Phytosterols: Beneficial Effects

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Abstract

Phytosterols (PS) are plant sterols and stanols widely distributed in plant sources that resemble cholesterol in terms of structure and physiological functions. The cholesterol-lowering capacity of PS is well documented in animal and human studies. However, recent studies suggest that the beneficial effects of PS are not only limited to their hypocholesterolemic capacity as they can also act as immunomodulatory, anti-inflammatory, and antidiabetic agents. Further, there is a growing body of evidence which supports that they play an important role in the prevention of other diseases such as cancer and atherosclerosis. Nevertheless, the mechanisms by which PS exert their beneficial functions, the physiological relevance of PS, and their potential adverse effects are not yet fully understood. Therefore, the main aim of this chapter is to provide a contemporaneous overview of the beneficial properties of PS, their mechanism of action, and safety.

Keywords

anticancer • antidiabetic • cardiovascular diseases • cholesterol • immunomodulation • inflammation • molecular mechanism • phytostanols • phytosterols • side effects

Abbreviations

ABC	ATP-binding cassette transporter
ACAT	Acyl-CoA: cholesterol *O*-acyltransferase
CHD	Cardiovascular heart disease
GST	Glutathione-*S*-transferase(s)
HDL	High-density lipoprotein
HMG-CoA	3-Hydroxy-3-methyl-glutaryl-coenzyme A
hs-CRP	High-sensitive C-reactive protein
IDL	Intermediate-density lipoprotein
IL	Interleukin
LDL	Low-density lipoprotein
LDL-c	Low-density lipoprotein cholesterol
LTB_4	Leukotriene B4
LXR	Liver X receptor
NPC1L1	Niemann-Pick C1-like 1 protein
PS	Phytosterol(s)
PSO	Phytosterol oxide(s)
TICE	Transintestinal cholesterol efflux
TNF-α	Tumor necrosis factor alpha
VLDL	Very-low-density lipoprotein

1 Introduction

Phytosterols (PS) are plant sterols or stanols found in plants. Plant sterols belong to the triterpene family and differ from cholesterol by having a methyl or ethyl group in C24. Plant stanols, on the other hand, are the saturated form of the plant sterols (Fig. 113.1). PS are present in free or conjugated form as fatty-acyl esters, hydroxycinnamate steryl esters, steryl glycosides, or acylated steryl glycosides. The main function of plant sterols/stanols is to stabilize plant membranes and serve as precursors in the synthesis of steroidal saponins, alkaloids, and other steroids [1].

PS are widely distributed in plants and plant-containing foods. The most abundant of which are β-sitosterol, campesterol, and stigmasterol [2]. Table 113.1 shows the most common sources of PS. Vegetable oils are considered to be the major sources of PS and their esters [2]. Other good dietary sources include legumes, plant seeds, cereals, and cereal-milling products [3–6]. It is estimated that the dietary intake of PS ranges between 150 mg day^{-1} in western-style diets to 500 mg day^{-1} in diets rich in vegetable dietary habits [7, 8].

The capacity of both plant sterols and stanols to reduce blood cholesterol is well documented [10–12]. However, their precise mechanism of action is not yet fully defined. Current research is providing new insights on the mechanisms of action of PS as well as potential new roles in other physiological benefits. In addition, the safety of these products when they are used at high doses has recently been challenged. Therefore, the main objective of the present review is to discuss current evidences regarding not only the bioactive properties of PS and their mechanism of action but also their potential undesirable effects.

2 Metabolism and Physiological Effects

A large number of studies have provided consistent evidence on the beneficial physiological effects of PS, especially their hypocholesterolemic capacity [10, 13–15]. Thus, the use of PS as functional food components or dietary supplements has become of great interest and led to the development of a wide variety of functional foods and nutraceutical products [16, 17].

2.1 Phytosterol Bioavailability

Like cholesterol, PS are absorbed in the proximal part of the small intestine after being incorporated into mixed micelles. Compared to cholesterol, the intestinal absorption of PS is low. While 40–60% of dietary cholesterol is absorbed, only about 5% of the PS are absorbed [18]. In addition, the efficiency of PS absorption is critically dependent on the structure of both sterol nucleus and side chain. For instance, the rate of plant sterol absorption was investigated in a human study

