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REVIEW ARTICLE

Phytosterols and phytosterolemia: gene–diet interactions

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Abstract Phytosterol intake is recommended as an adjunctive therapy for hypercholesterolemia, and plant sterols/stanols can reduce cholesterol absorption at the intestinal lumen through the Niemann-Pick C1 Like 1 (NPC1L1) transporter pathway by competitive solubilization in mixed micelles. Phytosterol absorption is of less magnitude than cholesterol and is preferably secreted in the intestinal lumen by ABCG5/G8 transporters. Therefore, plasma levels of plant sterols/stanols are negligible compared with cholesterol, under an ordinary diet. The mechanisms of cholesterol and plant sterols absorption and the whole-body pool of sterols are discussed in this chapter. There is controversy about treatment with statins inducing further increase in plasma non-cholesterol sterols raising concerns about the safety of supplementation of plant sterols to such drugs. In addition, increase in plant sterols has also been reported upon consumption of plant sterol–enriched foods, regardless of other treatments. Rare mutations on ABCG5/G8 transporters affecting cholesterol/non-cholesterol extrusion, causing sitosterolemia with xanthomas and premature atherosclerotic disease are now known, and cholesterol/plant sterols absorption inhibitor, ezetimibe, emerges as the drug that reduces phytosterolemia and promotes xanthoma regression. On the other hand, common polymorphisms affecting the NPC1L1 transporter can interfere with the action of ezetimibe. Gene–diet interactions participate in this intricate network modulating the expression of genetic variants on specific phenotypes and can also affect the individual response to the hypolipidemic treatment. These very interesting aspects promoted a great deal of research in the field.

Keywords Plant sterols · Phytosterolemia · Gene–diet interaction · Statins · Ezetimibe · Cardiovascular disease

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Sterols structure and biology

Sterols and phospholipids are the main components of cell membrane, and they are crucial for the maintenance of cell properties, serving not only as a barrier, separating the cell from the external environment, but also are responsible for ions and metabolites transport into and out of the cell, cell interaction, communication and regulation processes. The proper permeability, fluidity, and rigidity of the cell membrane are ensured by these lipid components [8]. Cholesterol is the sterol predominantly found in humans, and although essential for life, when in excess, mainly as LDL-cholesterol (LDL-C), there is a higher incidence of premature vascular diseases [11].

Plant sterols are very similar in their structure to cholesterol, differing from the former by the presence of one or two methyl or ethyl groups in the molecule's side chain (Fig. 1). These non-cholesterol sterols are present in foods of plant origin, mainly in vegetable oils, nuts, and seeds; the dietary intake of plant sterols depends on the type of diet consumed, but is very similar to the cholesterol ingestion, varying from 150 to 450 mg/day, with higher consumption in vegetarians. They have a similar biological role when compared to cholesterol in animals, as an essential part of cell membrane. Plant stanols are less consumed than sterols (usually about 30–50 mg/day, on a regular diet, if no supplementation is added); a source of sitostanol is tall oil derived from conifers. Stanols also

