Interindividual variability in the cholesterol-lowering effect of supplementation with plant sterols or stanols

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Special Article

Plant sterols and stanols: Physiology, Metabolism and Inter-individual variability in the cholesterol-lowering effect of supplementation with plant sterols/stanols

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Abbreviations: ABCA1, ATP-binding cassette subfamily A member 1; ABCB4, ATP-binding cassette subfamily B member 4; ABCG5/G8, ATP-binding cassette subfamily G member 5/8; ACAT2, Acyl coenzyme A: cholesterol acyltransferase 2; APOE, Apolipoprotein E; CETP, Cholesterol ester transfer protein; CYP7A1, Cholesterol 7 α-hydroxylase; FCH, familial combined hyperlipidemia; FH, familial hypercholesterolemia; FSR, fractional cholesterol synthesis rate; HDL(-C), high-density lipoprotein (cholesterol); LDL(-C), low-density lipoprotein (cholesterol); MDR2, multidrug resistance 2; MetS, Metabolic syndrome; NPC1L1, Niemann-Pick C1 Like 1 protein; SCARB1, scavenger receptor BI; SOAT2, sterol O-acyltransferase 2; STRIP, Special Turku Coronary Risk Factor Intervention Project

Abstract

Low-density lipoprotein cholesterol (LDL-C) plays a causal role in atherosclerosis. One way to reduce LDL-C levels is to inhibit cholesterol absorption. Plant sterols and stanols compete with cholesterol for absorption in the intestine and induce an average decrease in LDL-C by 5 to 15% in a dose-dependent manner. However, a decrease does not occur in all individuals. This review focuses on the inter-individual variability in response to supplementing the diet with plant sterols and stanols. Dietary plant sterols and stanols have no significant effect on substantial numbers of subjects. Higher responses, in absolute value and percentage of LDL-C, are observed in subjects with higher cholesterol absorption and a lower rate of cholesterol synthesis. Some data evidenced the influence of genetics on the response to plant sterols and stanols. Further studies on large populations are required to extend these conclusions about genetic influences.

Keywords: plant sterols, cholesterol transporters, intestinal absorption, LDL-cholesterol, individual response, genetics

Introduction

Plant sterols are natural compounds found in plants, especially seeds and oilseeds. They have a similar structure to cholesterol, but differ in the side chain at carbon 24 and in the position and configuration of the double bond structure.[1] **(Figure 1)**. Stanols are produced by the hydrogenation of plant sterols, with no double bond at carbon 5. Plant sterols and plant stanols have a cholesterol lowering effect that was first identified in 1951,[2] and sitosterol was introduced as a lipid-lowering therapeutic agent by Eli Lilly in 1957.[1] Its low aqueous solubility and low bioavailability have made it a forgotten and abandoned agent. The esterification of plant sterols and stanols has not only improved their solubility, but given a renewed interest in these molecules used in functional foods. These compounds compete with cholesterol for absorption in the intestine[3] and induce an average decrease in low-density lipoprotein cholesterol (LDL-C) of 5 to 15% in a dose-dependent manner.[4]

There is inter-individual variability in the response to diverse interventions, and the metabolic response to plant sterols and stanols in the diet is no exception. In this review, we consider the range of this variability, and examine the metabolic and genetic factors that have been studied in an attempt to clarify this variability.

Nature and sources of plant sterols and stanols

Plant sterols are the botanical analogs of cholesterol, and are found in the parts of plants rich in fibers and fats. The main sources of plant sterols are nuts, oilseeds, cocoa butter, legumes, and grains. Vegetables and fruits contain also small amounts of plant sterols **(Table 1)**.[3,5-7] Plant stanols are naturally found in some cereals (rice, maize, wheat) and plant products, for example the wood of conifers such as pine and spruce.

More than 200 types of plant sterols and stanols have been identified[1,8] but the most abundant in the diet are sitosterol (about 60%), and campesterol (about 20%), then brassicasterol, stigmasterol, sitostanol and campestanol.[9,10]

In nature, plant sterols may be free, or esterified with fatty acids or with ferulic acid, or glycosylated with a hexose (usually glucose) or a 6-fatty-acyl hexose, on carbon 3 **(Figure 1)**. These glycosylated classes are frequently not measured, because their measurement needs an additional acid hydrolysis cleaving the glycosyl link or direct analysis of intact glycosides. The exclusion of glycosylated derivatives underestimates the total amount of plant sterols by up to 37%.[5]

In addition, the analysis of the main plant sterols excludes minor sterols.[5] It is unclear to what extent the glycosylated plant sterols are bioactive in humans because they are not hydrolyzed by pancreatic enzymes in vitro.[11]

Role of plant sterols and stanols in cholesterol metabolism

Cholesterol is involved in many biological functions as a cell membrane component and as a precursor of steroid hormones, bile acids and vitamin D, but can play a deleterious role in diseases like atherosclerosis. Cholesterol homeostasis depends on a complex balance between absorption and synthesis. This reflects the limited role of dietary cholesterol on plasma cholesterol and its very controversial role in cardiovascular risk.[12] The absorption of cholesterol is carried out by many systems and receptors, allowing a redundancy of mechanisms. Like plant sterols, plasma cholestanol, the 5α-dihydro derivative of cholesterol, is a marker of cholesterol absorption under normal conditions.[13,14]

