Effect of phytosterols/stanols on LDL concentration and other surrogate markers of cardiovascular risk

J.-M. Bard a, b, *, F. Paillard c, J.-M. Lecerf d

a UFR des sciences pharmaceutiques et biologiques, bureau 254, centre de recherche en nutrition humaine, EA 2160 - IUMIL - institut universitaire Mer et Littoral - FR3473 CNRS, université de Nantes, BP 53508, 9, rue Bias, 44035 Nantes cedex 1, France
b Institut de cancérologie de l'Ouest, boulevard Jacques-Monod, 44805 Saint-Herblain, France
c Centre de prévention cardio-vasculaire, pôle TVM, CHU Ponchaillou, 35033 Rennes, France
d Service de nutrition, Institut Pasteur de Lille, 1, rue du Professeur-Calmette, 59019 Lille cedex, France

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Abstract

Plant sterols and stanols are well-known to reduce LDL-cholesterol (LDL-C) concentrations. It is generally accepted that supplementation with 2 g/day of sterols/stanols leads to a 10% reduction in LDL. However, most of the clinical trials supporting this conclusion were of short-term duration, and the results of longer interventions are scanty. In four studies, interventions lasting >6 months were carried out and the LDL-C-lowering effects were maintained over this longer duration, although some results suggest that a reduced effect may be observed with sterols, while stanols maintain their effect. In any case, the data are too limited to be definitive. In a free-living population as well as in multiparametric interventional studies, however, the LDL-C-lowering effect has been confirmed, although to a lesser extent than in clinical studies. In the absence of data on cardiovascular morbidity and mortality, data for surrogate markers of cardiovascular risk could be considered adequate alternatives. Several studies have been conducted on this basis, but their results failed to demonstrate any favourable effects. The present report summarizes the different results obtained in long-term studies, and in those comparing the effects of sterols and stanols on lipids and other surrogate markers of cardiovascular risk.

Keywords: Plant sterols; LDL-cholesterol; Cardiovascular risk markers

1. Introduction

Plant sterols and stanols share a steroid chemical structure with cholesterol. The main differences between these compounds are found on the lateral chain and the presence or not of an unsaturated Δ5 bond. This bond is saturated in stanols and unsaturated in sterols. This structure communally shared with cholesterol leads to inhibition of dietary cholesterol intestinal absorption and increased cholesterol in faeces. As a consequence, the synthesis of hepatic apolipoprotein (apo) B/E receptors increases, leading to increased clearance of circulating low-density lipoprotein (LDL) and decreased plasma LDL concentrations. Hepatic cholesterol synthesis also increases, which limits the hypcholesterolaemic effect, but the overall effect is generally a reduction of LDL-cholesterol (LDL-C). In agreement with this mechanistic hypothesis, clinical studies have shown a mean lowering effect of 8–15% in circulating LDL-C when plant sterols/stanols are added to the usual diet at a dose of 1.5–3 g/day [1, 2]. Food authorities such as the European Food Safety Authority (EFSA) consider this effect convincing and have accepted the claim that adding these compounds to food may help to reduce LDL-C in the general population. However, most of the clinical studies have been of short-term durations of between 3 and 6 weeks, whereas a long-term effect is required to support any reduction in cardiovascular disease risk. The objective of the present report is to summarize the data so far on the efficacy of plant sterols/stanols on reducing circulating LDL.
focusing on the long-term effects of these compounds on other surrogate markers of cardiovascular risk.

2. Expected efficacy of plant sterols/stanols on LDL-C

In a review published in 2003, Katan et al. [3] pooled the data of 41 trials comparing the effects of adding plant sterols/stanols with a placebo in lowering LDL-C. This meta-analysis showed that 2 g/day of these compounds reduced LDL by 10%. They also demonstrated a dose–effect relationship with an LDL reduction plateau of 10% observed with doses > 2 g/day and about half this effect with 1 g/day. There was little additional effect with doses > 2.5 g/day, which produced an estimated maximum of 11.3%. Most of the trials included in this meta-analysis were of short-term duration (<8 weeks), and the dose of plant sterols/stanols was usually divided up into two or three portions throughout the day. Yet, one trial used a single daily intake and apparently obtained the same range of results as the other trials. However, in a more recent meta-analysis of 59 randomized clinical trials [4], the authors concluded that plant sterols given as a single dose had no effect on LDL-C. Another meta-analysis of 84 trials [5] reported a strong tendency towards slightly lower efficacy with a single dose vs multiple daily doses.

