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Review

Phytosterols as anticancer compounds

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Phytochemicals have been proposed to offer protection against a variety of chronic ailments including cardiovascular diseases, obesity, diabetes, and cancer. As for cancer protection, it has been estimated that diets rich in phytochemicals can significantly reduce cancer risk by as much as 20%. Phytosterols are specific phytochemicals that resemble cholesterol in structure but are found exclusively in plants. Phytosterols are absorbed from the diet in small but significant amounts. Epidemiological data suggest that the phytosterol content of the diet is associated with a reduction in common cancers including cancers of the colon, breast, and prostate. The means by which dietary phytosterols may be achieving these effects is becoming clearer from molecular studies with tumorigenic research models. Phytosterols affect host systems potentially enabling more robust antitumor responses, including the boosting of immune recognition of cancer, influencing hormonal dependent growth of endocrine tumors, and altering sterol biosynthesis. In addition, phytosterols have effects that directly inhibit tumor growth, including the slowing of cell cycle progression, the induction of apoptosis, and the inhibition of tumor metastasis. This review summarizes the current state of knowledge regarding the anticancer effects of phytosterols.

Keywords: Anticancer / Cancer / Food / Nutrition / Phytosterols

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1 Introduction

Phytochemicals have been proposed to offer protection against a variety of chronic ailments including cardiovascular diseases, obesity, diabetes, and cancer. As for cancer protection, it has been estimated that diets rich in phytochemicals can reduce cancer risk by 20% [1]. Just what these specific phytochemicals are and how they exert their anticancer effects are really uncertain. However, answers to these questions are beginning to be learned. This is in large part because of scientific research, both population studies in which controlled or observed diets are statistically evaluated for their linkage to the incidences of various cancers and laboratory studies in which specific phytochemical compounds are examined for effects in animal and tissue culture models of cancer initiation and cancer progression.

Phytosterols are specific phytochemicals that resemble cholesterol in structure but are found exclusively in plants. Phytosterols have been studied both for their cholesterol-lowering effects and their anticancer properties. This review summarizes the current state of knowledge regarding the anticancer effects of phytosterols, starting with an examination of phytosterol sources in specific foods, phytosterol structures, and metabolism in humans and then moving on to review the experimental evidence supporting their anticancer properties, and finally describing the cellular and signal transduction processes that dietary phytosterols have within human tissues that might account for their anticancer properties.

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Abbreviations: BPH, benign prostatic hypertrophy; **NK,** natural killer; **NPC1L1,** Niemann–Pick C1-Like 1 protein; **OR,** odds ratio

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2 Structures, sources, and metabolism of phytosterols

2.1 Phytosterol structures

Phytosterols are derivatives of the parent molecule 4-desmethyl sterol [2]. More than 200 phytosterols exist naturally

Table 1. Variations in the structure of sterols

Sterol	Number of carbons	Side chain double bonds
Cholesterol	27	0
Campesterol	28	0
β-Sitosterol	29	0
Stigmasterol	29	1

in the plant kingdom and many are found in edible foodstuffs [3]. The most common phytosterols in the human diet are β-sitosterol, campesterol, and stigmasterol (Fig. 1). The concentrations of these phytosterols vary among food groups but a typical distribution of phytosterols in common plant foods as reported in the Food Chemical Codex monograph for sterols consists of 50–65% β-sitosterol, 10–40% campesterol, and 0–35% stigmasterol [4]. Minor sterols, including brassicasterol and avenasterols, can compose up to a few percent of sterols in specific foods such as rapeseed, pistachio, and ginseng oils. The chemical differences among the common 4-desmethyl sterols reside in the number of carbon atoms in the carbon-17 branch chain (either 8 or 10) and in the presence or absence of a double bond at position 22 (Table 1). All phytosterols contain one double bond at carbon-5. Saturation of this double bond either enzymatically by plants *in vivo* or by hydrogenation in industry, results in the formation of stanols. The two most common stanols are β-sitostanol and campestanol.

