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PLANT STEROLS - A DIETARY APPROACH FOR EFFECTIVE BLOOD LIPID LOWERING AS PART OF A HEART HEALTHY DIET

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ABSTRACT: ***Plant sterols (PS) are naturally occurring compounds found in foods of plant-based origin. Despite their structural similarity with cholesterol, PS are not absorbed in significant quantities; their intestinal absorption is less than 2% as compared to 30-60% for cholesterol. PS partly inhibit intestinal cholesterol absorption, which is the underlying mechanism of action responsible for their cholesterol-lowering effect. The cholesterol-lowering action of PS was already known in the 1950s and to date several meta-analyses have summarised the evidence for their total and LDL-cholesterol (LDL-C) lowering effect in intervention studies with different populations consuming a variety of plant sterol-enriched foods. The effect is dose-dependent with an intake around 2 g/day resulting in a reduction in LDL-C of about 10% on average, while doses above 3 g/day do not add much additional benefit. The cholesterol-lowering effect of PS is established within a few weeks and is maintained over longer periods as established in long-term efficacy studies lasting up to 85 months. The effect of PS is additive to that of a lipid-lowering diet and is also effective on top of treatment with lipid-lowering drugs like e.g. statins. PS-enriched foods can contribute to increasing the effectiveness of a heart healthy diet in lowering LDL-C and offer a valuable addition to coronary heart disease risk reduction strategies. PS and stanols, the saturated counterparts of PS, are among the first food compounds for which the European Food and Safety Authority approved a disease risk reduction health claim.***

KEY WORDS: Atherosclerosis, Cholesterol lowering efficacy, Healthy diet, Mechanism of action, LDL-cholesterol, Plant sterols.

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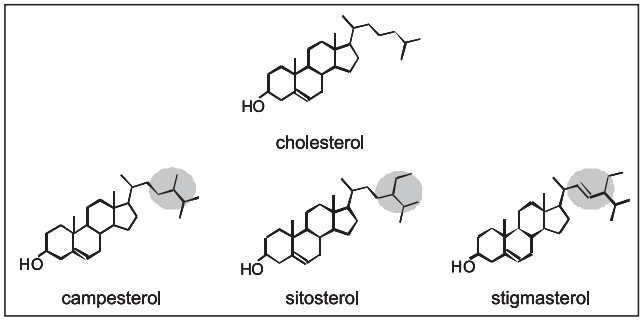
INTRODUCTION

Plant sterols (PS), including sitosterol, campesterol and stigmasterol, are naturally occurring compounds structurally similar to cholesterol yet differing in their side chain configuration (Figure 1). PS have a similar structure and cellular function to cholesterol, but are not synthesised in mammals and are derived solely from dietary sources. PS occur naturally in all foods of plant origin and are found in everyday foods like vegetable oils (especially unrefined oils), nuts, seeds, cereal grains, legumes, vegetables and fruits.

FIGURE 1. Chemical structure of cholesterol and the most biologically relevant plant sterols.



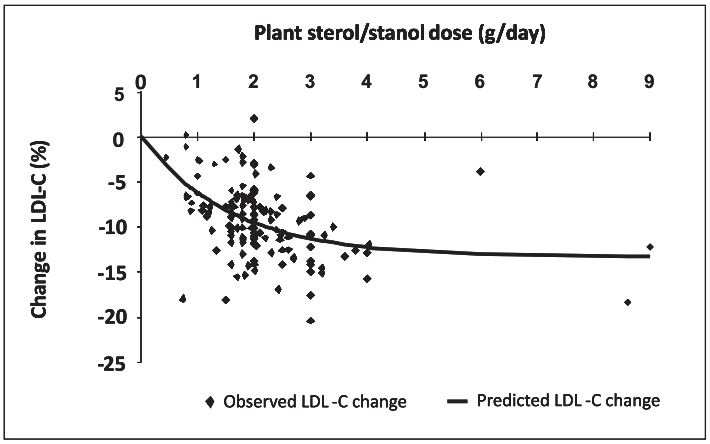
The average daily intake of PS with habitual diets varies between 150 to 400 mg/day (Katan et al., 2003), whilst vegetarians consume between 500 mg and 1 g/day (Vuoristo and Miettinen, 1994). The daily intake of stanols, the saturated form of PS, is in the magnitude of about 25 mg/day (Normen et al., 2001). The main food sources contributing to the daily PS intake are bread, in particular fibre-rich bread, and vegetable oils and fats (Normen et al., 2001).

