Lipid-lowering Activity of Natural and Semi-Synthetic Sterols and Stanols

Dhiaa A Taha[1], Ellen K Wasan[2], Kishor M Wasan[2] and Pavel Gershkovich[1]

[1]School of Pharmacy, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK.[2] College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

ABSTRACT - Consumption of plant sterols/ stanols has long been demonstrated to reduce plasma cholesterol levels. The objective of this review is to demonstrate the lipid-lowering activity and anti-atherogenic effects of natural and semi-synthetic plant sterols/ stanols based on evidence from cell-culture studies, animal studies and clinical trials. Additionally, this review highlights certain molecular mechanisms by which plant sterols/ stanols lower plasma cholesterol levels with a special emphasis on factors that affect the cholesterol-lowering activity of plant sterols/stanols. The crystalline nature and the poor oil solubility of these natural products could be important factors that limit their cholesterol-lowering efficiency. Several attempts have been made to improve the cholesterol-lowering activity by enhancing the bioavailability of crystalline sterols and stanols. Approaches involved reduction of the crystal size and/or esterification with fatty acids from vegetable or fish oils. However, the most promising approach in this context is the chemical modification of plant sterols /stanols into water soluble disodium ascorbyl phytostanyl phosphates analogue by esterification with ascorbic acid. This novel semi-synthetic stanol derivative has improved efficacy over natural plant sterols/ stanols and can provide additional benefits by combining the cholesterol-lowering properties of plant stanols with the antioxidant potential of ascorbic acid.

This article is open to **POST-PUBLICATION REVIEW**. Registered readers (see "For Readers") may **comment** by clicking on ABSTRACT on the issue's contents page.

_____

INTRODUCTION

Phytosterols are integral components of plant cell membranes. They are structurally similar to cholesterol with some differences in the D-ring side chain (1-3). The term phytosterols is widely used to describe both plant sterols, which have a double bond at position 5 of the sterol ring, and plant stanols in which the double bond is saturated (4, 5) (Figure 1). Phytosterols are extracted from vegetable oils such as corn oil, rapeseed oil, soybean oil, sunflower oil, olive oil and tall oil either in free forms or as fatty acid esters (6). They are also present to a lower extent in nuts, seeds, fruits and vegetables (7). The most abundant plant sterols are sitosterol, campesterol and stigmasterol. They constitute approximately 65, 30, and 3% of dietary sterol intakes, respectively (3, 8). Stanols, on the other hand, are less abundant in nature (8, 9) and can be produced commercially by hydrogenation of plant sterols (10). Sitostanol and campestanol are the most common plant stanols. The dietary intake of plant sterols ranges from 150 to 450 mg/day and can be as high as 600 mg/day in vegetarians (11). In contrast, the dietary intake of plant stanols is usually only about 50 mg/day (9, 12). Plant sterols are poorly absorbed from gastrointestinal tract (0.4-5%) and the absorption of plant stanols is even lower (0.02-0.3%) (13, 14).

Due to the chemical similarity with cholesterol, plant sterols block cholesterol absorption. Therefore, these compounds are of interest as therapeutic agents to reduce plasma cholesterol. However, the use of plant sterols/stanols as supplements or ingredients for functional foods is restricted by their crystalline nature, low solubility in fats, insolubility in water, high melting point of 140–150 °C, in addition to poor palatability (15-18). Several attempts have been made to enhance the bioavailability of crystalline sterols by reducing the crystal size or by the use of microcrystalline suspensions (18, 19). Other approaches involve modification of phytosterols by esterification with fatty acids from vegetable (20, 21) or fish oils (22, 23), which leads to synthesis of semisynthetic sterols. Such modification can provide an easy way of introducing the daily amount of phytosterols required for optimal reduction of cholesterol (17).

Corresponding Author: Pavel Gershkovich, School of Pharmacy, Centre for Biomolecular Sciences The University of Nottingham, University Park Nottingham, UK, NG7 2RD; Email: pavel.gershkovich@nottingham.ac.uk

Esterification not only changes the physical properties, but also can improve the efficacy of plant sterols since it allows greater solubilisation within food products and optimal dispersion within mixed micelles during intestinal absorption of lipids (17, 24). The first commercial application of fatty acid esterification of plant stanols was in the form of margarine (Benecol®) in 1995 in Finland by Raisio Benecol Ltd (12, 17). These compounds have been approved as food additives in the US and Canada since then, and are now found in products as diverse as juice, spreads, dressings, margarines and dietary supplements in capsule or tablet form (Table 1).

The objective of this review is to demonstrate the lipid-lowering activity and anti-atherogenic effects of natural and semi-synthetic plant sterols/ stanols based on evidence from cell-culture studies, animal studies and clinical trials. Additionally, this review highlights molecular mechanisms by which plant sterols/ stanols lower plasma cholesterol levels with a special emphasis on factors that affect the cholesterol-lowering activity of plant sterols/stanols.

Cholesterol-lowering effect of phytosterols

The cholesterol-lowering effects of phytosterols was demonstrated for the first time in chicks in the early 1950s by Peterson and co-workers (25-

27). This initial observation was soon confirmed in humans by Pollak (28) who reported a significant reduction in plasma total cholesterol levels of about 28% in 26 healthy subjects fed plant sterols at a dose ranging from 5.7-10 g/day for 2 weeks. Thereafter, a series of experiments have been conducted on a number of animals including rabbits (28), mice (29), rats (30, 31) and hamsters (32). Results from these studies have shown that plant sterols and stanols significantly reduce total plasma cholesterol, LDL-cholesterol (LDL-C) and liver cholesterol concentrations. In most of the early studies, plant sterols were used in crystalline, un-esterified (or free) form, which is not well absorbed following oral administration, and therefore doses in excess of 5 g/day were necessary to achieve satisfactory cholesterol-lowering effects (18). However, more recently, the use of phytosterol-enriched foods or supplements allowed dose reduction as a result of enhanced bioavailability. Several randomized placebo-controlled trails have been carried out to evaluate the effect of various phytosterol containing products on serum lipid and lipoprotein concentrations (Table 2). Recent clinical trials have suggested that the LDL-C lowering effects of phytosterols can be achieved with 2-3 g/day, while higher intakes produce little additional benefit (Figure 2) (9, 33, 34).
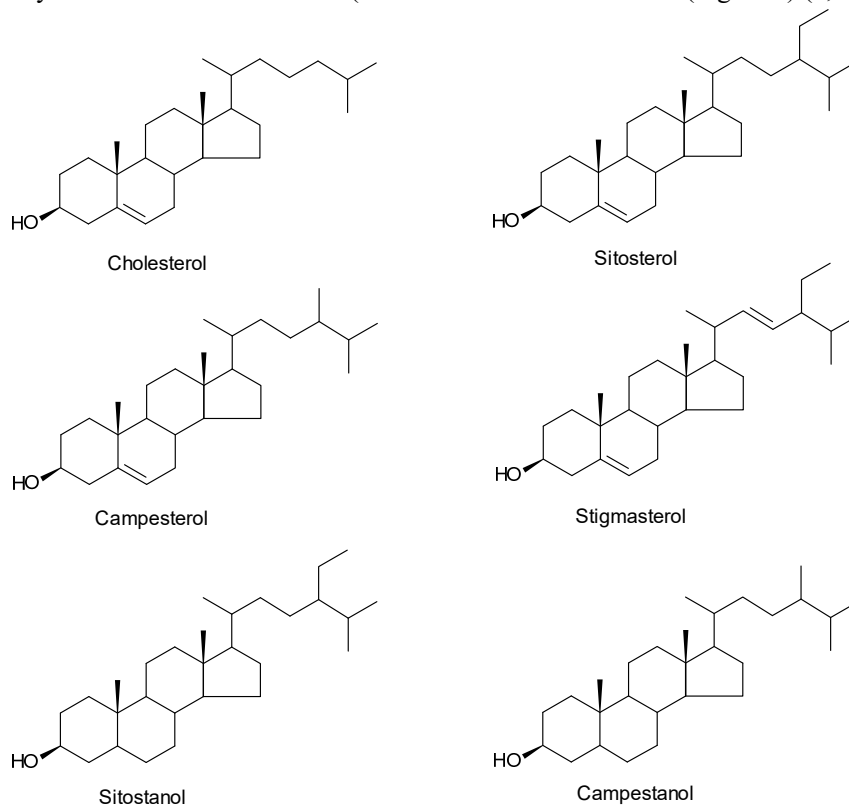

Figure 1. Chemical structures of plant sterols/ stanols.

Table 1. Composition of commercially available products of plant sterols, sterols and their esters.

| Product's brand name	Manufacturer	Products	Type of phytosterols	Amount of phytosterol per serving
Benecol®	Raisio plc.	Yogurt Yogurt drinks Dairy Free Fruit & Soya Drinks Spreads	Tall oil and vegetable oil stanol esters	0.8g/ pot (120 g) 2 g/ bottle (67.5 g) 2 g/ bottle (65.5 g) 0.8 g/ tablespoon
Flora Pro.Activ®	Unilever	Skimmed milk drink Spreads Mini-drink	Vegetable oil sterol esters	0.75 g/ 250 mL 1.25 g/ 2 teaspoons (10 g) 2 g/ bottle (100 g)
Promise Activ™ (Formerly Take Control®)	Unilever	Mini-drink Spreads	Unprocessed sterols, primarily sitosterol from soybean oil	2 g/ 3ounce serving 1.7 g/ tablespoon
Lifetime®	Lifeline Food Co.	Cheese	Vegetable sterol esters	0.65 g/ portion (28g)
CocoaVia®	Mars Inc.	Chocolates	Canola oil sterol esters	1.1 g/ bar
Yoplait Healthy Heart Yogurt™	General mills	Yogurt	Vegetable oil sterol esters	0.4 g/ 6 ounce, single-serving carton.
Premium Heart Wise®	Minute Maid	Orange Juice	Cargill CoroWise® plant sterols	1 g/ serving (240 mL)
Smart Balance® HeartRight	Smart Balance Inc.	Fat free milk	Cargill CoroWise® plant sterols	0.4 g/ cup (240 mL)
Reducol™	Forbes Medi-Tech Inc.	Phytosterol powder for incorporated into various food products including margarine, cheese, milk, yoghurt drinks and cooking oil	Plant sterols and stanols	NA
Listrin™ Plant Sterols	Innovia Nutraceuticals	Tablets	β-sitosterol β-sitostanol	225 mg of β-sitosterol and 79 mg of β-sitostanol/ tablet
Ultra™ Plant Sterols	Vitabiotics	Tablets	β-sitosterols	450 mg/ tablet
Heart health plant sterols	Boots pharmaceuticals	Capsules	Plant sterol esters	650 mg/ capsule

NA: not applicable.
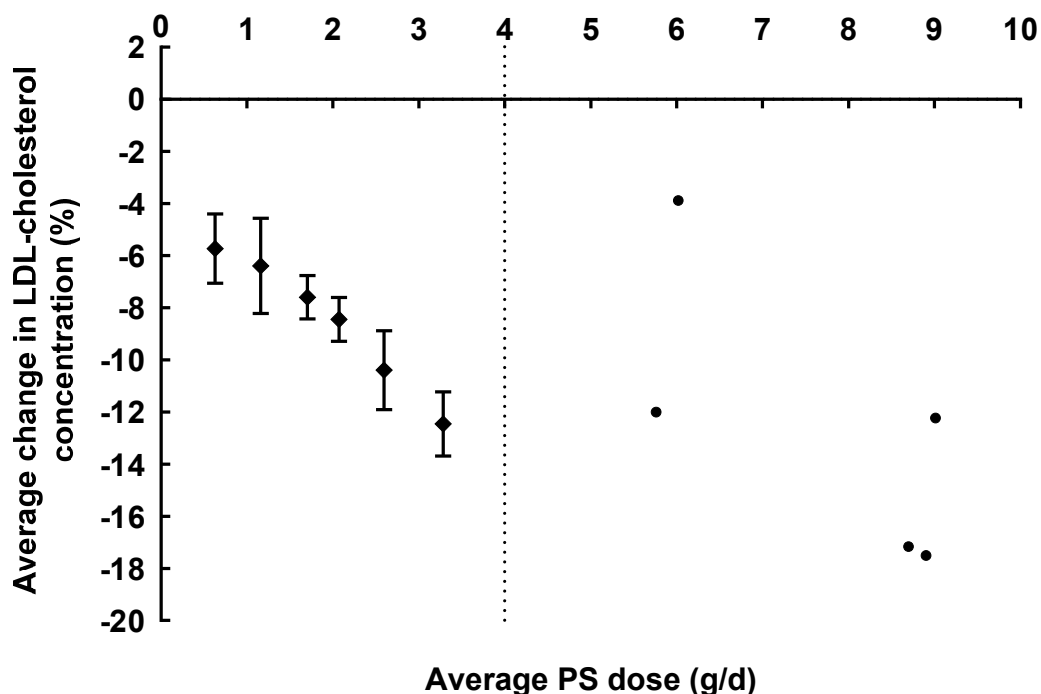
Figure 2. Effects of different dose ranges of phytosterols on average change in LDL-C concentrations. Filled diamonds represent pooled results from multiple clinical trials; filled circles represent results of single high-dose studies that were not pooled as these were scarce and scattered across a wide range of doses. PS; Phytosterol .Values are expressed as means, with 95% CI represented by vertical bars. Adapted from Ras *et al*. (33) with the permission of Cambridge University Press.

Several meta-analyses have been performed to evaluate the cholesterol-lowering effects of plant sterols and stanols (50). A meta-analysis of 41 clinical trials have shown that daily intake of 2 g/d of plant sterols /stanols reduced the LDL-C by 10% with little additional reduction at higher doses (9). Another meta-analysis of 84 clinical trials has reported an 8.8% reduction in serum LDL-C with a daily phytosterol intake of 2.15 g with no statistically significant differences were reported between plant sterols, stanols, or their esterified derivatives (34). Most recent meta-analysis have shown an average LDL-C lowering effect of 12% with a daily intake of 3 g (33). No alterations in serum HDL-cholesterol (HDL-C) concentrations have been reported in most animal and human studies (24, 51-53). However, in subjects with familial hypercholesterolemia (54) and type 2 diabetic patients (55), plant sterol consumption was associated with an increase in serum HDL-C levels.

Mechanisms of cholesterol-lowering effect of plant sterols and stanols

The precise mechanisms of action by which plant sterols/ stanols decrease plasma cholesterol levels remain obscure. Figure 3 summarizes the different mechanisms of action proposed for the cholesterol-lowering effects of plant sterols and stanols.

