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Journal of Nutritional Biochemistry 14 (2003) 362–369

THE JOURNAL OF Nutritional Biochemistry

Reviews: Current Topics

# Metabolic effects of plant sterols and stanols (Review)

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**Abstract**

High serum LDL cholesterol concentration is a major risk factor for cardiovascular complications. This risk can be lowered by diet. In this respect foods containing plant sterol or stanol esters can be useful for mildly- and hypercholesteraemic subjects. Plant sterols and stanols, which are structurally related to cholesterol, decrease the incorporation of dietary and biliary cholesterol into micelles. This lowers cholesterol absorption. Furthermore, these components increase ABC-transporter expression, which may also contribute to the decreased cholesterol absorption. Consequently, cholesterol synthesis and LDL receptor activity increase, which ultimately leads to decreased serum LDL cholesterol concentrations. Animal studies have further shown that these dietary components may also lower atherosclerotic lesion development. Plant sterols and stanols also lower plasma lipid-standardized concentrations of the hydrocarbon carotenoids, but not those of the oxygenated cartenoids and tocopherols. Also, vitamin A and D concentrations are not affected. Although absorption of plant sterols and stanols (0.02-3.5%) is low compared to cholesterol (35-70%), small amounts are found in the circulation and may influence other physiological functions. However, there is no consistent evidence that plant sterols or stanols can change the risk of colon or prostate cancer, or immune status. In conclusion, plant sterols and stanols effectively reduce serum LDL cholesterol and atherosclerotic risk. In addition potential effects of plant sterols and stanols on other metabolic processes remain to be elucidated. 

*Keywords:* Plant sterol; Plant stanol; Atherosclerosis; Sterol metabolism; Diet

## 1. Introduction

Increased cholesterol concentrations in the low-density lipoproteins (LDLs), a well-known modifiable risk factor for atherosclerosis [1], can be lowered by changing the fatty acid composition of the diet [2]. Recent dietary recommendations, however, also emphasize the possibility of lowering LDL cholesterol levels through consumption of products enriched with plant sterols or stanols [2]. Plant sterols and stanols, which are mainly present in nuts, vegetable oils, seeds, cereals and beans, are structurally related to cholesterol, but are characterized by an extra ethyl (sitosterol) or methyl group (campesterol) in the side chain [3,4]. They cannot be synthesized by humans, and all plant sterols and stanols in the human body therefore originate from the diet. Sitosterol, campesterol and stigmasterol are the most common plant sterols in nature [5]. Sitostanol and campestanol are saturated plant sterols, which are found in nature in much smaller amounts than plant sterols. Because of their cholesterol-lowering effects, these components are incorporated nowadays into a wide variety of food products, referred to as functional foods.

This review first describes different aspects of cholesterol metabolism, and in the second part describes the effects of plant sterols and stanols on sterol metabolism and atherosclerosis risk. Finally, the effects of plant sterols and stanols on other metabolic processes in the human body are discussed.

### *1.1. Cholesterol metabolism*

#### *1.1.1. Intestinal cholesterol absorption and lipoprotein metabolism*

In the intestinal tract, esterified cholesterol from the diet is first hydrolyzed. Absorption of free cholesterol now depends on mixed micelles: mixtures of free cholesterol, mono- and di-acylglycerols, fatty acids, phospholipids, and bile salts. The transfer of free cholesterol from the mixed micelles through the apical brush border membrane into the enterocyte is not completely understood [6]. This process was thought to be driven by passive diffusion [3], but recent findings suggest the existence of a specific, saturatable cholesterol transporter in the intestinal mucosa facilitating the transport of cholesterol into the cell [7].

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0955-2863/03/$ – see front matter 
doi:10.1016/S0955-2863(03)00002-0

Within the enterocyte, free cholesterol is esterified by acyl-coenzyme A cholesterol acyltransferase (ACAT), incorporated into chylomicrons, which are subsequently excreted into the circulation and converted into a chylomicron-remnant by the action of lipoprotein lipase [8]. The liver then takes up these chylomicron-remnants and forms very-low density lipoproteins (VLDLs). VLDL can subsequently be converted into intermediate lipoproteins (IDL), and IDL into low-density lipoproteins (LDL). LDL particles contain most of the cholesterol and can be - like the IDL - cleared from the circulation by the LDL receptor. Free cholesterol, however, can also be transported back into the intestinal tract through ATP-binding cassette (ABC) transporters, such as ABCA1, ABCG5 and ABCG8 [9].