The cholesterol-lowering properties of PS were already

discovered in the early 1950s (Pollak, 1953). Since then, a vast number of studies both in animals and humans have demonstrated their total (TC) and LDL-cholesterol (LDL-C)-lowering effect. Several reviews and meta-analyses have summarized the wealth of evidence that administration of PS in the form of enriched conventional foods are effective in lowering TC and LDL-C in humans (Berger et al., 2004; Katan et al., 2003; Law, 2000; Moreau et al., 2002). More recently, meta-analyses have further quantified the cholesterol-lowering effect of PS and stanols and their dose-response relationship (AbuMweis et al., 2008; Demonty et al., 2009; Talati et al., 2010).

Since diet and lifestyle changes are advocated as the first steps towards improving plasma cholesterol concentrations for the prevention of cardiovascular disease (CVD), the incorporation of PS and stanols is recommended by national and international authorities such as the International Atherosclerosis Society (IAS, 2003) and the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III guidelines (NCEP, 2002) as an additional dietary option as part of a heart healthy diet.

FIGURE 2. Continuous dose-response relationship of the LDL-cholesterol lowering effect of plant sterol and stanols. Based on Demonty et al (2009). Randomised controlled intervention studies meeting specific inclusion criteria and published until July 2007 were included in the meta-analysis.



CHOLESTEROL-LOWERING EFFICACY OF PLANT STEROLS

Dose-response of the LDL-cholesterol lowering efficacy of plant sterols

The dose-response of the LDL-C lowering effect of PS and stanols has been described in several meta-analyses by either estimating the mean LDL-C reduction for a mean dose or for ranges of doses (Katan et al., 2003; AbuMweis et al., 2008; Talati et al., 2010) or by establishing a continuous dose-response relationship that would allow predicting the effect of a given dose (Demonty et al., 2009). Meta-analyses that expressed the LDL-C reduction in % change from baseline LDL-C concentrations showed that, on average, 2 g/day PS or stanols (the equivalent dose expressed as free sterols based on 3.3 g/day PS or stanol esters) lowered LDL-C concentrations by about 9-10% (Demonty et al., 2009; Katan et al., 2003). The LDL-C lowering effect of PS and stanols was shown to be similar (Demonty et al., 2009; Katan et al., 2003; Talati et al., 2010). The effect appeared to taper off at intakes of 2.5-3 g/day, with little additional benefit at higher intakes (Katan et al., 2003; Demonty et al., 2009) (Figure 2). In contrast, two recent high dose plant stanol studies, out of which one was a dose-response study (Gylling et al., 2010; Mensink et al., 2010), seem to suggest that LDL-C reductions would not taper off at about 3 g/day and that larger LDL-C reductions (-17%) than estimated from the dose-response curve could be achieved by using doses as high as 9 g/day. However, even when considering these two recent studies, the number of available data at high intakes (>5 g/day) remains very limited. In addition, the reported inter-study variability in the LDL-C lowering effects achievable at such high doses is expected to be as large as the variability observed at lower intakes. For example, 15-20% reductions in LDL-C were reported in studies with PS or stanol intakes in the range of 1.5-3 g/day (Alhassan et al., 2006; Cater et al., 2005; Hayes et al., 2004; Jones et al., 1999); these reductions are larger than the true estimate obtained from the totality of evidence. More studies would thus be needed to establish the real LDL-C lowering effect of high doses of PS and stanols, and it would be premature to conclude that intakes beyond 2-3 g/day should be recommended for enhanced LDL-C lowering.

LDL-C lowering efficacy of plant sterols in the long term

Most evidence for the LDL-C lowering efficacy of PS is provided by interventions lasting 4-8 weeks. Nevertheless, there is evidence that the LDL-C lowering effect of PS is maintained in the long term. Studies in which the LDL-C lowering effect of PS and stanols was investigated over a period of >6 months are listed in Table 1. Overall, the LDL-C reductions observed after 6 months to 1.5 years are in the expected range for the doses used, considering that the inter-trial variability was similar to the variability observed in the shorter term studies. Some authors hypothesized that the efficacy of PS could potentially decrease over time due to a down regulation of bile acid synthesis via the slight increase in plasma PS concentrations following enriched-food consumption. However, this hypothesis was drawn from the outcomes of a study which had no control treatment (O'Neill et al., 2004), and is not supported by other published data. In fact, repeated measurements over a 1-year period of PS consumption showed similar LDL-C reductions after 7 months, 10 months and 1 year, confirming that the effect of PS is maintained over time (Hendriks et al., 2003). In the longest intervention study (1.5 years) to date, the overall evidence showed similar LDL-C lowering effects of PS and stanols in statin-users and did also not show differences in bile acid synthesis markers between the PS and stanol groups (De Jong et al., 2008). In addition,