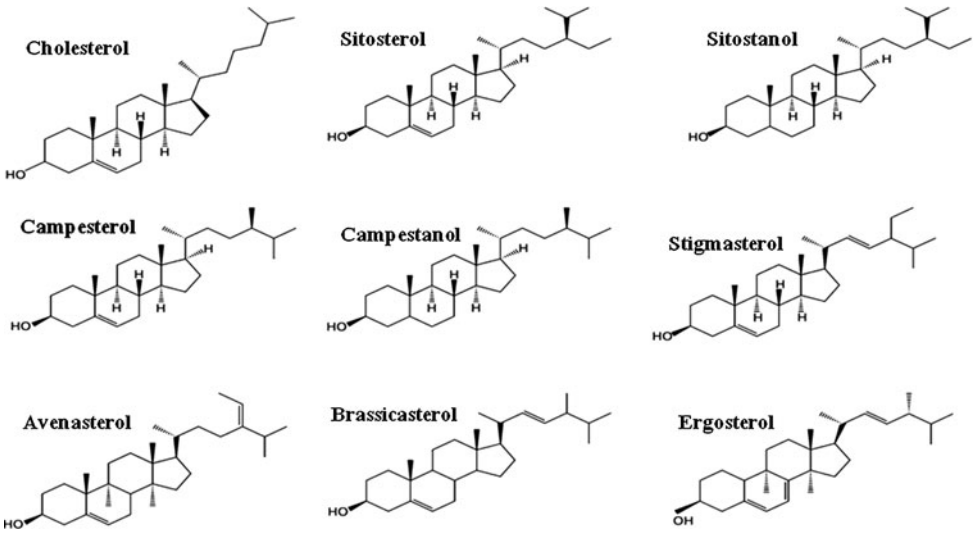

Fig. 1 Representation of chemical structures of cholesterol, plant sterols, and stanols. Stanols lack the Δ5 double bond in the B-ring. Saturation of sterols gives rise to stanols

differ from cholesterol in their structure of the side chains; they are saturated sterols, which lack the Δ5 double bond in their B-ring. Therefore, sitostanol results in the saturation of sitosterol, the most commonly occurring plant sterol, whereas campestanol results in the saturation of campesterol. There are differences in the interactions between phytosterols and phospholipids in membrane models. An elegant study from Hac-Wydro et al. [27], evaluating the interactions between phytosterols and phospholipids in model membranes demonstrated that the structure of sterols (cholesterol, β-sitosterol, and stigmasterol) does not affect the stoichiometry of the most stable complexes formed with particular phospholipids, but influences their stability. The strongest interactions were found for cholesterol/phospholipids mixtures, while the weakest were for mixed systems containing stigmasterol.

Regulation of cholesterol/non-cholesterol absorption and extrusion on enterocytes

To understand how sterols ingestion can affect plasma levels, the process of sterols absorption and extrusion has to be known (Fig. 2). Briefly, cholesterol and plant sterols when ingested are solubilized by biliary acid to form micelles, which are stable structures. Cholesterol and plant sterols are compounds of similar structure, but sterol molecule presents higher molecular weight and is more hydrophobic, what increases its affinity for intestinal micelles, displacing cholesterol from micelles, thus limiting the available quantity of cholesterol to be absorbed. The sterols in the micelles are therefore transferred to the enterocyte surface, where Niemann-Pick C1 Like 1 (NPC1 L1) protein has a crucial role in the transport of these compounds to the cytoplasm and endoplasmic reticulum. Once in the cytoplasm, cholesterol is esterified by acyl-cholesterol acyl transferase (ACAT2) to form chylomicrons that are secreted in the lymph. The absorption of cholesterol in humans is effective, and approximately 50% of the cholesterol in the intestinal tract is absorbed. Most of the cholesterol absorbed through this pathway reaches the circulation and part is extruded by ABCG5/G8 transporters back to the intestinal lumen. The opposite occurs with phytosterols, which are preferably excreted. NPC1L1 is the site of action of ezetimibe, a sterol absorption inhibitor.

Campesterol or sitosterol concentrations and the ratios between these compounds and cholesterol reflect the efficiency of cholesterol absorption. On the other hand, lathosterol to cholesterol ratio is a good index of endogenous cholesterol synthesis. There is a reciprocal relationship between cholesterol synthesis and absorption, and those individuals who synthesize less amounts of cholesterol are usually those with high absorption levels, and vice versa, to ensure a precise cholesterol homeostasis control.