#### *1.1.2. Cellular cholesterol homeostasis*

Cells tightly regulate their intracellular free cholesterol concentrations through ABC transporter mediated cholesterol efflux, LDL-receptor mediated lipoprotein uptake and endogenous cholesterol synthesis [1,3]. Transcription of ABC transporters depends on intracellular oxysterol concentrations. These oxysterols originate from the oxidation of redundant cellular cholesterol and are potent ligands for the Liver X receptor (LXR). LXR activity may ultimately upregulate transcription of ABC transporters and increase cellular cholesterol efflux.

Gene expression of the LDL-receptor and of HMG CoA reductase - a key enzyme for cholesterol synthesis - is controlled by sterol regulatory element-binding proteins (SREBPs) [10]. When intracellular free cholesterol levels are low, the amount of SREBP increases [11], resulting in increased transcription of the LDL-receptor and HMG CoA reductase genes. In this way, cellular cholesterol influx and synthesis is raised. SREBP transcription and activity is also affected by LXR [12].

### *1.2. Effects of plant sterols and stanols on sterol metabolism*

#### *1.2.1. Absorption of plant sterols and stanols*

Plant sterols are poorly absorbed in the intestine (0.4-3.5%), while absorption of plant stanols (0.02-0.3%) is even lower [13]. For comparison, cholesterol absorption ranges between 35 and 70%. Gender differences may exist as absorption of plant sterols and stanols in female rats appeared to be higher than in male rats [14]. Interestingly, plant stanols may also lower plant sterol absorption and vice versa [15].

A reason for the low absorption of plant sterols and stanols might be that plant sterols and stanols are poorly esterified, possibly due to the low affinity of ACAT for these components [16]. As merely esterified sterols are incorporated into chylomicrons, absorption of the unesterified plant steros and stanols is consequently low. The difference in absorption between plant sterols and stanols is reflected by their serum concentrations. On regular diets as well as on plant sterol- or stanol-enriched diets, serum plant sterol concentrations are 10-30 times higher than plant stanol concentrations [13].

It has been suggested that the extent and rate of plant sterol or stanol absorption depends on the side chain length and the presence of the $\Delta 5$ double bond (saturation) [17,18]. Other factors like mutations and polymorphisms in the ABCG5 or ABCG8 gene may change the intestinal absorption of plant sterols or stanols [19]. For example, mutations in either the ABCG5 or ABCG8 gene may lead to sitosterolemia. This rare autosomal recessive disorder results in accumulation of plant sterols and stanols [19,20] and can lead to severe atherosclerosis already at a very young age. Furthermore, the use of statins results in higher plasma cholesterol-standardized sterol levels [21], but whether this is due to an increased plant sterol absorption or to a decreased clearance of sterols is not known [22,23]. In hypercholesterolemic patients, the cholesterol-lowering effects of statins and plant sterols or stanols are additive [23,24]. This suggests that these patients may need a lower dose of statins, when consuming plant sterol or stanol enriched margarines. In rats, increased amounts of plant sterols were found in adrenal glands, intestinal epithelia and ovaries after intake of a diet enriched with plant sterols [14,25]. In studies with rabbits, campesterol and sitosterol were also found in trace amounts in the aorta, muscle, skin subcutaneous adipose tissue and liver after intake of a diet enriched with plant sterols [26]. In addition, after consumption of plant stanols, the deposition of these compounds in rats' tissues is almost negligible compared with plant sterols [27].

#### *1.2.2. The effects of plant sterols and stanols on intestinal cholesterol absorption*

Different mechanisms have been suggested to explain the cholesterol-lowering activity of plant sterols and stanols (Figure 1) [28]. Firstly, plant sterols or stanols may displace cholesterol from mixed micelles [29], because they are more hydrophobic than cholesterol. This replacement causes a reduction of micellar cholesterol concentrations and consequently lowers cholesterol absorption. Furthermore, plant sterols or stanols might reduce the esterification rate of cholesterol in the enterocyte [30] and consequently the amount of cholesterol excreted via the chylomicrons. However, in Caco2-cells no effect of sitosterol on ACAT activity was found [31].