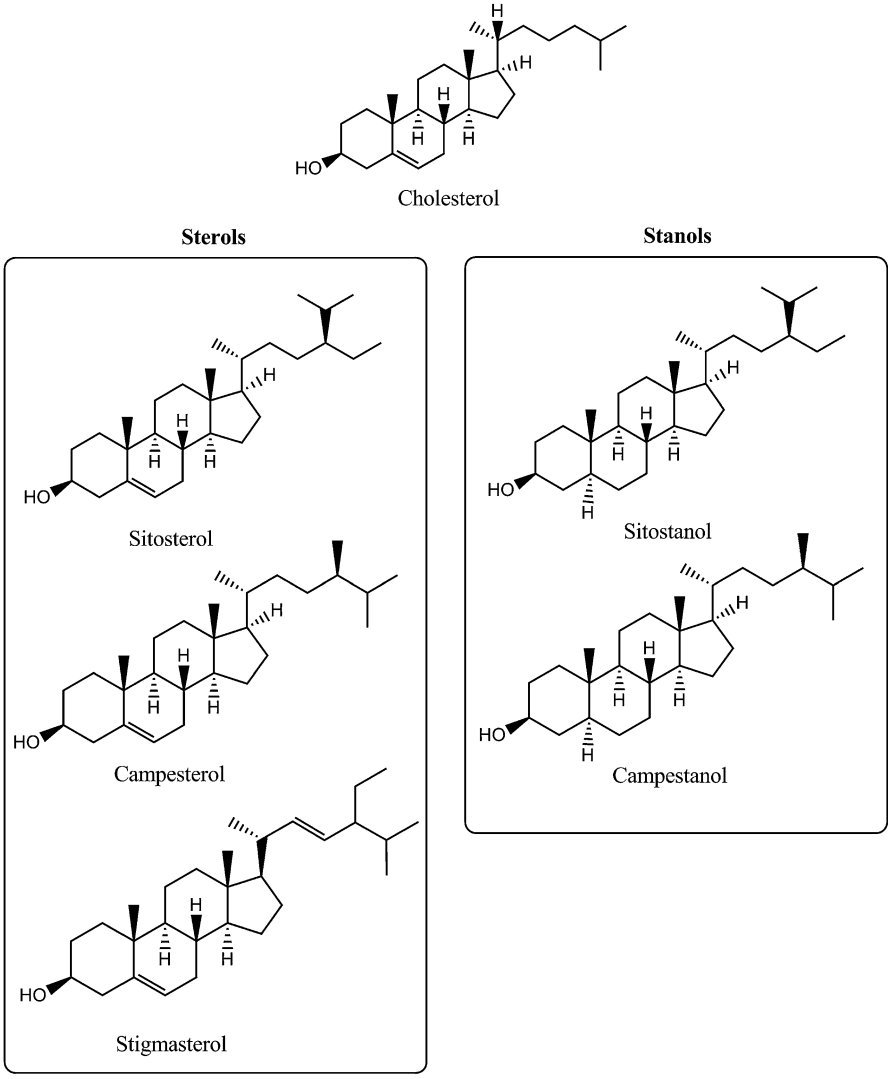


Fig. 113.1 Structure of cholesterol and representative sterols and stanols

using deuterium-labeled PS. The absorption rates of the different plant sterols were 1.9% and 0.5% for campesterol and β-sitosterol, respectively, whereas that of stanols were 0.16% for campestanol and 0.04% for sitostanol [19]. Plasma concentrations of PS are normally very low due to the low absorption of these compounds but it can be very variable among populations mainly due to the differences in dietary habits. Plasma concentrations of campesterol range from 6.9 to 27.9 μmol L^{-1}, whereas those of sitosterol range from 2.8 to 16.0 μmol L^{-1} [20].

Table 113.1 Phytosterol content from different food sources

	Total phytosterols (mg 100 g^{-1})
Vegetable oils[a]	
Corn oil	686–1,400
Rapeseed oil	250–878
Soybean oil	203–328
Olive oil	114–162
Palm oil	49–79
Grain and cereals	
Corn	66–178
Rye	77–113
Barley	59–83
Wheat	45–83
Nuts	
Peanuts	220
Almonds	143
Walnuts	108
Fruits	
Avocado	75
Orange	23–24
Grape	4–20
Apple	13–18
Banana	12–16
Vegetables	
Olives	50
Broccoli	4–50
Cauliflower	31–40
Carrots	16–30

[a]The range includes the content from crude and refined oils
Adapted from references [2, 3, 5, 6, 9]

2.2 Hypocholesterolemic Effect

An elevated concentration of plasma cholesterol is considered one of the most important risk factors for the development of coronary heart disease (CHD) [21]. The hypocholesterolemic effect of PS was first demonstrated in the 1950s [22]. Since then, the capacity of both plant sterols and stanols to reduce blood cholesterol has been well documented [10–12]. Moreover, a large number of clinical studies has confirmed their efficiency as cholesterol-lowering agents in humans (see Ref. [15] and [23] for exhaustive summary of clinical trials).

The beneficial effects of PS on cholesterol levels are usually shown after a period as short as 2–3 weeks of intervention and remain stable for at least 1 year of continuous treatment [15]. In humans, the absorption of cholesterol can be reduced by 30–40% after consumption of 1.5–2.0 g day^{-1} [15, 24]. Doses of 0.8–4.0 g of PS day^{-1} have been efficient in reducing LDL-cholesterol (LDL-c) concentration

by 10–15% [10]. However, a dose of 2.0 g day^{-1}, which can result in a reduction of plasma LDL-c of 10%, has been proposed as optimal [25]. Higher doses than 2.0 g day^{-1} are in general not recommended as they do not show additional reductions in cholesterol levels and may lead to undesirable side effects [16], although this aspect remains controversial [26]. In addition, it has been suggested that in some cases, PS exert beneficial effects on other lipid variables, such as increasing HDL-cholesterol, decreasing triglycerides levels, and decreasing the ratio of apolipoprotein B/apolipoprotein A1 [27].

It is still a matter of controversy whether plant sterols and stanols are equally efficient in reducing cholesterol levels [28]. Some studies have shown that despite their different bioavailability, there is no clinical relevance with regard to their effect on total cholesterol, LDL-c, HDL-cholesterol, or triglyceride levels [25, 29]. Nevertheless, other authors have suggested that the differences in efficacy between plant sterols and plant stanols remain in the long-term interventions rather than in the short-term studies [30]. For instance, in a recent meta-analysis of randomized placebo-controlled trials, decreases in LDL-c concentrations were dose-dependent for plant stanols but not for sterols. Similarly, intakes of plant stanols higher than 2 g day^{-1} have been associated with additional and dose-dependent reductions in LDL-c [26]. Yet, this effect remains questionable [29]. It has been proposed that the difference in efficiency between these two compounds may be explained by the fact that plant stanols may reside longer in the intestine due to their lower absorption [31].

Several factors can influence the overall effect of plant sterols and stanols. Naumann et al. [32] reported that men are slightly more sensitive to PS intake than women, although the responsible mechanism for this difference is unknown. Another factor that may influence the efficiency of PS involves the baseline levels of plasma lipids [25, 32, 33]. For example, subjects with high and very high baseline levels showed stronger reductions in LDL-c levels than subjects with levels near optimal clinical concentrations [25, 33]. However, these effects could not be reproduced by other authors [34]. Similarly, controversial effects are seen on the improvement of other lipid parameters as HDL-cholesterol concentration increases in subjects with low baseline levels and decreases in those subjects with initially high levels [32]. These differences suggest that people with an unfavorable ratio of total to HDL-cholesterol would especially benefit from PS consumption.

