DOI 10.1002/mnfr.201100722 *Mol. Nutr. Food Res.* 2012, *56*, 1058–1072

REVIEW

Effects of plant sterols and stanols on intestinal cholesterol metabolism: Suggested mechanisms from past to present

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Received: October 27, 2011
Revised: February 27, 2012
Accepted: April 3, 2012

Plant sterols and stanols are natural food ingredients found in plants. It was already shown in 1950 that they lower serum low-density lipoprotein cholesterol (LDL-C) concentrations. Meta-analysis has reported that a daily intake of 2.5 g plant sterols/stanols reduced serum LDL-C concentrations up to 10%. Despite many studies, the underlying mechanism remains to be elucidated. Therefore, the proposed mechanisms that have been presented over the past decades will be described and discussed in the context of the current knowledge. In the early days, it was suggested that plant sterols/stanols compete with intestinal cholesterol for incorporation into mixed micelles as well as into chylomicrons. Next, the focus shifted toward cellular processes. In particular, a role for sterol transporters localized in the membranes of enterocytes was suggested. All these processes ultimately lowered intestinal cholesterol absorption. More recently, the existence of a direct secretion of cholesterol from the circulation into the intestinal lumen was described. First results in animal studies suggested that plant sterols/stanols activate this pathway, which also explains the increased fecal neutral sterol content and as such could explain the cholesterol-lowering activity of plant sterols/stanols.

Keywords:
Cholesterol / Mechanism of action / Mixed micelles / Plant stanols / Plant sterols

1 Introduction

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide. It is well established that lifestyle—and particularly our diet—plays an important role in the prevention and treatment of CVD [1]. A major target for dietary interventions is reducing the increased serum low-density lipoprotein cholesterol (LDL-C) concentrations [2].

A meta-analysis summarizing the results of 26 clinical trials of cholesterol-lowering agents clearly showed a risk reduction of nonfatal occlusive vascular events by about one-fifth for each 1 mmol/L reduction in serum LDL-C concentration 1 year after randomization. More specifically, a serum LDL-C reduction of 1, 2, or 3 mmol/L lowered the risk by 22, 40, and 50%, respectively [4]. Despite these impressive risk reductions, there is still an ongoing discussion whether these effects are causally related to the reduction in LDL-C concentrations. It is even questioned whether the cardioprotective effects of statins are causally related to their serum LDL-C lowering effects or rather to their pleiotropic effects, such as improving endothelial function, increasing vascular nitric oxide bioavailability, and reducing oxidative stress [5]. In this respect, LaRosa [6] clearly showed that it is not important how LDL-C is lowered. Combining the results of all the currently available intervention studies showed that lowering serum LDL-C to decrease the risk for a nonfatal

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Abbreviations: **ABCA1**, ATP-binding cassette transporter A1; **ABCG5**, ATP-binding cassette transporter G5; **ABCG8**, ATP-binding cassette transporter G8; **ACAT-2**, acylcoenzyme A cholesterol acyltransferase-2; **ANXA2**, annexin2; **Caco2**, colorectal adenocarcinoma; **CAV1**, caveolin1; **CVD**, cardiovascular disease; **FMD**, flow-mediated vasodilatation; **HMG-CoA**, 3-hydroxy-3-methyl-glutaryl-Coenzyme A; **LiSA**, ligand sensing assay; **LXR**, liver X receptor; **MTP**, microsomal triglyceride transfer protein; **NPC1L1**, Niemann-Pick C1 like 1 protein; **NTD**, N-terminal domain; **Ox-LDL**, oxidized LDL; **RCT**, reverse cholesterol transport; **SREBP2**, sterol response element binding protein 2; **TAG**, triacylglycerol; **TICE**, transintestinal cholesterol excretion

myocardial infarct and coronary heart disease death by diet is as valuable as lowering serum LDL-C by, for example, statins and 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors.

Foods enriched with fatty acid esters of plant sterols or stanols, i.e. plant sterol or stanol esters are well known for their serum LDL-C lowering effect [7, 8], which is not transient, as shown in an 85-week intervention study [9]. The effectiveness of these compounds is further supported by the fact that they are nowadays incorporated into national and international guidelines such as the National Cholesterol Education Program guidelines. These guidelines encourage a daily incorporation of 2 g plant sterols or stanols into a healthy diet low in saturated fatty acids to reduce CVD risk for subjects with elevated LDL-C concentrations. In this case, addition of plant sterols and stanols can lower serum LDL-C concentrations up to 10% [2].