observational data provide support for the long-term effectiveness of PS- and stanol-enriched spreads. In the 5-year long Doetinchem cohort study (Wolfs et al., 2006), TC concentrations were significantly reduced by 0.3 mmol/L, corresponding to a 5% reduction, in PS-enriched spread users vs. non users. Also in the Dutch Community intervention study (De Jong et al., 2007), the difference in TC concentrations over 5 years between PS-enriched spread users and non users was 0.34 mmol/L, corresponding to a reduction in TC of 5% and an estimated reduction in LDL-C of 6%. The cholesterol reduction observed in spread users for an average consumption of 1.3 g/day of PS in both observational studies is in line with the expected effect based on the dose-response curve established mainly from short term studies (Demonty et al., 2009). Overall, these findings confirm that PS exert in the real-life setting and over years similar LDL-C lowering effects as obtained from short-term intervention studies.

TABLE 1. LDL-cholesterol (LDL-C) lowering effect of plant sterol or stanol-enriched foods in dietary intervention studies lasting for 6 to 19 months. [a]based on comparing endpoint data as reported, [b]open label follow-up, not placebo-controlled, reduction based on % change from baseline, [c]plant stanol intake was 2.6 g/day for the first 6 months; at 6 months, half of subjects continued with 2.6 g/day whereas the other half received 1.8 g/day, [d]calculated from reported data by subtracting the %change from baseline in the control group from that in the plant sterol/stanol group.

Study	Dose(g/day)	Duration	Control-adjusted LDL-C reduction (%)
Christiansen et al., 2001	1.5 (plant sterols)	6 months	-11.3[a]
	3.0 (plant sterols)	6 months	-10.6[a]
Amundsen et al., 2004	1.5 (plant sterols)	~6 months	-11.0[b]
Miettinen et al., 1995	2.6[c] (stanols)	12 months	-15.0[d]
	1.8[c] (stanols)	12 months	-8.6[d]
Hendriks et al., 2003	1.6 (plant sterols	~7 months	-6.8[d]
		~10 months	-6.2[d]
		12 months	-6.5[d]
De Jong et al., 2008	2.5 (plant sterols)	~10 months	-11.6
	2.5 (stanols)		-8.7
	2.5 (plant sterols)	~19 months	-8.7
	2.5 (stanols)		-13.1

Impact of food format and modalities of intake on the cholesterol-lowering efficacy of plant sterol-enriched foods

From a practical point of view, defining the conditions that favour the optimal efficacy of PS-enriched foods is important for making appropriate recommendations to consumers. Two recent meta-analyses have investigated the potential impact of food format and frequency of intake on LDL-C reductions achieved with consumption of PS and stanols (Abumweis et al., 2008; Demonty et al., 2009). Abumweis et al. (2008) suggested that PS or stanols consumed in fat spreads, mayonnaise and salad dressings, and milk and yoghurt would lead to larger pooled average LDL-C reductions (-0.32 to -0.34 mmol/L) than other food formats (-0.20 mmol/L). In the meta-analysis by Demonty et al. (2009), no significant difference was detected between the dose-response curves established for fat-based vs. non fat-based and dairy vs. non dairy foods. Together with the fact that some dairy foods are low in fat, these data suggest that fat content is not a crucial characteristic for ensuring optimal efficacy of PS-enriched foods. Frequency of intake may have a modest impact on the LDL-C lowering efficacy of PS: pooled average LDL-C reductions were lower (-6.1% for an average dose of 1.76 g/day) for single daily intakes than multiple (2 or 3) daily intakes (-8.9% for an average dose of 1.81 g/day) (Demonty et al., 2009). In addition, consumption with or without a meal may have an impact on efficacy as consumption of a once-a-day yoghurt drink with lunch was shown to lower LDL-C concentrations more markedly than consumption on an empty stomach, 30 minutes before breakfast (Doornbos et al., 2006). As yoghurt drinks are intended for single daily intakes and may be consumed as a snack without a meal, it is possible that intake occasion may have partly contributed to the lower efficacy reported for single daily intakes when compared to multiple daily intakes.