Plant sterols and stanols come into competition with free cholesterol in the micelles of lecithin and bile salts, which causes a reduction in cholesterol solubilization. When the concentration of plant sterols/stanols is high, intestinal cholesterol loses almost all

97 solubility.[15-17] The affinity of plant sterols/stanols for micelles exceeds that of cholesterol.
98 Accordingly, the addition of plant stanols in a test meal reduces the absorbable cholesterol of
99 micelles (measured by duodenal aspiration in humans) which leads to decreased intestinal
100 absorption.[16]

101 The mechanism of action of plant sterols and stanols is not limited to the intestinal
102 lumen. They are picked up with cholesterol by the intestinal mucosa via the membrane
103 transporter Nieman Pick Disease C1 Like1 (NPC1L1) and can be absorbed into the systemic
104 circulation. However, the majority of plant sterols and stanols are expelled from the
105 enterocytes into the intestinal lumen by the ATP-binding cassette subfamily G members 5 and
106 8 (ABCG5 and ABCG8) heterodimeric pumps. Plant sterols and stanols ejected from the
107 enterocyte are excreted in the feces with unabsorbed and non-micellar cholesterol, catabolized
108 as coprostanol by the intestinal microbiota. Alteration of the micellar solubilization of
109 intestinal cholesterol seems to be the only mechanism of action of plant sterols and stanols
110 that is unequivocally established. Nevertheless, other mechanisms involving modifications in
111 cholesterol esterification, cholesterol efflux or bile acid synthesis, composition and excretion,
112 have been discussed.[11,17-21]

113 Acyl coenzyme A: cholesterol acyltransferase 2 (ACAT2), also known as sterol O-
114 acyltransferase 2 (SOAT2), is a key step in the metabolism of cholesterol, allowing its
115 enterocyte esterification, followed by its incorporation into chylomicrons and export.[22]
116 ACAT2 esterifies cholesterol much more efficiently than sitosterol (between 7.2 and 60 times,
117 as estimated from different studies).[18,23] Because of this strong preference, there is no
118 evidence that plant sterols are esterified by ACAT2. Consequently, plant sterols are not
119 incorporated into chylomicrons and most of them are ejected into the intestinal lumen. It is
120 also possible that a certain fraction of plant sterols may be incorporated into high-density
121 lipoprotein (HDL) particles after basolateral efflux through ATP-binding cassette A1

(ABCA1) transporter.[24] In animals, it has been shown that plant sterols and stanols reduced the enzymatic activity of ACAT2, causing a reduction in the net absorption of dietary cholesterol.[17] The reduced activity of ACAT2 could be explained by a diminished trafficking of cholesterol from the plasma membrane to the endoplasmic reticulum.[17]

Plant sterols and stanols also induce the expression of ABCA1, a cholesterol transporter, *in vitro*.[19,20] ABCA1 regulates the efflux of cholesterol and phospholipids from the cell to the extracellular space. However, ABCA1 carries out its functions in the basolateral membrane of enterocytes, and not in the apical membrane, where it could excrete cholesterol into the lumen.

A diet rich in plant sterols alters the expression of more than 130 hepatic genes and their transcripts involved in the regulation of sterol metabolism in mice.[21] Nevertheless, it is possible that such changes are due to the inhibition of cholesterol absorption or reductions in plasma cholesterol, and are not a direct effect of plant sterols.

The addition of sitosterol (1 g) to a test meal containing cholesterol (500 mg) reduced cholesterol absorption by an average of 42%.[25] In another experimental protocol where both a liquid diet and sitosterol or sitostanol were infused in the intestinal lumen, sitosterol and sitostanol reduced cholesterol absorption by 50% and 85% respectively.[26] This mimics the effect of a deficit in hepatic cholesterol, and causes an increase in the expression of LDL receptors. This higher expression of LDL receptors induces an increase in the hepatic uptake of cholesterol and a decrease in plasma LDL-C. A compensatory increase in the synthesis of cholesterol takes place: the synthesis of whole body cholesterol (as measured through the incorporation in cholesterol of deuterium from water marked with deuterium) increases from 38 to 53%.[27] Even at high doses (from 1.6 up to 8.6 g/d), plant sterols and stanols cause a decrease in the synthesis and excretion of bile acids,[28] which could explain the reduction in the cholesterol-lowering effect after a month. The decrease in bile acid synthesis is caused by

increased net absorption of plant sterols from the diet leading to higher plasma levels.[28] Nevertheless, a short term experimental study in an ileostomy model showed that a modest increase in plant sterols (584 mg/d vs. 258 mg/d), increased synthesis and excretion of bile acids.[29] Stanols do not lead to changes in the composition of bile acids, or the percentage of biliary cholesterol.[28,30] This might be due to the fact that stanol absorption and plasma levels are much lower than those of plant sterols. (See *infra*).