2.2 Sources of phytosterols

Phytosterols exist within plants in both esterified and free alcohol forms. The *in vivo* esterification process involves condensation with either a fatty acid, ferulic acid, or a sugar molecule to the hydroxyl group on carbon-3 of the phytosterol molecule. There is an uncertainty as to what proportion of phytosterols exists as esters in nature. Some investi-

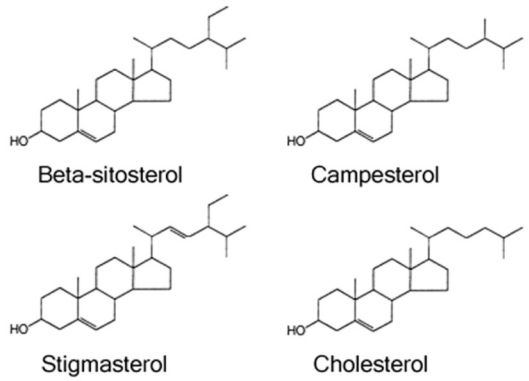

Figure 1. Structures of common dietary phytosterols and cholesterol.

gations have reported that more than half of sterols in some edible plant oils are esterified [5]. Other reports show that free sterols compose from 32 to 94% of the total sterol content of edible oils [6]. The extent of sterol esterification is affected by several factors including the time of the growth season during which analyses are performed, the processing methods used in extracting and refining the oils, seed stratification, and the variety of the plant source. The existence of the free form, particularly after human ingestion, is significant since the free sterols are usually associated with cell membranes, a location in which they may influence the functional properties of membranes. Esterified sterols on the other hand constitute a storage form of phytosterols and are usually associated with triglycerides in intracellular and extracellular lipid depots.

Phytosterols are enriched in high lipid content plant foods such as nuts, legumes including peanuts, and seeds such as sesame seeds. The concentrations of total phytosterols in specific foods are given in Table 2. Fruits and grains also contain abundant phytosterols (1–50 mg/100 gm) although at lower concentrations as compared to nuts, legumes, and seeds [7]. Some grain products such as wheat

Table 2. Concentration (mg/100 g) of major phytosterols in high source foods

	β-Sitosterol	Campesterol	Stigmasterol	Total phytosterol
Oils				
Olive oil	117	5	ND	145
Peanut oil	153	23	13	189
Soybean oil	221	58	67	346
Sesame oil	367	77	28	473
Nuts, legumes, seeds				
Walnut	114	5	2	121
Peanuts	47–133	6–18	7–10	64–161
Almond	143	5	5	153
Sesame seeds	231	53	22	306

Different sources of peanuts have variable amounts of phytosterol content. Data are from Awad *et al.* [8] and Phillips *et al.* [66]. ND = not detected.

germ, wheat bran, and cornflakes also have significant levels of phytosterols [7]. The food production and refining processes can result in a reduction in phytosterol content of plant products. For example, refined olive oil has 81% of the phytosterol content compared to virgin pressed olive oil [8]. Similarly, refined peanut oil has only 67% of the phytosterol content of unrefined peanut oil [8].

Phytosterols are effective in reducing cholesterol absorption from the gut [9]. Thus, some commercial food products are engineered to be enriched in phytosterols and are potential sources of phytosterols in the human diet. These food products are marketed to help lower serum cholesterol levels and to reduce the risk of heart disease. For example, Take Control® Spread contains 1650 mg plant sterol esters including 1000 mg free sterols *per* serving and Benecol® Spread contains 850 mg plant sterol esters with 500 mg free sterols *per* serving. Other enriched products are currently being developed and/or marketed in Europe and the United States.

2.3 Absorption of phytosterols

In humans, the only source of phytosterols is the diet and there is only limited absorption of dietary phytosterols. Whereas approximately 45–55% of dietary cholesterol is absorbed, less than 20% of dietary campesterol and 7% of dietary β-sitosterol are absorbed. Estimates of the bioavailability of dietary sterols have been approximated from controlled intestinal perfusion studies with healthy normal men [10]. Under these experimental settings, sterol bioavailability averaged 33% for cholesterol, 10% for campesterol, 5% for stigmasterol, and 4% for β-sitosterol.

The physiological process of sterol absorption was made clearer by studies on the mechanism of action of the drug ezetimibe. Ezetimibe is marketed as *Zetia* or as *Vitorin* when in combination with simvastatin. Ezetimibe reduces sterol absorption and is used in the treatment of hypercholesterolemia and sitosterolemia [11]. These studies revealed that dietary cholesterol and phytosterols are absorbed by the same transporter which is localized to the proximal jejunum on the surface of the absorptive enterocytes. This common sterol transporter has been identified as Niemann–Pick C1-Like 1 protein (NPC1L1) [12]. The transporter takes up cholesterol as well as phytosterols from micelles within the lumen of the small intestine and then moves them through a subapical endosomal sorting compartment. Cholesterol progresses through the ER where it is esterified and transferred to chylomicrons headed for systemic circulation in the lymph and bloodstream [13]. Ezetimibe and its congeners bind specifically and with high affinity to native enterocyte membranes and to recombinant NPC1L1 but not to enterocyte membranes derived from NPC1L1 knockout mice [14]. Mice deficient in NPC1L1 exhibited an approximate 70% reduction in sterol absorption, with the residual uptake being insensitive to ezetimibe [14].