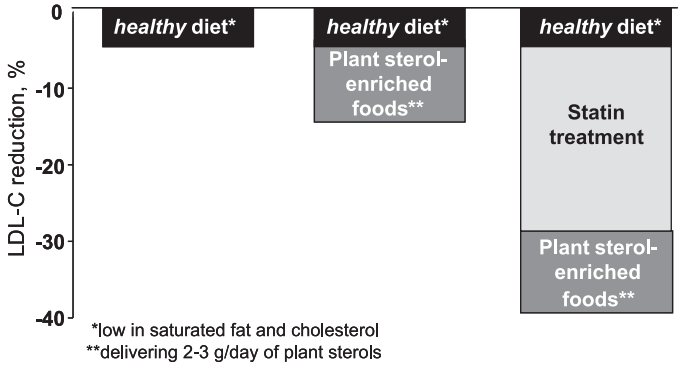
Combination of plant sterols with other cholesterol-lowering approaches

Various clinical studies have investigated the LDL-C lowering efficacy of PS when combined with a healthy diet, i.e. low in total and saturated fat and/or dietary cholesterol. Three studies that evaluated the cholesterol-lowering efficacy of PS on top of a healthy diet in (mildly) hypercholesterolemic subjects (Jones et al., 1999; Cleghorn et al., 2003; Chen et al., 2009) showed that switching to a healthy diet reduced LDL-C by 4.5-8.9%, while adding 1.5-3.3 g/day of PS increased the effect up to a total LDL-C decrease of 12-24%. Thus the LDL-C lowering effects attributable to PS were 6.8-15.5%. Considering the inter-study variability, these effects are in the expected range for the PS doses used (Figure 2), suggesting that the effect of PS is fully additive to that of a healthy diet. The use of PS and stanols is therefore recommended to complement a healthy diet low in saturated fat and cholesterol (NCEP, 2002; IAS, 2003).

By combining PS with other cholesterol-lowering compounds or foods, further reductions in LDL-C can be achieved. The Portfolio diet, which includes, next to a PS-enriched spread, other plant-based cholesterol-lowering

ingredients such as viscous dietary fibres, soy protein and almonds, has been shown to reduce LDL-C by 29.6% within one month in hypercholesterolemic subjects (Jenkins et al., 2005). This effect was similar to that observed with a first generation statin (Jenkins et al., 2005). On the long term (1 year), the overall LDL-C lowering effect observed with the Portfolio diet in free-living subjects was smaller, on average 13%, but LDL-C reductions of >20% were achieved in the most compliant subjects (Jenkins et al., 2006). By eliminating PS from the diet, it was more recently shown that the PS effect (-9%) is in fact the major contributor to the LDL-C lowering benefit of the Portfolio diet (Jenkins et al., 2008).

FIGURE 3. Plant sterols have an additive LDL-cholesterol lowering effect to a healthy diet and to lipid lowering medication.



Reference: Based on Chen et al., 2009; Cleghorn et al., 2003; Jones et al., 1999; Katan et al., 2003; Edwards and Moore,2003

Consumption of PS may be recommended to patients already receiving statin therapy to further lower LDL-C concentrations (Katan et al., 2003). A large multi-centre study designed to evaluate whether PS and statins have additive or interactive LDL-C lowering effects showed that statin alone, PS alone and the combination reduced LDL-C by 32%, 8%, and 39%, respectively, indicating a clear additive effect (Simons, 2002). A recent meta-analysis of 8 studies where 1.8-6.0 g/day of PS or stanols was consumed for 4-14 weeks, mainly in margarine and spreads, confirmed that the LDL-C lowering effect of PS is independent of statin use (Scholle et al., 2009). Overall, the statin/PS combination lowered LDL-C by 0.34 mmol/L (corresponding to a relative decrease of 8.5% when assuming a baseline LDL-C of 4.0 mmol/L) vs. statin alone, with no difference in efficacy between PS and stanols, and regardless of additional diet modification. This LDL-C lowering effect is similar to that found in meta-analyses that included mainly studies with subjects not receiving statin therapy Abumweis et al., 2008; Demonty et al., 2009). The efficacy of PS in patients on statin therapy has also been shown in the longer term (85 weeks) (De Jong et al., 2008). In fact, the additional LDL-C lowering effect obtained with PS is comparable to that achievable by doubling the statin dose (Roberts, 1997). The LDL-C lowering effect of PS was shown to be additive also to fibrates (Nigon et al., 2001). However, an additive effect was not shown when PS were combined with ezetimibe (Jakuli et al., 2005), possibly due to the similar mechanism of action of PS and ezetimibe, i.e. inhibition of cholesterol absorption.