The question of the influence of the food carrier on efficacy is also a subject of debate. AbuMweis et al. [4] concluded that reductions in LDL-C were greater when plant sterols were incorporated into fat-rich foods (such as mayonnaise, salad dressing or yoghurt) rather than other foods. Yet, Demonty et al. [5] found no differences between fat and non-fat or dairy and non-dairy products, whereas a significant difference between solid and liquid foods was observed, albeit only for doses > 2 g/day. At this relatively high intake, the maximum estimated LDL-C-lowering effect of solid foods was 5.2% greater than that of liquid foods.

3. Clinical studies of long-term effects of sterols/stanols

Table 1 is a brief summary of the results obtained in long-term clinical studies. One double-blind study [6] randomized 153 subjects with moderate hypercholesterolemia into three groups after a run-in period of 6 weeks, during which the participants consumed a control rapeseed-oil margarine low in sitostanol. A group of 51 subjects continued eating the control margarine for an additional 6 months, while the 102 others switched to a sitostanol-enriched margarine for a final intake of 2.6 g/day of sitostanol. After this 6-month period, 51 volunteers from this group switched to a margarine providing 1.8 g/day of sitostanol for an additional 6 months, while the remaining 51 subjects continued to consume 2.6 g/day. The control group continued with the standard rapeseed-oil margarine for the same period.

At the initial study randomization, LDL-C concentrations were 4.04 ± 0.1 mmol/L (1.59 ± 0.04 g/L) in the control group, 3.89 ± 0.1 mmol/L (1.53 ± 0.04 g/L) in the group consuming 1.8 g/day of sitostanol from months 6 to 12, and 4.06 ± 0.1 mmol/L (1.60 ± 0.04 g/L) in the group eating 2.6 g/day of sitostanol from months 6 to 12. Ingesting 2.6 g/day of sitostanol lowered LDL-C by 10.4% and 14.1% at months 6 and 12, respectively, while the control group increased their LDL-C by 0.9% and 1.1% at the same time points. The changes between randomization and study time points differed significantly (P < 0.001). The reduced intake from 2.6 g/day to 1.8 g/day led to stable LDL-C levels [3.48 ± 0.08 mmol/L (1.37 ± 0.03 g/L) at month 6 vs 3.51 ± 0.08 mmol/L (1.38 ± 0.03 g/L) at month 12], while continuation of the 2.6 g/day was accompanied by a further decrease [3.58 ± 0.1 mmol/L (1.41 ± 0.04 g/L) at month 6 vs 3.40 ± 0.08 mmol/L (1.34 ± 0.03 g/L) at month 12]. Indeed, at the end of the 12-month period, the three groups differed significantly (P < 0.001). Triglycerides and high-density lipoprotein cholesterol (HDL-C) were not affected by intake of sitostanol.

At the end of 12 months, the subjects switched back to the control margarine for an additional 2-month period, at the end of which LDL-C returned to baseline values.

Another double-blind randomized study [7] evaluated the safety and efficacy of an enriched spread containing sterol esters at a dose of 1.6 g/day for 1 year in 185 healthy volunteers with a mean cholesterol level of 5.90 ± 0.98 mmol/L at baseline. After the year-long period of consumption of the spread, LDL-C was reduced by 6% compared with the control diet not supplemented by sterol esters.

An open study of 37 children, aged 7–13 years, with heterozygous familial hypercholesterolaemia and 22 of their parents, aged 32–51 years, evaluated the efficacy of a spread providing 1.2 g/day (children) and 1.5 g/day (parents) of plant sterols for 26 weeks [8]. Baseline LDL-C was 5.31 ± 1.49 mmol/L in children and 4.10 ± 1.22 mmol/L in parents, and was reduced by 11.4% (P < 0.001) in children and 11% (P = 0.012) in parents.