Another beneficial effect of PS is based on their ability to decrease serum concentrations of triacylglycerols especially in people with high serum concentrations [32]. This effect may be attributed to a reduction in the synthesis of very-low-density lipoproteins (VLDL), which are the main transporters of this type of lipids [35].

2.3 Combination with Other Therapies

In order to increase the effectiveness of PS in the reduction of CHD-associated factors, PS can be used in combination with other drugs or bioactive substances [23]. For instance, PS have been used in combined therapies with statins (3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) inhibitors) [36], with

Table 113.2 Summary of intervention studies of PS combined with other therapies

Combined therapy	Study design	Time[a]	Conclusions	References
Stable statin treatment + plant sterols (2.0 g day^{-1})	Double-blind, randomized trial (55 patients on stable statin treatment)	6 weeks	No further cholesterol reduction observed	[40]
Stable statin treatment + plant sterols or plant stanol (2.5 g day^{-1} each)	Double-blind, randomized trial (54 patients on stable statin treatment)	85 weeks	Increased reduction of LDL-c for both plant sterols (8.7%) and plant stanols (13.1%) compared to statin treatment alone	[41]
Ezetimibe (10 mg day^{-1}) + phytosterols (2.0 g day^{-1})	Double-blind, randomized trial (40 mildly hypercholesterolemic subjects)	4 weeks	No therapeutic benefit over ezetimibe	[42]
Ezetimibe (10 mg day^{1}) + phytosterols (2.5 g day^{-1})	Double-blind, randomized, placebo-controlled, triple crossover study (21 mildly hypercholesterolemic subjects)	3 weeks	Enhanced reduction of LDL-c from 16% (ezetimibe alone) to 22% (combined therapy)	[43]
Saturated-fat dairy and whole wheat cereal diet + diet containing viscous fibers (5–10 g day^{-1}), soy foods (25 g soy protein day^{-1}), and almonds (estimated total PS intake of 1–3 g day^{-1})	Randomized crossover trial (34 hypercholesterolemic subjects)	4 weeks	LDL-c reduction of 29%, which was similar to that of statins	[44]
Omega-3 polyunsaturated fatty acids provided as sunola (1.4 g day^{-1}) or fish oils (1.4 g day^{-1}) + plant sterols (1.4 g day^{-1})	Randomized, double-blind, 2 × 2 factorial design (60 hyperlipidemic subjects)	3 weeks	Reduction of inflammatory markers: hs-CRP, TNF-α, IL-6, and LTB_4. Increased levels of adiponectin. Higher CHD risk reduction	[38]
Fish oil (2.0 g day^{-1}) + plant sterols (2.0 g day^{-1})	Randomized, double-blind, 2 × 2 factorial design (200 hypercholesterolemic subjects)	4 weeks	The combination lowered triglycerides by 15% compared to control but no significant interaction between PS and n-3 on plasma cholesterol	[45]

(*continued*)

Table 113.2 (continued)

Combined therapy	Study design	Time[a]	Conclusions	References
Oat β-glucan (5.0 g day^{-1}) + plant stanols (1.5 g day^{-1})	Randomized, controlled, 3-period crossover study (40 mildly hypercholesterolemic subjects)	4 weeks	Slightly further reduction of LDL-c of combined treatment (9.7%) compared to oat β-glucan alone (5.1%)	[39]

Abbreviations: *LDL-c* LDL-cholesterol, *hs-CRP* High-sensitive C-reactive protein, *TNF-α* tumor necrosis factor alpha, *IL-6* interleukin-6, LTB_4 leukotriene B4
[a]Refers to the time of combined treatment

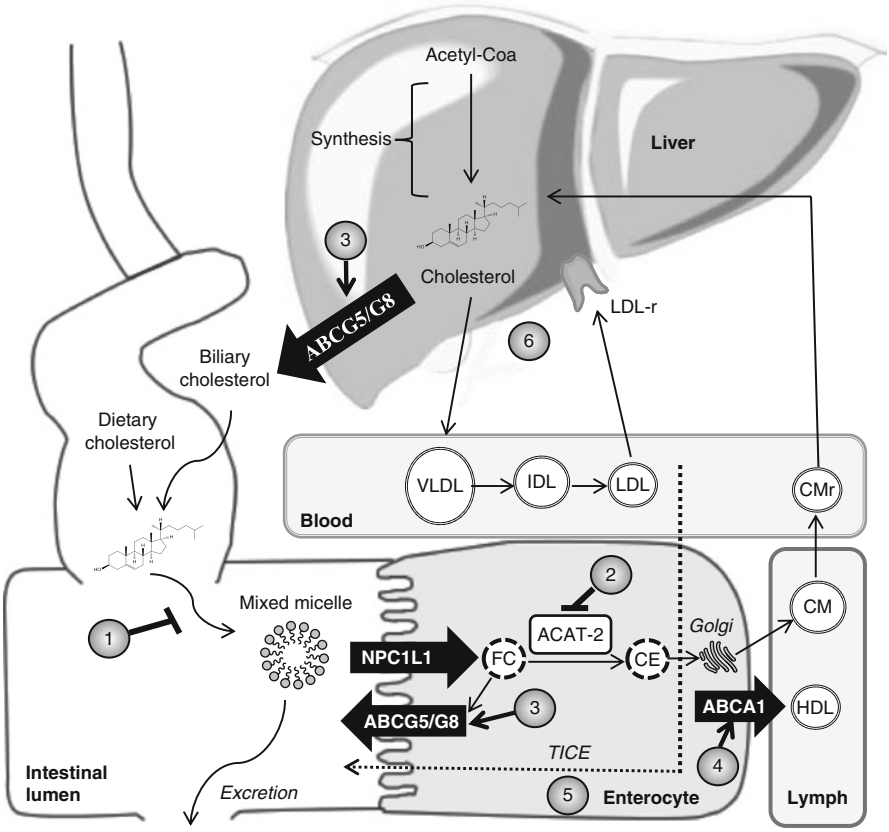
ezetimibe (inhibitor of cholesterol transporter of Niemann-Pick C1-like 1 (NPC1L1) that blocks the intestinal absorption of both biliary and dietary cholesterol) [37], with n-3 polyunsaturated fatty acids [38], and with different fibers [39], among others. A summary of clinical studies is shown in Table 113.2.