Taken together, these data show that PS are effective in lowering LDL-C when used alone in the diet, as part of a heart healthy diet low in saturated fat and/or cholesterol, in combination with other cholesterol-lowering food components such as in the Portfolio diet, and as adjuvant to cholesterol-lowering medication (Figure 3).

PLANT STEROL EFFECT ON INTESTINAL CHOLESTEROL ABSORPTION AND ABSORPTION OF PLANT STEROLS

Partial inhibition of intestinal absorption of endogenous (dietary) and exogenous (biliary) cholesterol is the main mechanism of action responsible for the cholesterol-lowering effect of PS. As a consequence, fecal excretion of cholesterol is increased. Several human studies in which the effect of dietary PS intake on intestinal cholesterol absorption has been directly measured showed that a daily PS intake of about 2 g reduced cholesterol absorption by 30-40%, subsequently leading to about 10% lowering of plasma LDL-C (Normen et al., 2004)

Several underlying mechanisms contribute to the overall inhibition of intestinal cholesterol absorption by PS (Trautwein et al., 2003). The key mode of action is considered to be the displacement of cholesterol by PS from the dietary mixed micelles due to their limited capacity to embody sterols (Mel'nikov et al., 2004). Stimulation of bile flow by food intake is a crucial step for the formation of dietary mixed micelles. This plays an important role in the overall mechanism of action and consequently for optimal cholesterol-lowering efficacy. For instance, ingestion of a (fatty) meal stimulates bile flow, resulting in a release of (endogenous) biliary cholesterol into the gut lumen, which increases the likelihood for PS to compete with cholesterol for micellisation. There is also emerging evidence that PS interfere with transporter-mediated processes of cholesterol uptake (Trautwein et al., 2003).

Both cholesterol and PS are taken up from the intestine by the same uptake mechanism involving the NPC1L1 protein transporter, a membrane protein expressed in intestinal cells (Wang, 2007). However, despite their structural similarity with cholesterol, intestinal absorption of PS is low. Most recent data have shown that PS are not absorbed in significant quantities with absorption being less than 2% for sitosterol and campesterol as compared to 30-60% for cholesterol (Bosner et al., 1999; Ostlund et al., 2002). This is because a high proportion of the ingested PS taken up into the intestinal cells is actively excreted back into the intestinal lumen by the two ABC-transporter proteins ABCG5 and ABCG8 (Oram and Vaughan, 2006).

PLANT STEROL PLASMA CONCENTRATIONS IN COMPARISON TO PLASMA CHOLESTEROL CONCENTRATIONS

The difference in absorption efficiency between cholesterol and PS is also reflected in the circulating plasma concentrations which are about 200-times lower for the sum of sitosterol and campesterol as compared to plasma TC concentrations (Table 2). Plasma PS concentrations in the general population are typically 20-100 times lower than concentrations found in subjects with homozygous sitosterolemia (also called phytosterolemia). In these individuals, plasma PS concentrations are in the range of 290-966 mmol/L (Sudhop and von Bergmann, 2004), which accounts for 7-16% of the total circulating sterols in plasma, while PS account for <1% of total sterols in normal healthy subjects. The increased circulating PS concentrations seen in sitosterolemic subjects are caused by a functional mutation in the ABCG5/G8 transporters leading to both an increased absorption from the intestine and a reduced elimination from bile. Sitosterolemia is frequently associated with hypercholesterolemia; however, in sitosterolemic subjects, plasma cholesterol concentrations vary widely and may even not be elevated (Jessup et al., 2008). The presence of premature atherosclerosis in the absence of severely elevated cholesterol concentrations in some sitosterolemic subjects has led to the speculation that elevated PS concentrations may directly promote atherosclerosis development. However, it is currently not well understood by which mechanisms PS might promote the formation and progression of atherosclerotic plaques in these subjects (Jessup et al., 2008).

TABLE 2. Circulating plasma cholesterol and plant sterol concentrations in the general population*. *Based on data from 45 published studies between 1986 and 2005 (Chan et al., 2006), **Range for total cholesterol not reported.