Plant sterols and stanols are components that are naturally present in plants. Like cholesterol, they exist mainly in a free and an esterified form. When incorporated as functional food ingredient, plant sterols and stanols are frequently esterified with a fatty acid ester to increase the solubility in the food matrix [10]. The rate of absorption of cholesterol and plant sterols/stanols is very different. About 40–60% of cholesterol is absorbed, whereas plant sterols/stanols are absorbed for 15% or less, depending on the specific isoform [11–13].

Four meta-analyses have shown significant reductions in LDL-C concentrations after consumption of foods enriched with plant sterol or stanol esters [7, 14–16]. In contrast with these four nonlinear dose–response curves, Mensink et al. [17] found a clear linear relationship between plant stanol intake and reductions in LDL-C up to 9 g/day. Compared with the control group, the reductions in serum LDL-C concentrations after a daily consumption of 3, 6, and 9 g were 7.5, 12, and 17.4%, respectively. Comparable findings were reported by Gylling and colleagues [18], in which a 17.4% reduction in serum LDL-C was found after a daily consumption of 8.8 g plant stanols provided as their fatty acid esters for a period of 10 weeks. In this respect, the most recent meta-analysis from Musa-Veloso et al. [8] suggested that consumption of plant stanols above the currently recommended 2 g/day is associated with an additional and dose-dependent reduction in serum LDL-C concentration. They included 113 publications and one unpublished study report and found that the maximal reduction in LDL-C was 16.4% after plant stanols and 8.3% after plant sterols consumption at daily doses ranging from 0.8 to 8.8 g and 0.19 to 9 g, respectively. Remarkably, there are no studies comparing the LDL-C lowering activity of high doses (>4 g/day) of plant stanols and sterols head-to-head. However, such a clinical trail is needed to further explore the efficacy and possible differences between plant sterols and stanols at higher intakes.

Although the LDL-C lowering effect of food enriched with plant sterol and stanol esters is sustained and widely accepted, the discussion whether the type of food (food matrix) influences its efficacy is still ongoing [7, 16]. Besides the type of food carrier used, the frequency of intake seems to be important as well [7, 19]. Furthermore, Abumweis et al. [16] concluded that the time of intake is also crucial, since consumption before or with breakfast only failed to reduce serum LDL-C, while the expected serum LDL-C lowering effect was observed when plant sterols were consumed together with a main meal being either lunch or dinner.

To better understand all these discrepancies between the individual studies, understanding the effects of plant sterol/stanol esters on (intestinal) cholesterol metabolism is essential. Therefore, the main objective of this review is to focus on past and recent findings, and on assumptions and more or less accepted explanations of the mechanisms underlying the plant sterol/stanol ester induced serum LDL-C lowering effect. For this, we will provide an historical overview of these compounds, starting in the 1950s until now. Based on these findings, we will try to conclude whether we can predict their use to reduce atherosclerotic lesion formation.

2 Suggested mechanisms over the years

Inhibition of intestinal cholesterol absorption is an interesting target to lower concentrations of LDL and other apoB100 containing lipoprotein fractions. Cholesterol absorption is a multistep process, in which the most important steps are (1) cleavage of (dietary) sterol/stanol esters into free sterols/stanols in the intestinal lumen, (2) the solubilization of unesterified cholesterol into the emulsified fat phase and the mixed micelles in the lumen, (3) the transport of cholesterol through mucosal barriers such as the unstirred water layer and the brush border membrane. After (4) uptake and (5) (re)esterification by acylcoenzyme A cholesterol acyltransferase-2 (ACAT-2) inside the enterocyte, cholesterol is (6) incorporated into chylomicrons by involvement of the microsomal triglyceride transfer protein (MTP), and (7) released into the lymph. Over the years, almost every single step has been discussed for its potential involvement in lowering intestinal cholesterol absorption via plant sterol or stanol ester consumption. We will now recapitulate the chronology of the different paradigms in explaining the reduction in intestinal cholesterol absorption by plant sterol and stanol esters.

2.1 The early days

The very first studies, mentioning a role for plant sterols in the regulation of serum cholesterol concentrations, were published by Peterson et al. in 1951. Chickens were fed a diet containing 0.5–1% soybean sterols, 0.5–1% cholesterol, or a mixture of both compounds. Significant reductions in hepatic and plasma cholesterol concentrations were found in chickens fed a diet supplemented with the soybean sterols [20]. In the following studies [21], again in chickens, the effects of plant sterols on atherosclerotic lesion formation were evaluated. The extent and severity of the lesions decreased after administration of soybean sterols in cholesterol-fed

Table 1. An overview of the mechanisms contributing to the cholesterol-lowering activity of plant sterols/stanols from past to present