The bulk of phytosterols are transported by the same transporter as cholesterol; however, the normal low net absorption of phytosterols is the result of their selective and rapid efflux back into the intestinal lumen. This efflux is mediated by the ATP-binding cassette cotransporters ABCG5 and ABCG8 [15–17]. ABCG5 and ABCG8 are expressed on the apical surface of enterocytes as well as in hepatocytes. Experimental support for this phytosterol efflux model comes in part from the observation that Abcg5-null mice have significantly elevated circulating levels of β-sitosterol (37-fold) and campesterol (7.7-fold) compared to wild-type controls [18]. Interestingly, the reduction in cholesterol absorption normally promoted by phytosterols is not associated with any change of expression of intestinal LXR target genes nor is it influenced by ABCG5 deficiency. ABCG8 has also been implicated in phytosterol efflux from independent epidemiologic and genetic studies. Studies of dyslipidemic individuals on the Micronesian island of Kosrae indicate clear evidence of a strong founder effect for a novel *ABCG8* mutation [19]. Heterozygotes and homozygotes for the mutated ABCG8 allele exhibit increased plasma phytosterol levels. Thus, it is clear that phytosterols are specifically absorbed by the NPC1L1 transporter and that a significant portion of the absorbed phytosterols is rapidly returned to the jejunum *via* the ABCG5/ABCG8 efflux pump.

Despite the mechanisms for rapid efflux of absorbed dietary phytosterols, significant levels of phytosterols do circulate in the blood of normal, healthy individuals who consume phytosterol-enriched diets. The concentration of plasma β-sitosterol in humans ranges from 0.005 to 0.024 mmol/L, depending on diet; whereas in sitosterolemic patients, the mean baseline circulating levels of β-sitosterol and campesterol average 0.50 and 0.27 mmol/L, respectively [11, 19]. It is not clear whether circulating phytosterols are esterified similarly to cholesterol. About two-thirds of serum cholesterol is esterified by lecithin:cholesterol acyltransferase. In one animal study, dietary phytostanols had no effect on lecithin:cholesterol acyltransferase activity [20].

Phytosterols from breads and cereals are bioavailable. In a study of 35 healthy subjects designed to measure the effects on serum lipids of a high phytosterol diet, plasma concentrations of β-sitosterol and campesterol increased significantly while subjects were on the defined diet [21]. The study monitored sterol levels during a 2 wk baseline period during which subjects consumed diets of phytosterol-free foods and then after a 12 wk period during which subjects consumed phytosterol-enriched foods, averaging 6.6 g phytosterol intake/day. At baseline, plasma levels of β-sitosterol and campesterol were 3.32 ± 1.47 and 3.14 ± 1.53 mg/L and increased to 5.00 ± 1.86 and 6.62 ± 2.48 mg/L, respectively, after 12 wk on the high phytosterol diet. A separate study asked whether plant food-based diets might increase serum phytosterol levels in postmenopausal

women [22]. In this study, subjects consuming experimental diets high in nuts, seeds, and soy for 11–16 wk, without caloric changes, exhibited increase in serum β-sitosterol up to 20%, confirming both the bioavailability of dietary phytosterols as well as the ability of diet to affect circulating phytosterol levels.

2.4 Distribution and excretion of phytosterols

In safety evaluations of phytosterol esters, rats dosed with radiolabeled cholesterol and phytosterols confirmed that at least 25% of the cholesterol dose and 3–6% of the β-sitosterol dose are absorbed [23]. The overall distribution of β-sitosterol was similar to that of cholesterol with the adrenal cortex, intestinal epithelia, spleen, bone marrow, and ovaries (in females) showing the greatest retention of β-sitosterol. Despite being distributed to the ovaries, there is little evidence that phytosterols are estrogenic. Phytosterols do not bind with any significant affinity to the human estrogen receptor and have minimal estrogenic activity in *in vitro* assays (see Section 4.3). The portion of dietary phytosterols that is absorbed and distributed to the body is ultimately eliminated in the bile [23].