The combination of n-3 polyunsaturated fatty acids with PS is having an emerging interest; it has been reported that it is effective in reducing the levels of inflammation markers [38, 46] and cardiovascular risk factors including total cholesterol and triglyceride concentrations, pro-aggregatory factors, eicosanoid, and thromboxane A2 levels [47]. Moreover, Micallef et al. [38] estimated that sunola or fish oils (4.0 g day^{-1}), which are rich in n-3 fatty acids, were more efficient in reducing cancer risk when administered in combination with 2.0 g day^{-1} of PS than when administered alone.

Nevertheless, despite that the use of PS with other therapies seems promising, clinical evidence remains scarce and far from being conclusive.

3 Mechanism of Action

The cholesterol-lowering activity of PS has been attributed to several mechanisms which are mainly ascribed to their structural similarities with cholesterol. Traditionally, the main mechanism accounted for the cholesterol-lowering capacity of PS has been attributed to the competitive solubilization into mixed micelles between cholesterol and PS at the intestinal level [48]. However, several studies have provided evidence to support that the cholesterol-lowering activity is also related to mechanisms other than interference with cholesterol incorporation into micelles [15, 28, 49, 50]. Although the physiological relevance of all these mechanisms is not clear, the metabolic effects of PS are commonly attributed to lower absorption of cholesterol and changes in intestinal cholesterol efflux and lipoprotein homeostasis. These mechanisms are summarized in Scheme 113.1.



Scheme 113.1 *Schematic overview of cholesterol metabolism and main proposed mechanisms of action of phytosterols*. *1*. The absorption of dietary and/or biliary cholesterol is reduced by competition with PS for incorporation into mixed micelles. *2*. Esterification of free cholesterol in the enterocyte is reduced by competition with PS for ACAT-2 enzyme. *3*. Upregulation of the heterodimer ABCG5/G8 by PS can increase intestinal and hepato-biliar secretion. *4*. Upregulation of ABCA1 by PS can increase the incorporation of sterols into nascent HDL. *5*. Increased cholesterol excretion via TICE. *6*. Although it is not directly mediated by PS, the lower levels of hepatic cholesterol can lead to a lower VLDL secretion and upregulation of LDL receptor, which improves the clearance of plasma cholesterol. Abbreviations: *FC* free cholesterol, *CE* cholesterol esters, *ACAT-2* Acyl-CoA: cholesterol O-acyltransferase 2, *CM* chylomicron, *CMR* chylomicron remnant, *TICE* transintestinal cholesterol efflux, *LDL* low-density lipoprotein, *IDL* intermediate-density lipoprotein, *HDL* high-density lipoprotein

3.1 Competitive Incorporation into Mixed Micelles

Intestinal cholesterol absorption begins with the incorporation of both dietary and biliary cholesterol into mixed micelles. A large number of studies have demonstrated the competition between cholesterol and PS for solubilization into micelles [48, 51–54], wherein the incorporation of PS into mixed micelles seems to be more

favorable than that of cholesterol [48, 54]. However, it remains questionable whether sterols and stanols can compete for micellar incorporation differently. In vitro studies using micelle preparations have shown that cholesterol can be substituted in a similar extent by the most abundant phytosterols: campesterol, sitosterol, and sitostanol [54]. On the contrary, the effect of minor plant sterols (i.e., stigmasterol) is not clear. While some authors have reported that minor sterols have lower ability to decrease cholesterol solubility [53], others have not detected such differences [52]. Differences in the micellar system used may well be responsible for the discrepancies between studies.

3.2 Effect of PS at Intestinal Level

Once cholesterol is incorporated into the micelles, its uptake by enterocytes is actively mediated by transporters, mainly NPC1L1 protein, which is located in the brush border membrane [55, 56]. Cholesterol is then esterified with fatty acids by acyl-CoA: cholesterol *O*-acyltransferase 2 (ACAT-2), incorporated into chylomicrons and then secreted to the lymph through the basolateral membrane of the enterocyte [57]. Unesterified cholesterol can be secreted back to the intestinal lumen by ATP-binding cassette transporters G5 and G8 (ABCG5/ABCG8) in direct opposition to NPC1L1 [58]. Moreover, ABCA1 can mediate the incorporation of sterols into nascent high-density lipoproteins (HDL) which lead to their secretion into the lymph [59]. Alternatively, emerging evidence suggests that the proximal part of the small intestine is able to secrete cholesterol actively, a pathway called transintestinal cholesterol efflux (TICE), although its molecular mechanism has not yet been elucidated [60].