Plasma levels of plant sterols/stanols

Plant sterol/stanol concentrations in human plasma are less than 0.5% that of cholesterol, in spite of similar ingestion (cholesterol intake varies from 300 to 500 mg/day). This discrepancy relates to the fact that cholesterol is mainly synthesized by the liver and secreted in the bile (1,200–1,500 mg/day); in addition, plant sterols are far less absorbed in the small intestine (Fig. 2). Stanols are even less absorbed than plant sterols, with plasma levels almost indetectable (0.05% the amount of cholesterol) [86]. Normal serum contains small amounts of non-cholesterol sterols, including those reflecting cholesterol absorption (cholestanol, and the plant sterols campesterol, sitosterol,

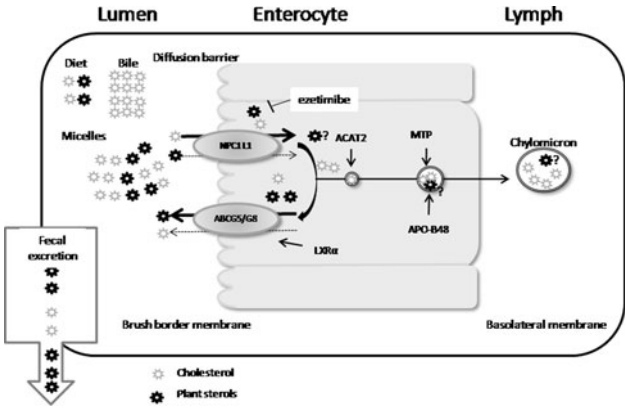

Fig. 2 Representation of cholesterol and non-cholesterol sterol intestinal absorption and extrusion. ABCG5/G8 = adenosine triphosphate (ATP)-binding cassette (ABC) transporter family (ABCG5 and ABCG8); ACAT2 = acyl-cholesterol acyl transferase; ApoB48 = apolipoprotein B48; LXRα = liver X receptor α; MTP = microsomal triglyceride transfer protein; NPC1L1 = Niemann-Pick C1 Like 1 protein

and avenasterol), and those that are precursors of cholesterol synthesis (cholestenol, desmosterol, and lathosterol) [47, 55]. The plasma levels of different sterols in 18 individuals with mild hypercholesterolemia were reported [51, 81]. For cholesterol levels of 236.5 mg/dl, campesterol, sitosterol, stigmasterol, and brassicasterol mean plasma levels were 0.474, 0.326, 0.011, and 0.047 mg/dl, respectively, without plant sterol supplementation. Non-cholesterol sterols' plasma levels, and the ratios of these sterols to cholesterol, can be assessed to evaluate cholesterol metabolism.

When foods with added plant sterols and stanols are consumed, approximately 0.5–2% of plant sterols and only 0.04–0.2% of the plant stanols are absorbed. However, ingestion of 18 g/day of plant sterols lowered blood cholesterol and promoted marked increases in plant sterol plasma levels, mainly of campesterol, that are more absorbed than sitosterol. However, this is not the usual amount recommended for consumption nowadays.

The concentrations of plant sterols in plasma of subjects consuming ~2.0 g of sterol ester products are within the range of 0.6–2.0 mg/dl [28, 83]. This range is 20–100 times lower than in patients homozygous for sitosterolemia (10–30 mg/dl), raising some concerns that the increase in plasma plant sterol levels attributable to plant sterol–enriched food products used for cholesterol-lowering purposes may in fact be atherogenic [24].

Statins reduce plasma levels of markers of cholesterol synthesis, such as desmosterol, squaleno, cholestanol, and lathosterol; however, they can increase the levels of markers of sterol absorption. The initial observations came from the early nineties, when pravastatin and lovastatin had shown to increase cholestanol and plant sterol absorption markers, such as sitosterol, campesterol, stigmasterol, avenasterol, brassicasterol, and ergosterol [78–80].