Absorption characteristics and plasma levels of plant sterols and stanols

The absorption rate of plant sterols and stanols depends on their chemical structure. The side chain group at carbon 24 (methyl for campesterol and campestanol, ethyl for sitosterol and sitostanol) and the presence of a double bond at carbon 5 have independent effects on absorption efficiency.[31] (**Figure 1**). Sterols with longer side chains and no double bond are less absorbed because of increased hydrophobicity and decreased micellar solubility. This explains why sterols are better absorbed than stanols, and why campesterol is better absorbed than sitosterol. Consequently, plasma levels of campesterol are higher than plasma sitosterol, although higher amounts of sitosterol are generally consumed. The net systemic absorption of plant sterols and stanols after a few days goes from 0.04% for sitostanol to 1.9% for campesterol.[31] In the general population, 3 to 16 μmol/L (0.12 to 0.66 mg/dL) of sitosterol and 7 to 28 μmol/L (0.28 to 1.12 mg/dL) of campesterol are found in plasma.[32] Concentrations of plant sterols are 15 to 30 times higher than those of stanols, but 200 times lower than those of cholesterol. The plasma concentrations of plant sterols are modulated by plant sterol intake. The percentage absorption of plant sterols is lower when intakes are higher, despite higher mass absorption.[8] With supplementation of 1.8 to 2.0 g/d plant sterols for 4 to 8 weeks in adults,[32] plasma campesterol increased from 52 to 99%, and sitosterol from 23 to 96%. When doubling the usual intake in children (132 mg/d vs. 65 mg/d), plasma

campesterol and sitosterol increased by 75% and 44% respectively.[33] Conversely, stanols (1.5 to 3.0 g/d for 4 weeks) reduced campesterol from 28 to 113% and sitosterol from 24 to 50%.[32]

Sitosterolemia is a rare recessive disease characterized by excessively high plasma plant sterol levels (30 to 100 times higher than normal values)[34,35] due to mutations in the *ABCG5* or *ABCG8* transporter genes.[36-38] These mutations cause abnormal retention of plant sterols and stanols. Heterozygous subjects are generally healthy, but have higher plasma plant sterol levels (+30 to +50%) than the general population.[34] Some genetic polymorphisms of *ABCG5* and *ABCG8* have been associated with variations in plasma plant sterol levels (see *infra*).

Apolipoprotein E (APOE) is a major component of all lipoprotein classes except LDL. It is involved in the removal of chylomicron and very-low-density lipoprotein remnants from the circulation. Three common isoforms of APOE, E2, E3 and E4 are encoded by the alleles ε2, ε3 and ε4, respectively. The most frequent is ε3. Some very rare isoforms have also been described. The ε4 allele (or E4 isoform) is generally associated with higher total cholesterol and LDL-C levels in the general population, and higher cholesterol absorption, with the ε2 allele (E2 isoform) having the opposite effects.[39] Elevated plasma plant sterol levels are observed in subjects with the *APOE* E4 isoform.[40]

Statins decrease endogenous cholesterol synthesis, but induce a compensatory increase in sterol absorption, including plant sterols, with higher plasma levels in statin users.[41,42]

Conversely, familial combined hyperlipidemia (FCH), obesity, metabolic syndrome (MetS), and type 2 diabetes with insulin resistance are associated with a decreased absorption of plant sterols.[40,43-45] This has been linked to increased expression of the *ABCB4* (*MDR2)* transporter gene involved in the biliary excretion of sterols in cases of insulinopenia or insulin resistance.[32]

The physiological intake of plant sterols and stanols

The dietary intake of plant sterols and stanols is low but not absent, roughly equivalent to that of cholesterol. However, cholesterol has essential physiological functions that cause the body to keep it stable by adjusting the absorption and synthesis. Conversely, plant sterols and stanols are at an extremely low plasma concentration and have no known role, which can account for the very powerful processes to eliminate them. The response to plant sterol and stanol treatment is lower in subjects with high basal cholesterol synthesis (generally low absorption) than in subjects with low basal synthesis (high absorption).[46] (See *infra*)

Their presence in the gut has an effect on the intestinal absorption of cholesterol and its plasma level even at very low doses.[47] The spontaneous level of intake of plant sterols and stanols is inversely correlated with LDL-C levels and intestinal cholesterol absorption.[48] Several studies are in favor of a non-negligible role of plant sterols and stanols in the cholesterol-lowering effect of some oils (e.g. corn) rich in plant sterols and stanols, especially when unrefined, beyond their fatty acid composition.[11,49,50]

General variability in the cholesterol lowering effect of plant sterols/stanols

A recent meta-analysis of randomized controlled studies (including 124 studies) clearly showed a dose-response effect of plant sterols and stanols.[4] For intakes of 1 to 4 g/d, LDL-C concentrations reduced on average by 6 to 12%. When plant sterols and stanols were analyzed separately, similar dose-response relationships were observed.

Nevertheless, there is inter-individual variability in the response to supplementing the diet with plant sterols and stanols. Some studies report that dietary plant sterols and stanols have no significant effects on substantial numbers of subjects.[46,51-54] In one study with a spread providing 0.8 g/d plant sterols, there was no lowering of LDL-C in 37% of the subjects after 3 weeks.[51] Similarly, 33% of subjects were non-responders after 4 weeks of consuming a

spread providing 2.0 g/d plant sterols,[52] and no change was observed in 20% of the subjects consuming 1.3 to 1.6 g/d plant sterols in enriched milk.[53] A 4-week cross-over study in hypercholesterolemic subjects assessing the effects of plant sterol doses of between 1.6 and 2.0 g/d (meta-analysis of three trials) showed an average lowering of LDL-C by -7.3±1.2%; however, 47 subjects (42%) were non-responders (LDL-C: +3.7±1.0%), and 66 were responders (-15.2±1.0%).[46] In this study, the responders were those with a decrease in LDL-C of 5% or more. Among hypercholesterolemic male subjects (n=82), 31 (38%) were non-responders to 2.0 g/d plant sterols (spread). Plasma total cholesterol increased by 4.4±1.2% and LDL-C by 9.4±5.9% in non-responders, whereas in responders, the total cholesterol decrease was 12.7±1.2% and that of LDL-C was 18.3±2.0%.[54]

Other than the factors linked to the characteristics of the trials, and the origin and the doses of the plant sterols and stanols, or the food matrix used, main sources of variation in these studies are the subjects themselves.