The effects of plant stanols on cholesterol absorption continue for at least several hours after ingestion. This was recently demonstrated by Plat et al [32], who showed that the decrease in serum cholesterol in subjects consuming margarines enriched with plant stanol esters once a day was similar to that in subjects who consumed the same amount of plant stanol esters divided over three servings a day. A possible explanation for this effect may be that plant stanols remain present in the intestine for a while. Another explanation may be that the stanols present inside the enterocytes

affect intestinal lipoprotein metabolism. In this respect, Plat and Mensink have recently shown in Caco-2 cells that plant stanols upregulate the expression of ABC-transporters in intestinal cells, which may result in an increased excretion of cholesterol by the enterocyte back into the lumen [33]. ABCA1 -/- mice showed increased cholesterol absorption [34], however not all ABCA1 knock out mice show increased cholesterol absorption, probably because of differences in genetic background [35].

*1.2.3. Effects on lipid and lipoprotein metabolism*

An earlier study has suggested that plant stanols are more effective than sterols [18]. However, three independent studies [36,37] have shown that plant sterol and stanol esters have comparable effects on serum LDL cholesterol.

In response to the decreased cholesterol absorption, cholesterol synthesis increases [38]. Also, LDL receptor mRNA and protein expression increases [39]. This will not only increase clearance of LDL, but also of IDL. As IDL is the precursor for LDL, this will lower LDL production, as indeed has been demonstrated by Gylling et al [15] using radio-labeled LDL. At a daily intake of 2-2.5 g of plant sterols or stanols, the lower cholesterol absorption, the higher LDL receptor expression and the higher endogenous cholesterol synthesis together result in an average reduction of LDL cholesterol of up to 14% [40]. The decrease of total serum cholesterol is completely accounted for by a reduction in LDL. At higher daily intakes, the additional effects on LDL are marginal [36]. Plant sterols and stanols have no effect on triacylglycerol or HDL cholesterol levels [41]. Interestingly, in diabetic patients an increase in HDL cholesterol levels was found, and this affects atherosclerosis in a positive way [38]. The reason for the positive effect remains unclear, but may be related to disturbances in lipoprotein metabolism as found in diabetic subjects, such as increased triacylglycerol concentrations or VLDL production. Effects of plant sterols and stanols in humans on hepatic VLDL production, however, are not known.

No consensus exists about the effects of plant sterols or stanols on bile metabolism [42]. In colectomized patients, biliary secretion of cholesterol, bile salts and phospholipids remained unchanged after consumption of a plant stanol ester-enriched margarine [43]. Consumption of plant sterols also did not change bile salt excretion, measured in ileostomy bags and feeces respectively [4,44]. In Apolipoprotein E*3-Leiden transgenic mice, however, plant stanol ester consumption reduced cholesterol saturation of bile resulting in less excretion of bile in bile salts [45]. Finally, a study in Wister rats did show a change in bile salt excretion after stigmasterol consumption [42].

*1.2.4. Effects on development and regression of atherosclerosis*

Although serum LDL cholesterol concentrations are a well-validated biomarker for atherosclerotic risk, only measurement of lesion development truly reflects the effects of a compound on atherogenesis but this is difficult to measure in humans. In this respect, small animal models are of great benefit to evaluate the potential of a dietary compound to affect lesion formation and to unravel the underlying mechanisms. For this purpose, normal rodents are not very suitable, as their plasma lipoprotein profiles are not human-like, while these rodents are also very resistant to the development of atherosclerosis. However, in transgenic mice with specific defects in lipid and lipoprotein metabolism - such as the LDL receptor-deficient mouse, lipoprotein concentrations are sensitive to dietary changes and drug treatment [46,47].

Whether the reduction in LDL cholesterol after consumption of plant sterol and stanol esters lowers cardiovascular risk in humans has formerly not been proven. Animal studies, however, are convincing and showed decreased plaque formation after consumption of plant sterols or stanols. In ApoE-deficient mice, a mixture of plant sterols and stanols reduced both atherosclerotic lesion size and complexity [48]. These effects were attributed to the cholesterol-lowering effects of plant sterols and stanols. In a second study, also in apoE-deficient mice [49], the prevention of plaque formation after plant sterol consumption was attributed to a reduction in the concentration of the atherogenic $\beta$-VLDL particles, resulting in a decreased foam cell formation. In New Zealand White (NZW) rabbits, pure sitostanol also reduced plaque formation [16]. Finally, in apoE*3-Leiden transgenic mice, plant stanol esters reduced atherosclerotic lesion size and severity mainly by lowering plasma levels of VLDL and IDL [50].