In a 4-month randomized trial comparing three groups of 50 hypercholesterolaemic subjects [9], one group followed a Mediterranean diet, the second group received 2 g/day of plant stanols in margarine and the third group received a control (non-supplemented) margarine. After 4 months, LDL-C was reduced by 14% in the stanol group and 7% in the Mediterranean diet group.

4. Clinical studies of differences between sterols and stanols

Some of the randomized studies with intakes lasting >6 weeks evaluated the difference between sterols and stanols. The results are summarized in Table 1. One study [10], which included patients with familial hypercholesterolaemia treated with statins and also taking plant sterol/stanol esters, suggested that the effect of sterols did not persist after 2 months, whereas stanols remained effective beyond that time. Another study of hypercholesterolaemic subjects treated with statins and lasting for 16 weeks [11] compared the effect of three margarines: a control margarine (n = 11); a margarine providing 2.5 g/day of plant sterol esters (n = 15); and a margarine containing 2.5 g/day of plant stanol esters (n = 15). This study concluded that the combined effect of plant sterols and stanols was −10.3% vs controls (P = 0.028) with no difference between sterols and stanols. The same team [12] then studied the same population and the same dietary intervention for 18 months. Total cholesterol was reduced by 6.8% (P = 0.005) in the group consuming sterols.
and by 8.8% ($P = 0.001$) in the group consuming stanols. This difference between sterols and stanols was not significant.

In yet another paper, the same group [13] suggested a progressive loss of effectiveness with sterols, while stanols remained effective in the long-term. Comparing the same three groups of dietary interventions for 85 weeks, they obtained LDL-C reductions of 11.6% ($P = 0.037$) after 45 weeks and 8.7% ($P = 0.08$) after 85 weeks with sterols ($n = 18$), while stanols ($n = 19$) maintained a reduction of 13.1% ($P = 0.006$) at 85 weeks, compared with the control margarine. The same team [14] also compared LDL-C after 85 weeks of intervention in a subgroup of this same population as a secondary objective of another clinical trial. Compared with baseline, the control group ($n = 11$) increased its LDL-C concentration by 5.3%, while LDL-C decreased by 9.2% and 9.6% in the sterol ($n = 11$) and stanol ($n = 8$) groups, respectively. Absolute changes between baseline and follow-up were significant among the three groups ($P = 0.006$), although the authors made no multiple comparisons.

Considering these results as a whole, it is difficult to conclude differences in efficacy between sterols and stanols, and further studies focusing on this point are needed. In fact, a meta-analysis published in 2009 [5] failed to establish any significant difference between sterols and stanols in terms of dose–response curves.

### 5. Multiparametric interventions

Two dietary interventional studies may help to clarify the effects of plant sterols/stanols on LDL-C as part of a global dietary recommendation. One was a trial using the Mediterranean diet as the main intervention, and compared three groups of subjects at high cardiovascular risk [15]. The first group was given Mediterranean diet advice and asked to take an additional dose of virgin olive oil. The second group was asked to add nuts to the Mediterranean diet advice, while the third group (controls) followed a low-fat diet. LDL-C at baseline was 3.54 ± 0.66 mmol/L, 3.24 ± 0.79 mmol/L and 3.15 ± 0.64 mmol/L in the three groups, respectively. The mean increase in dietary plant sterols after 1 year was 76 mg/day, 158 mg/day and 15 mg/day in the three groups, respectively. A significant 8.3% decrease in LDL-C ($P = 0.036$) was observed only in the nuts group, which had a higher plant sterol intake than the low-fat group. Moreover, the siatostol/sterol ratio, considered a marker of plant sterol intake, was significantly and independently associated with LDL-C reduction ($r = -0.256$, $P = 0.008$).

Another trial produced indirect information on the effect of plant sterols [16]. In this study, hyperlipidaemic subjects were advised to consume a diet rich in soybean protein, soluble fibre, almonds and plant sterols (1 g/1000 kcal) for 52 weeks. From week 52 to week 62, the same diet was maintained, but the additional intake of plant sterols was stopped. At the end of 52 weeks, LDL-C decreased by 15.4% ($P < 0.001$) whereas, at the end of 62 weeks (same diet, but without added plant sterol), this decrease was reduced to 9% ($P < 0.001$). The authors calculated that the decrease in LDL-C attributable to plant sterols was 6.3% ($P < 0.05$).