Role of the initial plasma cholesterol concentration

The first source of variation linked to the subject is the initial plasma cholesterol concentration. In a meta-analysis of four randomized trials with plant stanol esters, there was a highly significant effect ($P<0.001$) of baseline plasma LDL-C on the final response.[55] The lowering obtained with 2.0 g/d stanols was between -0.24 mmol/L and -0.17 mmol/L (-8.0 to -5.7%) for an initial LDL-C value of 3 mmol/L, between -0.32 mmol/L and -0.22 mmol/L (-8.0 to -5.5%) for an initial LDL-C value of 4 mmol/L, and between -0.41 mmol/L to -0.27 mmol/L (-8.2 to -5.4%) for an initial LDL-C of 5 mmol/L. Therefore, the effects of plant stanols as a percentage of the baseline LDL-C value were constant, such that the change increased in absolute value with the value at baseline. The authors calculated that with 3.55

mmol/L initial LDL-C, the variation would be -5.8%, -7.5% and -10.2% for stanol doses of 1.7 g/d, 2.2 g/d and 3.0 g/d, respectively.

Relationship with cholesterol synthesis and/or absorption

Other individual characteristics that modulate the LDL-C response to plant sterols and stanols in the diet are those which influence the absorption and endogenous synthesis of cholesterol. Generally subjects with high basal plasma plant sterol levels, an indirect measure of absorption efficiency, display large reductions in LDL-C in response to plant sterol therapy.[56] In the study by Rideout et al.,[46] the fractional cholesterol synthesis rate (FSR, assessed directly by stable isotope analysis) was 23% higher in non-responders than in responders. The lowering of the LDL-C was only 3.2% in the highest synthesis quartile whereas it was 12.3% in the lowest quartile. When selecting subjects with high vs. low cholesterol synthesis (assessed by the cholesterol precursor lathosterol to cholesterol ratio), MacKay et al.[57] confirmed that cholesterol synthesis rate predicted the responsiveness to 2.0 g/d plant sterols. In subjects defined as high synthesizers, the LDL-C lowering was 0.05 mmol/L vs. 0.29 mmol/L in low synthesizers. Zhao et al.[54] reported that responders had a lower synthesis rate than non-responders. In subjects with low basal plasma plant sterol levels, absorption rate was low and the FSR was high, and the opposite was observed in subjects with high plasma plant sterol levels. There was a trend for subjects with high basal levels to be responders, and for a higher frequency of non-responders among those with low levels (P=0.09). Post-experiment plasma plant sterol concentrations were highly positively correlated with absorption rates of campesterol and cholesterol, and negatively correlated with cholesterol FSR.[58]

Metabolic syndrome (MetS) is generally associated with high cholesterol synthesis and low cholesterol absorption.[43,45,59] In the study by MacKay et al.,[57] subjects with high

cholesterol synthesis and low response to plant sterols had higher body mass index and lower HDL-C, both characteristics associated with MetS. In a randomized parallel trial, 24 moderate hypercholesterolemic patients with MetS were compared to 24 moderate hypercholesterolemic patients without MetS. Enrichment of a "healthy" diet with plant sterols (2.0 g/d) for 3 months lowered LDL-C by 10.5% in patients without MetS but had no effect in patients with MetS.[60] The authors suggest that this difference is due to poor absorption efficiency, as demonstrated by lower campesterol and sitosterol concentrations (before and after diet) in patients with MetS than those without MetS. In particular, the plasma concentration of plant sterols following their consumption increased only by 20 to 30% in patients with MetS, but by 50 to 60% in controls. Nevertheless, these results contrast with those of another study[61]: plant sterol supplementation (4.0 g/d) of the habitual diet for 2 months lowered total cholesterol and LDL-C by 15.9% and 20.3%, respectively, in patients with MetS. The discrepancy between these two studies might be explained by the type of diet during the intervention period ("healthy" in one case, "westernized" in the other) and by the dose of plant sterols (2.0 g/d and 4.0 g/d). The second study also included 5 times more patients and therefore had greater statistical power. As noted in [62], the discrepancies in trials involving patients with MetS might also be due to the broad range in criteria that define MetS.