Except for effects on the plasma lipoprotein profile, *in vitro* studies suggest that plant sterols may affect plaque formation through other mechanisms as well. Awad et al [51] showed decreased growth and proliferation of vascular smooth muscle cells (VSMC), isolated from rats and cultured in the presence of sitosterol and campesterol. Also, prostacyclin release from VSMC in response to plant sterol administration was increased, which may cause vasodilation and decrease platelet aggregation.

Whether plant sterols and stanols induce regression of existing atherosclerotic plaques is less evident. Moghadasian et al [52] were not able to show regression of atherosclerotic plaques after feeding apoE-deficient mice a mixture of plant sterols and plant stanols. It was remarkable, however, that serum cholesterol levels in the sterol-treated animals were not reduced compared with those in the control group.

## 2. Other effects of plant sterols and stanols

In addition to the effects on lipid and lipoprotein metabolism, plant sterols and stanols may affect other metabolic processes (Figure 2). Although serum campesterol concentrations are about three times higher than sitosterol concen-

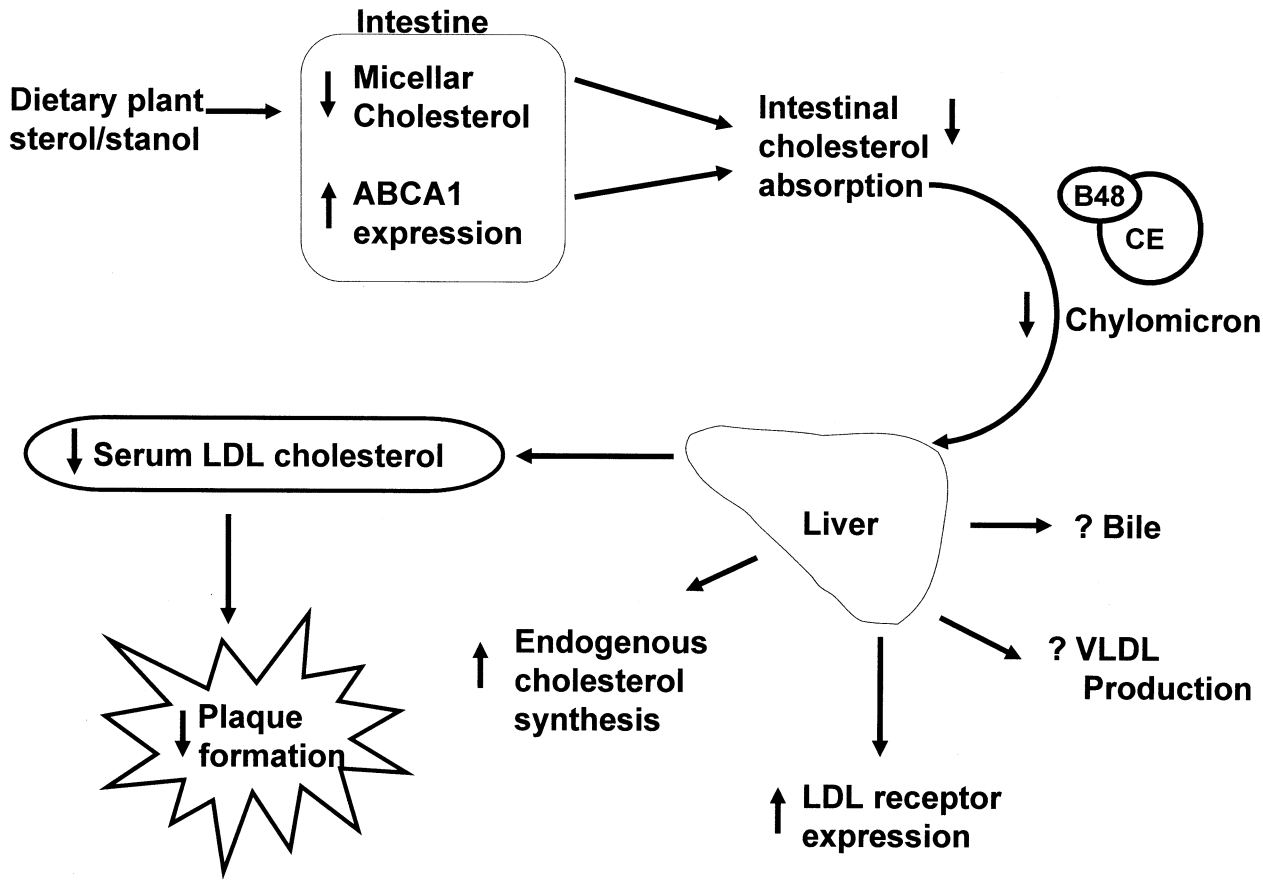


Fig. 1. Potential effects of plant sterols and stanols on lipid and lipoprotein metabolism. Animal and *in vitro* studies have shown that plant sterols and stanols reduce micellar cholesterol incorporation, and may increase intestinal ABCA1 expression. These changes result in a reduced intestinal cholesterol absorption and into a reduced cholesterol flux through chylomicrons to the liver. Consequently, endogenous cholesterol synthesis and LDL receptor expression in the liver increase. Effects on VLDL production, and bile composition and output are unknown. The overall effect of these metabolic changes is a reduction in serum LDL cholesterol concentrations. Animal studies have further shown that increasing the intake of plant sterol and stanols lowers plaque formation.

trations, most studies have focused upon metabolic effects of sitosterol.

### *2.1. Effects on fat-soluble antioxidants*

Since plant sterol and stanol esters reduce cholesterol absorption by decreasing the solubility of cholesterol in mixed micelles, it is interesting to examine the effects of these dietary components on the absorption of the fat-soluble antioxidants from the diet. In agreement with other studies, we have recently shown that daily consumption of 3.8 to 4.0 g plant stanols provided as fatty acid esters during a period of eight weeks significantly lowers serum concentrations