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were
significantly suppressed, and plasma estradiol concentrations in the follicular phase increased. These results were exciting, as similar responses have been seen with tamoxifen, when used as a prophylactic agent against breast cancer [136].

Since then, many other clinical trials have been conducted. Lu et al. have examined the modulation of soy feeding on these parameters in several studies. In one study, 10 healthy, cycling premenopausal women were given 36 ounces of soy milk (equivalent to 113–207 mg isoflavones/day) for 1 month. A 25% reduction in estradiol, and a 45% reduction in progesterone, were found, both highly significant (P<0.01, P<0.0001, respectively). No changes in LH or FSH were found [137]. However, in a randomized cross-over study, using soy protein powders with low isoflavone (64 mg/day), high isoflavone (128 mg/day) or control, for three cycles plus 9 days, the low isoflavone diet significantly decreased LH (P=0.003) and FSH (P=0.04), during the peri-ovulatory phase. The high isoflavone diet also reduced free thyroxine and dehydroepiandrosterone sulfate (DHEAS) during early follicular phase. Besides estrone, which decreased during midfollicular phase, no other significant changes in hormones or length of cycle were found [138]. Several other feeding studies have shown similar conflicting results [139–141]. Length of treatment and levels of soy do not seem to influence results.

Higher levels of sex hormone binding globulin have been associated with decreased breast cancer risk. This plasma protein binds to steroid hormones (estrogens and androgens), thereby “inactivating” them, i.e., only free steroids exert their effects. Therefore, SHBG is one mechanism through which steroidal activity can be modulated [142]. Significant positive correlation between urinary excretion of phytoestrogens and plasma SHBG, and negative correlation between SHBG and excretion of 16α-hydroxyestrone (see below) and estriol have been found [143]. These results suggested that isoflavones may affect uptake of sex hormones by regulation of plasma SHBG levels and influence biological activity. However, other studies, do not support this hypothesis [136,144].

Higher levels of two putative carcinogenic metabolites of estradiol have also been implicated in breast cancer risk. These are 4-hydroxyestrogen and 16α-hydroxyestrogen. Lower amounts of the anticarcinogenic metabolite 2-hydroxyestrogen are also associated with greater breast cancer risk. In order to determine whether a soy diet may alter the metabolism of 17β-estradiol to these products, eight women were placed on the soy milk-supplemented diet described above for one complete menstrual cycle. After a 4-month washout, they were then given an isoflavone-free soy milk supplement, providing less than 4.5 mg isoflavones/day. It was found that the isoflavone diet increased mean daily urinary excretion of 2-hydroxyestrone by 47% (P=0.03), but had no effect on 16α-hydroxyestrone production. Because the ratio of 2:16α also significantly increased, it was suggested that isoflavones, through increased metabolism of endogenous estrogens to the protective 2-hydroxy form, may be important in lowering 17β-estradiol levels [145].

The role of ethnicity has been a consideration for breast cancer development, as Asian women have significantly lower incidence. Hence, Wu et al. placed 20 premenopausal women, 10 Asian–American, 10 non-Asian American, on a 7-month soy intervention study. Asian soy foods, including tofu, soy milk and green soybean peas, providing 32 mg isoflavones/day, were added to a basal diet for three menstrual cycles. The intervention was found to significantly reduce estradiol in the luteal phase in the Asian women only. Moreover, these women had higher excretion levels of isoflavones, compared to their non-Asian counterparts. It was concluded that perhaps genetics does play a role in the metabolism of these compounds, and thus modulation of breast cancer risk factors [146]. Opposing this theory, is a study of 31 premenopausal Japanese women, fed 400 ml soymilk per day, providing 109 mg isoflavones, for three consecutive menstrual cycles. Although estrone and estradiol levels decreased 23 and 27%, respectively, in the soymilk group, these were not statistically significant [147]. Perhaps a longer study would have enabled these results to reach significance.

Another parameter that may be important in determining breast cancer risk is the profile of breast secretions. Petrakis et al. hypothesized that long-term consumption of commercial soy protein products would alter features of nipple aspirate fluid (NAF) of non-Asian women to resemble those previously characterized in Asian women. Twenty-four normal pre- and postmenopausal Caucasian women under-
went this year-long study. For months 1–3 and 10–12, they did not eat any soy products. Between months 4 and 9, these women consumed 38 g of soy protein isolate, (SPI) containing 38 mg of genistein. NAF volume, gross cystic disease fluid protein (GCDFP-15) concentration and NAF cytology were used as biomarkers of possible effects of soy protein isolate on breast. Other measurements taken were plasma concentrations of estradiol, progesterone, SHBG, prolactin, total cholesterol, HDL-C and triglycerides. Compliance was measured through urinary excretion of genistein and daidzein, and was excellent throughout the study [148].

Compared to months 1–3, there was a 2–6-fold increase in NAF volume in months 4–9 for all premenopausal women, and no change in postmenopausal subjects. No changes were seen in any of the plasma parameters for postmenopausal women. However, in the premenopausal group, plasma estradiol levels erratically increased during soy consumption, and there was a moderate decrease in GCDFP-15 levels. What was surprising to investigators, and of potential concern, was the cytological detection of epithelial hyperplasia found in seven of 24 women (29.2%) during months of SPI consumption. These results indicate that prolonged consumption of SPI has stimulatory effects on premenopausal breast, suggestive of an estrogenic stimulus [148]. These data were further supported by a randomized trial examining the effects of 60 g soy supplement on the proliferation rates of premenopausal, histologically normal breast epithelium of 48 women with benign or malignant breast disease. One group was given a normal diet, and the other given diet + soy supplement for 14 days. Biopsy samples of normal breast were labeled with [3H]thymidine to detect the number of cells in S-phase, and were measured for the proliferation antigen Ki67. The proliferation rate of the breast lobular epithelium significantly increased after the 14-day soy supplementation, when both day of cycle and age of patient were accounted for. PR levels also increased [149]. Increases in pS2 concentrations in NAF have also been found [150]. These studies, taken together, demonstrate that both short-term and long-term soy feeding may provide a stimulus for proliferation in the breast. The implications of this remain unknown.

Very few studies have looked at men. We examined the effects of feeding 33 g/day soy, providing 86 mg isoflavones/2000 kcal/day, to 31 men and postmenopausal women. Ex vivo hormone activity showed no alterations in androgen (through PSA) or estrogen (pS2) activities [151]. Many more studies must be done before we can decipher the data thus far, let alone determine the effects of other flavonoids in steroid hormone-dependent cancers.

5. Discussion

Many mechanisms have been proposed for how flavonoids may help prevent steroid hormone-dependent cancers and several have been validated in vitro and in animal models. These include modulation of steroid hormones, inhibition of proliferation, and anticarcinogenic and antioxidative activities. However, the physiological implications are questioned by most. For most animal models, doses tested are significantly higher than those that would exist in human plasma after consumption of whole foods. Nutraceutical or functional food administration has thus been suggested as possibilities for reaching such concentrations.

Randomized clinical trials are still in their infancy in this area. To date, only soy has been tested in this capacity, dealing specifically with breast cancer risk factors. Though several studies have been done, these are very conflicting. Little data exist on other flavonoids or other cancer types. At this point in time, we are still in the dark. We cannot be sure that consumption of soy will have positive, neutral or even negative effects of breast cancer risk. Advising women at high risk for breast cancer, or those who have this disease and want alternate therapies is not feasible at present.

References