In type 2 diabetic patients, a meta-analysis of 5 randomized, placebo controlled trials, showed that plant sterols and stanols (1.6 to 3.0 g/d) significantly reduced LDL-C (combined effect: -12.2%).[63] Although stanols seemed more effective than sterols, no significant heterogeneity between studies was found in the meta-analysis. Nevertheless, few studies were examined. Type 2 diabetes (insulin resistance) has been associated with high synthesis and low absorption of cholesterol.[40,59] Type 2 diabetic patients should therefore have a low LDL-C response to plant sterol supplementation. Since it does not seem to be the case, Plat et al.[62] raised the question of the value of surrogate markers (plasma plant sterols and cholesterol

292 precursors) in the affected population. Plant stanols were effective for LDL-C lowering in
293 patients with type 1 diabetes[64]; of note, type 1 diabetes is characterized by low synthesis and
294 high absorption of cholesterol.[65]

295 **Genetic factors**

296 *Monogenic diseases*

297 Healthy subjects heterozygous for the mutations of *ABCG5* or *ABCG8*, responsible for
298 sitosterolemia in homozygotes, might have a particular response to plant sterol therapy. This
299 possibility has been tested, although sample sizes are small due to the rarity of the mutations.
300 In a study of both (obligate) heterozygote parents of a patient with sitosterolemia, LDL-C
301 decreased by 11% after 4 weeks on a diet containing 3.3 g/d plant sterols (margarine), and
302 plasma concentrations of plant sterols increased by 83 to 139%.[66] These subjects were also
303 obese and hypercholesterolemic. In 12 obligate heterozygotes from 2 families (with 2
304 different mutations), a low fat diet, poor in saturated fatty acids and cholesterol, led to a
305 decrease in LDL-C of 11.2% after 6 weeks and 16% after 12 weeks. The addition of 2.2 g/d
306 plant sterol esters to this diet led to an additional 5.9% decrease.[67] The LDL-C response is
307 similar to that of subjects who do not carry the mutations. Seven heterozygotes and 10
308 controls received portions of margarine containing 2.0 g of plant sterols, 2.0 g of stanols or a
309 control margarine daily for 6 weeks each.[68] Similar decreases in total and LDL-C were
310 observed in both groups. The changes in plasma concentrations of plant sterol expressed as
311 percentages did not differ between the two groups. With sterols in the diet, plasma plant
312 sterols increased by 14.5 µmol/L (0.59 mg/dL, +23%) in heterozygotes, and by 4.9 µmol/L
313 (0.20 mg/dL, +20.5%) in controls. With stanols, plasma plant sterols decreased by 34.2
314 µmol/L (1.40 mg/dL, -54.2%) in heterozygotes and by 12.2 µmol/L (0.50 mg/dL, -50.6%) in
315 controls.[68] Similar changes in LDL-C were observed in 10 individuals heterozygous for the

316 *ABCG8* S107X mutation (-10.7%) and 15 controls (-9%) following the change from placebo
317 during 4 weeks to 1.6 g/d plant sterols. Cholesterol absorption was decreased and synthesis
318 increased similarly in both groups (stable isotope measurements).[69]

319 In conclusion, inclusion of plant sterol and stanols in the diet leads to a relative
320 decrease in total cholesterol or LDL-C that is similar both in individuals heterozygous for
321 sitosterolemia mutations and in control subjects (normal healthy subjects from other studies).
322 With plant sterols in the diet, the relative increase in plasma plant sterols (as a percentage of
323 baseline levels) was similar in heterozygotes and control subjects (although the absolute value
324 is much larger). With dietary stanols, the percentage decrease in plasma plant sterols was also
325 similar in the two groups, but again the change in absolute values was different.
326 **(Supplementary table S1 online only)**

327 The effects of these products have been studied in cases of monogenic familial
328 hypercholesterolemia (FH), an inherited autosomal dominant disorder of the lipoprotein
329 metabolism caused by mutations on the LDL-receptor gene. A meta-analysis of 4 studies of
330 patients heterozygous for a LDL-receptor gene mutation was performed in 2006.[70] Three of
331 these studies were in children.[71-73] The meta-analysis demonstrated that the decrease in total
332 cholesterol and LDL-C was similar in heterozygous FH patients and in other
333 hypercholesterolemic subjects after 2.3±0.5 g/d plant sterols or stanols. The effects of 1.6 to
334 2.0 g/d (depending on body weight) plant sterols for 12 weeks were compared between
335 different groups of hypercholesterolemic children. The groups studied included FH
336 heterozygotes, cases of FCH and cases with unknown cause of hypercholesterolemia.[74] The
337 decrease in LDL-C was slightly smaller in FH heterozygotes (-10.7%) than in groups with
338 FCH (-14.2%) or undetermined hypercholesterolemia (-16.0%). However, the number of
339 subjects included was small, and consequently the difference observed is probably not
340 significant. **(Supplementary table S1 online only)**

APOE polymorphism

As already mentioned, the E4 isoform (or ε4 allele) has been associated with higher total cholesterol and LDL-C in the general population, and higher cholesterol absorption. Consequently, a number of studies have investigated whether the effects of plant sterols and stanols are greater in carriers of E4 than other subpopulations.[75-83] (**Figure 2**, **supplementary table S2 online only**).