Competition with intestinal cholesterol for incorporation into mixed micelles

In vitro studies have demonstrated that plant sterols/ stanols reduce cholesterol solubility in bile salt micelles in the presence or absence of oleic acid and monoolein (12, 57-62). Phytosterols are more hydrophobic than cholesterol and have higher affinity to mixed micelles than cholesterol (1, 13). Ikeda and colleagues have shown that the presence of sitosterol at equimolar amounts with cholesterol in a binary mixture reduces the micellar solubility of cholesterol (63). Another study by Slota and co-workers demonstrated that the addition of β-sitosterol to the mixed micelles mixture consisting of 10 mM taurocholate and 6 mM oleate reduced cholesterol solubility to a greater extent than would be expected from an equimolar replacement of cholesterol by β-sitosterol (Figure 4) (59).
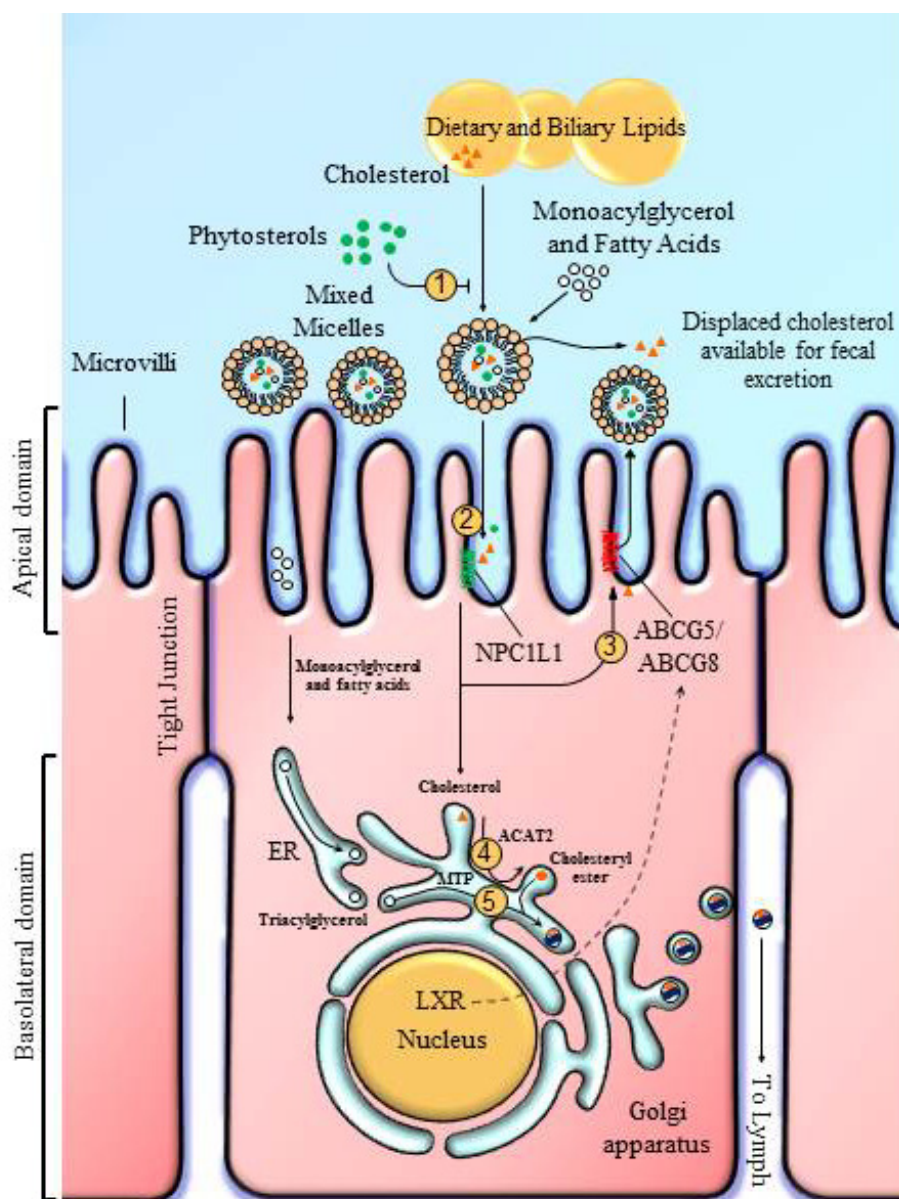
Figure 3. Schematic diagram summarizing the mechanisms of action of plant sterols/ stanols. Proposed mechanisms involve: 1) competition with cholesterol for incorporation into mixed micelles; 2) interference with cholesterol binding to brush border membranes via Neimann-Pick C1-Like1 transporter (NPC1L1); 3) Activation of nuclear liver-X receptor (LXR) resulting in up-regulation of cholesterol efflux transporter genes (ATP binding cassette ABCG5/ABCG8) which mediates the efflux of cholesterol back to the lumen; 4) inhibition of cholesterol esterification via acyl CoA-cholesterol acyltransferase-2 (ACAT2); and 5) interference with cholesterol incorporation into nascent chylomicrons via microsomal triglyceride transfer protein (MTP). ER= endoplasmic reticulum. Adapted from Ikonen (56) with the permission from Nature Publishing Group, Nature Reviews Molecular Cell Biology.

Evidence from *in vivo* studies in rats have indicated that intragastric administration of equimolar amounts of cholesterol and sitosterol as a single emulsified lipid meal significantly inhibits lymphatic absorption of cholesterol by 57% in 24 hr (Figure 5). However, when equimolar amounts of cholesterol and sitosterol were incorporated together in phospholipid bile-salt micelles, no inhibition of lymphatic cholesterol absorption was reported following administration. The simultaneous presence of both cholesterol and sitosterols in the mixed micelles before administration could abolish the ability of sitosterol to compete with cholesterol for incorporation into micelles (63).
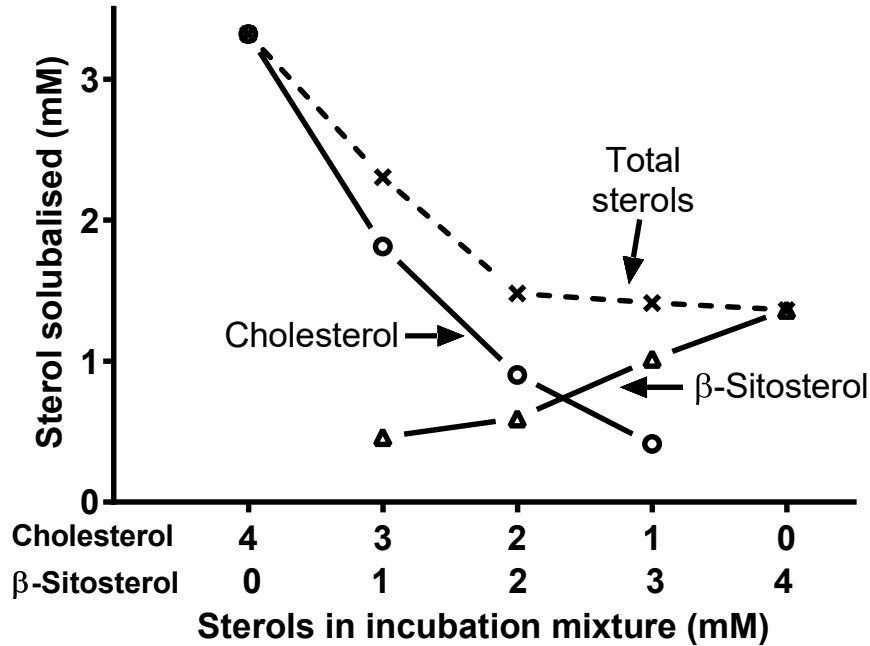
Figure 4. Solubility of cholesterol and β-sitosterol in mixed micellar solutions containing 10 mM taurocholate and 6 mM oleate. β-sitosterol reduces the solubility of cholesterol below concentration that would be expected by an equimolar replacement of cholesterol by β-sitosterol. Adapted from Slota *et al*. (59) with the permission of BMJ Publishing Group Ltd.
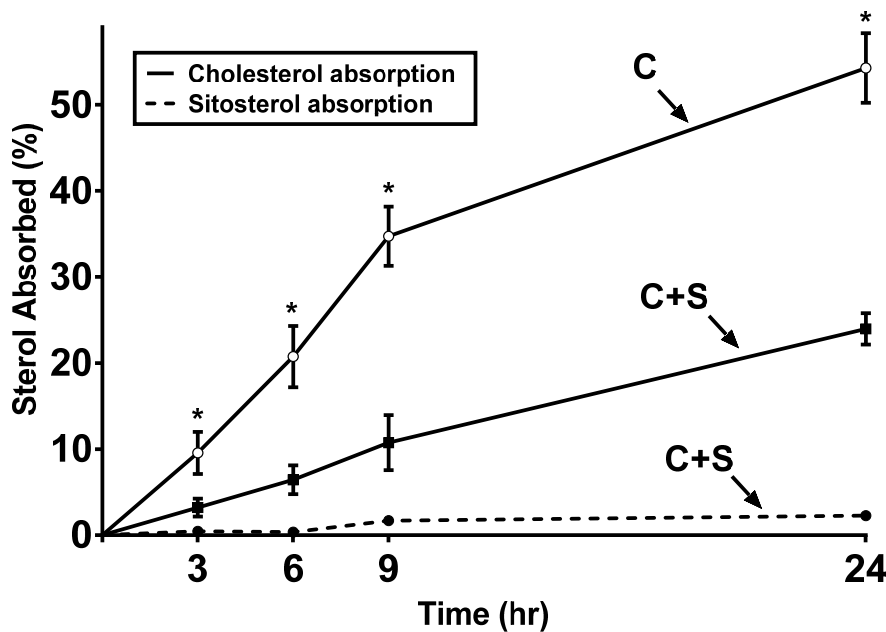

Figure 5. inhibitory effect of sitosterol consumption on exogenous cholesterol absorption in lymph fistula rats. Animals were administered intragastric emulsion containing 25 mg [^{3}H] cholesterol alone (C) or plus 25 mg of sitosterol (C+S). Cholesterol radioactivity was measured following the collection of lymph at different time points over 24 hr. Data were expressed as mean ± SE for 6 replicates. * Significant difference of C vs. C+S, $p<0.05$. Adapted from Ikeda *et al*. (63) with the permission of the American Society for Biochemistry and Molecular Biology.

Effects of plant sterols and stanols on cholesterol trafficking and metabolism

Several *in vitro* and *in vivo* studies have suggested that plant sterols/stanols may exert their lipid-lowering effects by mechanisms other than inhibition of cholesterol incorporation into mixed micelles (64-73). Some researchers have demonstrated a significant reduction in plasma cholesterol levels in chicks (71) and hamsters (70) following subcutaneous administration of low doses of soya sterols. Therefore, one can assume that the reduction of plasma cholesterol levels might be not only due to the inhibition of intestinal cholesterol absorption, but also to other factors affecting cholesterol metabolism.

A few studies have suggested that plant sterols / stanols may competitively inhibit cholesterol uptake by brush boarder membrane of intestinal mucosal cells (63). It has been demonstrated that both cholesterol and sitosterol can bind to rat brush border membranes *in vivo* (74), rat brush border membranes of jejunal loops *in situ* (75), isolated rat brush border membranes (63, 74) or isolated jejunal villus cells (76). Although the affinity of sitosterol to brush border membranes in each case was lower than that of cholesterol, high doses of sitosterol have been suggested to inhibit cholesterol binding (63). Recently, it has been shown that the uptake of cholesterol from intestinal lumen into the enterocytes is mediated by specific interactions with the luminal N-terminal domain (NTD) of the NPC1L1 protein (77). While the luminal NTD specifically binds cholesterol and mediates their endocytosis, plant sterols have not been shown to interact with this terminal domain and therefore competition with dietary cholesterol for intestinal absorption is an unlikely mechanism of plant sterols/ stanols in lowering plasma cholesterol levels (63, 77, 78). In line with this, some studies have demonstrated that plant sterols reduce plasma cholesterol without affecting intestinal Niemann-Pick C1-like 1 protein gene expression (79-82).

Other suggested mechanisms involve local activation of nuclear liver-X receptor (LXR) resulting in induction of expression of a number of target genes (73, 83). Among those genes, the ATP binding cassette (ABCA1 and ABCG5/ABCG8) efflux transporter genes have been shown to be involved in intestinal cholesterol excretion (64, 73, 84). Increased expression of ABCA1 have been reported *in vitro* in Caco-2 cells following incubation with mixed micelles enriched with sitostanol or cholesterol plus sitostanol (64). Furthermore, feeding mice plant sterols has been shown to reduce fractional cholesterol absorption and to stimulate fecal cholesterol excretion via a non-biliary route. These effects were partially mediated by the Abcg5/Abcg8 heterodimer (84). However, some studies have suggested that plant sterols reduce intestinal cholesterol absorption and increase fecal neutral sterol excretion without inducing intestinal LXR target gene expressions (79, 81). These findings suggest that modulation of LXR gene expression cannot be considered as the main mechanism by which plant sterols reduce intestinal cholesterol absorption.

There is also some evidence that plant sterols/ stanols interfere with the cholesterol esterification process inside the enterocytes. This process is necessary for the incorporation of cholesterol into chylomicrons and is mediated by the activity of the enzyme acyl coenzyme A: cholesterol acyltransferase (ACAT). A significant drop in the activity of this enzyme has been reported in the jejunum and ileum of rabbits fed with β-sitosterol (65). In addition, down-regulation of ACAT has been demonstrated in hamsters that were fed high-fat diet enriched with β-sitosterol and stigmasterol (69). However, in an *in vitro* study, incubation of Caco-2 cells with micelles containing cholesterol and β-sitosterol has not been shown to alter the activity of ACAT enzyme (66). Instead, there was a decrease in the uptake of micellar cholesterol resulting in an attenuation of the influx of plasma membrane cholesterol and an inhibition of secretion of cholesteryl esters derived from the plasma membrane. Furthermore, β-sitosterol decreased cholesterol synthesis and HMG-CoA reductase activity despite the reduction in intracellular cholesterol concentration (66). In line with this, Δ^{22}-unsaturated phytosterols (stigmasterol, brassicasterol and ergosterol) have inhibited cholesterol synthesis by competitive inhibition of sterol Δ^{24}-reductase (an enzyme which catalyses the saturation of the double bond at C-24 during cholesterol biosynthesis) in mammalian cells (67). Another study have shown that stigmasterol (0.5% w/w) feeding of Wistar and WKY rats over 6 weeks resulted in significant reduction in HMG-CoA reductase activity by 44% and 77%, respectively (85).

Plant sterols/ stanols could also affect the expression of microsomal triacylglycerol transfer protein (MTP) which is involved in the assembly of very low density lipoprotein and chylomicrons (13). A study by Liang *et al*. (69) have shown that the cholesterol-lowering activity of sitosterol was associated with a decrease in the mRNA level of MTP in male golden Syrian hamsters.