Therefore, cholesterol absorption is a complex process that involves different molecular targets. Recent studies have proposed that PS can play an important role in this process [49, 50], although the mechanisms are not yet fully understood and further investigation is necessary. It has been postulated that LXR induction could explain the hypocholesterolemic action of PS [51, 61]. However, this hypothesis is still controversial [62]. For instance, although some authors have reported that PS can act as LXR ligands [51, 61] and regulate the expression of NPC1L1 and ABC transporters [58, 63], others could not confirm this role [49, 50]. Moreover, it has been reported that dietary PS decrease intestinal cholesterol absorption independently of changes in gene expression of intestinal NPC1L1 and ABC transporters [64, 65]. These observations do not rule out the possibility that the activity of these transporters could be altered by PS through posttranscriptional mechanism. Thus, several studies have used genetically engineered mice to examine the involvement of these transporters [66, 67]. However, these studies have shown that the reduction in cholesterol induced by PS is not influenced by the absence of the heterodimer ABCG5/G8 [66] and ABCA1 transporter [67].

Other mechanisms independent to LXR have also been proposed [49]. For instance, it is plausible that PS could reduce plasma cholesterol levels by competing with cholesterol for esterification in the enterocyte by ACAT-2 enzyme,

thus reducing its incorporation into chylomicrons [68]. In addition, in vitro experiments with CaCo-2 cells have demonstrated that PS can reduce endogenous cholesterol synthesis by inhibiting the expression of HMG-CoA, a rate-limiting enzyme in the synthesis of cholesterol [69]. However, other reports have reported contradictory results [70, 71]. Although its functional importance is less clear, it has been hypothesized that PS can also interfere with the expression of other mucosa proteins from the ANX family, such as ANXA2, which are involved in cholesteryl ester transport [49, 72]. Since ANXA2 mediates the internalization of cholesteryl esters from caveolae to internal membranes of the brush border [73], it has been postulated that PS may reduce cholesterol transport [49]. Nevertheless, there is yet no clinical evidence to confirm this hypothesis. Finally, it has been recently reported that PS can also increase cholesterol excretion via TICE [50], although further research is necessary to elucidate the molecular mechanisms.

In conclusion, although recent insights into the intestinal absorption of cholesterol have also provided new evidence regarding the potential action of PS, the molecular mechanisms of PS are still a field of debate and further research is necessary.

3.3 Effect of PS on the Liver

It is well known that the liver plays a critical role in cholesterol homeostasis. Hepatic cholesterol concentrations are a balance of its intestinal absorption, its synthesis, its degradation to bile acids, and its excretion with the bile or as VLDL [74]. Reduced cholesterol absorption results in multiple changes in lipid homeostasis in the liver.

Firstly, a decrease in hepatic cholesterol concentrations leads to an upregulation of LDL-receptor expression, ultimately leading to a decrease in plasma LDL-cholesterol [28]. In addition, lower hepatic cholesterol may lead to reduced liver secretion of VLDL [75, 76]. Like in the intestine, ABCG5 and ABCG8 transporters can also be upregulated by PS, which favors hepatic secretion of cholesterol into the intestinal lumen [49].

Finally, since the absorption of cholesterol is reduced by PS, this reduction would lead to a compensatory increase in de novo synthesis of cholesterol [71]. Indeed, cholesterol biosynthesis is upregulated after consumption of diets containing phytosterols, although this increment is insufficient to offset the beneficial effects of phytosterol [28, 49, 77].

4 Other Biological Activities

The beneficial effect of PS has been traditionally ascribed to their cholesterol-lowering properties. However, recent research reveals that their biological role has been underestimated. Several in vitro and in vivo studies have reported that PS can act as immunomodulatory and anti-inflammatory agents and reduce the risk of several diseases such as cancer [13, 78, 79].

4.1 Cancer Preventive Agents

The second major beneficial effect of PS is based on their role as cancer preventive agents [78, 80–82]. The activity of a large number of phytochemicals (alone or combined with other factors) as cancer preventives has been largely reviewed and discussed [80, 83]. Some authors have suggested that PS can reduce cancer risk, although there is still a lack of data in humans, similar to those manifested by other phytochemicals [81]. PS have been proposed to prevent cancer development through several mechanisms of action such as inhibition of carcinogen production, cancer-cell growth, angiogenesis, and promoting apoptosis [80]. Emerging evidence suggests that PS can consequently play an important role in the prevention of several types of cancer such as lung, stomach, prostate, ovarian, and breast cancer [80].

One of the first studies suggesting the preventive effect of PS on cancer showed that Seventh-Day Adventists, having a high dietary intake of PS, presented low rates of colon cancer [84]. The PS intake in this population could reach 344 mg day^{-1}, which was considerably high in comparison with the average intake of the USA population. This preventive effect was mainly attributed to the reduced bile acid excretion of this population after PS intake [85], as it is known that high levels of bile acids in the bowel can increase the risk of colon cancer [86]. In contrast, contradictory results have been reported with regard to decreased bile acid excretion due to PS intake [14]. Moreover, these findings are limited by the possible modulating effect coming from other components of the diet. Ileostomy studies are more accurate to determine their effect on reducing bile acid levels in the bowel as the variability attributed to side factors can be minimized or even eliminated [87]. Revision of the available studies on this subject reveals that the magnitude of the effect attributed to PS on the reduction of bile acid excretion can be highly dependent on other dietary factors that must be taken into account. In addition, the effect on bile acid excretion can vary according to the molecule of the PS studied [81].

Some authors have proposed that other mechanisms could be involved in the cancer preventive effect of PS. Awad and Fink [88] proposed a hypothesis based on the inhibition of cell growth through stimulation of apoptosis (programmed cell death). In vitro studies have also shown the inhibitory effect of certain PS on breast- and colon-cancer cell cultures and, in a lower extent, on prostate-cancer cells [88, 89]. Another proposed mechanism is based in the capacity of PS to stimulate the sphingomyelin cycle. For instance, sitosterol seems to have a clear in vitro modulatory effect on this cycle. The PS molecule can be incorporated into the cell wall, thus reducing sphingomyelin and increasing ceramide levels in the cell membrane, which can consequently increase cell apoptosis [89, 90]. Finally, changes in testosterone concentrations can also be accounted as an alternative mechanism involved in the prevention of prostate cancer [88]. It has been also reported that diets containing 2% PS reduce the activity of 5α-reductase in liver and prostate and thus the testosterone levels in plasma [91]. However, this hypothesis has not yet been confirmed in human studies.