One study[75] with 67 moderate hypercholesterolemic subjects yielded results in the expected direction: total cholesterol and LDL-C concentration in the plasma decreased by 7.5% and 10%, respectively in the intervention group. Subjects with phenotypes E4E4 or E3E4 were better responders to plant stanols (LDL-C:-11.8%) than E3E3 subjects (LDL-C:-6%). Plasma sitosterol and campesterol concentrations were lower and cholesterol precursors higher in E4E4 or E3E4 than E3E3 subjects, indicating a greater decrease in cholesterol absorption with a compensatory increase in synthesis. The same team[76] studied the effects of a small dose (700 mg/d) of plant sterols or plant stanols in 31 moderate hypercholesterolemic subjects. Four diets were tested: rapeseed mayonnaise, rapeseed + sitosterol, rapeseed + sitostanol and rapeseed + sitostanol esters. Serum total cholesterol and LDL-C reductions were small in the plant sterol-fed groups, and tended to be highest in the sitostanol ester group (-7%). The reduction in LDL-C (5%) was significant when sterol/stanol groups were combined. The decrease in LDL-C in E4 carriers (n=8) was 0.28 mmol/L (P<0.05) and in E3E3 subjects (n=15) 0.06 mmol/L (NS). The LDL-C decrease correlated with a decrease in absorption and an increase in endogenous synthesis, which was more pronounced in E4 carriers.

However, other studies do not find any greater effect of plant sterols/stanols in subjects carrying E4. In a study of 105 healthy subjects receiving either a placebo, or 2.0 g/d

or 3.0 g/d stanols for 4 weeks, there was no difference in plasma total cholesterol and LDL-C responses between subjects with E3 and those with E4.[77] A crossover trial was performed in normocholesterolemic children selected from the STRIP (Special Turku Coronary Risk Factor Intervention Project) cohort.[78] In the group carrying the E4 isoform, there was a decrease in LDL-C of 8.4% after consumption of a margarine containing plant stanols; the corresponding value for non-carriers was 7.6%. Nevertheless, cholesterol synthesis (assessed by assaying plasma sterol precursors) was increased only in E4 carriers and absorption was similarly lowered in both groups. In healthy adult subjects (crossover trial with a margarine providing 3.2 g/d plant sterols, for 3 weeks), the decrease in LDL-C was 12.2% in subjects not carrying E4 and 9.8% in E4 carriers (not significant).[79] In another crossover trial with 1.7 g/d plant sterols,[81] there was no statistically significant difference in total cholesterol and LDL-C response according to genotype. The response was significant only in E3E3 subjects, but the number of E3E4 subjects was small. A similar result was obtained with hypercholesterolemic patients subjected to a diet during 5 weeks (control margarine/+1.1 g/d plant sterols/+2.2 g/d plant sterols). There was a significant response in E3E3 subjects but not in E4 carriers.[82] Once again, this result might have been due to a lack of statistical power because of the small number of E4 subjects. Nevertheless, the greatest response in the study was observed in E2 carriers. In another study of 75 moderate hypercholesterolemic adult subjects, no difference in response was observed according to APOE isoforms after a period of 3 months on either a standard NCEP/ATIII diet, or the same diet + 2.0 g/d plant sterols and stanols in low-fat milk.[83] Again, the sample sizes were small (13 E4 subjects, 24 E3E3 subjects).

In the most recent study, a crossover trial was performed in 63 mildly hypercholesterolemic subjects (2 periods of 4 weeks with a margarine providing either 2.0 g/d plant sterols or no plant sterols).[84] The LDL-C decrease in *APOE* E3 carriers (-0.13 mmol/L, $P<0.05$, n=40) was smaller than in *APOE* E4 carriers (-0.31 mmol/L, $P<0.001$, n=23). In this

study, a higher response was found in subjects with low endogenous cholesterol synthesis (assessed by the lathosterol/cholesterol ratio), but the cholesterol synthesis phenotype was not associated with *APOE* polymorphism. Nevertheless, the authors of this study suggested as a potential mechanism that the enhanced LDL-C response to plant sterols/stanols may be due to the accelerated clearance of chylomicrons with E4 isoform, leading to a faster hepatic delivery of cholesterol.[84] Overall, these studies have not fully demonstrated the expected better response of E4 isoform carriers due to high basal LDL-C and high cholesterol absorption. The association of E4 isoform with cholesterol absorption has not always been found, and may depend on the amount of dietary cholesterol consumption.[85] Another explanation is that most of these studies lack statistical power due to small samples.

Polymorphisms of the genes encoding the sterol transporters ABCG5/G8 and NPC1L1

ABC transporters, and in particular ABCG5/G8, play a major role in intestinal sterol absorption. Mutations in the genes coding for these two proteins explain the high plasma plant sterol and cholesterol concentrations associated with sitosterolemia.[36-38] The associations between genetic polymorphisms of these transporters and plasma sterols have been studied. In 100 Japanese hypercholesterolemic subjects, the *ABCG8* 1285A/G (M429V) polymorphism was associated with higher plasma sitosterol levels and sitosterol/cholesterol ratio, and with lower lathosterol (cholesterol precursor).[86] The rare H variant of *ABCG8* D19H polymorphism has been consistently found to be associated with a higher risk of gallstone disease, and with lower cholesterol absorption (lower levels of plant sterols in plasma) and higher cholesterol synthesis (higher levels of sterol precursors).[87,88] Nevertheless, a meta-analysis of the associations of *ABCG5/G8* polymorphisms (859T/C (C287R) and 1810C/G (Q604E) in *ABCG5*, 1285A/G (M429V), 161G/A (C54Y), 1199C/A (T400K) and 1895C/T

413 (A632V) in *ABCG8*) found little evidence of any effect on plasma LDL-C concentrations.[88]
414 (**Supplementary table S3 online only**).