### 6. Studies in free-living populations

Data from epidemiological observational studies performed under free-living conditions appear to support plant sterol efficacy. One post-marketing trial within the Dutch Doetinchem Cohort Study [17] compared changes in plasma cholesterol levels in consumers of margarine enriched with either plant sterols ($n = 67$) or stanols ($n = 13$) with levels in non-users ($n = 81$).
The study’s first evaluation period preceded the launch of plant sterol/stanol-enriched products (1994–1998) and the second evaluation period was post-marketing (1999–2003). During the latter period, plant sterol consumption was 1.1 ± 0.6 g/day (n = 67) and plant stanol consumption was 0.6 ± 0.4 g/day (n = 13) in margarine users. Between the two evaluation periods, cholesterol was reduced by 4% in sterol consumers, but increased by 2% in non-users (P = 0.05 between groups), while stanol consumers experienced a 7.6% reduction in cholesterol (non-significant difference between groups).

This research was followed by an additional evaluation [18] of 3651 non-statin, non-plant sterol/stanol users in 1998–2002, who were examined again in 2003–2007. During this 5-year period, 169 subjects became plant sterol/stanol consumers, 203 became stanol users and 24 started taking both. Among the plant sterol/stanol consumers, only 9% reached the recommended dose of 2 g/day and 20% reached 1.5 g/day with 20 g of margarine. Cholesterol decreased by 0.16 mmol/L in the sterol/stanol users, 1.40 mmol/L in the stanol users and 1.64 mmol/L in the stanol plus plant sterol/stanol users; 98% of plant-fat consumers were taking sterols, and the effect was dose-related. It was calculated that 2 g of plant sterols decreased cholesterol by 0.25 mmol/L, or 5%, a figure slightly lower than that observed in randomized clinical trials.

One community-based interventional study led to an analysis of the effect of plant sterol/stanol marketing [19]. The study population was advised to increase physical activity, reduce dietary lipid intakes and quit smoking. As plant sterols/stanols were marketed in 1999 in the Netherlands, the comparison between the 1998 and 2003 evaluations offers an indirect indication of the effect of these compounds. On the basis of the 2003 questionnaire, subjects were classified into users of plant sterol/stanol-enriched margarine, users of hypolipidaemic drugs and plant sterol/stanol-enriched margarine, and non-users. The plant sterol (n = 99) and stanol (n = 16) users obtained it from 14 ± 9 g of margarine. Between 1998 and 2003, cholesterol was reduced by 17% in those taking hypolipidaemic drugs and by 29% in those taking drugs and plant sterols/stanols, and increased by 2% in non-users. However, the authors pointed out that the use of plant sterols/stanols did not reach the recommended dose. Although they concluded that the effect of these compounds on cholesterol was modest, it was still noteworthy that the additional 12% LDL-C decrease in users of both drugs and plant sterols/stanols was not negligible. Thus, plant sterols/stanols may have an additive effect to statins. Nevertheless, given the multiparametric nature of the intervention, it is difficult to attribute any specific effect to plant sterols/stanols.

7. Effects on other lipid markers of cardiovascular risk

Although not the primary endpoint of studies evaluating the effect of phytosterols/stanols on lipid metabolism, study data usually included effects on triglycerides and HDL cholesterol as secondary endpoints. Staying with studies of relatively long duration (>6 months), it can generally be assumed that concentrations of those lipid markers were not affected [6,7,11]. Only the study by Amundsen et al. in 2004 [8] showed a significant decrease in HDL cholesterol — specifically, 4.8% in children with familial hypercholesterolaemia and 10.6% of their parents — while triglyceride levels did not change.

8. Effects on surrogate markers of cardiovascular risk

8.1. Intima–media thickness

Few studies have evaluated the effects of phytosteroles/stanols on intima–media thickness (IMT) in humans. Gylling et al. [20] compared the effects of consuming margarine enriched with either phytosterols or phytostanols for 1 year. These margarines had no effect on IMT compared with a placebo. Similarly, Raitakari et al. [21] showed that the consumption of margarine enriched with phytostanols for at least 2 years also had no effect on IMT. In both studies, however, either cholesterol or LDL-C decreased with the margarines tested.