Lipid-lowering effects of plant sterols/stanols

Plant sterols and stanols have been known for a long time to reduce serum levels of LDL-C by competing with dietary and biliary cholesterol for intestinal absorption [38, 43, 50]. The first therapeutic agent described was β-sitosterol, used to treat hypercholesterolemia about 60 years ago [54]. The lipid-lowering effects of plant sterols and stanols have been demonstrated both in animals and in humans [54, 58, 59, 63]. This reduction applies to both dietary and biliary cholesterol. However, the intake of plant sterols and stanols from an ordinary diet is not sufficient to promote an effective reduction in cholesterol levels. Therefore, plant sterols and stanols have been added to food, as supplements, and present a more effective lipid-lowering capacity. When foods with added plant sterols and stanols are consumed, approximately 0.5–2% of plant sterols and only 0.04–0.2% of the plant stanols are absorbed. Evaluating sterol balance, plant sterols inhibit cholesterol absorption, the maximal effect being at an intake of 2–3 g/day; however, in familial hypercholesterolemia, intakes of 6 g/day of unesterified sitosterol promoted mild reductions in LDL-C, and association of 3 g with fibrates resulted in long-term benefits. [5]. Short-term treatment with the supplementation of 1.6 g of plant sterols reduced LDL-C by 9–15% [37, 38]. For stanols supplementation, the LDL-C-lowering effect was not followed by increase in plasma sitostanol [30], but other studies showed contradictory effects [21, 52]. The interpretation was that the physical state of plant stanols could be determinant of their efficacy. Sterols are more soluble (~3%) than stanols (<1%) in food preparations, e.g., margarine [71].

A systematic review of the data for the cholesterol-lowering effects of both plant sterols and stanols showed a dose–response relation up to about 2 g/day of sterol or stanol [40], with no further reduction in low-density lipoprotein cholesterol above this dose [51]; reductions in LDL-C were about 9 to 14% and were similar between plant sterols and stanols. However, the cholesterol-lowering effect of dietary plant sterol esters is less marked in longer term than in short-term studies, whereas plant stanol esters seem to maintain their efficacy [12, 51]; thus, these compounds can be used for the long-term management of hypercholesterolemia. In addition, stanol esters can reduce LDL-C levels in postmenopausal women by about 13%, suggesting their use as a component of non-drug therapy in these women. Plant stanols have additive LDL-C-lowering effect, when associated with ongoing statin therapy [12]. These observations make plant stanols and sterols

attractive dietary components to help in achieving LDL-C goals in patients requiring an LDL-lowering drug. For these reasons, the Adult Treatment Panel (ATP) of the National Cholesterol Education Program (NCEP) III [23] recommends the intake of 2.0 g of plant sterols or stanols daily as an adjunctive therapy to reduce cardiovascular risk. Sources of these sterols/stanols are mainly derived from industrialized foods, presented in spreads, like margarine, in yogurt, and in certain oils (tall and soy oil).

Are plant sterols/stanols levels a risk factor for cardiovascular disease?

Functional foods enriched with phytosterols have gained important role on strategies to lower cardiovascular risk [25], for competing with cholesterol absorption on enterocytes. Usually, the absorption of plant sterol is lower than cholesterol, around 5% for phytosterol compared to 55% for cholesterol [9, 31]. The low absorption of phytosterols is attributed to the active resecretion into the intestine, a process mediated by the ABCG5 and ABCG8 transporters. Both processes ensure a very low level of net retention of non-cholesterol sterols [65, 66], resulting in total plasma plant sterol levels of $\sim$1 mg/dl [45, 66] to around 200 mg/dl for cholesterol.

In contrast to the favorable effects of plant sterols on cholesterol serum levels, sitosterolemia is a rare autosomal recessive disorder, in which plant sterols, or phytosterols, are over absorbed and accumulate in tissues causing xanthomata and premature atherosclerotic disease [64].

Considering the pro-atherogenic role of high levels of phytosterols in sitosterolemia, the safety of raised sterol plasma levels has been debated, and concerns about the levels of plasma sterols/stanols in non-sitosterolemic individuals increasing the risk of cardiovascular disease were raised. Recent epidemiologic studies have shown that small increases in plant sterols' concentrations may be atherogenic, even in individuals that do not have sitosterolemia [4, 64, 74, 75]. In the Scandinavian Simvastatin Survival Study (4S) [68], study individuals with raised phytosterol levels at baseline did not show benefits on cardiovascular events when treated with simvastatin. These patients presented higher levels of phytosterols and lower levels of LDL-C during the study when compared to placebo-treated group with similar baseline sterol plasma levels. Later studies showed that with more potent statins, the reduction in markers of endogenous synthesis was followed by elevation in plasma plant sterols levels, proportional to the statin potency [19, 20, 48, 49].