Furthermore, PS have also been associated as agents capable to reduce angiogenesis and metastasis [80]. In vitro studies reveal that a reduction in the invasiveness and adhesiveness of cancer cells can be responsible, at least in part, of a lower metastasis capacity of cancer cells [92, 93]. However, further clinical studies are necessary to corroborate the anti-angiogenic properties of PS.

In a similar way than other phytochemicals, comparison between animal and human studies is controversial as it is difficult to extrapolate the effective doses necessary to reach clinical relevance. In general terms, extrapolation of doses from animals to human suggests that very high intakes would be necessary to reach significant effects.

4.2 Modulation of the Immune System and Anti-inflammatory Properties

Modulation of the immune system and reduction of inflammatory disorders have also been proposed as other beneficial effects of PS consumption [13, 79].

First evidences suggested that PS can modulate the immune system by improving the activity of T lymphocytes and natural killer cells [94]. Further clinical studies have investigated the immunomodulatory effect of PS under clinical trial situations [94, 95]. The findings reveal that PS consumption can improve the clinical recovery of pulmonary-tuberculosis patients and ameliorate the adverse effects caused by immune suppression induced by immunodeficiency viruses or stress [95]. It has been recently reported that β-sitosterol can enhance the action of vitamin D on the immune function of macrophages [96]. In addition, research conducted in human Jurkat T cells has revealed that campesterol, β-sitosterol, and β-sitostanol can suppress mitogen-induced IL-2 production in a dose-dependent manner [97]. This interaction with IL-2 could be useful for patients requiring immunosuppressive effects, although further research is needed to elucidate its clinical relevance.

The role of PS as anti-inflammatory agents is commonly ascribed to their capacity to modulate cytokine production. However, how this production is modulated remains unclear. Some authors have reported that PS can reduce the production of pro-inflammatory cytokines such as IL-6 or TNF-α [94, 98], whereas others have reported the opposite effect [96, 99]. Other results show that the induced production of cytokines IL-10, IL-4, and gamma interferon in Jurkan T cells is not altered by PS [97]; however, this observation is not devoid of controversy as this effect could not be confirmed in animals [100]. However, regardless of their mechanism, different animal and human studies provide a reasonable body of evidence supporting the anti-inflammatory properties of PS [13, 100–102].

Altogether, these beneficial effects on immune and inflammatory functions seem promising as they can also be involved in the development of other pathologies such as cancer or atherosclerosis. Nevertheless, current evidence is not consistent enough, and further research is necessary to elucidate the clinical implications of sterol supplementation.

4.3 Antidiabetic Effect

PS may play an important role in ameliorating obesity or diabetic-associated disorders [13], despite the evidence is scarce. Misawa et al. [103] reported that oral administration of two types of antidiabetic PS isolated from *Aloe vera* (lophenol and cycloartenol) improves hyperglycemia in Zucker diabetic fatty rats [103]. Moreover, these PS can downregulate the expression of hepatic genes involved in the expression of gluconeogenic enzymes (glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, Pepck) and upregulate that of β-oxidation enzymes such as peroxisome proliferator-activated receptor alpha (Ppar-α) [104]. Likewise, PS have been proposed to be one of the main compounds responsible of the antidiabetic effect of some plant extracts [13, 105], although further clinical evidence is necessary.

5 Side Effects

The hypocholesterolemic effect of PS at high doses is well known, and several nutraceutical products have been approved until now by the administrations of the EU, USA, and other countries. However, much less is known about the possible toxicity or undesirable side effects of this high intake.

5.1 Toxicity

It is generally accepted that consumption of PS is safe due to their negligible rate of absorption in the upper small intestine. However, it is still in debate whether toxicological effects could appear at very high and continuous intake of some plant sterols and stanols [16].

Several studies conducted in animals [106, 107] and humans [108, 109] did not find negative physiological effects at high and/or continuous intake of plant sterols and stanols. However, other reports have reported that some toxicological effects, mainly related to the possibility of anomalous accumulation of PS in some tissues, must be taken into account [110, 111]. For instance, the study of Lees et al. [111] on hypercholesterolemic patients treated with plant sterol preparations found high serum levels of campesterol (mean 16 mg dL^{-1}) in five patients, suggesting the possibility of iatrogenic atherosclerosis due to PS accumulation. However, another study conducted in rabbits fed with semi-purified diets containing sitosterol or sitostanol esters revealed that while serum cholesterol was present at levels of milligrams dL^{-1}, serum PS were present at levels of micrograms dL^{-1} [112]. In the same study, aortic cholesterol and PS were found in microgram and nanogram quantities, respectively, and no aortic lesions were observed. One of the major concerns regarding the PS accumulation in tissues is based on their accumulation in the brain. Jansen et al. [110] reported that increased circulating levels of plant sterols, as a result of intake of a plant sterol-enriched diet in wild-type mice or as

a consequence of ABCG5 or ABCG8 deficiency, was associated with elevated levels of plant sterols in the brain. More recently, Vanmierlo et al. [113] reported that mice fed with a plant sterol ester-enriched diet for 6 weeks displayed increased concentrations of plant sterols in serum, liver, and brain. In addition, the authors observed that after stopping plant sterol intake for a period of 6 months, brain PS levels remained unaffected. Interestingly, this accumulation was not found when animals received plant stanols. However, the clinical implications of these are still to be established.