3 Dietary phytosterols and cancer

3.1 Epidemiological studies

The National Cancer Institute's Surveillance, Epidemiology, and End Results program estimates that in the year 2006 there will be over 106 000 new cases of colon cancer, 214 000 new cases of breast cancer, and 234 000 new cases of prostate cancer in the United States (Cancer Facts & Figures, www.cancer.org 2006). It is against these common cancers that population and epidemiological studies have suggested dietary phytosterols to be strongly protective. Numerous population studies have shown that the incidences of colon, breast, and prostate cancers are relatively low in Asian countries; but when Asians relocate to western countries and consume more animal-based diets, the rates of these cancers increase significantly [24, 25]. These observational analyses and other controlled epidemiologic studies cited below confirm that people who consume predominantly plant-based, phytosterol-enriched diets have lower incidences of colon, breast, and prostate cancer.

Association studies within the Seventh-day Adventist community showed that Seventh-day Adventists experience lower rates of colorectal cancer as well as other cancers compared to the general population [26]. The life principles of Seventh-day Adventists promote ovolactovegetarian diets and dietary considerations may lie at the heart of the lower cancer incidence in this community. Dietary phytosterol intake for Seventh-day Adventists is estimated at 344 mg/day as compared to an average intake of 78 mg/day for the general American population. However, there are many factors besides phytosterol content associated with ovolactovegetarian diets, so it is not possible to conclude that it is the elevated phytosterol content of such diets that is the protective factor against colorectal cancers. It is possible that elevated phytoestrogens in the ovolactovegetarian diet may be directly protective or may synergize with phytosterols in reducing the incidence of colorectal and other cancers (see Section 4.1).

A series of case control studies carried out at major hospitals in Uruguay has investigated the role of dietary phytosterols in the risk of specific cancers [27–30]. These studies addressed the question of whether or not the amount of phytosterols in the diet was associated with the incidence of lung, breast, stomach, or esophageal cancer. These case control studies included from 100 to 500 newly diagnosed and histologically verified cases of specific cancers and frequency matched control patients of similar age, gender, residence, and urban/rural status. All patients were interviewed using food frequency questionnaires based upon 64 food items considered representative of local diet. Published food composition data were used to assess specific intakes of β-sitosterol, campesterol, stigmasterol, and total phytosterol. After controlling for major confounding factors, a potential protective role of dietary phytosterol was observed in all four major cancers. Total phytosterol intake was associated with specific protective effects in adenocarcinoma of the lung (odds ratio (OR) = 0.29, 95% CI = 0.14–0.63), breast cancer (OR = 0.41, 95% CI = 0.26–0.65), stomach cancer (OR = 0.33, 95% CI = 0.17–0.65), and esophageal cancer (OR = 0.21, 95% CI = 0.10–0.50).

McCann *et al*. [31] used the case control format to study the relationship between dietary phytochemical intake, including phytosterols, and the risk of ovarian cancer. The study included 124 women with incident, primary, histologically confirmed cases of ovarian carcinoma and 696 women serving as case controls and the frequency matched for age (40–85 years). Dietary intake of total phytosterol, β-sitosterol, campesterol, and stigmasterol were assessed using food frequency questionnaires and published food composition data. In rank analysis, the study found reduced ovarian cancer risk observed for the highest quintile intakes of stigmasterol (OR = 0.42, $p < 0.05$, 95% CI = 0.20–0.87). Despite no reduction in ovarian cancer risk related to intake of other phytosterols or total phytosterols, the ORs for the highest two quintiles of total phytosterol intake were 0.70 and 0.92, demonstrating a trend toward cancer protection. However, these odd ratios did not achieve levels of significance in this study.

There may be a potential preventative role for dietary β-sitosterol in patients with benign prostatic hypertrophy (BPH). BPH is not prostatic cancer, but rather an enlargement of the central area of the prostate and it is associated with excessive cellular proliferation in both the glandular and stromal elements. The results from two multicentered, randomized, double-blind, placebo-controlled clinical trials

have been reported [32, 33]. Patients with BPH in the active arms of the studies were given either 20 mg β-sitosterol three times a day [32] or 65 mg of β-sitosterol twice a day [33]. Both studies showed that supplemental phytosterols were associated with significant symptomatic improvements in prostate function compared to placebo. All clinical endpoints including modified Boyarsky scores, International Prostate Symptoms Scores, and urinary flow parameters were significantly improved in the β-sitosterol group without any relevant adverse side effects. Improvements compared favorably to those seen in clinical studies with α-receptor blocking agents or finasteride. However, no reduction in prostate volume was detected. Despite this, both studies clearly established the efficacy, safety and benefits of β-sitosterol therapy in the treatment of BPH.