A subgroup analysis of Finnish participants of the 4S study showed that subjects with a high cholesterol absorption rate and low cholesterol synthesis rate did not show an appropriate LDL-C-lowering effect or reduction in coronary events after treatment with simvastatin [48].

A number of studies have suggested that high serum plant sterol values may be associated with increased risk of coronary heart disease (CHD) [3, 24, 60, 76]. When plant sterols are added to a statin, further reduction in LDL-C can be observed. However, part of the beneficial effect can be lost by the increase in plasma plant sterols levels during statin treatment, and some authors have proposed high plant sterol plasma levels as novel risk factor for cardiovascular disease in non-sitosterolemic subjects. This aspect raised the interest on drugs that can both inhibit the absorption of cholesterol and plant sterols, such as ezetimibe, an intestinal sterol absorption inhibitor, that added (or not) to a statin could potentially protect against these deleterious effects [75].

Increase in plant sterols can be seen also upon consumption of plant sterol–enriched foods, regardless of other treatments. On the other hand, consumption of a diet naturally enriched in plant sterols, a measure of healthy dietary choices, increases plasma phytosterols levels, and these increased levels are believed to be a coronary heart disease risk factor. To address this apparent paradox, two large clinical transversal studies have shown an inverse relationship between phytosterol intakes on a regular diet and total and LDL-C concentrations, suggesting that moderate increases in phytosterolemia in non-sisterolemic subjects could not be harmful, but beneficial [56]. Escurriol et al. [22] evaluated baseline risk factors, phytosterol intake, and plasma non-cholesterol sterol levels in participants of a nested case–control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) Spanish cohort who developed CHD ($n = 299$) and matched controls ($n = 584$) who remained free of CHD after a 10-year follow-up. Sitosterol-to-cholesterol ratios increased across tertiles of phytosterol intake; HDL-cholesterol levels increased; and adiposity measures, cholesterol/HDL ratios, levels of glucose, triglycerides, and lathosterol decreased across plasma sitosterol tertiles. No differences between cases and controls regarding phytosterol intake or plasma sitosterol were observed.

The same group [14] investigated associations between plasma non-cholesterol sterols and metabolic syndrome components in 674 dyslipidemic patients and 361 healthy subjects participating in a prospective cohort study. In both dyslipidemic and healthy populations, metabolic syndrome was associated with increased plasma lathosterol, a cholesterol synthesis marker, and decreased plasma sitosterol, a marker of cholesterol absorption. Elevated plasma phytosterols were related to a lower frequency of cardiometabolic risk factors, suggesting an association with reduced risk of cardiovascular disease.

To conclude these controversial issues, there appears to be a protective effect of phytosterol intake from a natural diet in non-sitosterolemic individuals. Supplementation of phytosterols may increase plasma levels of phytosterols, especially if sterols, not stanols are added. However, the benefits of LDL-C-lowering outweigh the increase in plasma sterol concentrations [67, 84]. There is no clear answer to this issue yet as long-term studies are lacking. This sterol raising effect may be harmful in individuals who present higher absorption levels, such as in familial hypercholesterolemia [51], or in individuals under high-dose statins. Cholesterol/sterols absorption inhibitors, e.g. ezetimibe, can attenuate the effects of increased phytosterols.