Factors affecting the cholesterol-lowering activity of plant sterols and stanols:

Several factors have been shown to influence the hypocholesterolemic effects of plant sterols and stanols. In general, the LDL-C lowering effects of plant sterols are greater among subjects with higher baseline cholesterol levels compared to those with normal or borderline levels (50, 51, 86). Moreover, a strong correlation was reported between the cholesterol-lowering effects of plant sterols and age. More efficient reduction of LDL-C levels was observed in older than in younger subjects. However, when the LDL-C lowering effect of plant sterols were corrected for the baseline LDL-C levels, the differences between various age groups were non-significant (9, 10).

The time of intake of plant sterols and stanols is also another important factor affecting cholesterol-lowering activity. It has been suggested that plant sterols should be present simultaneously with cholesterol in the intestinal lumen for the maximum effectiveness (87). In general, plant sterols/ stanols are to be administered with or immediately after the main meal for the optimal cholesterol-lowering activity (78). However, when taken before breakfast, no significant reduction in serum LDL-C concentrations was reported (86). In line with this, Doornbos and co-workers observed a significantly higher reduction in LDL-C concentrations in subjects taking sterol-enriched yoghurt drink with or immediately after lunch, compared to those taking the drink before breakfast (88).

Incorporation of plant sterols into different food matrices or pharmaceutical formulations has also been suggested to alter the cholesterol-lowering capacity of plant sterols and stanols (48, 89). The physical state of the administered plant sterols is also a crucial factor affecting the partitioning of plant sterols and cholesterol over different phases in the intestinal contents (78, 87). A study by Clifton *et al*. (89) has demonstrated that plant sterol added to low-fat milk is almost three times more effective in lowering cholesterol levels than sterol-enriched bread and cereal products. Similarly, sitostanol administration in lecithin micelles at a dose of 700 mg has been shown to reduce cholesterol absorption more efficiently than 1 g sitostanol in crystalline powder form (90). On the other hand, phytosterol esters intake as capsules have been shown to be ineffective in reducing plasma LDL-C levels in subjects with mild to moderate hypercholesterolemia (48). In another study, however, the relative LDL-C lowering efficacy of plant sterols/ stanols provided as supplements (tablets/capsules) have not been shown to differ from that of phytosterols-enriched dietary products (16). Moreover, some clinical trials have demonstrated that the cholesterol-lowering efficacy of esterified sterols and stanols is independent of the food matrix (18, 39).

Other factors include whether the plant sterols/ stanols are taken as their free forms or as fatty acid esters. In this context, an early study by Mattson and co-workers has suggested that the esterified form of plant sterols, although has the advantage of being readily incorporated into dietary fat, is less effective in reducing intestinal cholesterol absorption compared with the free form (87). Another study in a thoracic duct cannulated rat model has shown that intra-gastric administration of un-esterified plant sterols was associated with significant reduction in lymphatic recovery of radiolabeled cholesterol compared with administration of sterol ester (Figure 6) (91).

An *in vitro* study has shown that intact plant sterol esters and their simulated hydrolysis products differentially affect the solubility of cholesterol in mixed bile-salt/phosphatidylcholine micelles. While free sterols and fatty acids reduce cholesterol solubility in a dose dependent manner, intact plant sterol esters do not incorporate into mixed micelles nor do they alter cholesterol solubility (57). The free plant sterols can displace cholesterol from the micellar phase, while the major part of the esterified forms accumulate in the oil phase (92, 93). Therefore, plant sterol esters need to be hydrolysed first to non-esterified forms in the intestinal lumen before they can be incorporated into and expel cholesterol from mixed micelles. The hydrolysis is catalysed by the enzyme pancreatic cholesterol esterase and is thought to be a rate limiting step for cholesterol absorption inhibition (91).

Contrary to these findings, a number of studies have reported a comparable cholesterol-lowering efficacy of esterified and un-esterified plant sterols in humans (9, 94) and hamsters (95). Such discrepancies could be explained by the rapid rate of hydrolysis of plant sterol esters in human intestinal lumen (92). Furthermore, un-hydrolysed plant sterol esters may affect cholesterol absorption by creating a lipid phase in which cholesterol becomes trapped (96). On the other hand, Nissinen and colleagues has shown that the free plant sterols could become partially esterified during the duodenal transit resulting in partial loss of their capacity to inhibit intestinal cholesterol absorption. Although, the authors were not able to provide clear explanation to the esterification of free plant sterols, reversed action

of pancreatic cholesterol esterase has been suspected (92).

The fatty acid moiety of phytosterols has been suggested to influence their cholesterol-lowering efficacies (96, 97). A study in hamsters has shown that consumption of phytosterol esters enriched with stearic acid, compared to linoleic acid, significantly reduced cholesterol absorption and total plasma and hepatic cholesterol concentrations (98). The enhanced efficacy of phytosterol stearate ester has been attributed to the ability of stearic acid itself to reduce intestinal cholesterol absorption and increase fecal neutral sterol excretion secondary to alteration of bile acid composition, resulting in lower plasma and liver cholesterol concentrations (99, 100). Another study in hyperlipidemic men and women has shown that combined supplementation of phytosterols with long-chain polyunsaturated fatty acids synergistically lowered plasma LDL-C and triglyceride concentrations with an improvement in HDL-C levels (101). Similarly, fish oil esters of plant sterols have demonstrated marked reduction in fasting and postprandial plasma triacylglycerol concentrations, in hyperlipidemic subjects, compared to sunflower oil esters of plant sterols (23).Several researchers

have claimed that plant stanols are more effective in reducing circulating cholesterol levels than plant sterols (102-109). This claim was supported by the finding that stanols reduce the *in vivo* micellar solubility of cholesterol more efficiently than sterols (62). The superiority of plant stanols over sterols in reducing cholesterol absorption and serum LDL-C levels has been reported in a number of animal studies (102, 109, 110) and in humans (105, 111, 112). In one study in humans, intestinal infusion of sitostanol was found to be more effective in reducing cholesterol absorption by 85% compared with 50% reduction in the case of sitosterol (105). In another study, sitostanol supplementation in children with severe familial hypercholesterolemia at a dose four-fold lower than that of sitosterol has been shown to be more effective in reducing elevated levels of LDL-C than sitosterol. The higher LDL-C lowering effects of sitostanol were associated with higher fecal neutral sterol excretion than sitosterol (111). On the other hand, comparable cholesterol-lowering activity and anti-atherogenic effects of plant sterols and stanols were reported by Pritchard *et al*. (113) in Apolipoprotein E (Apo E)-deficient mice over a 14 week experimental course.
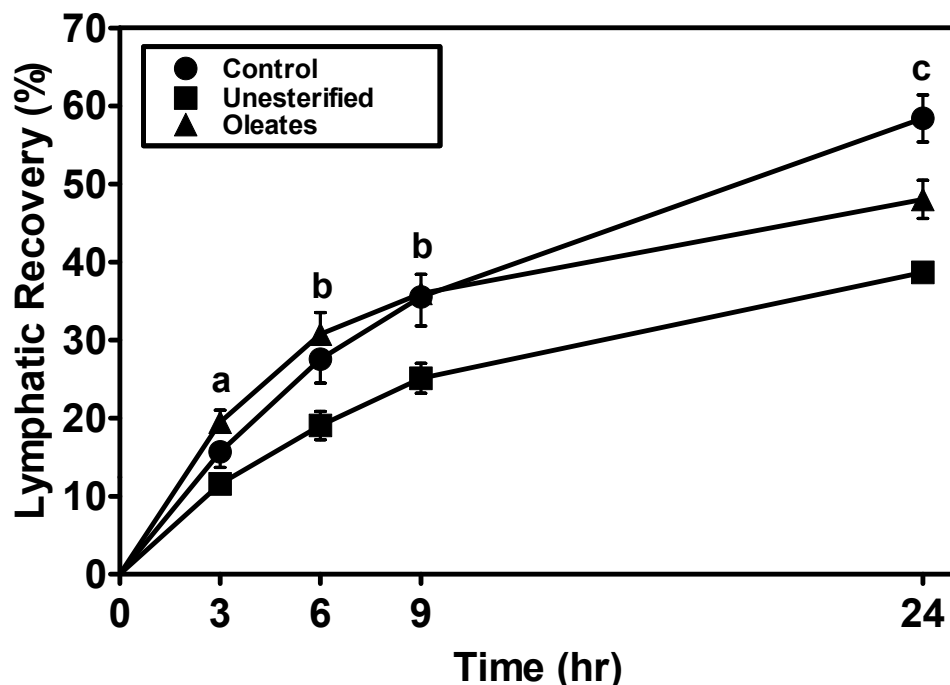

Figure 6. Effect of un-esterified plant sterols and plant sterol oleates on lymphatic recovery of radiolabeled cholesterol in thoracic duct cannulated rat model. Data are expressed as mean ± SE of 6-7 rats. Filled circle = 10 mg radiolabeled cholesterol, filled square = 10 mg cholesterol +10 mg un-esterified plant sterol, filled triangle= 10 mg radiolabeled cholesterol + 16.5 mg plant sterol oleate (equivalent to 10 mg plant sterol). a= significant difference of un-esterified sterols vs. sterol oleate at p<0.05; b= significant difference of un-esterified sterols vs. control and sterol oleate at p<0.05; c= significant difference between un-esterified sterols, sterol oleate and control at p<0.05. Adapted from Kobayashi *et al*. (91) with permission from J-STAGE.

Results from meta-analyses comparing the LDL-C lowering efficacy of plant stanols and sterols are conflicting (9, 114, 115). In an early meta-analysis, the pooled LDL-C reduction for plant stanols (10.1%; mean dose, 2.5 g/day) was not significantly different from that for plant sterols (9.7%; mean dose, 2.3 g/day) (9). In another meta-analysis, the efficacy of plant stanols and plant sterols was compared head-to-head at doses ranging from 0.6 to 2.5 g/day in healthy subjects and patients with hypercholesterolemia. Again, no statistically or clinically significant difference were reported between plant sterols and plant stanols in their ability to modify total plasma cholesterol, LDL-C, HDL-C or triglyceride levels (115). However, more recent meta-analysis has shown that the maximal LDL-C reduction achievable with plant stanols is approximately two-fold greater than that achieved with plant sterols (114).

Triglyceride-lowering effects of plant sterols and stanols
Numerous clinical and animal studies have shown that consumption of plant sterols and stanols was associated with significant reduction of plasma triglyceride levels (50, 116-118). Pooled analysis of twelve randomised controlled trails including 935 hypercholesterolemic subjects has shown that a daily consumption of 1.6 - 2.5 g of plant sterols/ stanols exerts a modest triglyceride-lowering effect of about 6% (0.12 mmol/L). The triglyceride-lowering effects of plant sterols are more pronounced in people with elevated baseline triglyceride levels (117). In metabolic syndrome patients with moderate hypertriglyceridemia, consumption of a plant stanol ester-enriched yogurt drink (2 g/d) for 8 weeks lowered serum triglyceride concentrations by 27%. In that study, the reduction in triglyceride levels was accompanied by decrease in hepatic production of both large (> 60 nm) and medium size (35-60 nm) VLDL particles (119). In another study, plant sterols supplementation (4 g/d) in patients with metabolic syndrome for 2 months period decreased triglyceride levels by 19.1% (36). The proposed mechanism behind the triglyceride-lowering effect of plant sterols is a reduction in triglyceride-rich VLDL particles production by the liver (118, 120). There is also similar evidence from experimental mouse models suggesting that consumption of plant sterols/ stanols was associated with significant reduction of plasma triglyceride levels with or without reduction of hepatic triglyceride levels (52, 116, 118). In these animal studies, the triglyceride-lowering effects

of plant sterols were accompanied by inhibition of hepatic VLDL secretion (118) and an increase in fecal excretion of the saturated fatty acids, palmitate and stearate, as a result of interference with intestinal fatty acid absorption (116).

Direct effects of plant sterols/ stanols on atherosclerotic lesions and endothelial function
The role of plant sterols/ stanols in prevention and treatment of cardiovascular and atherosclerotic diseases is mediated not only by their cholesterol-lowering properties but also by possessing anti-atherogenic activities (15).

Evidence from cell culture-based studies
The anti-atherogenic effects of phytosterols have been investigated in a number of *in vitro* studies (121-123). Results from these studies have shown that plant sterols suppress aortic endothelial cells and vascular smooth muscle cell proliferation (121-123), inhibit platelet aggregation (122) and stimulate prostacyclin (PGI_2) production (a potent inhibitor of platelet aggregation with vasodilator and anti-inflammatory actions) (121). The anti-inflammatory effects of phytosterols have been demonstrated *in vitro* in human aortic endothelial cells. Phytosterols reduced the expression of vascular and intracellular adhesion molecules (VCAM)-1 and (ICAM)-1 in TNF-α stimulated human aortic endothelial cells, attenuated the phosphorylation of nuclear factor-κB and inhibited the binding of monocytes to TNF-α stimulated human aortic endothelial cells (124). The concentrations of plant sterols used in these studies were distributed over wide ranges. In some studies the concentrations of plant sterols were relevant to the physiologically achieved plasma levels in humans (121-123). However, in other studies higher concentrations were employed (124).

Evidence from animal studies
Anti-atherogenic activity of plant sterols have been reported for the first time in chickens by Peterson and co-workers, who observed a significant reduction in the severity and extent of atherosclerotic lesions upon inclusion of soybean sterols in cholesterol rich diet (26). Soon thereafter, similar effects were reported in rabbits by Pollak (28). The beneficial effects of dietary sterol supplementations on endothelial and vascular smooth muscle cell functions have also been reported in atherosclerotic rat and mice models (22, 29, 30, 52, 125-127). Administration of plant sterols/ stanols to Apo E-deficient mice has been shown to decrease the size and severity

of atherosclerotic lesion (29, 125-127). Reduction in plasma fibrinogen concentrations (29) and pro-inflammatory cytokines production with an increase in anti-inflammatory cytokines production has also been reported in these animals (125). The anti-atherogenic effects of plant sterols/ stanols were found to be strongly correlated with the cholesterol-lowering effects (126) and unaffected by the increase in serum and tissue sterol/stanol concentrations (128). Plant sterols/ stanols were reported to inhibit the progression or even induce the regression of existing lesions in LDL receptor-deficient mice independent of changes in serum levels of plant sterols (128). A study on hamsters fed with an atherogenic diet has shown that dietary phytosterol supplementations significantly reduced aortic foam cell development, despite a moderate increase in plasma plant sterol concentrations (129). Furthermore, dietary sitostanol supplementation in hyperlipidemic rabbits at a concentration of 0.8% (w/w) for 2 months has been shown to significantly delay plaque formation in coronary arteries and the ascending aorta (130). In contrast, Moghadasian and colleagues reported no evidence of regression of pre-established atherogenic lesions in Apo E-deficient mice following 25 weeks of treatment with 2% (w/w) dietary phytosterol mixture (131).