Circulating sterols, mmol/L	Mean ± SD	Range
Total cholesterol	5.88 ± 0.87	-**
Campesterol	0.014 ± 0.005	0.007 to 0.028
Sitosterol	0.008 ± 0.003	0.003 to 0.016

The intake of PS-enriched foods by non-sitosterolemic subjects results in a modest increase in plasma PS concentrations next to the substantial LDL-C lowering effect. The intake of 2 g/day of PS results on average in about 7% TC lowering and 10% LDL-C lowering; these reductions in absolute terms are equivalent to 0.5 and 0.4 mmol/L, respectively. Plasma concentrations of the two main PS, sitosterol and campesterol, are increased by 20-100% and 40-100% respectively (Kritchevsky and Chen, 2005), which relates in absolute terms to a small increase of 0.02 mmol/L for the sum of these two PS. Furthermore, plasma PS concentrations in PS-enriched food users remain within the range of 0.01 to 0.05 mmol/L typically seen in healthy subjects. In addition, in a study that enrolled men with pre-defined high vs. low basal plasma PS concentrations, increases in plasma PS after a 4-week intake of a PS-enriched spread were not different between these two groups (Houweling et al., 2009).

SAFETY OF PLANT STEROL USE AND PUTATIVE HEALTH CONCERNS

PS have been used for their cholesterol-lowering properties and have been shown to be safe for half a century. Early studies showed efficacy and no toxicity of a wide range of daily PS intakes obtained from a variety of sources (as summarized by Kritchevsky and Chen, 2005). A series of safety studies have been conducted both for pharmaceutical formulations such as the drug Cytellin® as well as more recently for PS added to foods. For the latter, an extensive safety program has shown no evidence of genotoxicity, no effect on the reproductive system including estrogenicity, no toxicity in animal studies and no indication of adverse effects of high doses in human studies (Baker et al., 1999; Hepburn et al., 1999; Waalkens-Berendsen et al., 1999; Weststrate et al., 1999; Ayesh et al., 1999; Sanders et al., 2000; Wolfreys et al., 2002; Lea et al., 2004). Moreover, the use of PS and stanols has been recognised as safe for humans by the US Food and Drug Administration (FDA) and the Scientific Committee on Foods of the European Union, nowadays called European Food and Safety Authority (EFSA) (Katan et al., 2003; COMMISSION DECISION 2000/500/EC; COMMISSION DECISION 2004/335/EC; Scientific Committee on Food, 2002).

Effects on plasma carotenoid concentrations

As PS interferes with intestinal cholesterol absorption, and fat-soluble vitamins and carotenoids share the same absorption pathway, their absorption is likely to be affected as well. Several studies have shown that intakes of PS-enriched foods do not affect plasma concentrations of retinol, vitamin D and K, but lower plasma concentrations of vitamin E (tocopherols) and carotenoids have been reported (Katan et al., 2003). As tocopherols and carotenoids are transported in plasma by lipoproteins, usually their concentrations are standardised for plasma lipid concentrations. After such lipid standardisation, plasma concentrations of tocopherols, alpha-carotene, and lycopene were unaltered. Only the lipid standardised reductions in beta-carotene (-12% with 95% confidence interval: -17% to -7%) remained statistically significant, indicating that reductions in this carotenoid are likely due to decreased absorption (Katan et al., 2003). Nevertheless, carotenoid concentrations still remain within the normal inter-individual range and typical seasonal variations (10-40%) observed in the population (Lux and Naidoo, 1994). Furthermore, reductions in plasma beta-carotene do not increase over time as shown by similar lipid-adjusted reductions observed after 26 and 52 weeks (Hendriks et al., 2003). Studies with higher PS and stanol intakes of up to 9 g/day also showed no greater carotenoid reductions than with intakes of 2-3 g/day (Davidson et al., 2001; Clifton et al., 2004; Chen et al., 2009; Mensink et al., 2010).

In addition, the PS-related decreases in plasma carotenoid concentrations can be counterbalanced by consuming more fruits and vegetables such as the recommended 5 portions per day (Ntanios and Duchateau, 2002; Noakes et al., 2002; Colgan et al., 2004). In light of recent insights, it remains unclear what the health relevance of reduced plasma or serum beta-carotene concentrations would be, especially considering the unaltered vitamin A (retinol) concentrations. Indeed, the only established nutritional function of beta-carotene is being a precursor of vitamin A, suggesting that the modest decrease in plasma beta-carotene associated with PS-enriched food intake present no health risk, except in persons whose need for vitamin A is increased, such as pregnant and lactating women and young children. However, these population groups are not the target market group for foods enriched with PS.

Plasma plant sterols and atherosclerosis

No human study so far has directly investigated whether the cholesterol-lowering benefit of PS and stanols leads to a reduction in atherosclerotic lesion development and thus to a reduction in risk of coronary heart disease (CHD). Nevertheless, the relation between LDL-C lowering and reduced CHD incidence and mortality has been established from both dietary and pharmacological intervention studies (Baigent et al., 2005; Schaefer, 2002).