The limited number of controlled studies prevents any clear conclusion from being made regarding dietary phytosterols and any specific cancer prevention. Epidemiologic analyses and specific controlled studies have demonstrated a cancer protective effect of phytosterols, but other studies of diet and cancer have not supported these conclusions. One must look at the types of studies that were performed: case control studies, cohort studies and randomized placebo-controlled trials. Case control studies are less reliable than either cohort studies or randomized placebo-controlled trials. The determination of a statistical relationship between dietary phytosterol and a lower incidence of specific cancer, as seen in most of the published case control studies, does not necessarily mean that the former caused that latter. However, support for such a conclusion is strengthened by animal experimentation studies discussed below.

3.2 Animal studies

3.2.1 Models of colon cancer

Rodent models have been used to determine whether dietary phytosterols provide protection against colon cancer. Rats and mice fed diets with or without enriched phytosterols were administered chemical carcinogens and after specific time periods the incidence of colon cancer determined pathologically or by indirect measures of cell proliferation. The pioneering studies of Raicht *et al*. [34] showed that a 2% β-sitosterol mixture (95% β-sitosterol, 4% campesterol, and 1% stigmasterol) in the diet reduced to one-third the incidence of observable colon tumors induced by intracolonic administration of *N*-methyl-*N*-nitrosourea (MNU).

Subsequent studies assessed the effect of dietary phytosterols on colonic mucosal cell proliferation with the supposition that dysregulated proliferation underlies the development of colon cancer and thus may be considered a risk factor [35, 36]. Deschner *et al*. [36] noted a reduction in both the size of the proliferative compartment as well as the colonocyte labeling index within crypt columns in MNU-treated rats that were maintained on a diet containing 0.2% β-sitosterol. Similarly in mice fed diets supplemented with phytosterols (0.3–2%), there was observed a dose-dependent reduction in cholic acid-induced colonic cell proliferation [37]. Phytosterol feeding reduced the number of mucosal cells in the S phase as measured by labeling index assessment. Further demonstrating the antiproliferative effects of phytosterols in the colon cell, Awad *et al*. [38] used Sprague-Dawley rats and showed that a 2% phytosterol mixture in the diet inhibited cholic acid-induced cell proliferation in the proximal colon after as short a dietary intervention as 22 days. These rodent studies support a cancer protective model of dietary phytosterols with reduced colonic cell division in animals after challenge with proliferative carcinogenic stimuli. On the other hand, contrary results were obtained by the study of Quilliot *et al*. [39] who observed a lack of effect of dietary phytosterols on carcinogen-induced colon cancer in rats. It is possible that the experimental diet formulated with hydrogenated coconut oil and low protein might have affected the latter outcomes. More research is required.

3.2.2 Models of breast cancer

There have been several reports of experiments testing the efficacy of dietary phytosterols in inhibiting the proliferation and development of explanted human breast cancer cells in rodent hosts. The studies involved immune deficient mice lacking either both B and T cells (SCID mice) or just T cells (nude or athymic mice). Awad *et al*. [40] fed female SCID mice defined diets supplemented with 2% phytosterols, 2% cholesterol, or the 0.2% cholic acid vehicle. After 2 wk, the mice were injected with MDA-MB-231 estrogen receptor-negative human breast cancer cells into their inguinal mammary fat pads. After 8 wk on the respective diets, mice receiving phytosterols exhibited a 40% reduction in serum cholesterol along with a 20- to 30-fold increase in serum β-sitosterol and campesterol concentrations. At 8 wk, the tumor sizes in the animals fed the phytosterol diet were 33% smaller than those in animals fed the cholesterol diet. Pathological analysis indicated that the tumor cells had metastasized to lymph nodes and lungs in 71% of the cholesterol-fed animals compared to only 57% of the phytosterol-fed animals.

In similar experiments, using ovariectomized athymic mice that were injected with the MCF-7 estrogen receptor-positive human breast cancer cells, a 32–42% reduction in tumor size was observed in mice fed diets enriched in β-sitosterol [41]. The effects of the β-sitosterol diet were not attributable to an antiestrogenic action as the expression levels of the estrogen-responsive pS2 mRNA did not differ among groups. These studies suggest that dietary phytosterols inhibit breast cancer tumor growth through an action independent of estrogen.

3.2.3 Models of prostate cancer

In similar studies, using the same human tumor cell explant protocol in mice fed diets enriched or not enriched with

phytosterols, Awad *et al*. [42] examined the potential protective effect of phytosterols against proliferation and metastasis of PC-3 human prostate cancer cells in male SCID mice. At the end of the 8 wk feeding period, there was a 40–43% reduction in tumor size in animals fed the phytosterol diet *versus* the cholesterol diet and a 50% reduction in the rate of tumor metastasis to the lung, liver, and lymph nodes compared to those fed the cholesterol diet.