415 An intervention study on the effects of plant stanols according to 3 of these
416 polymorphisms[89] found that, after a stabilization period of 4 weeks and before the
417 experimental sequence, plasma LDL-C levels were higher in subjects with the QQ genotype
418 than in E carriers for the *ABCG5* Q640E polymorphism (3.04±0.75 vs. 2.70±0.81 mmol/L,
419 *P*=0.039). Subjects with the TT genotype of *ABCG8* T400K polymorphism had higher levels
420 of plasma campesterol and sitosterol than allele K carriers (16.9 and 6.6 µmol/L vs. 13.5 and
421 4.8 µmol/L respectively) (0.68 and 0.27 mg/dL vs. 0.54 and 0.20 mg/dL), suggesting higher
422 absorption efficiency in TT subjects. Nevertheless, at the end of the experiment, LDL-C had
423 decreased in all genotypes and no difference was found according to *ABCG5/G8* transporter
424 polymorphisms. In a study where the average effects of plant stanols and plant sterols were -
425 4.2% and -4.4% respectively relative to control values, *ABCG5/G8* polymorphisms were not
426 found to have any effect.[90] The interaction between *ABCG5/G8* transporter gene
427 polymorphisms and plant sterol absorption level has also been examined.[54]
428 Hypercholesterolemic subjects were ranked according to plant sterol absorption, as assessed
429 by a stable isotope method, and compared according to their basal concentrations of plasma
430 sterols. Subjects with high basal plant sterol concentrations showed high sterol absorption and
431 low cholesterol FSR. The response to plant sterols varied according to 1199C/A (T400K)
432 polymorphism and the basal plant sterol levels. Among subjects carrying the A allele
433 (400TK+KK), the LDL-C decline was 4 times larger in the group with high than low basal
434 plant sterol concentrations (-16.6±6.3% vs. -3.4±5.7%, $P<0.05$). No such interactions were
435 found for the other polymorphisms studied (D19H and Q604E). This study also investigated
436 another gene important for sterol absorption, *NPC1L1*. A haplotype involving the two
437 polymorphisms 872 C/G (L272L) and 3929 G/A (Y1291Y) was associated with a 2.4 greater

change in LDL-C: -13.4±3.0% for minor allele carriers vs. -3.9±2.9% (P<0.05) for common allele carriers. In the study by MacKay et al.,[84] where significant results for *APOE* were found, no significant association was found between *ABCG8* T400K polymorphisms and the response to plant sterols.

Other polymorphisms

Other factors, such as the scavenger receptor B1 (SR-B1 coded by the gene *SCARB1*) and apolipoprotein AIV (coded by *APOA4*), may influence the effects of dietary plant sterols/stanols. SR-B1 is a receptor for high density lipoproteins and might be involved in cholesterol absorption by enterocytes. Apolipoprotein AIV plays a role in chylomicron formation, allowing the sterols present in intestinal cells to be exported into lymphatic vessels. The consequences of genetic polymorphisms of these proteins (Gln^{360} →His, Thr^{347} →Ser for *APOA4*, *SCARB1* HaeIII) have been evaluated together with those of other proteins involved in lipoprotein intravascular remodeling (*CETP* TaqIB) and transport (*APOE* E2/E3/E4), and endogenous cholesterol synthesis (HMGCoA Reductase VNTR).[80] Three groups of normocholesterolemic subjects were included in a parallel trial for 8 weeks: control rapeseed margarine (n=42), the same margarine with 3.8 g/d plant stanols from vegetable oil (n=34), and the same margarine with 4.0 g/d plant stanols from wood (n=36). Before the intervention, plasma high density lipoprotein cholesterol and cholesterol synthesis (lathosterol/cholesterol ratio) were lower in *SCARB1* allele 2 carriers, and LDL-C was lower in *APOE* E2 carriers. Nevertheless, none of the polymorphisms studied were associated with the LDL-C response to plant stanols.[80]

In another study, two *CETP* polymorphisms were studied: TaqIB and I405V.[81] This crossover trial included 60 moderate hypercholesterolemic subjects (average 7 mmol/L) and involved 2 periods of 4 weeks, one with a margarine providing 2.8 g/d plant sterol esters, the

462 other with control margarine. The change in total cholesterol was -7.2%, -4.2% and -0.4% in
463 subjects with genotypes II (n=15), IV (n=27) and VV (n=9), respectively; the changes in
464 LDL-C were -9.5%, -6.3% and +4.8, respectively. Consequently in this experiment, 18% of
465 the subjects were non-responders (VV homozygotes). There was no difference in response
466 according to *CETP* TaqIB polymorphism.