Over 30 animal studies have investigated the effect of PS or stanols on experimental atherosclerosis in different models such as chickens, rabbits, Golden Syrian hamsters, and more recently, in various knockout mouse models (Kritchevsky and Chen, 2005). These studies have shown clear protective effects, such as reductions in arterial lipid accumulation and in the development of atherosclerosis, e.g. lesser plaque development or reduced lesion size, an inhibition of lesion formation and progression and even regression of existing lesions correlated with the cholesterol-lowering action of PS (Pollak and Kritchevsky, 1981; Ntanios et al., 2003; Moghadasian et al., 1997; Moghadasian et al., 1999a; Moghadasian et al., 1999b; Volger et al., 2001; Plat et al., 2006). The key findings related to the evidence from these animal studies are summarised in Table 3. Taken together, it was demonstrated that elevated plasma PS concentrations caused by feeding dietary PS alone or in combination with a statin have no atherogenic effects; they could delay or reduce atherosclerotic lesion formation or even induce plaque regression.

TABLE 3. Anti-atherosclerotic effects observed with plant sterol (PS) and stanol feeding in various animal models. *with respect to control group (where applicable), **as summarised by: Pollak and Kritchevsky, 1981

Study	Animal model	Dose (range)	Test compounds and source	Effects on Atherosclerosis development*
Ntanios et al., 2003	F1B Hybrid Syrian hamsters	0.24-2.84%	PS esters from vegetable oil	Foam cell formation (pre-form of atherosclerotic lesions) attenuated by PS feeding in dose-dependent manner and despite an increase in plasma PS concentrations
Moghadasian et al., 1997	C57BL/6J apo-E -deficient mice	2%	Tall oil sterol mix mainly containing PS	Reduced lesion formation after 18 wks
Moghadasian et al., 1999a	C57BL/6J apo-E knockout mice vs. wild type C57Bl/6J mice	2%	Tall oil sterol mix mainly containing PS	Reduced lesion formation after 20 wks
Moghadasian et al., 1999b	apo-E-deficient mice	2%	Tall oil sterol mix mainly containing PS	Reduced lesion size after 33 wks (following 18 wks induction phase) and additional 25 wks (regression phase) in the PS treated mice
Volger et al., 2001	apoE*3-Leiden transgenic mice	1%	Stanol esters from vegetable oil, from wood and mixture of vegetable oil and wood	Reduced extent and severity of lesions after 38 wks
Plat et al., 2006	LDL-receptor deficient mice	1-2%	Wood PS or stanol esters	Aortic lesion size was significantly decreased after 35 wks despite the up to 4- to 11-fold increase in plasma sitosterol and campesterol concentrations in mice fed PS/stanols alone or in combination with a statin. In mice fed an atherogenic diet for 33 wks, regression of existing lesions was found after additional 12 wks feeding of PS/ stanols
17 studies**	Rabbits	0.2-3%	Mainly sitosterol or PS mixes from soy and vegetabole oils	Reduced development of lesions
7 studies**	Chickens	1-5%	Mainly sitosterol or PS mixes from soy, cottonseed, corn or tall oil	Reduced lesion formation

Further, in a study with ABCG5/G8 and LDL-receptor double knockout mice fed a standard Chow diet, no or only a few atherosclerotic lesions were found after 7 months, despite greatly elevated plasma PS concentrations. Even in severely hypercholesterolemic mice with inactivated LDL-receptor activity, the increase in plasma PS did not lead to greater aortic lesion development (Wilund et al., 2004). In a study with wild-type mice, increased plasma PS concentrations after PS feeding were correlated with impaired endothelial vasorelaxation and increased cerebral lesion size after cerebral artery occlusion (Weingärtner et al., 2008). Surprisingly, PS feeding did not reduce plasma cholesterol concentrations in these wild-type mice, suggesting that this model may not be the most appropriate to study the effects of PS. In the same study (Weingärtner et al., 2008), apo-E knockout mice fed PS showed significantly reduced atherosclerotic lesion formation compared to mice fed a control diet, although the observed reduction in lesion formation and size was larger in mice treated with ezetimibe, a cholesterol-absorption inhibitor.