4 How do phytosterols inhibit cancer development?

The development of cancer within animals usually entails multiple molecular events or steps. These steps often increase cell proliferation within tissues and include: accelerated passage of cells through checkpoints within the cell cycle, impaired responses to normal apoptotic signals or to other stimulators of programmed cell death, overproduction of growth regulatory hormones, enhanced metastasis of cancerous lesions, and alteration in host immune responses. These altered functions have a central underlying biochemistry in that they all involve cellular signal transduction. One of the basic questions in the research to understand how phytosterols attenuate cancers is to define the mechanisms by which phytosterols affect cellular and signal transduction processes.

4.1 Cell cycle effects

The loss of normal checkpoint control of cell cycle progression is recognized as a critical step in the development of cancer [43]. Dividing cells normally arrest at multiple checkpoints in the cell cycle, allowing damaged DNA to be repaired before progressing on to DNA replication and mitosis. In transformed or neoplastic cells, normal cell cycle progression is almost universally aberrant. It is becoming increasingly clear that many natural and dietary phytochemicals may exert anticancer effects by inhibiting dysregulated cell cycle progression and by promoting apoptosis. Phytosterols are among this group of phytochemicals that affect cell cycle progression and induce cellular apoptosis.

Several phytochemicals have been implicated in affecting cell cycle progression and kinetics. Natural flavonoids exert antineoplastic effects by targeting dysregulated cell cycle progression [44]. Inhibitory effects of natural flavonoids can be seen on cyclins, cyclin-dependent kinases, and their inhibitors, p53, Rb proteins, check-point kinases, and other proteins controlling G1/S and G2/M transitions in the cell cycle [44]. Isothiocyanates in cruciferous vegetables inhibit the proliferation of tumor cells in part by promoting cell cycle arrest and apoptosis [45]. The principal effect of dietary isothiocyanates on the cell cycle is to arrest progression at the G2/M phase accompanied by and perhaps caused by down regulation or inhibition of cyclin B1, cdc2, and cdc25C [45]. Allyl sulfur compounds in garlic, garlic extracts, and garlic oils suppress tumor cell proliferation and this is correlated with the induction of the hyperphosphorylation of cell cycle proteins and the hyperacetylation of histones [46]. Diallyl disulfide, an organosulfur in processed garlic, decreases cdc2/cyclin B1 complex formation, shifts the hyperphosphorylation state of cdc2, and inhibits cdc2 kinase activity. Resveratrol produced by grapevine skin and found in large amounts in red wines inhibits carcinogenesis in part by affecting the promotion and progression of the cell cycle [47]. Resveratrol inhibits the growth of human prostate DU145 cancer cell growth coincident with its inhibition of D-type cyclins and cyclin-dependent kinase 4 expression and the induction of the p53 tumor suppressor and the p21 Cdk inhibitor [48]. Other dietary agents such as butyrate and sulforaphane inhibit histone deacetylase activity resulting in disruption of the cell cycle and induction of apoptosis through derepression of P21 and BAX genes [49].

Phytosterols also affect cell cycle kinetics. In tissue culture studies of MDA-MB-231 human breast carcinoma cells, β-sitosterol induced cell cycle arrest at the G2/M transition [50]. After continuous β-sitosterol supplementation, 43% of the breast carcinoma cells were in G2 compared to 12 and 24% of cells maintained in cholesterol or vehicle-supplemented media, respectively. Similar effects were observed with human prostate adenocarcinoma PC3 cells: 32% of β-sitosterol-treated PC3 cells were in G2 phase where only 16% of vehicle-treated cells were in G2. In a study of the effects of β-sitosterol on the growth of HCT 116 human colon cancer cells, Choi *et al*. [51] observed a dose-dependent inhibition of growth and an increase in the sub-G1 cell population. Almost as a rule, these dietary agents are not potent inhibitors of cell cycle progression in the way that strong antineoplastic drugs are, but rather they act as weak inhibitors that over prolonged periods of exposure subtly regulate the activity of proteins and the expression of genes involved in cell growth and apoptosis.

4.2 Apoptosis

Programmed cell death or apoptosis is a highly conserved physiological cell suicide response essential for mammalian homeostasis. Apoptosis involves cascades of enzymatic events including apoptosis-related cysteine proteases called cytosolic aspartate-specific proteases or caspases [52]. Depending upon the triggers of apoptosis and which initiator caspases are involved, apoptotic pathways are termed either extrinsic or intrinsic.