467 Cholesterol 7α-hydroxylase (CYP7A1) is the rate limiting enzyme in the bile acid
468 synthesis pathway, the major way to remove cholesterol from the body. The -204A>C
469 (rs3808607) polymorphism of the *CYP7A1*gene promoter has been studied.[91] The C allele is
470 functional, enhancing promoter activity and reducing the binding of transcription inhibitors of
471 the *CYP7A1* gene. Two trials (4 and 8 weeks duration, 3.2 and 2.0 g/d plant sterols) were
472 pooled, including 67 subjects (males and females, mean age 42 years). In subjects carrying the
473 C allele (n=36), the change in total cholesterol was -0.43 mmol/L vs. 0.14 mmol/L in AA
474 subjects. The lathosterol/cholesterol ratio (cholesterol synthesis marker) increased more in C
475 carriers than in AA subjects (0.75 vs. 0.10).[91] The very recent study by MacKay et al.
476 confirmed this effect of the *CYP7A1* gene polymorphism.[84] In this crossover trial with 2.0 g/d
477 plant sterols in mildly hypercholesterolemic subjects, the C allele was associated with a
478 statistically significant LDL-C response in a dose-dependent fashion (-0.05, -0.22, and -0.46
479 mmol/L in AA, CA, and CC genotypes respectively). Interestingly, the C allele was more
480 frequent in the group of subjects with low cholesterol synthesis on the basis of
481 lathosterol/cholesterol ratio. Nevertheless the genotype was not significantly associated with
482 FSR measured by an isotopic technique. There was a trend for an interaction between
483 *CYP7A1* and *APOE* genotypes, with the lowest response observed in subjects carrying the
484 combination E3-AA. These interesting results need to be confirmed with a larger sample. An
485 alternative approach to large samples of unselected subjects may be to select individuals on

the basis of the most relevant genotypes for *APOE* and *CYP7A1*. The same study did not show a significant effect of *CETP* I405V polymorphisms.

Conclusion

There is some evidence for substantial diversity in the individual response to dietary supplementation with plant sterols or stanols, and in particular the absence of response in 20-42% of subjects. One of the factors responsible for the variability is the initial LDL-C concentration. The decrease in LDL-C is apparently constant as a percentage, and is therefore higher in absolute value in subjects with high basal plasma LDL-C. Also, the effect is positively correlated with the rate of intestinal absorption of sterols and negatively correlated with the rate of endogenous cholesterol synthesis. Trials with *APOE* genotyping are not concordant. The first studies indicated a higher efficiency in E4 isoform carriers, but subsequent investigations suggest that there were no such differences, or even that there was a high efficiency in E2 and no apparent effect in E4 carriers. Polymorphisms of *ABCG5/G8* do not seem to play an important role. Only one study showed a greater change in subjects carrying the A allele (K) of *ABCG8* T400K with high sterol absorption levels. Few studies have been performed with polymorphisms of other lipid metabolism genes. Nevertheless, polymorphisms of *NPC1L1*, *CETP* (I405V) and *CYP7A1* have been reported to have significant effects, but only the results with *CYP7A1* have been replicated once. These results need to be replicated. Indeed, more generally, all of the relevant studies lack statistical power; therefore, new studies with larger populations are needed to assess the relationship between genetic polymorphisms and dietary supplementation with plant sterols and stanols. Moreover, inter-individual variability in responsiveness to any nutrient or food component cannot be explained using just one polymorphism, rather a combinatorial approach should be used. To this end, a score combining polymorphisms of genes involved in cholesterol metabolism might be calculated.[92] By analogy, the intake of sugar-sweetened beverages was associated

511 with adiposity in interaction with a genetic predisposition score calculated on the basis of 32
512 BMI-associated loci.[93] Future research towards this direction would enhance our
513 understanding on the role of genetics in cholesterol lowering responsiveness to plant
514 sterols/stanols.
515
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517 *Declaration of interest.* The authors have no relevant interests to declare.
518 **SUPPORTING INFORMATION**
519 The following Supporting Information is available through the online version of this article at
520 the publisher's website:
521 *Table S1* Response to dietary plant sterols/stanols in subjects with mutations for
522 sitosterolemia and familial hypercholesterolemia
523 *Table S2* Response to dietary plant sterols/stanols in subjects according to *APOE*
524 polymorphism
525 *Table S3* Experimental protocols of studies on plant sterols/stanols according to sterol
526 transporter polymorphisms
527
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795

796 **Table 1**: Plant sterol content (mg/100 g) in a selection of foods (from [3,5])

797

Food	Plant sterol content
Fruits and vegetables [3]	
Broccoli (frozen)	44
Green peas (frozen)	25
Orange	24
Apple	13
Cucumber	6
Tomato	5
Cereals [3]	
Wheat bran	200
Swedish knackebrot	89
Wholemeal bread	53
Rolled oats	39
Wheat bread	29
Fats and oils [3] (except *)	
Corn oil	912
Rapeseed (canola) oil	668
Liquid margarine	522
Sunflower oil	213
Spreadable butter	153
Olive oil	154
Peanut butter*[5]	146
Nuts and seeds [5]	
Almond	199
Cashew	150
Hazelnut	121
Macadamia nut	187
Peanut	135
Pecan	157
Pistachio	279

Sesame seed	400
Sunflower seed kernel	270
Walnut	113

Plant sterol content:

for reference [3] = sum of sitosterol, campesterol, stigmasterol, sitostanol and campestanol

for reference [5] = sum of sitosterol, campesterol, stigmasterol, Δ5-avenasterol, sitostanol, campestanol, other sterols

803 **Figure legends**

804 **Figure 1: Structure of sterols and stanols**

805

806 **Figure 2: LDL-C change (%) in response to dietary plant sterols/stanols according to**

807 ***APOE* polymorphism**

808 Differences significant only for reference [75],[76] and [84]. Changes in % were found in the articles

809 except for references [80, 82,83,84] (calculated by the authors of the present review). More details

810 are given in Supplementary table S2 (online only).

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