of various cartenoids and tocopherols [53]. A part of this reduction can be explained by a decreased number of LDL particles in the circulation, which transport these fat-soluble antioxidants. Therefore, results are normally standardized for serum lipid concentrations. However, even after standardization, the concentrations of the hydrocarbon carotenoids ($\alpha$-carotene, $\beta$-carotene and lycopene) are lowered. Interestingly, we have demonstrated that the reduction of the lipophylic hydrocarbon carotenoids after plant stanol ester consumption- was associated with the lowered cholesterol absorption, whereas changes in oxygenated carotenoids (lutein, zeaxanthin and cryptoxanthin) and in tocopherols were mainly associated with lowered serum LDL cholesterol concentrations [53]. This suggests that plant sterols and stanols interfere in particular with the incorporation into the mixed micelles and/or absorption of the lipophylic hydrocarbon carotenoids. The clinical importance of the reductions in plasma carotenoids is not known, but certainly warrants attention. Furthermore, during the eight-week intervention period of this study, plasma retinol (vitamin A) and vitamin D concentrations were not affected [53]. These findings suggest that absorption or synthesis of these fat-soluble vitamins, that are not transported by lipoproteins, is not affected. Effects on vitamin K concentrations are not clear. Hendriks et al [41] showed a dose-dependent reduction of vitamin K concentrations of 5.4%, 7.4% and even 18% at a daily intake of 0.8 g,1.6 g and 3.2 g of plant sterols respectively, for 3.5 weeks. However, eight weeks of 3.8 g vegetable oil based plant stanols or 4.0 g wood based plant stanols had no effect on vitamin K dependent blood coagulation factors and fibrinolytic parame-

ters [54]. In addition, daily consumption of 4.5 g plant stanol esters for eight weeks did not influence warfarin anticoagulant therapy, suggesting that vitamin K dependent heamostasis is not substantially affected after plant stanol consumption [55]. This suggests that, at least in short term studies, vitamin K status is not affected by consumption of plant stanols.

### *2.2. Effects on colon and prostate cancer*

Soybeans are, among other potentially bio-active compounds, rich in plant sterols. Populations with high intakes of soybean-based products, such as Seventh-day Adventists and Mexican Taramuhara Indians, have lower cancer mortality rates, especially of the colon and the prostate [56,57]. It is therefore tempting to suggest that plant sterols reduce the risk for certain cancers.