Phytosterols, in particular β-sitosterol, induce apoptosis and stimulate ceramide cascades in transformed cell lines *in vitro* [53]. Significant induction of cellular apoptosis following β-sitosterol supplementation has been observed in MDA-MB-231 hormone-insensitive human breast adeno-

carcinoma cells, in metastatic LNCaP hormone-sensitive human prostate adenocarcinoma cells, in HT-29 human colon adenocarcinoma, and in PC-3 hormone-insensitive human prostate adenocarcinima cells [53]. Experimental observations suggest that activation of sphingomyelinase and the sphingomyelin cycle may be responsible for the reduction in sphingomyelin and in the production of ceramide and both processes may play causative roles in β-sitosterol-mediated apoptosis in transformed cells. Other phytosterols including diosgenin and solamargine are potent inducers of apoptosis in human erythroleukemia HEL and K562 cell lines and human hepatocytic Hep3B cells [54, 55].

Phytosterols contain a double bond and are susceptible to cellular oxidation, forming products including 7β-hydroxysitosterol, 7-ketositosterol, and numerous sitosterol epoxides. Phytosterol oxides, including β-sitosterol oxide, were shown to be cytotoxic in primary cultures of mouse macrophage as indicated by lactate dehydrogenase leakage, reduced cell viability, and reduced mitochondrial dehydrogenase activity [56].

4.3 Hormonal effects

There is conflicting evidence as to whether or not phytosterols have estrogenic activity. Besides the structural similarity as being sterols, phytosterols could affect endogenous estrogen levels through receptor competition, alterations in enterohepatic recirculation, by effects on bile acid metabolism, by altering estrogen reabsorption, or through competition with cholesterol as a substrate for steroid hormone synthesis.

The results of a study of human breast adenocarcinoma MCF-7 cells which measured the effects of phytosterols on proliferation and which included determinations of reporter gene assays and measurements of specific binding to recombinant human estrogen receptors α and β suggested that phytosterols may be functioning as weak estrogen receptor modulators and might function *in vivo* as endocrine disruptors [57]. However, other studies failed to show any estrogenic potential of phytosterols. Short-term *in vitro* tests of phytosterol activities as well as standard safety evaluations of phytosterol esters failed to demonstrate estrogenic activity of phytosterols or phytostanols [58, 59].

4.4 Effects on tumor metastasis

In vitro models of metastasis using measurements of cell invasion, migration, and adhesion have been used to assess the metastatic potential of tumors and the influence of potential therapeutic agents on metastasis. Invasion of human breast cancer MDA-MB-231 cells through Matrigel and their adherence to plates coated with collagens, fibronectins, and laminins were significantly inhibited by prior treatment with β-sitosterol [50]. A potential implication of this work is that dietary phytosterols might offer protection from breast cancer metastasis by inhibiting cell invasion of the basement membrane. In animal studies, SCID mice maintained on a 2% mixed phytosterol diet not only exhibited fewer and smaller tumors after implanting human prostate PC-3 cancer cells, but also showed only half the rate of lymph node and lung metastasis [42]. Similar results were observed in phytosterol-fed SCID mice implanted with human breast cancer MDA-MB-231 cells [40].

Angiogenesis, the promotion of new blood vessel growth, is necessary for tumor growth and the metastatic advance of cancers. The discovery of endogenous inhibitors of angiogenesis and the observed inhibitory effects of phytosterols on cancer metastasis suggest that dietary phytosterols may promote the expression or activity of these endogenous inhibitors [60]. This represents another promising area of translational research with dietary phytosterols.

4.5 Effects on immune function

The description of dietary phytosterols as immunomodulating compounds arose from the initial observations that mixtures of sterols and sterolins enhanced the cellular responsiveness of T lymphocytes both *in vitro* and *in vivo* [61]. The proprietary sterol mixture augmented immune responses involving natural killer (NK) cells.

Bouic *et al*. [62] first reported studies indicating that the mixture of β-sitosterol and its glucoside derivative stimulated blood lymphocyte proliferation *in vitro*. The proliferation was accompanied by a profile of cytokine secretion suggestive of a selective effect on T_{H1} helper cells. Secretion of the T_{H1} helper cell-selective IL-2 and INFγ was increased whereas that of the T_{H2} helper cell-selective IL-4 remained unchanged. Additional studies demonstrated greatly enhanced lytic and cytotoxic activities of NK cells against transformed cell lines after NK cell preincubation with the β-sitosterol–glucoside mixture. The enhanced cytotoxic activity was speculated to be due to the secretion of IL-2 and IFNγ promoted by β-sitosterol. These experimental studies were extended to select clinical studies including the management of pulmonary tuberculosis, the inhibition of immune stress in marathon runners, management strategies for allergic rhinitis and sinusitis, and the management of HIV-infected patients [61, 63]. No adverse events have been reported and the clinical results are reported to be promising.