Plasma plant sterols and CHD risk

A number of observational studies (Glueck et al., 1991; Sutherland et al., 1998; Rajaratnam et al., 2000; Sudhop et al., 2002; Assmann et al., 2006) suggested that not only pathologically high (50- to 100-fold) plasma PS concentrations as seen in sitosterolemic subjects, but also slightly (1.2- to 2-fold) elevated PS concentrations might be associated with an increased risk of cardiovascular events independently of the plasma cholesterol concentration. This has caused some concerns about a possible relationship between plasma PS and CHD risk. More recent observational studies with larger sample size and relying on multivariate analyses adjusting for established CHD risk factors have however not confirmed these findings and hence do not support an association between plasma PS concentrations and CHD risk (Wilund et al., 2004; Pinedo et al., 2007; Windler et al., 2009; Fassbender et al., 2008, Silbernagel et al., 2009; Silbernagel et al., 2010; Escurriol et al., 2010).

More specifically, Silbernagel et al. (2009 and 2010) published their findings on the association between cholesterol absorption markers like plasma non-cholesterol sterols (cholestanol, campesterol, sitosterol) and cholesterol synthesis markers like plasma lathosterol and the severity of CHD in the cohort of the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. All non-cholesterol sterols were positively correlated with TC. Moreover, an increase in cholesterol absorption markers, such as the ratios of cholestanol to cholesterol and campesterol to cholesterol, were associated with the severity of CHD, while an increase in the ratio of the cholesterol synthesis marker lathosterol to cholesterol was associated with a lower CHD severity. It was concluded that there is a modest association of high cholesterol absorption and low synthesis with increased CAD severity and that an atherogenic role of plasma PS themselves is considered unlikely as the association was also shown for cholestanol. Plasma sitosterol and campesterol concentrations are typically used as markers for intestinal cholesterol absorption efficiency. Therefore, higher plasma PS concentrations are closely related to more cholesterol being absorbed (Tilvis and Miettinen, 1986). As CHD and its severity have repeatedly been associated with more effective cholesterol absorption and lower cholesterol synthesis (Miettinen et al, 1990; Rajaratman et al., 2000; Silbernagel et al., 2009; Silbernagel et al., 2010), it seem plausible that the association between plasma PS concentrations and the risk of CHD is not reflecting a causal relationship, but may simply reflect the already reported association between increased cholesterol absorption and increased risk of CHD.

RECOMMENDATION FOR USE OF PLANT STEROLS AND APPROVED HEALTH CLAIMS

Consuming 2 g/day of PS or stanols in addition to a heart healthy diet to further lower elevated LDL-C is among the recommendations of the NCEP ATPIII (NCEP, 2002), the American Heart Association (Lichtenstein et al., 2006) and the International Atherosclerosis Society (IAS, 2003). Regional Heart Foundations and Atherosclerosis Societies also recognise the benefits of consuming PS-enriched foods for lowering plasma TC and LDL-C and their contribution to reducing the risk of developing CHD. The US FDA approved in 2000 a health claim stating that foods containing PS may reduce the risk of heart disease. More recently in 2008, PS and stanol-enriched foods were amongst the first food compounds for which the EFSA approved a disease risk reduction health claim confirming their cholesterol lowering properties and that high cholesterol is a major risk factor in the development of CHD. The health claim needs to state that the beneficial effect is obtained with a daily intake of 1.5-2.4 g PS or stanols and the magnitude of the expected effect only applies for fat-based spreads, dairy products, mayonnaise and salad dressings, thus for food formats for which the evidence of a cholesterol-lowering benefit of PS and stanols has been most widely established.

The need for having dietary options such as PS-enriched foods for plasma cholesterol-lowering is apparent in view of data showing that nearly two-thirds of the European adult population (men and women) have blood TC concentrations above the desirable level of 200 mg/dL (5.2 mmol/L) (Tolonen et al., 2005). Leading societies such as the International Atherosclerosis Society (IAS, 2003) and the Joint Task Force of the European Society of Cardiology, the European Atherosclerosis Society, the European Society of Hypertension, the International Society of Behavioural Medicine, the European Society of General Practice/Family Medicine, and the European Heart Network (Wood et al., 1998) emphasise the importance of plasma cholesterol as a major risk factor in the development of CHD. In fact, there is convincing evidence from epidemiological studies and randomised controlled clinical trials for a causal relationship between elevated LDL-

C and CHD risk and evidence is also available showing that the risk of CHD can be reduced by cholesterol-lowering therapy including dietary intervention strategies. A reduction in LDL-C of about 10% as achievable with the regular intake of 2 g/d PS via enriched foods could be expected to reduce CHD risk by up to 20%, an important contribution to the prevention of CHD (Katan et al., 2003; NCEP, 2002).

CONFLICT OF INTEREST DISCLOSURE

All authors are employed by Unilever R&D, The Netherlands. Unilever markets globally a range of plant sterol-enriched foods.

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