Evidence from clinical trials
It has been reported that consumption of 4 g/d of wood-based stanol esters by healthy subjects resulted in an increase in the activity of antithrombin-III, a potent inhibitor of coagulation, without further effects on other coagulation and fibrinolytic parameters (132). However, clinical evidence for the anti-atherogenic effects of plant sterols is lacking. Consumption of margarine enriched with plant sterols/ stanols have not been shown to affect intima-media thickness (133), nor to improve arterial elasticity (134) or endothelium-dependent vasodilatation despite the effective reduction in total and LDL-C levels (133-138). Furthermore, several studies have shown a lack of effect of plant sterols/ stanols on markers of inflammation and endothelial dysfunction [C-reactive protein (CRP), monocyte chemotactic protein (MCP)-1, interleukin-6 (IL-6), CD 40 ligand (CD40L), E-selectin, intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1] (139, 140). In addition, markers of oxidative stress and antioxidant status have not been shown to be affected by phytosterol intakes (139, 141, 142). Nevertheless, reduction in circulating

concentrations of oxidized LDL have been reported in few studies (143).

Short and long term safety and toxicity of sterols and stanols
Evidence from clinical trials and experimental models have indicated a good safety profile of plant sterols/stanols (144). However, long-term use has raised concerns of a potential increase in the risk of cardiovascular events, probably due to elevation of plasma and tissue plant-sterol concentrations (145, 146). High plasma plant-sterol levels could be detrimental in sitosterolemia (a rare genetic disorder caused by genetic mutation of ABCG5/ABCG8 transporter genes and characterised by high plasma levels of sitosterol and campesterol, premature atherosclerosis and increased risk of coronary heart and aortic valve disease) (1).

A number of studies have suggested that phytosterols consumption could be a risk factor of cardiovascular disease (147-150). Despite that, currently available data could neither confirm an increased cardiovascular risk with plant sterols nor rule it out (144, 151). It has been suggested that plant sterols may induce inflammation and reduce cholesterol efflux from macrophages, conditions that are directly implicated in the development of atherosclerosis (145). A study by Weingärtner *et al*. (152) have shown that food supplementation with plant sterol impairs endothelial function, exacerbates atherogenesis and ischemic brain injury in mice and increases tissue sterol concentrations in humans. Vergès and Fumeron (151) recently reviewed studies examining the relationship between plasma phytosterol levels and cardiovascular disease events. They reported conflicting results of a potential connection between elevated plasma plant-sterol levels and cardiovascular risk. Some studies suggest a positive association while other show no or even inverse correlation. Although the review included a total of 225,437 subjects from different case control, prospective and genome-wide association studies, it was difficult to draw a firm conclusions even though the large prospective trails and genome wide association studies have suggested a positive correlation which cannot be ruled out.

Daily consumption of 3.24 g of phytosterols over a period of 3.5 weeks produced no significant alteration in serum levels of alkaline phosphatase, alanine transaminase, aspartate transaminase and gamma- glutamate transaminase (41). Few clinical trials have shown that the use of high doses of plant sterols/ stanols was

associated with a modest reduction in levels of carotenoids, fat-soluble vitamins and antioxidants (40, 94, 153-155). Similar changes were reported in rats treated with high doses of stanol ester for 13 weeks (156). However, when the levels of these vitamins were adjusted to the lowered LDL-C concentrations, the reduction was not significant for vitamin E, D and K (9, 41, 106), while plasma levels of α-, β -carotene and lycopene remained low (9, 41, 153, 157). The effects of plant sterols on fat soluble vitamins can be overcome by maintaining adequate daily intake of fruits and vegetables (1, 9, 158).

Although consumption of plant sterols have been associated with increased plant sterol levels in erythrocyte membranes, no detrimental effects have been reported in humans with normal sterol metabolism (157, 159-161). However, increased RBC fragility with episodes of haemolysis have been reported in patients with sitosterolemia (162, 163). The increased RBC fragility by plant sterols have also been shown to shorten the life span of stroke-prone spontaneously hypertensive (SHRSP) rats (164).

Some studies have suggested potential estrogenic effects of plant sterols/stanols (165-167). However, these assumptions have been disproved by extensive safety evaluations using series of *in vitro* and *in vivo* experiments (168, 169). Indeed, consumption of plant sterol in humans have not been shown to affect hormone levels in males (free and total testosterone) and females (luteinizing hormone, follicle stimulating hormone, β-estradiol and progesterone) (157) and plant sterols do not bind to oestrogen receptors (168).

A promising semisynthetic stanol: disodium ascorbyl phytostanyl phosphate (FM-VP4).
Commercially available plant sterols and stanols are semisynthetic, hydrophobic fatty acid esters and their use as food additives are limited to oil-based foods such as margarines (170). Many

attempts have been made to improve the lipid-lowering activity of plant sterols by modification of their chemical structure to increase their micellar incorporation capacity. One of such well characterized phytostanol analogue is disodium ascorbyl phytostanyl phosphates, also known as FM-VP4 (21). Disodium ascorbyl phytostanyl phosphate is a novel hydrophilic stanol analogue that can be formulated into a wide range of delivery vehicles (171). FM-VP4 is composed of a mixture of two components; disodium ascorbyl sitostanyl phosphate and disodium ascorbyl campestanyl phosphate (Figure 7). FM-VP4 is produced by hydrogenation of sitosterol and campesterol to form sitostanol and campestanol followed by esterification with ascorbic acid by a phospodiester bond (172). FM-VP4 has an improved therapeutic efficacy over plant sterols/ stanols, most probably resulting from the combined cholesterol-lowering properties of the plant stanols and the antioxidant potential of ascorbic acid (173).

The lipid-lowering effects of FM-VP4 have been reported in a number of experimental animals and human studies. Evidence from previous studies has suggested that FM-VP4 is more effective in lowering plasma cholesterol levels than plant sterols/ stanols (159, 174, 175). In an early study, Apo E-deficient mice have been used to evaluate the lipid-lowering effects and anti-atherosclerotic properties of FM-VP4. The Apo E-deficient mouse is particularly popular model because of its propensity to spontaneously develop atherosclerotic lesions similar to human counterparts even on a standard chow diet (176). In that study, the incorporation of FM-VP4 into animal's diet at a dose of 0.5% (w/w) or drinking water at a dose of 0.5% (w/v) significantly reduced total plasma cholesterol levels and aortic atherosclerotic lesion by 75% over the 12 weeks study period. Transient reduction in plasma triglyceride levels has also been reported in the first 8 weeks of FM-VP4 treatment.

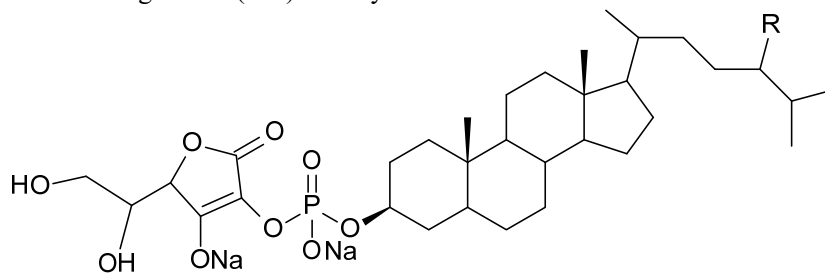**Figure 7.** Chemical structure of disodium ascorbyl phytostanyl phosphate; R=CH$_3$ (disodium ascorbyl campestanyl phosphate), R= C$_2$H$_5$ (disodium ascorbyl sitostanyl phosphate).
Lipid-lowering effects and anti-atherosclerotic activity of FM-VP4

On the other hand, administration of un-esterified plant stanol with or without ascorbic acid is associated with less significant reduction of total plasma cholesterol and no triglyceride-lowering effects (72).

In another study, 4 weeks' administration of FM-VP4 to gerbils at a dose of 1 to 2% (w/w) significantly reduced total plasma cholesterol and LDL-C levels without affecting plasma triglyceride or HDL-C levels (170). However, a longer period of administration (8 weeks) with a higher dose of 4% (w/v) in drinking water resulted in a significant reduction of total plasma triglyceride levels and a significant increase in HDL-C as well (177). Administration of 2% (w/w) FM-VP4 for 12 continuous weeks in mice fed either with a high fat or a low fat diet significantly reduced total plasma cholesterol levels by 32% and 45%, respectively, compared with animals that received no treatment (178). In hamsters, the cholesterol-lowering effect of FM-VP4 was found to be three-fold greater than that of free stanols. Five weeks of feeding hamsters with 0.71% or 1.43% (w/w) FM-VP4 (corresponding to 0.5 or 1% free plant stanol)

reduced plasma cholesterol levels by 34% and 46%, respectively. On the other hand, free stanols reduced plasma cholesterol levels by only 14%. The non-HDL-C levels were reduced by 39 and 54% in animals supplemented with 0.71% or 1.43% (w/w) FM-VP4, respectively. Moreover, plasma triglyceride levels were reduced by 42% and 49%, respectively, compared to hamsters that were fed un-esterified stanols (159, 174). Interestingly, the cholesterol-lowering effects of FM-VP4 were associated with a significant reduction in abdominal fat and less body weight gain in diet-induced obese mice (178) and hamsters (174).

It has been reported that a single oral gavage of FM-VP4 with [3H] cholesterol co-administered in Intralipid® fat emulsion to rats significantly reduced intestinal [3H] cholesterol absorption and decreased plasma [3H] cholesterol level in a dose-dependent manner. The cholesterol-lowering effects of FM-VP4 have been attributed to displacement of cholesterol from bile salts micelles and inhibition of cholesterol uptake by enterocytes (Figure 8) (179, 180).
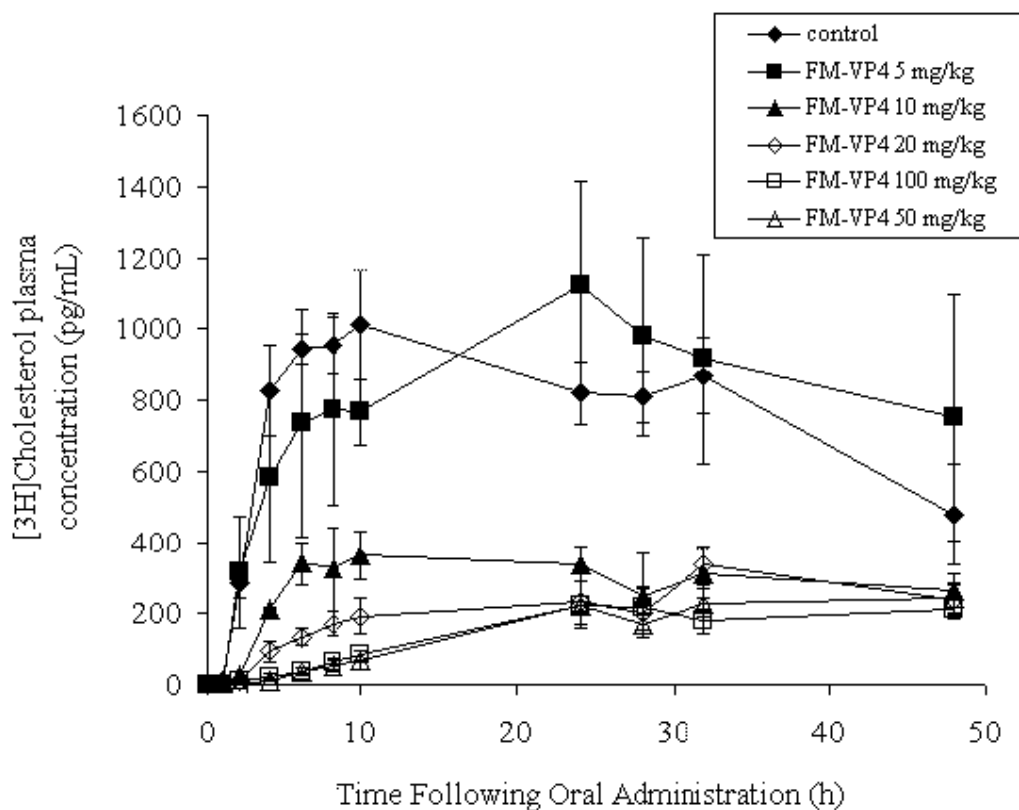

Figure 8. [3H]-Cholesterol plasma concentration-versus-time curve on a linear graph following a single oral dose of [3H]-cholesterol (227 ng/ml), unlabelled cholesterol (1 mg/ml) and FM-VP4 (0, 5, 10, 20, 50 and 100 mg/kg) co-administered in 10% Intralipid® to fasting Sprague Dawley male rats. Data are shown as mean ± SD. Adapted from Wasan *et al*. (180) with the permission from Canadian Society for Pharmaceutical Sciences.

Results from clinical trials in humans have shown that administration of FM-VP4 at a dose of 400 mg/day reduced LDL-C by 6.5% compared with baseline levels over four weeks' treatment period. Similar reduction in LDL-C levels was reported with plant sterol and stanol esters but at doses 2-3 times higher than that of FM-VP4 (175).

Mechanism of action of disodium ascorbyl phytostanyl phosphate
The exact mechanisms of action of disodium ascorbyl phytostanyl phosphate have not been fully elucidated yet. FM-VP4 exerts its cholesterol-lowering effect by acting locally within the gastrointestinal tract. Following oral administration, only a small proportion of FM-VP4 is absorbed and becomes available in the systemic circulation and the vast majority is temporarily retained within the gastrointestinal tract and then removed via the feces (180).

It has been suggested that FM-VP4 could inhibit cholesterol incorporation into mixed micelles and thereby reduce the extent of cholesterol solubilisation and absorption into intestinal epithelial cells (175, 181). FM-VP4 actually has a significant advantage over other plant sterols/stanols in that it has greater solubility in GI mixed micelles. This advantage is achieved by incorporation of hydrophilic ascorbyl residue to the hydrophobic campestanol and sitostanol tail through a phosphodiester bond. The unique amphiphilic structure of FM-VP4 allows self-assembly into micelle structures in aqueous solutions in the absence of bile salt (175, 182).