5.2 Sitosterolemia (Phytosterolemia)

It has been estimated that a maximum of 5% of the plant sterol intake is absorbed, resulting in very low levels in plasma (0.5 mg dL^{-1},representing less than 0.5% total neutral sterols in plasma) [16]. However, there is a very restricted group of patients presenting a rare autosomal recessive disease called phytosterolemia [114]. These subjects can absorb up to 60% of the dietary plant sterols whereas the rate of absorption of cholesterol seems to be normal [115, 116]. This disease is characterized by mutations occurring in ABCG genes controlling the efflux of PS at intestinal level and the delivery of PS by the liver [117, 118]. Several studies reported that these patients have PS plasma levels from 18 to 72 mg dL^{-1}, which represents 7–30% of total neutral sterols in plasma [119].

With this disease there is an accumulation of PS not only in plasma but also in adipose tissue, skin, aorta, and other tissues. As a result, main symptoms of this disease include xanthomatosis and atherosclerosis [120]. This accumulation is not only related by hyperabsorption of PS but also by impaired biliary secretion. Some authors have found that phytosterolemia patients present around 20% reduction of PS biliary excretion and around 50% reduction of the whole-body cholesterol synthesis [30, 121, 122]. It has been also found that in sitosterolemic patients, the hepatic conversion of cholesterol to bile acids is blocked, which can result in cholesterol accumulation and atherosclerosis [115].

New interest in the development of sitosterolemia has arisen from the fact that several studies found relationships between anomalous high levels of PS in plasma and CHD in non-sitosterolemic subjects [120]. For instance, Glueck et al. [123] found that plasma cholesterol levels of 7 mmol L^{-1} and 40 μmol L^{-1} of PS were associated with a higher deposition of PS in the aorta in seven subjects. Likewise, Stalen et al. [124] reported that lethal atherosclerosis is related to increases in plasma PS levels. Therefore, PS levels in phytosterolemia patients must be carefully controlled.

5.3 Phytosterol Oxides

Although cholesterol oxides and their biological effects have been studied for many years [125, 126], much less is known about the biological effects of phytosterol

oxides (PSO). However, the approval to supplement several food products with high doses of PS has generated the need to determine in which extent PSO can be present or formed in these products and whether they can constitute a risk for the consumer. As PS are very closely related to cholesterol in their molecular structure, it seems logical to hypothesize that they can undergo similar oxidative reactions. Thus, hydroxy-, keto-, epoxy-, and triol-derivatives of sterols would be expected to be the most abundant oxides found in food products. In contrast, plant stanols are lacking the nucleus double bound, which indicates that their oxidative pathways would not be analogous to those of cholesterol when they are subjected to processes involving reactive oxygen and free radical species, as well as irradiation and heating [127]. For stanols, the main ways of oxidation involve enzymatic reactions, which can affect the side chain [128].

The attribution of biological effects to PSO is controversial as studies reporting reliable values of their concentrations in food products are scarce and very recent [126, 129]. In addition, the values reported for PSO from food, plasma, or tissues are not easy to compare between studies due to the different analytical methods used for quantification, the lack of availability of PSO standards, the similarity of their structure, and their presence in trace amounts [130]. Thus, a better validation and standardization of analytical procedures is the main concern for future research in this field as higher amount of reliable results would help to elucidate the biological effects of PSO.

In phytosterol-enriched spreads, the content of PSO ranges from 12 to 68 $\mu g\ g^{-1}$ [131, 132]. This would correspond to a possible ingestion of less than 1.7 mg PSO day^{-1}, according to the recommended daily intake of 20–25 g of spread. Abramsson-Zetterberg et al. [133] estimated that in the Swedish adult population, the intake of PSO, which are originally from heated vegetable oil, should be less than 0.7 mg day^{-1}. This value would be clearly lower than that estimated for the intake of cholesterol oxides (3.0 mg per day) in the context of a low-cholesterol intake population (<300 mg of cholesterol day^{-1}) [127]. The total amount of PSO in the body is still controversial, and correlations between PSO intakes and PSO levels are difficult to establish as PSO levels in the body not only come from dietary sources but also from endogenous formation [134–136]. The first reliable data regarding plasma PSO levels were reported by Plat et al. [137]. These authors were able to detect sitostanetriol and 7-keto, 7α- and 7β-OH, and α-epoxy derivatives from β-sitosterol in phytosterolemia patients, although they did not correlate these levels with adverse health effects. Some other studies were able to measure PSO in plasma of healthy volunteers [132, 138]. In all cases, β-sitosterol oxides seem to be predominant, which suggests that the absorption of β-sitosterol oxides is higher than that of other PS oxides or that the oxidation of circulating β-sitosterol in plasma is higher than that of other PS [127].

Several studies have reviewed the biological effects of PSO [126, 127, 130], although it remains questionable whether PSO can exert undesirable or beneficial effects. Some authors have proposed that PSO can be pro-inflammatory, pro-atherosclerotic, and cytotoxic [139, 140]. In contrast, others have reported beneficial effects, including modulation of cholesterol homeostasis and anti-inflammatory,

lipid-lowering, and antidiabetic properties [126, 127, 130]. Moreover, for a long time, oriental traditional folk medicine has been using extracts from plants belonging to the genera Euphorbia, Urtica, and Bombyx for the treatment of some cancer-type pathologies. Recently, it has been reported that, in these extracts, PSO are the most abundant steroid compounds [141]. It has been demonstrated that PSO can induce cell death by apoptosis in different type of cell models [126]. Some observations suggest that the biological effects of PSO are similar to that of cholesterol oxides, but being five times less active [142]. Therefore, although PSO can modulate the human metabolism in some extent, it is difficult to conclude whether they are responsible of any relevant toxic effect.