8.2. Endothelial function

Celermajer et al. [22] clearly demonstrated that endothelium-dependent vasodilation is a relevant predictor of future cardiovascular risk. Yet, on analyzing the effects of margarines enriched with phytosterols or phytostanols on endothelial function, several studies have all shown a lack of improvement in endothelium-dependent vasodilation despite significant decreases in LDL-C.

In a study of 200 untreated hypercholesterolaemic subjects, the consumption of margarine enriched with phytostanols for 3 months failed to reveal any improvement in endothelium-dependent vasodilation despite an average 9% decrease in LDL-C [23]. These results were confirmed by the above-mentioned Raitakari et al. [21] study, which also found no improvement in endothelium-dependent vasodilation after at least 2 years’ consumption of margarine enriched with phytostanols.

In another study of untreated hypercholesterolaemic subjects, no improvement in endothelium-dependent vasodilation was observed following the consumption of margarines fortified with phytosterols/stanols, despite a significant decrease in LDL-C [24]. The above-mentioned study by Gylling et al. [20], which lasted longer (12 months) and involved slightly hypercholesterolaemic untreated subjects, also failed to show any improvement after the consumption of margarine enriched with either phytosterols or phytostanols.

In hypercholesterolaemic children, the use of margarine enriched with phytosterols for 4 weeks had no effect on endothelium-dependent vasodilation, despite a mean 14% decrease in LDL-C (vs a placebo) [25]. Likewise, the consumption of margarine enriched with phytostanols for 6 weeks in hypercholesterolaemic children did not improve endothelium-dependent vasodilation, despite a mean 9% decrease in LDL-C (vs a placebo) [26].

A study in type 1 diabetes patients using margarine enriched with phytostanols for 12 weeks failed to reveal any improvement
in endothelium-dependent vasodilation despite a mean 16% decrease in LDL-C (vs placebo) [27]. It should be noted, however, that the number of patients included in this study was small (n = 11 in the phytosterol group and n = 8 in the placebo group).

8.3. Plasma markers of endothelial dysfunction and inflammation

Markers of chronic inflammatory processes and endothelial dysfunction are also valid predictors of cardiovascular risk. However, consumption of margarine enriched with phytosterols/stanols for 16 weeks in 45 patients taking statins did not alter their circulating concentrations of either adhesion molecules [intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, E-selectin] or inflammatory markers [C-reactive protein (CRP) and monocyte chemoattractant protein (MCP)-1] [11].

Several other studies have similarly shown a lack of effect of phytosterols and phytostanols (intakes of 0.8–2.4 g/day) on circulating concentrations of adhesion molecules and inflammatory cytokines [28–32]. One showed a significant decrease in plasma CRP concentration in 72 hyperlipidaemic subjects after they consumed 2 g/day of phytosterols [33], while another showed that CRP concentrations tended to decrease (P = 0.07) with intakes ≥ 3 g/day of phytosterols, whereas 1.6 g/day had no effect [34].

8.4. Oxidative stress

The effect of phytosterols/stanols on oxidized LDL is still controversial. A study of 105 healthy subjects, who for 4 weeks consumed margarine providing 2 g/day of phytostanols or 3 g/day of phytosterols or a control margarine, found a 20% reduction of oxidized LDL [35]. Another study also reported a decrease in concentrations of oxidized LDL following the consumption of 0.8 g/day of phytosterols, although this effect was no longer significant when LDL-C was accounted for, suggesting that the decrease in concentration of oxidized LDL may have been due to LDL reduction [31]. Other studies, however, found that phytosterols/stanols have no effect on LDL oxidation [11,32,36]. In one recent study, neither phytosterols nor phytostanols altered concentrations of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) or markers of DNA oxidation or lipids [11].