Genetics of phytosterol absorption and extrusion

Phytosterolemia or sitosterolemia: ABCG5/G8 mutations

Sitosterolemia (also known as phytosterolemia, MIM 210250) is a genetic autosomic recessive disease. It occurs in consequence of a complete mutation in two adjacent, oppositely oriented genes that encode new members of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family (ABCG8 and ABCG5) located in a head-to-head organization on human chromosome 2p21 [6, 41, 44, 53]. These genes encode ATP-binding cassette (ABC) proteins that belong to the G family and may work together to pump sterols (cholesterol and plant sterols) from the brush border of enterocytes into the intestinal lumen and from the liver into bile, limiting the raise of plasmatic levels of plant sterols [41, 64, 66]. Described by Bhattacharyya and Connor [7], sitosterolemia is a rare inherited disorder that is characterized mainly by excessive absorption and high plasma levels of plant sterols and with normal or only moderately increased cholesterol levels. The major clinical manifestations include tendon and tuberous xanthomas that involve Achilles and patellar tendons, extensor tendons of the hand and the skin of the elbows, knees and premature atherosclerosis [7, 70].

Chemically, increased amounts of plant sterols, such as sitosterol campesterol and stigmasterol are found in the plasma, erythrocytes, and xanthomas. The presence of the homozygous mutation Gly574Arg in the *ABCG8* gene has been found previously in the Amish-Mennonite patients where a founder effect has been reported [73].

Mannucci et al. [46] described a novel non-sense mutation in exon 10 of the *ABCG5* gene in a 10-year-old girl showing clinical and biochemical features of sitosterolemia that when moved to Europe and received vegetables and olive oil developed xanthomas. They studied a broad spectrum of the *ABCG5/ABCG8* mutations causing sitosterolemia and highlighted the correlations between such gene mutations, biochemical phenotype, and the development of cardiovascular disease. The proband was homozygous for a single-nucleotide mutation in exon 10 of the *ABCG5* gene, presenting a C to T transition at nucleotide 1336 of the coding sequence, resulting in the premature termination of the ABCG5 protein at amino acid 446 (Arg446X). Her mother and brother were homozygous for the same mutation, all featuring elevated plasma β-sitosterol levels. However, the father was heterozygous and presented normal β-sitosterol levels. This mutation was not found in healthy normolipidemic subjects.

Solcà et al. [73] performed an haplotype analyses using microsatellite markers spanning 4.9 cM on chromosome 2 and showed that a patient with sitosterolemia of Swiss-German origin and the two Amish-Mennonite probands were identical for markers D2S2174, D2S1761, D2S4009, D2S4014, D2S4015, and D2S4016 encompassing the STSL locus. Other studies reported the same observations [42], and mutations in two tandem *ABC* genes, *ABCG5* and *ABCG8*, encoding sterolin-1 and 2, respectively, are now known to be mutant in sitosterolemia. Heterozygous individuals do not develop the disease, but present raised plasma phytosterol concentrations [64–66].

Caucasians seem to carry mutations in *ABCG8* gene, whereas Chinese, Japanese, and Indian (20% of known cases) patients seem to have mutations in *ABCG5* [44]. However, the prevalence of heterozygous mutations in the population is not known [39].

Polymorphisms in the ABCG5/G8 genes affecting LDL-C and plant sterols levels

In addition to the mutations causing the rare sitosterolemia, common polymorphisms of potential interest, as genetic variants that may influence the lipid profile, have been described by Hubácek et al. [33] in non-sitosterolemic individuals. Missense polymorphisms (Gln604Glu in the *ABCG5,* and Asp19His, Tyr54Cys, Thr400Lys, and Ala632Val in the *ABCG8*) were examined, and the Thr400Lys in the *ABCG8* gene was associated with changes in lipid levels in response to reduced dietary animal fat and cholesterol intake.

Zhao et al. [88] studying the effects of supplementation of plant sterols in 82 hypercholesterolemic men characterized by high versus low basal plasma plant sterol concentrations consuming spreads with or without 2 g/day of plant sterols observed that for the *ABCG8* 1289 C > A (T400 K) polymorphism, the A allele carriers with high basal plasma plant sterol concentrations demonstrated a 3.9-fold greater reduction in serum low-density lipoprotein cholesterol than their low basal plasma plant sterol concentration counterparts.