5.4 Decrease in Plasma Levels of Carotenoids

Several studies have reported associations between high doses of plant sterol and stanol ester intakes and decrease in plasma carotenoid concentrations. The first problem in understanding the relevance of this effect is that no standard levels of plasma carotenoids can be clearly established [16]. Several studies comparing high and low intakes of PS have reported a parallel decrease in plasma carotenoid levels when high amounts of PS are consumed. Kritchevsky [16] compiled nine different studies in humans that were administered with pure stanols (between 0.8 and 3.2 g day^{-1}), pure sterols (between 0.8 and 3.6 g day^{-1}), wood-derived stanols (2.3 g day^{-1}), or vegetable-derived stanols (2.6 g day^{-1}) during a variable period. Results of these studies showed that in all cases, relevant dose-dependent decreases in plasma total cholesterol and LDL-c were found, which was correlated with a parallel decrease in carotenoid concentrations. The reduction was up to 20% in some cases. However, results between studies are not easy to compare as the carotenoids determined are not always the same. Even though, most of these intervention studies showed that the decrease in plasma carotenoid levels, which was accompanied by α-tocopherol decreases, disappeared when values were corrected for total cholesterol [143]. In other cases, this decrease in carotenoid and tocopherol was not observed [34]. In addition, certain studies reported that basal plasma levels of these carotenoids can be maintained by supplementing with carotenoids a high-PS diet [144]. Given these evidences, Kritchevsky [16] explained the disparity of results from different studies by differences in the carotenoid and vitamin levels present in the basal diets.

It is worth mentioning that reductions in plasma carotenoids up to 10–20% of plasma carotenoids could not be associated with negative physiological effects [145, 146]. However, it has been hypothesized that a reduction on carotenoid plasma levels could induce some disturbance in the antioxidative pathways associated with the prevention of cancer development [81]. Glutathione-*S*-transferases (GST) are enzymes with antioxidant properties which can be partly involved in the prevention of some cancers [147]. In prostate cancer, a reduction of GST1 expression may promote the susceptibility to the carcinogenic effect of chemicals, while induction of GST (i.e., by carotenoid supplementation) in early-stage prostate

cancer can be a useful as a protective strategy [148]. Data existing at this moment reveal that only a slight reduction of GTS activity can be observed after PS intake [81]. Moreover, no clinical data showing a direct relationship between PS intake and risk of prostate cancer is available.

Nonetheless, since there is a parallel reduction in plasma cholesterol and carotenoid levels, it is advised to supplement the diet with these microcomponents in order to avoid the risk of side effects.

5.5 Other Safety Aspects

Other unfavorable effects have been proposed for PS intake at high doses. For instance, it is well known that PS intake increases the excretion of coprostanol and cholesterol [149]. Some recent data have shown that this increased excretion can promote the development of colon cancer [150]. However, this association was found in studies where the population consumed low-quality, high-fat diets. Therefore, it is difficult to investigate the direct relationship between high levels of PS intake and a higher colon cancer risk [81]. Further, some authors have suggested that PS could also participate as promoters of colon cancer by a possible mutagenic effect on gut bacteria. Studies on the mutagenic potential effect of PS and their esters have been conducted in bacterial in vitro cell gene mutation and in vivo rat mutagenicity assays [151]. Negative results have been obtained in all cases for PS, phytosterol esters, and for several metabolites of cholesterol (4-cholesten-3-one and 5β-cholestan-3-one). Some authors have also suggested that a high intake of PS and their esters can lead to higher levels of oxides, which can accelerate lipid autoxidation [81]. However, there is not enough information to conclude on the relevance of this oxidation process in promoting mutagenicity.

On the other hand, some studies, mainly performed in animals, have shown certain interesting and noteworthy effects of high PS intakes. Studies conducted in stroke-prone spontaneously hypertensive rats (SHRSP) demonstrated that dietary cholesterol and PS clearly affect the development of stroke and the survival of rats after the stroke [152]. According to these authors, certain level of plasma cholesterol is needed after the stroke in order to maintain the integrity of red blood cells. In contrast, high levels of PS in plasma can lead to a higher fragility of these cells and a shorter life span.

In addition, another safety aspect of relevant interest is the existence of a population of hypercholesterolemic patients with normal diets that also show hyperphytosterolemia [153]. This fact was observed in a study conducted in the USA with 595 subjects that constituted the top serum cholesterol quintile among a total population of 3,472 subjects. Approximately a 3.5% of these subjects showed anomalous high campesterol and stigmasterol levels, being considered "hypercholesterolemic-hyperphytosterolemic." This fact was associated with a personal or familiar history of premature CHD (<55 years old). However, a relevant fact reported in a follow-up study was that the levels of phytosterolemia can be normalized in this type of patients by eating a low plant sterol diet [123].

Other studies have reported similar observations [154, 155], supporting the conclusion that subjects with low endogenous cholesterol synthesis are at increased risk for coronary heart disease if they do not restrict PS intake in their diets. For these subjects, a careful control of the ratio of cholesterol/plant sterols in the diet and in plasma is recommended.

6 Conclusion

CHD is one of the main causes of mortality in developed countries. Elevated blood cholesterol concentration is known to be a major risk in the development of CHD. LDL-c in plasma can be reduced by 10% by consuming 2 g day^{-1} of PS. Although it has been suggested that higher doses can lead to undesirable side effects, the consumption of PS is generally considered safe. Current research is providing valuable insights with regard to the pathways associated to the beneficial effects of PS, although their exact mechanisms are not yet fully understood. Recently, some studies have suggested that these compounds may be able to prevent diseases such as cancer, diabetes, and inflammatory and immune disorders. Nevertheless, more studies are necessary to confirm their clinical relevance. The combination of PS with other bioactive compounds, drugs, or therapies also seems promising as their beneficial effects can be complementary or synergic.

Therefore, the incorporation of PS into functional foods or nutraceutical products can be of relevant interest not only to reduce the risk of CHD but also to prevent the development of many other diseases.

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