Bile acids may promote colon cancer, and an indirect effect of plant sterol consumption via a lowered bile secretion has been suggested as the mechanism [58]. However, *in vitro* cell studies clearly suggested that a possible protective effect of sitosterol on colonic epithelial cell proliferation, as a marker for carcinogenesis, was independent of cholic acid concentrations [59]. However, sitosterol inhibited the growth of HT-29, a human colon cancer cell line [60]. In an *in vivo* study, a diet supplemented with sitosterol decreased the number of mice carrying benign colon tumors, although the proportion of malignant tumors was higher in mice consuming sitosterol than control mice [56]. Finally, in a recent prospective cohort study, Normen et al [61] could not demonstrate a relationship between plant sterol intake and the risk for colon and rectal cancers in humans.

It has also been suggested that sitosterol may beneficially affect prostate cancer. Indeed, it was found that men taking 20 mg sitosterol three times a day showed symptomatic improvement of benign prostatic hyperplasia. Clinical improvement was indicated by increased urine flow and decreased residual urinary volume, but no relevant reduction in prostatic volume was observed [62]. Others have found similar beneficial clinical results in men taking 65 mg a day of a sitosterol extract [63]. However, these results are remarkable, as such doses of sitosterol are negligible compared with the normal intake of 163 ± 63 mg in Western diets [61]. Therefore, it is arguable if the reported results can be explained by the slightly increased intake of sitosterol.

In conclusion, there is no strong and consistent evidence that sitosterol intake affects either colon or prostate cancer risk.

### *2.3. Effects on membrane properties*

Plant sterols and stanols are incorporated into cellular membranes [64,65] and may consequently influence membrane properties. Although Mora et al [66] showed no effects of sitosterol on the membrane fluidity of human keratinocytes, Ratnayake et al [65] recently showed deleterious effects of increased incorporation of plant sterols into red blood cell membranes of stroke-prone spontaneously hypertensive rats. Interestingly, the life span of these rats was shortened, probably because plant sterols replaced cholesterol in the membrane of the red blood cells, which made the erythrocytes less deformable [65] and more fragile [67]. In this way the transport of red blood cells through the smallest capillaries may be impaired. Further, stiffer red blood cell membranes may cause more sheer stress on the microvessel wall, which may induce vessel wall injury. Similar findings have been reported for plant stanols [68]. Whether the effects of plant sterols and stanols in rats on red blood cell deformability can be extrapolated to humans is not known.

### *2.4. Effects on the immune system*

Effects of plant sterols and stanols on immune function have not been studied in detail, but some studies do suggest that these compounds may have an effect. For example, plant sterol supplementation lowered serum interleukin-6 (IL-6) concentrations and the cortisol/dihydroepiandrosterone sulfate (DHEA) ratio after marathon running, suggesting a lower inflammatory response [69]. Also, the increase in the number of white blood cells was smaller in the intervention group than in the control group, indicating that the plant sterol group was less immune-suppressed [69]. In contrast, feeding plant sterols to rats, increased the plant sterol/cholesterol ratio in liver microsomes, which was associated with an increased n-6/n-3 ratio, possibly caused by changes in desaturase activities [70]. It has been suggested that immune function is negatively related to the n-6/n-3 ratio [71].

## 3. Conclusion

Plant sterols and stanols effectively lower serum LDL cholesterol levels and may therefore play an important role in atherosclerotic lesion development. However, future research is necessary to determine the exact effect of plant sterols and stanols on atherosclerotic lesion development and plaque regression. It is clear that plant sterols and stanols are useful for mildly and hypercholesterolemic subjects as an addition to the diet or to cholesterol lowering medication such as statins. The reduced intestinal absorption of cholesterol leads to an increase in cholesterol synthesis and to an increased LDL receptor expression. Effects on VLDL production are unknown, and whether bile formation and/or composition is changed is also not clear. In most studies serum HDL cholesterol and triacylglycerol concentrations remained unchanged. Patients with mutations in ABCG5 and ABCG8, suffering from the rare (only 40 patients are diagnosed worldwide [72]) inheritable disease sitosterolemia, are characterized by extremely high serum plant sterol and stanol concentrations, indicating the