Phytosterols also affect macrophage function. β-Sitosterol reduced nitric oxide release induced by phorbol ester from RAW 264.7 macrophages, potentially being correlated with the impairment of inducible nitric oxide synthase levels and with NF-κB activation [64]. The growth of $P388D_1$/MAB macrophages in culture and release of the proinflammatory PGE_2 were also inhibited by β-sitosterol [65].

4.6 Signal transduction effects

The mechanisms underlying the cellular changes induced by phytosterols most likely involve altered signal transduction. One of the most interesting changes induced by phytosterol treatment of transformed and cancerous cells is an increase in sphingolipid turnover and ceramide metabolism. Tumorigenesis has been associated with changes in sphingomyelin metabolism [66]. The sphingolipid metabolites ceramide and sphingosine are cellular inducers of cell cycle arrest and apoptosis; whereas sphingosine-1-phosphate is a regulator of cell activation and proliferation [67, 68].

Ceramide is one of the central components of sphingolipid metabolism and evidence supports its role as a second messenger in promoting cell cycle arrest, senescence, and apoptosis [69–72]. Ceramide is generated from sphingomyelin by the action of sphingomyelinases and from sphingosine through the addition of a fatty acid by the enzyme, ceramide synthase. Alkaline sphingomyelinase in the human colon appears to play an antiproliferative and anti-inflammatory role through its generation of ceramide [73]. Reduction in alkaline sphingomyelinase activity has been reported in human colon cancers and mutations in the enzyme exist in transformed human colon cancer cell lines [74, 75].

Treatment of the human colon tumor HT-29 cells with β-sitosterol stimulated apoptosis and this was accompanied by a reduction in cellular sphingomyelin and an increase in ceramide levels [76]. Radiolabel tracer studies indicated that *de novo* ceramide synthesis is also increased by β-sitosterol, suggestive of enhanced sphingomyelin turnover. Similar results were observed with human prostate cancer LNCaP cells [77]. The significant anticancer properties of β-sitosterol may be mediated at least in part by enhanced sphingomyelin turnover and an increased generation of ceramide.

5 Conclusions

Phytosterols are absorbed from the diet in small but significant amounts and may reach serum concentrations in the low micromolar range. Epidemiological data suggest that phytosterols in the diet are associated with a reduction in common cancers including cancers of the colon, breast, and prostate. The means by which dietary phytosterols may be achieving these effects is becoming clearer from molecular studies with tumorigenic research models. Phytosterols may affect host responses enabling a more significant antitumor response. In addition, phytosterols may have effects that directly inhibit tumor growth. Phytosterols affect the cell cycle and promote apoptosis perhaps through their induction of sphingolipid metabolism and stimulation of ceramide formation. Influences of phytosterols on the immune and hormone systems may also contribute to their health effects. Potential mechanisms by which phytosterols influence tumorigenesis are shown in Fig. 2.

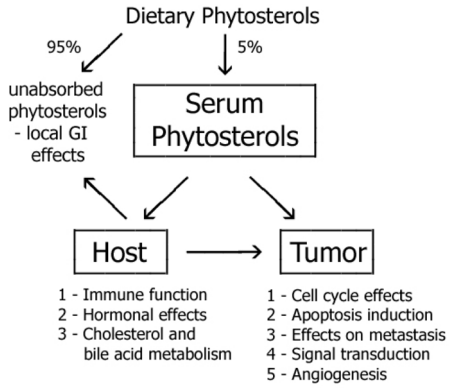

Figure 2. Mechanism by which dietary phytosterols function as anticancer compounds. Absorbed phytosterols may act on host systems to affect tumor surveillance or act directly or indirectly on tumors to affect tumor cell biology. Host influences include boosting immune recognition of cancer, influencing hormonal-dependent growth of endocrine tumors, and altering sterol biosynthesis. Effects on tumors include slowing of cell cycle progression, induction of apoptosis, inhibition of tumor metastasis, altered signal transduction, and induction or activation of endogenous antiangiogenic factors. Unabsorbed phytosterols may have local effects on tumors of the gastrointestinal tract.

6 References

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