In this context, a study using an *in vitro* lipolysis model has shown that disodium ascorbyl phytostanyl phosphate significantly reduced cholesterol distribution into the micellar phase, displacing it to the sediment phase and making it unavailable for absorption. This model mimics the digestion process that happens in the physiological environment of the upper gastrointestinal tract. In this model, the distribution of cholesterol into the oil, aqueous and sediment phases after ultracentrifugation provides insight into its probable fate of cholesterol in the intestine when consumed with FM-VP4. Cholesterol distributed into the aqueous phase is most readily available for absorption; cholesterol recovered in the oil phase is most likely to have a delayed absorption; while cholesterol found in sediment is most likely to be excreted. Because of its structural similarity to bile salts, FM-VP4 has been suggested to act as a surfactant and thus compete with bile salts for adsorption into lipid surface. However, the lack of hydroxyl groups prevents it from proper interaction with pancreatic lipase and/or co-lipase, thus hinders the lipid digestion process (181). Pancreatic lipase/ co-lipase mediate partial hydrolysis of the triacylglycerol into monoacylglycerol and non-esterified fatty acids which is required for dietary cholesterol to be absorbed as emulsified substrate by intestinal cells (183). Therefore, the inhibitory effect of FM-VP4 on lipid digestion could be attributed to its competition with bile salts during the lipolysis process (181).

The other suggested mechanism involves competition between FM-VP4 and cholesterol for uptake by intestinal epithelial cells. This mechanism has been illustrated by an *in vitro* cholesterol uptake study using cells derived from the duodenal and jejunal segments of rat small intestine (184). In that study, rat intestinal epithelial crypt cells (IEC-6) were incubated with [^{3}H] cholesterol micelles in the presence of increasing concentrations of FM-VP4 and the extent of cholesterol radioactivity associated with cell monolayers was determined at the end of incubation period. Results showed that 50 μM of FM-VP4 reduced [^{3}H] cholesterol accumulation into IEC-6 cells by 60% within 150-30 min of exposure. Similarly, cholesterol has been shown to inhibit the accumulation of radiolabeled FM-VP4 by 62 % within 60 min of exposure suggesting that FM-VP4 and cholesterol may compete for the same binding site on IEC-6 cells (184). Comparable results were reported in another study using Caco-2 cells. Interestingly, the inhibition of cholesterol accumulation by FM-VP4 was independent of pancreatic lipase activity, P-glycoprotein- mediated cholesterol influx or cholesterol incorporation into micelles (171).

Some reports have suggested that FM-VP4 could affect the expression of regulatory genes that are involved in controlling cholesterol homeostasis (171, 185). Mendez-Gonzalez and co-workers have reported transcriptional changes in hepatic and intestinal genes that are involved in regulating cholesterol and bile acids metabolism in female mice fed with an FM-VP4-enriched diet. They showed that FM-VP4 up-regulates the expression of hepatic LXRα, ABCA1, ABCG5/ABCG8, SR-B1 and HMG-CoA reductase genes. In addition, the study showed that FM-VP4down-regulates several farnesoid-X receptor (FXR) target genes and induces intestinal reabsorption of bile acids, resulting in an increase in the hepatic and intestinal bile acid contents and reduction of fecal bile acid output. Although these

changes were associated with significant reductions in intestinal cholesterol absorption as well as reductions in plasma and liver cholesterol concentrations, it is not clear whether such changes were due to direct actions of FM-VP4 or promoted as compensatory mechanism to its cholesterol-lowering effects (185).

Safety of FM-VP4

A clinical study of 100 subjects demonstrated that administration of FM-VP4 at doses up to 800 mg/day for 4 weeks is safe and well tolerated. Mild adverse effects were reported including dizziness, headache and fatigue (175). These adverse effects were resolved spontaneously. Phase 1 clinical trial has shown that FM-VP4 had no effects on blood pressure or heart rate. However, a slight reduction of systolic blood pressure was reported in phase 2 trial after 4 weeks of treatment with no changes in diastolic blood pressure. Furthermore, the pre- and post-dose ECG findings were normal in both phases of the trial. Plasma levels of fat soluble vitamins (A and E) were unaffected by 4 weeks treatment of FM-VP4 at a dose up to 800 mg/day (175). No apparent hepatic or renal toxicities have been reported with FM-VP4 administration in both experimental animals (178) and humans (175). Assessment of liver enzyme activities (aspartate aminotransferase; AST and plasma alanine aminotransferase; ALT) and renal function (plasma creatinine) revealed no significant changes following 12 weeks of feeding mice with 2% (w/w) FM-VP4 (178) or eight weeks of feeding gerbils with 2% - 4% FM-VP4, either incorporated into the diets or drinking water (177). Five weeks of feeding hamsters with 0.7% - 1.4 % (w/w) of FM-VP4 has not been shown to affect erythrocyte fragility (159). No mutagenic activity has been reported with FM-VP4 (186).

CONCLUSIONS

Based on abundant evidence from scientific literature, plant sterols/ stanols and their semisynthetic analogues have promising roles in prevention and treatment of elevated plasma cholesterol levels and atherosclerotic cardiovascular disease. The enhanced safety profile of plant sterols/ stanols allows them to be used either as an adjuvant or alternative therapy especially for those patients who are intolerant to statin therapy. Several factors have been suggested to affect the cholesterol-lowering activity of plant sterols/stanols. Modifying these factors could be valuable in optimizing their

efficacy. Synthesis of semisynthetic sterols/stanols by esterification with ascorbic acid improves the cholesterol-lowering efficacy of plant sterols and stanols and expands their capacity to lower elevated plasma triglyceride levels. FM-VP4 is a promising synthetic stanol that has shown substantially increased efficacy compared to other sterols and stanols.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Gupta A, Savopoulos C, Ahuja J, Hatzitolios A. Role of Phytosterols in Lipid-Lowering: Current Perspectives. QJM. 2011;104(4):301-308.
2. Roche Y, Gerbeau-Pissot P, Buhot B, Thomas D, Bonneau L, Gresti J, Mongrand S, Perrier-Cornet J-M, Simon-Plas F. Depletion of Phytosterols from the Plant Plasma Membrane Provides Evidence for Disruption of Lipid Rafts. FASEB J. 2008;22(11):3980-3991.
3. Moreau RA, Whitaker BD, Hicks KB. Phytosterols, Phytostanols, and Their Conjugates in Foods: Structural Diversity, Quantitative Analysis, and Health-Promoting Uses. Prog Lipid Res. 2002;41(6):457-500.
4. MacKay DS, Jones PJ. Phytosterols in Human Nutrition: Type, Formulation, Delivery, and Physiological Function. Eur J Lipid Sci Technol. 2011;113(12):1427-1432.
5. Carr TP, Ash MM, Brown AW. Cholesterol-Lowering Phytosterols: Factors Affecting Their Use and Efficacy. Nutr Dietary Suppl. 2010;2:59-72.
6. Verleyen T, Forcades M, Verhé R, Dewettinck K, Huyghebaert A, De Greyt W. Analysis of Free and Esterified Sterols in Vegetable Oils. J Am Oil Chem Soc. 2002;79(2):117-122.
7. Weihrauch JL, Gardner JM. Sterol Content of Foods of Plant Origin. J Am Diet Assoc. 1978;73(1):39-47.
8. Patel MD, Thompson PD. Phytosterols and Vascular Disease. Atherosclerosis. 2006;186(1):12-19.
9. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R. Efficacy and Safety of Plant Stanols and Sterols in the Management of Blood Cholesterol Levels. Mayo Clin Proc. 2003;78(8):965-978.

10. Law MR. Topic in Review: Plant Sterol and Stanol Margarines and Health. West J Med. 2000;173(1):43-47.

11. Vuoristo M, Miettinen TA. Absorption, Metabolism, and Serum Concentrations of Cholesterol in Vegetarians: Effects of Cholesterol Feeding. Am J Clin Nutr. 1994;59(6):1325-1331.

12. Thompson GR, Grundy SM. History and Development of Plant Sterol and Stanol Esters for Cholesterol-Lowering Purposes. Am J Cardiol. 2005;96(1):3-9.

13. Calpe-Berdiel L, Escolà-Gil JC, Blanco-Vaca F. New Insights into the Molecular Actions of Plant Sterols and Stanols in Cholesterol Metabolism. Atherosclerosis. 2009;203(1):18-31.

14. Weingärtner O, Böhm M, Laufs U. Controversial Role of Plant Sterol Esters in the Management of Hypercholesterolaemia. Eur Heart J. 2009;30(4):404-409.

15. Berger A, Jones P, Abumweis SS. Plant Sterols: Factors Affecting Their Efficacy and Safety as Functional Food Ingredients. Lipids Health Dis. 2004;3(5):1-19.

16. Shaghaghi MA, Abumweis SS, Jones PJ. Cholesterol-Lowering Efficacy of Plant Sterols/Stanols Provided in Capsule and Tablet Formats: Results of a Systematic Review and Meta-Analysis. J Acad Nutr Diet. 2013;113(11):1494-1503.

17. Wester I. Cholesterol-Lowering Effect of Plant Sterols. Eur J Lipid Sci Technol. 2000;102(1):37-44.

18. Salo P, Wester I. Low-Fat Formulations of Plant Stanols and Sterols. Am J Cardiol. 2005;96(1):51-54.

19. St-Onge M-P, Jones PJ. Phytosterols and Human Lipid Metabolism: Efficacy, Safety, and Novel Foods. Lipids. 2003;38(4):367-375.

20. Lichtenstein AH, Deckelbaum RJ. Stanol/Sterol Ester–Containing Foods and Blood Cholesterol Levels a Statement for Healthcare Professionals from the Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. Circulation. 2001;103(8):1177-1179.

21. Sachs-Barrable K, Darlington JW, Wasan KM. The Effect of Two Novel Cholesterol-Lowering Agents, Disodium Ascorbyl Phytostanol Phosphate (Dapp) and Nanostructured Aluminosilicate (Nsas) on the Expression and Activity of P-Glycoprotein within Caco-2 Cells. Lipids Health Dis. 2014;13(1):153.

22. Russell JC, Ewart HS, Kelly SE, Kralovec J, Wright JL, Dolphin PJ. Improvement of Vascular Dysfunction and Blood Lipids of Insulin-Resistant Rats by a Marine Oil-Based Phytosterol Compound. Lipids. 2002;37(2):147-152.

23. Demonty I, Chan Y-M, Pelled D, Jones PJ. Fish-Oil Esters of Plant Sterols Improve the Lipid Profile of Dyslipidemic Subjects More Than Do Fish-Oil or Sunflower Oil Esters of Plant Sterols. Am J Clin Nutr. 2006;84(6):1534-1542.

24. Nestel P, Cehun M, Pomeroy S, Abbey M, Weldon G. Original Communications-Cholesterol-Lowering Effects of Plant Sterol Esters and Non-Esterified Stanols in Margarine, Butter and Low-Fat Foods. Eur J Clin Nutr. 2001;55(12):1084-1090.

25. Peterson D. Effect of Soybean Sterols in the Diet on Plasma and Liver Cholesterol in Chicks. Exp Biol Med. 1951;78(1):143-147.

26. Peterson D, Nichols C, Shneour E, Gaffey H, Robbins R, Peek N. Some Relationships among Dietary Sterols, Plasma and Liver Cholesterol Levels, and Atherosclerosis in Chicks One Figure. J Nutr. 1952;47(1):57-65.

27. Peterson D, Shneour E, Peek N, Gaffey H. Dietary Constituents Affecting Plasma and Liver Cholesterol in Cholesterol-Fed Chicks. J Nutr. 1953;50(2):191-201.

28. Pollak O. Successful Prevention of Experimental Hypercholesteremia and Cholesterol Atherosclerosis in the Rabbit. Circulation. 1953;7(5):696-701.

29. Moghadasian MH, McManus BM, Godin DV, Rodrigues B, Frohlich JJ. Proatherogenic and Antiatherogenic Effects of Probucol and Phytosterols in Apolipoprotein E–Deficient Mice Possible Mechanisms of Action. Circulation. 1999;99(13):1733-1739.

30. Vaskonen T, Mervaala E, Krogerus L, Karppanen H. Supplementation of Plant Sterols and Minerals Benefits Obese Zucker Rats Fed an Atherogenic Diet. J Nutr. 2002;132(2):231-237.

31. Laraki L, Pelletier X, Debry G. Effects of Dietary Cholesterol and Phytosterol Overload on Wistar Rat Plasma Lipids. Ann Nutr Metab. 1991;35(4):221-225.

32. Ntanios FY, MacDougall DE, Jones PJ. Gender Effects of Tall Oil Versus Soybean Phytosterols as Cholesterol-Lowering Agents in Hamsters. Can J Physiol Pharmacol. 1998;76(7-8):780-787.

33. Ras RT, Geleijnse JM, Trautwein EA. Ldl-Cholesterol-Lowering Effect of Plant Sterols and Stanols across Different Dose Ranges: A Meta-Analysis of Randomised Controlled Studies. Br J Nutr. 2014;112(02):214-219.

34. Demonty I, Ras RT, van der Kniap HCM, Duchateau G, Meijer L, Zock PL, Geleijnse JM, Trautwein EA. Continuous Dose-Response Relationship of the Ldl-Cholesterol-Lowering Effect of Phytosterol Intake. J Nutr. 2009;139(2):271-284.

35. Plana N, Nicolle C, Ferre R, Camps J, Cos R, Villoria J, Masana L. Plant Sterol-Enriched Fermented Milk Enhances the Attainment of Ldl-Cholesterol Goal in Hypercholesterolemic Subjects. Eur J Nutr. 2008;47(1):32-39.

36. Sialvera T, Pounis G, Koutelidakis A, Richter D, Yfanti G, Kapsokefalou M, Goumas G, Chiotinis N, Diamantopoulos E, Zampelas A. Phytosterols Supplementation Decreases Plasma Small and Dense Ldl Levels in Metabolic Syndrome Patients

on a Westernized Type Diet. Nutr Metab Cardiovasc Dis. 2012;22(10):843-848.

37. Sierksma A, Weststrate JA, Meijer GW. Spreads Enriched with Plant Sterols, Either Esterified 4, 4-Dimethylsterols or Free 4-Desmethylsterols, and Plasma Total-and Ldl-Cholesterol Concentrations. Br J Nutr. 1999;82(04):273-282.

38. Maki KC, Davidson MH, Umporowicz DM, Schaefer EJ, Dicklin MR, Ingram KA, Chen S, McNamara JR, Gebhart BW, Ribaya-Mercado JD. Lipid Responses to Plant-Sterol-Enriched Reduced-Fat Spreads Incorporated into a National Cholesterol Education Program Step I Diet. Am J Clin Nutr. 2001;74(1):33-43.

39. Mensink RP, Ebbing S, Lindhout M, Plat J, van Heugten MMA. Effects of Plant Stanol Esters Supplied in Low-Fat Yoghurt on Serum Lipids and Lipoproteins, Non-Cholesterol Sterols and Fat Soluble Antioxidant Concentrations. Atherosclerosis. 2002;160(1):205-213.