Increased oxidation of phytosterols has nevertheless been reported. Indeed, a highly significant increase in oxysterosterols (7α-OH-sitosterol) was found in patients with phytosterolaemia as well as in lipid emulsions enriched with phytosterols [37]. In addition, an even greater increase in oxidation of sitosterol ex vivo has been observed [37]. Another study of a cultured cell line derived from macrophages reported that oxysterosterols had cytotoxic effects similar to those of cholesterol oxides [38]. At present, the consequences of an increase in oxystersterol concentrations are not known.

8.5. Erythrocytes

Unlike data based on hypertensive rats predisposed to stroke, the data from human studies show that foods rich in phytosterols/stanols have no effect on the fragility of erythrocytes [7,39–41]. In adult humans, the campesterol content of erythrocytes did not increase after consumption of phytosterol [39], whereas consumption of phytosterols in children increased phytosterol/cholesterol ratios in red blood cells [42].

8.6. Microcirculation

Although cardiovascular diseases are generally considered to affect the large vessels, some studies suggest they may be related to alterations in the microcirculation. In fact, several studies have shown a positive association between the diameter of retinal venules and markers of atherosclerosis [43–46]. It has also been shown that subjects with larger venule diameters had higher carotid plaque scores and more aortic calcifications [43].

A recent placebo-controlled study evaluated the effects of phytosterol (2.5 g/day) and phytostanol (2.5 g/day) ester consumption for 85 weeks on the diameter of retinal vessels in 30 stable hypercholesterolaemic patients taking statins [14]. A non-significant increase was found in venule diameter in those who consumed phytosterols, but not phytostanols, with a positive association between plasma concentrations of campsterol (standardized for cholesterol) and diameters of retinal cells (r = 0.39, P = 0.033). No association between plasma concentrations of phytostanols and the diameter of retinal venules was reported. The difference in diameter (2.3 μm) observed, following the consumption of phytosterols, according to the authors, may be regarded as significant with regards to the observational studies cited above. These changes in venule diameter were independent of plasma cholesterol, LDL, HDL and triglycerides.

9. Discussion

Randomized clinical studies clearly support a beneficial effect of plant sterols/stanols on circulating concentrations of LDL-C. However, most of these studies were short-term, and the results of long-term studies are somewhat scanty. However, the rare long-term studies suggest that the effects of these compounds are maintained over time, and a few such reports suggest that plant stanols may have more sustained long-term effects than plant sterols, although this point clearly needs further investigation. The issue appears to be of particular interest when taking into consideration that the effect in a free-living population may be less pronounced, most likely because the efficacious dose is rarely achieved. However, although the efficacy of these compounds on LDL concentration is not in doubt, their effect on cardiovascular risk remains unknown. In fact, LDL-C is causally associated with atherosclerosis, and many interventions aiming at lowering LDL-C have been consistently and eventually associated with a decrease in cardiovascular events. Thus, lowering LDL-C has become a major goal of cardiovascular disease prevention. However, the vast majority of these studies have involved statins, for which the level of evidence is very high.
and consistent, whereas other interventions aiming at improving lipid profiles, including reducing LDL-C, have failed to demonstrate any cardiovascular benefits. The latter include niacin [47], fibrates [48], cholesteryl ester transfer protein (CETP) inhibitors [49,50] and omega-6 polyunsaturated fatty acids (PUFA) in the Sydney study [51]. Thus, an observed LDL-C reduction, however desirable, cannot unequivocally be considered favourable in the absence of confirmation coming from a cardiovascular events study. Such studies would be extremely useful to better establish the benefits of plant sterols/stanols.

In the absence of such studies, the effect of plant sterols/stanols on surrogate markers of cardiovascular risk may be worthy of consideration. Unfortunately, the available results for such parameters so far are not particularly convincing. The total effect of plant sterols/stanols on atheromatous plaque has not been corroborated in humans by the two studies that evaluated IMT. Similarly, plant sterols/stanols do not appear to affect either endothelial function or the fragility of red blood cells. Although plasma concentrations of oxidized LDL may decrease, the effect can mostly be explained by decreases in LDL-C. In addition, a potentially deleterious effect of phytosterols on the diameter of retinal venules has been observed in an intervention study and has also been supported by others.

Considering all these arguments, any recommendation for the more widespread use of plant sterols/stanols in the general population for cardiovascular prevention has yet to be substantiated.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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