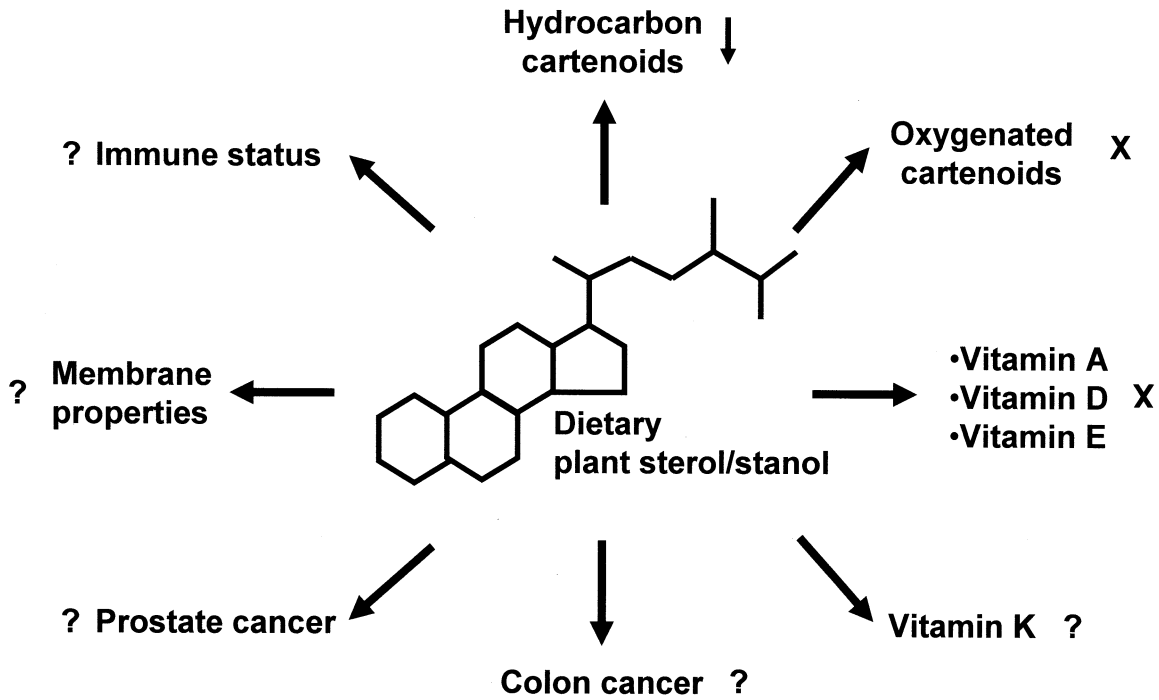


Fig. 2. Potential metabolic effects of plant sterols and stanols not related to lipid and lipoprotein metabolism. Human studies have shown that plant sterols and stanols lower lipid-standardized plasma concentrations of the lipophylic hydrocarbon carotenoids ($\alpha$-carotene, $\beta$-carotene and lycopene), but not those of oxygenated cartenoids (lutein, zeaxanthin, cryptoxanthin) or vitamin E. Absolute vitamin A and D concentrations do not change, while effects on plasma vitamin K concentrations are less clear. Further, there is no convincing evidence that plant sterols or stanols change colon and prostate cancer risk. Rat studies suggest that plant sterols and stanols diminish erythrocyte membrane deformability and increase erythrocyte fragility. These findings have not been confirmed in human studies. Finally, effects of plant sterols and stanols may influence immune status, but again effects are not clear.

relevance of ABC transporters in sterol metabolism. In addition, ABC-transporter expression in Caco2-cells is up-regulated when plant stanols are added, which may result in an increased cholesterol excretion of the enterocyte and thus may contribute to the cholesterol lowering properties of plant stanols. Although usually only small amounts of plant sterols and stanols are absorbed, effects on other metabolic pathways must be considered. Plant sterol and stanol consumption only seems to affect serum concentration of lipophylic and oxygenated cartenoids and tocopherols, but not of other fat-soluble antioxidants. Furthermore, the effects of sitosterol consumption on colon or prostate cancer risk are not really evident. On the other hand plant sterol and stanol consumption seem to decrease cell deformability and increase cell fragility at least in animal studies. It is not clear however, whether these results can be extrapolated to humans. Finally, conflicting effects of plant sterols and stanols on immune function have been shown in different studies. All these issues that are not only important for plant sterol and stanol enriched products, but also for functional foods in general, should be addressed in carefully controlled studies.

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