40. Weststrate J, Meijer G. Plant Sterol-Enriched Margarines and Reduction of Plasma Total-and Ldl-Cholesterol Concentrations in Normocholesterolaemic and Mildly Hypercholesterolaemic Subjects. Eur J Clin Nutr. 1998;52(5):334-343.

41. Hendriks H, Weststrate J, Van Vliet T, Meijer G. Spreads Enriched with Three Different Levels of Vegetable Oil Sterols and the Degree of Cholesterol Lowering in Normocholesterolaemic and Mildly Hypercholesterolaemic Subjects. Eur J Clin Nutr. 1999;53(4):319-327.

42. McPherson TB, Ostlund RE, Goldberg AC, Bateman JH, Schimmoeller L, Spilburg CA. Phytostanol Tablets Reduce Human Ldl-Cholesterol. J Pharm Pharmacol. 2005;57(7):889-896.

43. Goldberg AC, Ostlund RE, Bateman JH, Schimmoeller L, McPherson TB, Spilburg CA. Effect of Plant Stanol Tablets on Low-Density Lipoprotein Cholesterol Lowering in Patients on Statin Drugs. Am J Cardiol. 2006;97(3):376-379.

44. Woodgate D, Chan CH, Conquer JA. Cholesterol-Lowering Ability of a Phytostanol Softgel Supplement in Adults with Mild to Moderate Hypercholesterolemia. Lipids. 2006;41(2):127-132.

45. Carr TP, Krogstrand KLS, Schlegel VL, Fernandez ML. Stearate-Enriched Plant Sterol Esters Lower Serum Ldl Cholesterol Concentration in Normo-and Hypercholesterolemic Adults. J Nutr. 2009;139(8):1445-1450.

46. Maki KC, Lawless AL, Reeves MS, Dicklin MR, Jenks BH, Shneyvas E, Brooks JR. Lipid-Altering Effects of a Dietary Supplement Tablet Containing Free Plant Sterols and Stanols in Men and Women with Primary Hypercholesterolaemia: A Randomized, Placebo-Controlled Crossover Trial. Int J Food Sci Nutr. 2012;63(4):476-482.

47. Maki KC, Lawless AL, Reeves MS, Kelley KM, Dicklin MR, Jenks BH, Shneyvas E, Brooks JR.

Lipid Effects of a Dietary Supplement Softgel Capsule Containing Plant Sterols/Stanols in Primary Hypercholesterolemia. Nutrition. 2013;29(1):96-100.

48. Ottestad I, Ose L, Wennersberg MH, Granlund L, Kirkhus B, Retterstøl K. Phytosterol Capsules and Serum Cholesterol in Hypercholesterolemia: A Randomized Controlled Trial. Atherosclerosis. 2013;228(2):421-425.

49. McKenney JM, Jenks BH, Shneyvas E, Brooks JR, Shenoy SF, Cook CM, Maki KC. A Softgel Dietary Supplement Containing Esterified Plant Sterols and Stanols Improves the Blood Lipid Profile of Adults with Primary Hypercholesterolemia: A Randomized, Double-Blind, Placebo-Controlled Replication Study. J Acad Nutr Diet. 2014;114(2):244-249.

50. Naumann E, Plat J, Kester AD, Mensink RP. The Baseline Serum Lipoprotein Profile Is Related to Plant Stanol Induced Changes in Serum Lipoprotein Cholesterol and Triacylglycerol Concentrations. J Am Coll Nutr. 2008;27(1):117-126.

51. Wu T, Fu J, Yang Y, Zhang L, Han J. The Effects of Phytosterols/Stanols on Blood Lipid Profiles: A Systematic Review with Meta-Analysis. Asia Pac J Clin Nutr. 2009;18(2):179-186.

52. Volger OL, van der Boom H, de Wit EC, van Duyvenvoorde W, Hornstra G, Plat J, Havekes LM, Mensink RP, Princen HM. Dietary Plant Stanol Esters Reduce Vldl Cholesterol Secretion and Bile Saturation in Apolipoprotein E* 3-Leiden Transgenic Mice. Arterioscler Thromb Vasc Biol. 2001;21(6):1046-1052.

53. Párraga I, López-Torres J, Andrés F, Navarro B, del Campo JM, García-Reyes M, Galdón MP, Lloret Á, Precioso JC, Rabanales J. Effect of Plant Sterols on the Lipid Profile of Patients with Hypercholesterolaemia. Randomised, Experimental Study. BMC Complement Altern Med. 2011;11(1):73.

54. Ketomäki A, Gylling H, Miettinen TA. Non-Cholesterol Sterols in Serum, Lipoproteins, and Red Cells in Statin-Treated Fh Subjects Off and on Plant Stanol and Sterol Ester Spreads. Clinica chimica acta. 2005;353(1):75-86.

55. Gylling H, Miettinen T. Serum Cholesterol and Cholesterol and Lipoprotein Metabolism in Hypercholesterolaemic Niddm Patients before and During Sitostanol Ester-Margarine Treatment. Diabetologia. 1994;37(8):773-780.

56. Ikonen E. Cellular Cholesterol Trafficking and Compartmentalization. Nat Rev Mol Cell Biol. 2008;9(2):125-138.

57. Brown AW, Hang J, Dussault PH, Carr TP. Phytosterol Ester Constituents Affect Micellar Cholesterol Solubility in Model Bile. Lipids. 2010;45(9):855-862.

58. Nagadome S, Okazaki Y, Lee S, Sasaki Y, Sugihara G. Selective Solubilization of Sterols by Bile Salt Micelles in Water: A Thermodynamic Study. Langmuir. 2001;17(14):4405-4412.

59. Slota T, Kozlov N, Ammon H. Comparison of Cholesterol and Beta-Sitosterol: Effects on Jejunal Fluid Secretion Induced by Oleate, and Absorption from Mixed Micellar Solutions. Gut. 1983;24(7):653-658.

60. Armstrong MJ, Carey MC. Thermodynamic and Molecular Determinants of Sterol Solubilities in Bile Salt Micelles. J Lipid Res. 1987;28(10):1144-1155.

61. Matsuoka K, Kajimoto E, Horiuchi M, Honda C, Endo K. Competitive Solubilization of Cholesterol and Six Species of Sterol/Stanol in Bile Salt Micelles. Chem Phys Lipids. 2010;163(4):397-402.

62. Ikeda I, Tanabe Y, Sugano M. Effects of Sitosterol and Sitostanol on Micellar Solubility of Cholesterol. Journal of Nutritional Science and Vitaminology. 1989;35(4):361-369.

63. Ikeda I, Tanaka K, Sugano M, Vahouny G, Gallo L. Inhibition of Cholesterol Absorption in Rats by Plant Sterols. Journal of Lipid Research. 1988;29(12):1573-1582.

64. PLAT J, MENSINK RP. Increased Intestinal Abca1 Expression Contributes to the Decrease in Cholesterol Absorption after Plant Stanol Consumption. FASEB J. 2002;16(10):1248-1253.

65. Field FJ, Mathur SN. Beta-Sitosterol: Esterification by Intestinal Acylcoenzyme A: Cholesterol Acyltransferase (Acat) and Its Effect on Cholesterol Esterification. J Lipid Res. 1983;24(4):409-417.

66. Field FJ, Born E, Mathur SN. Effect of Micellar Beta-Sitosterol on Cholesterol Metabolism in Caco-2 Cells. J Lipid Res. 1997;38(2):348-360.

67. Fernández C, Suárez Y, Ferruelo A, Gómez-Coronado D, Lasuncion M. Inhibition of Cholesterol Biosynthesis by Δ22-Unsaturated Phytosterols Via Competitive Inhibition of Sterol Δ24-Reductase in Mammalian Cells. Biochem J. 2002;366:109-119.

68. Ho SS, Pal S. Margarine Phytosterols Decrease the Secretion of Atherogenic Lipoproteins from Hepg2 Liver and Caco2 Intestinal Cells. Atherosclerosis. 2005;182(1):29-36.

69. Liang YT, Wong WT, Guan L, Tian XY, Ma KY, Huang Y, Chen Z-Y. Effect of Phytosterols and Their Oxidation Products on Lipoprotein Profiles and Vascular Function in Hamster Fed a High Cholesterol Diet. Atherosclerosis. 2011;219(1):124-133.

70. Vanstone CA, Raeini-Sarjaz M, Jones PJ. Injected Phytosterols/Stanols Suppress Plasma Cholesterol Levels in Hamsters. J Nutr Biochem. 2001;12(10):565-574.

71. Konlande JE, Fisher H. Evidence for a Nonabsorptive Antihypercholesterolemic Action of Phytosterols in the Chicken. J Nutr. 1969;98(4):435-442.

72. Lukic T, Wasan KM, Zamfir D, Moghadasian MH, Pritchard PH. Disodium Ascorbyl Phytostanyl Phosphate Reduces Plasma Cholesterol Concentrations and Atherosclerotic Lesion Formation in Apolipoprotein E-Deficient Mice. Metabolism. 2003;52(4):425-431.

73. Plat J, Nichols JA, Mensink RP. Plant Sterols and Stanols: Effects on Mixed Micellar Composition and Lxr (Target Gene) Activation. J Lipid Res. 2005;46(11):2468-2476.

74. Ikeda I, Tanaka K, Sugano M, Vahouny G, Gallo L. Discrimination between Cholesterol and Sitosterol for Absorption in Rats. Journal of lipid research. 1988;29(12):1583-1591.

75. Ikeda I, Sugano M. Some Aspects of Mechanism of Inhibition of Cholesterol Absorption by B-Sitosterol. Biochimica et Biophysica Acta (BBA)-Biomembranes. 1983;732(3):651-658.

76. Child P, Kuksis A. Uptake of 7-Dehydro Derivatives of Cholesterol, Campesterol, and Beta-Sitosterol by Rat Erythrocytes, Jejunal Villus Cells, and Brush Border Membranes. J Lipid Res. 1983;24(5):552-565.

77. Zhang J-H, Ge L, Qi W, Zhang L, Miao H-H, Li B-L, Yang M, Song B-L. The N-Terminal Domain of Npc1l1 Protein Binds Cholesterol and Plays Essential Roles in Cholesterol Uptake. J Biol Chem. 2011;286(28):25088-25097.

78. Smet ED, Mensink RP, Plat J. Effects of Plant Sterols and Stanols on Intestinal Cholesterol Metabolism: Suggested Mechanisms from Past to Present. Mol Nutr Food Res. 2012;56(7):1058-1072.

79. Calpe-Berdiel L, Escolà-Gil JC, Ribas V, Navarro-Sastre A, Garcés-Garcés J, Blanco-Vaca F. Changes in Intestinal and Liver Global Gene Expression in Response to a Phytosterol-Enriched Diet. Atherosclerosis. 2005;181(1):75-85.

80. Jain D, Ebine N, Jia X, Kassis A, Marinangeli C, Fortin M, Beech R, Hicks KB, Moreau RA, Kubow S. Corn Fiber Oil and Sitostanol Decrease Cholesterol Absorption Independently of Intestinal Sterol Transporters in Hamsters. J Nutr Biochem. 2008;19(4):229-236.

81. Plösch T, Kruit JK, Bloks VW, Huijkman NC, Havinga R, Duchateau GS, Lin Y, Kuipers F. Reduction of Cholesterol Absorption by Dietary Plant Sterols and Stanols in Mice Is Independent of the Abcg5/8 Transporter. J Nutr. 2006;136(8):2135-2140.

82. Field FJ, Born E, Mathur SN. Stanol Esters Decrease Plasma Cholesterol Independently of Intestinal Abc Sterol Transporters and Niemann-Pick C1-Like 1 Protein Gene Expression. J Lipid Res. 2004;45(12):2252-2259.

83. Kaneko E, Matsuda M, Yamada Y, Tachibana Y, Shimomura I, Makishima M. Induction of Intestinal Atp-Binding Cassette Transporters by a Phytosterol-Derived Liver X Receptor Agonist. J Biol Chem. 2003;278(38):36091-36098.

84. Brufau G, Kuipers F, Lin Y, Trautwein EA, Groen AK. A Reappraisal of the Mechanism by Which Plant Sterols Promote Neutral Sterol Loss in Mice. PLoS ONE. 2011;6(6):e21576.

85. Batta AK, Xu G, Honda A, Miyazaki T, Salen G. Stigmasterol Reduces Plasma Cholesterol Levels

and Inhibits Hepatic Synthesis and Intestinal Absorption in the Rat. Metabolism. 2006;55(3):292-299.

86. AbuMweis SS, Barake R, Jones PJ. Plant Sterols/Stanols as Cholesterol Lowering Agents: A Meta-Analysis of Randomized Controlled Trials. Food Nutr Res. 2008;52.

87. Mattson FH, Grundy SM, Crouse JR. Optimizing the Effect of Plant Sterols on Cholesterol Absorption in Man. Am J Clin Nutr. 1982;35(4):697-700.

88. Doornbos A, Meynen E, Duchateau G, Van Der Knaap H, Trautwein E. Intake Occasion Affects the Serum Cholesterol Lowering of a Plant Sterol-Enriched Single-Dose Yoghurt Drink in Mildly Hypercholesterolaemic Subjects. Eur J Clin Nutr. 2006;60(3):325-333.

89. Clifton P, Noakes M, Sullivan D, Erichsen N, Ross D, Annison G, Fassoulakis A, Cehun M, Nestel P. Cholesterol-Lowering Effects of Plant Sterol Esters Differ in Milk, Yoghurt, Bread and Cereal. Eur J Clin Nutr. 2004;58(3):503-509.

90. Ostlund RE, Spilburg CA, Stenson WF. Sitostanol Administered in Lecithin Micelles Potently Reduces Cholesterol Absorption in Humans. Am J Clin Nutr. 1999;70(5):826-831.

91. Kobayashi M, Hamada T, Goto H, Imaizumi K, Ikeda I. Comparison of Effects of Dietary Unesterified and Esterified Plant Sterols on Cholesterol Absorption in Rats. J Nutr Sci Vitaminol (Tokyo). 2008;54(3):210-214.

92. Nissinen MJ, Vuoristo M, Gylling H, Miettinen TA. Respective Hydrolysis and Esterification of Esterified and Free Plant Stanols Occur Rapidly in Human Intestine after Their Duodenal Infusion in Triacyl-or Diacylglycerol. Lipids. 2007;42(7):603-612.

93. Nissinen M, Gylling H, Vuoristo M, Miettinen TA. Micellar Distribution of Cholesterol and Phytosterols after Duodenal Plant Stanol Ester Infusion. Am J Physiol Gastrointest Liver Physiol. 2002;282(6):G1009-G1015.