Sitosterolemia and Ezetimibe

Ezetimibe is a selective inhibitor of the Niemann-Pick C1 Like 1 (NPC1L1) intestinal protein that blocks the transport of cholesterol and phytosterols from diet and biliary sources [2, 16, 77]. This effect is a consequence of interference in normal function of NPC1L1 protein that is responsible for the sterols transport [1, 34]. The therapeutic responses to the ezetimibe seem to have a genetic basis, with wide inter-individual variations [29, 82].

Association of statins with ezetimibe lowers plasma phytosterol levels in individuals with sitosterolemia and hypercholesterolemia [19, 20] by producing synergistic effects. This combination blocks liver endogenous synthesis (statins effect) and also blocks exogenous sterols absorption through the NPC1L1 pathway (ezetimibe activity), thus lowering both LDL-C and plant sterols [19].

Niemann-Pick C1 Like 1

There is heterogeneity in cholesterol absorption, which varies from 29 to 80% in healthy individuals [26]. Cholesterol absorption is a heritable trait in humans, and only recently its genetic basis has been unraveled: a sterol transporter protein named Niemann-Pick C1L1 [2]. This protein is highly expressed in small intestine (on the enterocyte surface) and liver (on the hepatocyte canicular membrane); in the latter, it can pump free cholesterol to the liver cells [77]. Interestingly, Seedorf et al. [69] proved that macrophages also express NPC1L1, indicating the important role in the transport of free cholesterol. NPC1L1 is responsible for the intestinal phytosterol absorption [87] since these sterols are drastically reduced in deficient NPC1L1 mice [20] and in patients with sitosterolemia that were treated with ezetimibe [67].

NPC1L1 transports sterols in a non-equalitarian way; the uptake of cholesterol is 60% greater than sitosterol [85]. The affinity of the sterols' transporter differs, and the intestinal absorption preference is cholesterol > cholestanol > campesterol > sitosterol > campestanol > sitostanol. These differences in sterols absorption can contribute to the different plasma sterols concentrations [87]. The cholesterol-lowering effects of plant sterol appear to be due to at least 2 separate mechanisms; the disruption of cholesterol solubilization in micelles by plant sterols [35] and the inhibition of NPC1L1 expression in the small intestine, which can block the entry of cholesterol into enterocytes, by transporter saturation [36].

Intestinal NPC1L1 mRNA expression is downregulated in cholesterol/cholate-fed wild-type and NPC1L1 heterozygous mice. NPC1L1 contains a sterol-sensing domain like many other proteins involved in cholesterol metabolism [18]. Sterol regulatory elements (SREs) and a Ying Yang-1 binding site are present in the promoter region of NPC1L1, both of which have been implicated in sterol regulation of mRNA expression. The downregulation of intestinal NPC1L1 in the presence of the high cholesterol/cholate diet is consistent with the SCAP/SREBP complex staying intact, preventing the bHLH-Zip domain binding to the SRE in the *NPC1L1* promoter [32, 62].

NPC1L1 polymorphisms and gene–diet effects on LDL-C and plant sterols concentrations

Serum levels of LDL-C are strongly influenced by genetic polymorphisms, which contribute to intestinal efficacy of cholesterol absorption. Cohen et al. [15] observed that *NPC1L1* polymorphisms in non-sitosterolemic individuals contribute to the variability of sterol absorption and to serum levels of LDL-C. The *NPC1L1* SNPs (single-nucleotide polymorphisms) that are associated with lower levels of cholesterol were five times more frequent in Afro-Americans, thus showing that *NPC1L1* polymorphisms contribute in an important way to the variability of sterol plasma levels [29].

On the other hand, genetic variation in *NPC1L1* can affect the response of LDL-C to plant sterol supplementation. Rudkowska et al. [61], in a case–control study, found that *NPC1L1* rs4720470 polymorphism was associated with non-responsiveness to plant sterol intervention. In a Chinese study, with 224 participants, the C allele at −762 position of the *NPC1L1* gene was more common in persons of Chinese ethnicity, where carriers of −762C allele had a higher promoter activity and were associated with higher serum total cholesterol and LDL-C levels [13].