94. Richelle M, Enslen M, Hager C, Groux M, Tavazzi I, Godin J-P, Berger A, Métairon S, Quaile S, Piguet-Welsch C. Both Free and Esterified Plant Sterols Reduce Cholesterol Absorption and the Bioavailability of B-Carotene and A-Tocopherol in Normocholesterolemic Humans. Am J Clin Nutr. 2004;80(1):171-177.

95. Meijer GW, Bressers MA, de Groot WA, Rudrum M. Effect of Struture and Form on the Ability of Plant Sterols to Inhibit Cholesterol Absorption in Hamsters. Lipids. 2003;38(7):713-721.

96. Ash MM, Hang J, Dussault PH, Carr TP. Phytosterol Stearate Esters Elicit Similar Responses on Plasma Lipids and Cholesterol Absorption but Different Responses on Fecal Neutral Sterol Excretion and Hepatic Free Cholesterol in Male Syrian Hamsters. Nutr Res. 2011;31(7):537-543.

97. Guderian DM, Rasmussen HE, Wray CA, Dussault PH, Carr TP. Cholesterol-Lowering Properties of Plant Sterols Esterified with Beef Tallow Fatty Acids in Hamsters. Nutr Res. 2007;27(5):283-288.

98. Rasmussen HE, Guderian DM, Wray CA, Dussault PH, Schlegel VL, Carr TP. Reduction in Cholesterol Absorption Is Enhanced by Stearate-Enriched Plant Sterol Esters in Hamsters. J Nutr. 2006;136(11):2722-2727.

99. Cowles RL, Lee J-Y, Gallaher DD, Stuefer-Powell CL, Carr TP. Dietary Stearic Acid Alters Gallbladder Bile Acid Composition in Hamsters Fed Cereal-Based Diets. J Nutr. 2002;132(10):3119-3122.

100. Schneider CL, Cowles RL, Stuefer-Powell CL, Carr TP. Dietary Stearic Acid Reduces Cholesterol Absorption and Increases Endogenous Cholesterol Excretion in Hamsters Fed Cereal-Based Diets. J Nutr. 2000;130(5):1232-1238.

101. Micallef MA, Garg ML. The Lipid-Lowering Effects of Phytosterols and (N-3) Polyunsaturated Fatty Acids Are Synergistic and Complementary in Hyperlipidemic Men and Women. J Nutr. 2008;138(6):1086-1090.

102. Ikeda I, Kawasaki A, Samezima K, Sugano M. Anti-Hypercholesterolemic Activity of Beta-Sitostanol in Rabbits. J Nutr Sci Vitaminol (Tokyo). 1981;27(3):243-251.

103. Heinemann T, Pietruck B, Kullak-Ublick G, Von Bergmann K. Comparison of Sitosterol and Sitostanol on Inhibition of Intestinal Cholesterol Absorption. Agents Actions Suppl. 1988;26:117-122.

104. Heinemann T, Leiss O, Von Bergmann K. Effect of Low-Dose Sitostanol on Serum Cholesterol in Patients with Hypercholesterolemia. Atherosclerosis. 1986;61(3):219-223.

105. Heinemann T, Kullak-Ublick G-A, Pietruck B, Von Bergmann K. Mechanisms of Action of Plant Sterols on Inhibition of Cholesterol Absorption. Eur J Clin Pharmacol. 1991;40(1):S59-S63.

106. Gylling H, Miettinen TA. Cholesterol Reduction by Different Plant Stanol Mixtures and with Variable Fat Intake. Metabolism. 1999;48(5):575-580.

107. Miettinen TA, Vanhanen H. Dietary-Sitostanol Related to Absorption, Synthesis and Serum Level of Cholesterol in Different Apolipoprotein E Phenotypes. Atherosclerosis. 1994;105(2):217-226.

108. Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. Reduction of Serum Cholesterol with Sitostanol-Ester Margarine in a Mildly Hypercholesterolemic Population. N Engl J Med. 1995;333(20):1308-1312.

109. Sugano M, Morioka H, Ikeda I. A Comparison of Hypocholesterolemic Activity of Beta-Sitosterol and Beta-Sitostanol in Rats. J Nutr. 1977;107(11):2011-2019.

110. Sugano M, Kamo F, Ikeda I, Morioka H. Lipid-Lowering Activity of Phytostanols in Rats. Atherosclerosis. 1976;24(1):301-309.

111. Becker M, Staab D, Von Bergmann K. Treatment of Severe Familial Hypercholesterolemia in Childhood with Sitosterol and Sitostanol. J Pediatr. 1993;122(2):292-296.

112. Heinemann T, Axtmann G, Bergmann Kv. Comparison of Intestinal Absorption of Cholesterol with Different Plant Sterols in Man*. Eur J Clin Invest. 1993;23(12):827-831.

113. Pritchard PH, Li M, Zamfir C, Lukic T, Novak E, Moghadasian MH. Comparison of Cholesterol-Lowering Efficacy and Anti-Atherogenic Properties of Hydrogenated Versus Non-Hydrogenated (Phytrol™) Tall Oil-Derived Phytosterols in Apo E-Deficient Mice. Cardiovasc Drugs Ther. 2003;17(5-6):443-449.

114. Musa-Veloso K, Poon TH, Elliot JA, Chung C. A Comparison of the Ldl-Cholesterol Lowering Efficacy of Plant Stanols and Plant Sterols over a Continuous Dose Range: Results of a Meta-Analysis of Randomized, Placebo-Controlled Trials. PLEFA. 2011;85(1):9-28.

115. Talati R, Sobieraj DM, Makanji SS, Phung OJ, Coleman CI. The Comparative Efficacy of Plant Sterols and Stanols on Serum Lipids: A Systematic Review and Meta-Analysis. J Am Diet Assoc. 2010;110(5):719-726.

116. Rideout TC, Harding SV, Jones PJ. Consumption of Plant Sterols Reduces Plasma and Hepatic Triglycerides and Modulates the Expression of Lipid Regulatory Genes and De Novo Lipogenesis in C57bl/6j Mice. Mol Nutr Food Res. 2010;54(S1):S7-S13.

117. Demonty I, Ras RT, van der Knaap HC, Meijer L, Zock PL, Geleijnse JM, Trautwein EA. The Effect of Plant Sterols on Serum Triglyceride Concentrations Is Dependent on Baseline Concentrations: A Pooled Analysis of 12 Randomised Controlled Trials. Eur J Nutr. 2013;52(1):153-160.

118. Schonewille M, Brufau G, Shiri-Sverdlov R, Groen AK, Plat J. Serum Tg-Lowering Properties of Plant Sterols and Stanols Are Associated with Decreased Hepatic Vldl Secretion. J Lipid Res. 2014;55(12):2554-2561.

119. Plat J, Brufau G, Dallinga-Thie GM, Dasselaar M, Mensink RP. A Plant Stanol Yogurt Drink Alone or Combined with a Low-Dose Statin Lowers Serum Triacylglycerol and Non-Hdl Cholesterol in Metabolic Syndrome Patients. J Nutr. 2009;139(6):1143-1149.

120. Plat J, Mensink RP. Plant Stanol Esters Lower Serum Triacylglycerol Concentrations Via a Reduced Hepatic Vldl-1 Production. Lipids. 2009;44(12):1149-1153.

121. Awad A, Smith A, Fink C. Plant Sterols Regulate Rat Vascular Smooth Muscle Cell Growth and Prostacyclin Release in Culture. PLEFA. 2001;64(6):323-330.

122. Zhao J, Zhang C, Xu D, Huang G, Xu Y, Wang Z, Fang S, Chen Y, Gu Y. The Antiatherogenic Effects of Components Isolated from Pollen Typhae. Thromb Res. 1990;57(6):957-966.

123. Rubis B, Paszel A, Kaczmarek M, Rudzinska M, Jelen H, Rybczynska M. Beneficial or Harmful Influence of Phytosterols on Human Cells? Br J Nutr. 2008;100(6):1183.

124. Loizou S, Lekakis I, Chrousos GP, Moutsatsou P. B-Sitosterol Exhibits Anti-Inflammatory Activity in Human Aortic Endothelial Cells. Mol Nutr Food Res. 2010;54(4):551-558.

125. Nashed B, Yeganeh B, HayGlass KT, Moghadasian MH. Antiatherogenic Effects of Dietary Plant Sterols Are Associated with Inhibition of Proinflammatory Cytokine Production in Apo E-Ko Mice. J Nutr. 2005;135(10):2438-2444.

126. Moghadasian MH, McManus BM, Pritchard PH, Frohlich JJ. "Tall Oil"–Derived Phytosterols Reduce Atherosclerosis in Apoe-Deficient Mice. Arterioscler Thromb Vasc Biol. 1997;17(1):119-126.

127. Volger OL, Mensink RP, Plat J, Hornstra G, Havekes LM, Princen HMG. Dietary Vegetable Oil and Wood Derived Plant Stanol Esters Reduce Atherosclerotic Lesion Size and Severity in Apoe*3-Leiden Transgenic Mice. Atherosclerosis. 2001;157(2):375-381.

128. Plat J, Beugels I, Gijbels MJ, de Winther MP, Mensink RP. Plant Sterol or Stanol Esters Retard Lesion Formation in Ldl Receptor-Deficient Mice Independent of Changes in Serum Plant Sterols. J Lipid Res. 2006;47(12):2762-2771.

129. Ntanios FY, van de Kooij AJ, de Deckere EA, Duchateau GS, Trautwein EA. Effects of Various Amounts of Dietary Plant Sterol Esters on Plasma and Hepatic Sterol Concentration and Aortic Foam Cell Formation of Cholesterol-Fed Hamsters. Atherosclerosis. 2003;169(1):41-50.

130. Ntanios FY, Jones PJ, Frohlich JJ. Dietary Sitostanol Reduces Plaque Formation but Not Lecithin Cholesterol Acyl Transferase Activity in Rabbits. Atherosclerosis. 1998;138(1):101-110.

131. Moghadasian MH, Godin DV, McManus BM, Frohlich JJ. Lack of Regression of Atherosclerotic Lesions in Phytosterol-Treated Apo E-Deficient Mice. Life Sci. 1999;64(12):1029-1036.

132. Plat J, Mensink RP. Vegetable Oil Based Versus Wood Based Stanol Ester Mixtures: Effects on Serum Lipids and Hemostatic Factors in Non-Hypercholesterolemic Subjects. Atherosclerosis. 2000;148(1):101-112.

133. Raitakari O, Salo P, Ahotupa M. Carotid Artery Compliance in Users of Plant Stanol Ester Margarine. Eur J Clin Nutr. 2008;62(2):218-224.

134. Raitakari OT, Salo P, Gylling H, Miettinen TA. Plant Stanol Ester Consumption and Arterial Elasticity and Endothelial Function. Br J Nutr. 2008;100(03):603-608.

135. Hallikainen M, Lyyra-Laitinen T, Laitinen T, Moilanen L, Miettinen TA, Gylling H. Effects of Plant Stanol Esters on Serum Cholesterol Concentrations, Relative Markers of Cholesterol Metabolism and Endothelial Function in Type 1 Diabetes. Atherosclerosis. 2008;199(2):432-439.

136. Hallikainen M, Lyyra-Laitinen T, Laitinen T, Ågren JJ, Pihlajamäki J, Rauramaa R, Miettinen TA, Gylling H. Endothelial Function in Hypercholesterolemic Subjects: Effects of Plant Stanol and Sterol Esters. Atherosclerosis. 2006;188(2):425-432.

137. Jakulj L, Vissers MN, Rodenburg J, Wiegman A, Trip MD, Kastelein JJ. Plant Stanols Do Not Restore Endothelial Function in Pre-Pubertal Children with Familial Hypercholesterolemia Despite Reduction of Low-Density Lipoprotein Cholesterol Levels. J Pediatr. 2006;148(4):495-500.

138. Gylling H, Hallikainen M, Raitakari OT, Laakso M, Vartiainen E, Salo P, Korpelainen V, Sundvall J, Miettinen TA. Long-Term Consumption of Plant Stanol and Sterol Esters, Vascular Function and Genetic Regulation. Br J Nutr. 2009;101(11):1688-1695.

139. De Jong A, Plat J, Bast A, Godschalk R, Basu S, Mensink R. Effects of Plant Sterol and Stanol Ester Consumption on Lipid Metabolism, Antioxidant Status and Markers of Oxidative Stress, Endothelial Function and Low-Grade Inflammation in Patients on Current Statin Treatment. Eur J Clin Nutr. 2008;62(2):263-273.

140. Gagliardi A, Maranhão R, de Sousa H, Schaefer E, Santos R. Effects of Margarines and Butter Consumption on Lipid Profiles, Inflammation Markers and Lipid Transfer to Hdl Particles in Free-Living Subjects with the Metabolic Syndrome. Eur J Clin Nutr. 2010;64(10):1141-1149.

141. Bañuls C, Martínez-Triguero ML, López-Ruiz A, Morillas C, Lacomba R, Víctor VM, Rocha M, Hernández-Mijares A. Evaluation of Cardiovascular Risk and Oxidative Stress Parameters in Hypercholesterolemic Subjects on a Standard Healthy Diet Including Low-Fat Milk Enriched with Plant Sterols. J Nutr Biochem. 2010;21(9):881-886.

142. Hansel B, Nicolle C, Lalanne F, Tondu F, Lassel T, Donazzolo Y, Ferrières J, Krempf M, Schlienger J-L, Verges B. Effect of Low-Fat, Fermented Milk Enriched with Plant Sterols on Serum Lipid Profile and Oxidative Stress in Moderate Hypercholesterolemia. Am J Clin Nutr. 2007;86(3):790-796.

143. Homma Y, Ikeda I, Ishikawa T, Tateno M, Sugano M, Nakamura H. Decrease in Plasma Low-Density Lipoprotein Cholesterol, Apolipoprotein B, Cholesteryl Ester Transfer Protein, and Oxidized Low-Density Lipoprotein by Plant Stanol Ester-Containing Spread:: A Randomized, Placebo-Controlled Trial. Nutrition. 2003;19(4):369-374.

144. Gylling H, Plat J, Turley S, Ginsberg HN, Ellegård L, Jessup W, Jones PJ, Lütjohann D, Maerz W, Masana L. Plant Sterols and Plant Stanols in the Management of Dyslipidaemia and Prevention of Cardiovascular Disease. Atherosclerosis. 2014;232(2):346-360.

145. Lottenberg AM, Bombo R, Ilha A, Nunes VS, Nakandakare ER, Quintão EC. Do Clinical and Experimental Investigations Support an Antiatherogenic Role for Dietary Phytosterols/Stanols? IUBMB life. 2012;64(4):296-306.

146. Mellies MJ, Ishikawa T, Glueck C, Bove K, Morrison J. Phytosterols in Aortic Tissue in Adults and Infants. J Lab Clin Med. 1976;88(6):914-921.

147. Glueck CJ, Speirs J, Tracy T, Streicher P, Illig E, Vandegrift J. Relationships of Serum Plant Sterols (Phytosterols) and Cholesterol in 595 Hypercholesterolemic Subjects, and Familial Aggregation of Phytosterols, Cholesterol, and Premature Coronary Heart Disease in Hyperphytosterolemic Probands and Their First-Degree Relatives. Metabolism. 1991;40(8):842-848.

148. Sudhop T, Gottwald BM, von Bergmann K. Serum Plant Sterols as a Potential Risk Factor for Coronary Heart Disease. Metabolism. 2002;51(12):1519-1521.

149. Assmann G, Cullen P, Erbey J, Ramey DR, Kannenberg F, Schulte H. Plasma Sitosterol Elevations Are Associated with an Increased Incidence of Coronary Events in Men: Results of a Nested Case-Control Analysis of the Prospective Cardiovascular Münster (Procam) Study. Nutr Metab Cardiovasc Dis. 2006;16(1):13-21.

150. Thiery J, Ceglarek U, Fiedler GM, Leichtle A, Baumann S, Teupser D, Lang O, Baumert J, Meisinger C, Loewel H, Doering A. Elevated Campesterol Serum Levels - a Significant Predictor of Incident Myocardial Infarction: Results of the Population-Based Monica/Kora Follow-up Study 1994 to 2005. Circulation. 2006;114(18):884-884.

151. Vergès B, Fumeron F. Potential Risks Associated with Increased Plasma Plant-Sterol Levels. Diabetes Metab. 2015;41(1):76-81.

152. Weingärtner O, Lütjohann D, Ji S, Weisshoff N, List F, Sudhop T, Von Bergmann K, Gertz K, König J, Schäfers H-J. Vascular Effects of Diet Supplementation with Plant Sterols. J Am Coll Cardiol. 2008;51(16):1553-1561.

153. Clifton PM, Noakes M, Ross D, Fassoulakis A, Cehun M, Nestel P. High Dietary Intake of Phytosterol Esters Decreases Carotenoids and Increases Plasma Plant Sterol Levels with No Additional Cholesterol Lowering. J Lipid Res. 2004;45(8):1493-1499.

154. Hallikainen M, Sarkkinen E, Uusitupa M. Effects of Low-Fat Stanol Ester Enriched Margarines on Concentrations of Serum Carotenoids in Subjects with Elevated Serum Cholesterol Concentrations. Eur J Clin Nutr. 1999;53(12):966-969.

155. Tuomilehto J, Tikkanen M, Högström P, Keinänen-Kiukaanniemi S, Piironen V, Toivo J, Salonen J, Nyyssönen K, Stenman U, Alfthan H. Safety Assessment of Common Foods Enriched

with Natural Nonesterified Plant Sterols. Eur J Clin Nutr. 2009;63(5):684-691.

156. Turnbull D, Whittaker MH, Frankos VH, Jonker D. 13-Week Oral Toxicity Study with Stanol Esters in Rats. Regul Toxicol Pharmacol. 1999;29(2):216-226.

157. Hendriks H, Brink E, Meijer G, Princen H, Ntanios F. Safety of Long-Term Consumption of Plant Sterol Esters-Enriched Spread. Eur J Clin Nutr. 2003;57(5):681-692.

158. Noakes M, Clifton P, Ntanios F, Shrapnel W, Record I, McInerney J. An Increase in Dietary Carotenoids When Consuming Plant Sterols or Stanols Is Effective in Maintaining Plasma Carotenoid Concentrations. Am J Clin Nutr. 2002;75(1):79-86.

159. Ebine N, Jia X, Demonty I, Wang Y, Jones PJ. Effects of a Water-Soluble Phytostanol Ester on Plasma Cholesterol Levels and Red Blood Cell Fragility in Hamsters. Lipids. 2005;40(2):175-180.

160. Jenkins DJ, Kendall CW, Faulkner D, Vidgen E, Trautwein EA, Parker TL, Marchie A, Koumbridis G, Lapsley KG, Josse RG. A Dietary Portfolio Approach to Cholesterol Reduction: Combined Effects of Plant Sterols, Vegetable Proteins, and Viscous Fibers in Hypercholesterolemia. Metabolism. 2002;51(12):1596-1604.

161. Jenkins DJ, Kendall CW, Marchie A, Faulkner D, Vidgen E, Lapsley KG, Trautwein EA, Parker TL, Josse RG, Leiter LA. The Effect of Combining Plant Sterols, Soy Protein, Viscous Fibers, and Almonds in Treating Hypercholesterolemia. Metabolism. 2003;52(11):1478-1483.

162. Wang C, Lin HJ, Chan T-k, Salen G, Chan W-C, Tse T-F. A Unique Patient with Coexisting Cerebrotendinous Xanthomatosis and B-Sitosterolemia. Am J Med. 1981;71(2):313-319.

163. Bhattacharyya AK, Connor WE. B-Sitosterolemia and Xanthomatosis: A Newly Described Lipid Storage Disease in Two Sisters. J Clin Invest. 1974;53(4):1033.

164. Ratnayake WM, L'Abbé MR, Mueller R, Hayward S, Plouffe L, Hollywood R, Trick K. Vegetable Oils High in Phytosterols Make Erythrocytes Less Deformable and Shorten the Life Span of Stroke-Prone Spontaneously Hypertensive Rats. J Nutr. 2000;130(5):1166-1178.

165. Malini T, Vanithakumari G. Effect of Beta-Sitosterol on Uterine Biochemistry: A Comparative Study with Estradiol and Progesterone. Biochem Mol Biol Int. 1993;31(4):659-668.

166. Mellanen P, Petänen T, Lehtimäki J, Mäkelä S, Bylund G, Holmbom B, Mannila E, Oikari A, Santti R. Wood-Derived Estrogens: Studies in Vitro with Breast Cancer Cell Lines and in Vivo in Trout. Toxicol Appl Pharmacol. 1996;136(2):381-388.

167. MacLatchy DL, Vanderkraak GJ. The Phytoestrogen B-Sitosterol Alters the Reproductive Endocrine Status of Goldfish. Toxicol Appl Pharmacol. 1995;134(2):305-312.

168. Baker V, Hepburn P, Kennedy S, Jones P, Lea L, Sumpter J, Ashby J. Safety Evaluation of Phytosterol Esters. Part 1. Assessment of Oestrogenicity Using a Combination of in Vivo and in Vitro Assays. Food Chem Toxicol. 1999;37(1):13-22.

169. Turnbull D, Frankos VH, Leeman W, Jonker D. Short-Term Tests of Estrogenic Potential of Plant Stanols and Plant Stanol Esters. Regul Toxicol Pharmacol. 1999;29(2):211-215.

170. Wasan KM, Najafi S, Peteherych KD, Pritchard PH. Effects of a Novel Hydrophilic Phytostanol Analog on Plasma Lipid Concentrations in Gerbils. J Pharm Sci. 2001;90(11):1795-1799.

171. Ramaswamy M, Yau E, Wasan KM, Boulanger KD, Li M, Pritchard PH. Influence of Phytostanol Phosphoryl Ascorbate, Fm-Vp4, on Pancreatic Lipase Activity and Cholesterol Accumulation within Caco-2 Cells. J Pharm Pharm Sci. 2002;5(1):29-38.

172. Ng AW, Lukic T, Pritchard PH, Wasan KM. Development and Characterization of Liposomal Disodium Ascorbyl Phytostanyl Phosphates (Fm-Vp4). Drug Dev Ind Pharm. 2004;30(7):739-758.

173. Thornton SJ, Wong I, Neumann R, Kozlowski P, Wasan KM. Dietary Supplementation with Phytosterol and Ascorbic Acid Reduces Body Mass Accumulation and Alters Food Transit Time in a Diet-Induced Obesity Mouse Model. Lipids Health Dis. 2011;10:107.

174. Ebine N, Demonty I, Jia X, Jones P. Plant Stanol Ascorbate Esters Reduce Body Weight Gain through Decreased Energy Absorption in Hamsters. Int J Obes. 2006;30(5):751-757.

175. Vissers MN, Trip MD, Pritchard PH, Tam P, Lukic T, de Barse M, Kastelein JJ. Efficacy and Safety of Disodium Ascorbyl Phytostanol Phosphates in Men with Moderate Dyslipidemia. Eur J Clin Pharmacol. 2008;64(7):651-661.

176. Meir KS, Leitersdorf E. Atherosclerosis in the Apolipoprotein E–Deficient Mouse a Decade of Progress. Arterioscler Thromb Vasc Biol. 2004;24(6):1006-1014.

177. Wasan KM, Najafi S, Wong J, Kwong M, Pritchard PH. Assessing Plasma Lipid Levels, Body Weight, and Hepatic and Renal Toxicity Following Chronic Oral Administration of a Water Soluble Phytostanol Compound, Fm-Vp4, to Gerbils. J Pharm Pharm Sci. 2001;4(3):228-234.

178. Looije NA, Risovic V, Stewart DJ, Debeyer D, Kutney J, Wasan KM. Disodium Ascorbyl Phytostanyl Phosphates (Fm-Vp4) Reduces Plasma Cholesterol Concentration, Body Weight and Abdominal Fat Gain within a Dietary-Induced Obese Mouse Model. J Pharm Pharm Sci. 2005;8(3):400-408.

179. Wasan KM, Zamfir C, Pritchard PH, Pederson RA. Influence of Phytostanol Phosphoryl Ascorbate (Fm-Vp4) on Insulin Resistance, Hyperglycemia, Plasma Lipid Levels, and

Gastrointestinal Absorption of Exogenous Cholesterol in Zucker (Fa/Fa) Fatty and Lean Rats. J Pharm Sci. 2003;92(2):281-288.

180. Wasan KM, Peteherych KD, Najafi S, Zamfir C, Pritchard PH. Assessing the Plasma Pharmacokinetics, Tissue Distribution, Excretion and Effects on Cholesterol Pharmacokinetics of a Novel Hydrophilic Compound, Fm-Vp4, Following Administration to Rats. J Pharm Pharm Sci. 2001;4(3):207-216.

181. Zhao J, Gershkovich P, Wasan KM. Evaluation of the Effect of Plant Sterols on the Intestinal Processing of Cholesterol Using an in Vitro Lipolysis Model. Int J Pharm. 2012;436(1):707-710.

182. Wasan KM, Choo E, Sivak O, Wallis S, Letchford K, Burt HM, Stewart DJ, Lukic T. Determining the Critical Micelle Concentration of a Novel Lipid-Lowering Agent, Disodium Ascorbyl Phytostanyl Phosphate (Fm-Vp4), Using a Fluorescence Depolarization Procedure. Drug Dev Ind Pharm. 2004;30(7):725-730.

183. Young S, Hui D. Pancreatic Lipase/Colipase-Mediated Triacylglycerol Hydrolysis Is Required for Cholesterol Transport from Lipid Emulsions to Intestinal Cells. Biochem J. 1999;339:615-620.

184. Wasan KM, Yau E, Boulanger KD, Ramswamy M, Pritchard PH. Effects of Disodium Ascorbyl Phytostanol Phosphates (Fm-Vp4) on Cholesterol Accumulation within Rat Intestinal Cells. AAPS PharmSci. 2003;5(1):55-61.

185. Mendez-Gonzalez J, Süren-Castillo S, Calpe-Berdiel L, Rotllan N, Vazquez-Carrera M, Blanco-Vaca F. Disodium Ascorbyl Phytostanol Phosphate (Fm-Vp4), a Modified Phytostanol, Is a Highly Active Hypocholesterolaemic Agent That Affects the Enterohepatic Circulation of Both Cholesterol and Bile Acids in Mice. Br J Nutr. 2010;103(02):153-160.

186. Ng AW, Lukic T, Pritchard PH, Wasan KM. Development of Novel Water-Soluble Phytostanol Analogs: Disodium Ascorbyl Phytostanyl Phosphates (Fm-Vp4): Preclinical Pharmacology, Pharmacokinetics and Toxicology. Cardiovasc Drug Rev. 2003;21(3):151-168.

Table2. Randomized clinical trials on plant sterols/stanols showing the lipid lowering effects of different products.

Study	Study design	Supplementation format	Type of phytosterol	Dose* (g/day)	Duration (Weeks)	TC reduction (%)	LDL-C reduction (%)	TG reduction (%)
Weststrate and Meijer, (40)	Balanced incomplete Latin square	Margarine	Esterified soya bean-oil sterols	3.3	3.5	-8.3	-13	NA
Hendriks et al. (41)	Balanced incomplete Latin square	Spread	Esterified soya bean-oil sterols	0.83 1.61 3.24	3.5 3.5 3.5	-4.9 -5.9 -6.8	-6.7 -8.5 -9.9	-8.4 -6.1 -4.8
Sierksma et al. (37)	Crossover	Spread	Non-esterified soya bean oil sterols	0.8	3	-3.8	-6	-5
Maki et al. (38)	Parallel	Reduced-fat spread	Esterified plant sterols	2.2	5	-6.6	-8.1	-10.4
Mensink et al. (39)	Parallel	Low-fat yoghurt	Esterified plant stanols	3	4	- 8.7	- 13.7	-2.7
Plana et al. (35)	Parallel	Low-fat fermented milk	Esterified plant sterol/ stanols	1.6	6	-7.99	-10.49	-6.58
Sialvera et al. (36)	Parallel	Yogurt drink	Non-esterified plant sterols	4	8.5	-15.9	-20.3	-19.1
McPherson et al. (42)	Parallel	Tablets	Soy stanol-lecithin	1.26	6	-4.8	-10.4	5.3
Goldberg et al. (43)	Parallel	Tablets	Soy stanol-lecithin	1.8	6	-5.7	-9.1	-0.3
Woodgate et al. (44)	Parallel	Softgel capsules	Esterified phytostanols	1.6	4	-8	-9	0.6
Carr et al. (45)	Parallel	Capsules	Stearate-enriched phytosterol esters	1.8	4	NA	-11	2.8
Maki et al. (46)	Crossover	Tablets	Non-esterified plant sterol/ stanols	1.8	6	-2.8	-4.9	4.2
Maki et al. (47)	Crossover	Softgel capsules	Esterified plant sterol/ stanols	1.8	6	-7.4	-9.2	-9.1
Ottestad et al. (48)	Crossover	Softgel capsules	Esterified plant sterols	2	4	-1.8	-2.7	-9.1
McKenney et al. (49)	Crossover	Softgel capsules	Esterified plant sterol/ stanols	1.8	6	-3.5	-4.3	-3.2

*Phytosterol dose was expressed as free sterol/stanol equivalents; NA indicates that data are unavailable.