Common polymorphisms of sterol transporter genes can also affect baseline circulating plasma plant sterols concentrations [10, 17] and the responses to plant sterol supplementation. The promoter region SNPs at positions −18 and −133 could affect alternative splicing and produce NPC1L1 isoforms with properties that may differ from those of the full-length gene product [88]. A 4-week crossover study with 82 hypercholesterolemic men characterized by high versus low basal plasma plant sterols concentrations consuming spreads with or without supplementation of 2 g/day of plant sterols showed that for the *NPC1L1* haplotype of 872 C > G (L272L) and 3929 G > A (Y1291Y), individuals carrying mutant alleles showed a 2.4-fold greater reduction in LDL-C levels, compared to wild-type counterparts. In this study, genetic and metabolic biomarkers could predict inter-individual lipid level responsiveness to plant sterols intervention and thus would be useful for tailoring cholesterol-lowering strategies.

NPC1L1 gene: statin interactions

Polisecki et al. [57] recently examined whether five variants (−133A > G, −18A > C, L272L, V1296 V, and U3_28650A > G) at the *NPC1L1* gene would affect lipid levels, prevalence and incidence of coronary heart disease (CHD), and the lipid-lowering response to pravastatin in 5,804 elderly participants from the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) study and followed on average for 3.2 years. After adjustment for gender, homozygous carriers of the minor alleles at four *NPC1L1* sites (−18A > C, L272L, V1296 V, and U3_28650A > G, minor allele frequencies 0.15–0.33) had 2–8% higher LDL-C levels at baseline than homozygous carriers of the common alleles and also presented a 50–67% increased risk of developing fatal or non-fatal CHD on trial events, regardless of the treatment regimen. Therefore, the higher incidence of new CHD, death, or non-fatal MI in carriers of minor alleles did not appear to be modified by statin treatment, indicating that pravastatin is not effective in CHD risk reduction in this subgroup of patients.

NPC1L1 gene–ezetimibe interactions

Two studies addressed whether polymorphisms on *NPC1L1* could affect LDL-C levels in response to treatment with ezetimibe [29, 72], showing that inter-individual variation on LDL-C levels could be related to certain NPC1L1 haplotypes. Subjects that did not carry the common *NPC1L1* haplotype 1735C-25342A-27677T were found to have a greater reduction in plasma LDL-C with ezetimibe than those with at least one copy of this haplotype.

Conclusions and perspectives

One of the main goals of the cardiovascular therapy is to reduce atherosclerotic cardiovascular disease. LDL-C is a major risk factor and the primary target of hypolipidemic therapy. Maximal diet therapy combined with effective drugs has been proposed to attain LDL-C goals. Plant sterols or stanols (~2.0 g/day) are recommended as adjunctive therapy to a diet designed for a maximal LDL-C reduction. The beneficial effects of plant sterols/stanols are observed and are magnified when added to a statin; on average, the addition of plant sterols to a statin doubles the effect of the statin alone. However, plant sterol plasma levels can increase in subjects taking plant sterols and these increases are especially marked in patients with familial hypercholesterolemia who are taking high-dose statins [51]. In addition, increase in plant sterols can be seen also upon consumption of plant sterol–enriched foods, regardless of other treatments. Phytosterols have been recently identified in atheromatous plaques obtained from individuals with apparently normal absorption of plant sterols raising the possibility that phytosterols are a novel atherosclerotic risk factor. Plant stanols are also effective lipid-lowering agents, when supplemented, and are less absorbed than sterols. However, long-term prospective studies testing these interventions are lacking. Gene–diet interactions seem to play a role modulating cholesterol/plant sterols absorption and secretion. These influences may interfere with the response to drugs such as statins or ezetimibe and can discriminate individuals who are good responders to inhibition of synthesis, absorption, or both mechanisms. The evaluation of precursors of cholesterol synthesis and markers of absorption, and especially their ratios can give us information on the intricate metabolism of sterols in humans and may be used as markers of cardiovascular disease.

Conflict of interest None.

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