Era	Target	Proposed mechanism
The early days: the 1950s	Intestinal cholesterol absorption	No suggested mechanism
The mixed micelle era: the 1960s	Intestinal cholesterol absorption	Competition for incorporation into mixed micelles
The cellular era: the 1960s and 1970s	Intestinal cholesterol absorption	Competition for incorporation into chylomicrons
The transporter era: >2000	Intestinal cholesterol absorption	Transporters: NPC1L1-ABCG5/ABCG8–ABCA1 Activation of LXR target genes
The era of new discoveries: >2006	Cholesterol excretion	TICE

chickens. The observation that soybean sterols lowered the serum cholesterol concentration was confirmed in other species by Pollak and co-workers. For this, rabbits were fed a diet with cholesterol, sitosterol, or a mixture of both in different proportions. Clear inhibition of hypercholesterolemia and prevention of atherosclerosis was achieved by feeding the proper amount of plant sterols. In rabbits, sixfold excess of sitosterol over cholesterol was needed, whereas threefold excess was effective in chickens [21,22]. Already in these early days, the hypocholesterolemic effect of plant sterols was confirmed in patients [23]. However, the underlying mechanism was completely unknown, but was thought to be related to effects on intestinal cholesterol absorption (Table 1 and Fig. 1A) [22,23].

2.2 The mixed micelle era

Intestinal luminal cholesterol consists of two distinct pools derived from, respectively, endogenous and exogenous cholesterol. The contribution of these two pools to the amounts of cholesterol available for uptake and consequent appearance in serum is not equal. Sklan et al. [24] showed in chickens that endogenous cholesterol is more rapidly and more completely absorbed as compared to exogenous cholesterol. When chickens were fed a cholesterol-free, low-fat diet, the duodenum and the upper part of the jejunum are the main sites of cholesterol absorption. Addition of cholesterol into the diet resulted in a distal shift of the predominant site of cholesterol absorption toward the jejunum. Moreover, this shift was accompanied by an increased secretion of endogenous cholesterol as well as bile acids into the duodenum [25]. In contrast to endogenous cholesterol, which is mainly secreted through the bile already in micelles, dietary cholesterol must first be cleaved by specific esterases. Dietary cholesterol is predominantly present in its esterified form and only free cholesterol is incorporated into the mixed micelles to become available for absorption [24]. Altogether, these findings may contribute to the preferential absorption of endogenous over exogenous cholesterol.

The question is how plant sterol and stanol esters interfere with intestinal cholesterol uptake and whether there is a difference between the effects of plant sterols and stanols on endogenous and exogenous cholesterol absorption. As cholesterol and plant sterols/stanols are practically water insoluble, they have to be solubilized into micelles before absorption can occur. However, the capacity of micelles to solubilize lipophylic water-insoluble molecules is limited. During the 1960s of the previous century [26], it became more or less generally accepted that plant sterols and stanols competed with dietary cholesterol for incorporation into mixed micelles (Fig. 1B). As plant sterols/stanols are more hydrophobic than cholesterol, it was speculated that they displaced cholesterol from the mixed micelles [27] or in other words, plant sterols/stanols lowered the solubility of cholesterol within the mixed micelles [28,29]. More into detail, Armstrong and Carey et al. [30] have suggested that noncholesterol sterols are less easily dissociated from mixed micelles, thereby limiting the micellar solubilization of cholesterol. This could be explained by the increased hydrophobicity of plant sterols/stanols compared with cholesterol, resulting in a lower solubility but a higher affinity for micelles. This micelle concept was elegantly shown by Ikeda et al. [27]. In that study, rats were fed a diet containing 0.5% cholesterol alone or 0.5% cholesterol plus an equal amount of sitosterol or sitostanol for 10 days, directly followed by analysis of the composition of the intestinal aqueous micellar phase. Compared with rats fed cholesterol alone, the solubility of cholesterol in the aqueous micellar phase was 24% lower for the rats fed cholesterol plus sitosterol and 53% for those fed cholesterol plus sitostanol. The difference between sitosterol and sitostanol was not statistically significant. There was also no difference between sitosterol and sitostanol in the in vitro experiments. Following experiments—still focusing on micellar composition—tried to unravel whether plant sterols and stanols were equally effective or not. It was found that the recovery of sitostanol in the feces was almost complete, whereas the recovery of sitosterol ranged between 85 and 92% [31]. In agreement, Hassan and Rampone [32] showed that only 2% of sitostanol was found in the lymph of Sprague-Dawley rats compared with 36% for cholesterol, reflecting the poor absorption of sitostanol. They proposed an inverse relationship between the intestinal absorption of plant sterols/stanols and their ability to inhibit cholesterol absorption. To explain the potentially higher efficacy of plant stanols on cholesterol absorption, Heinemann and colleagues [33] suggested that hydrogenation enhanced the hydrophobicity, resulting in a higher affinity for binding

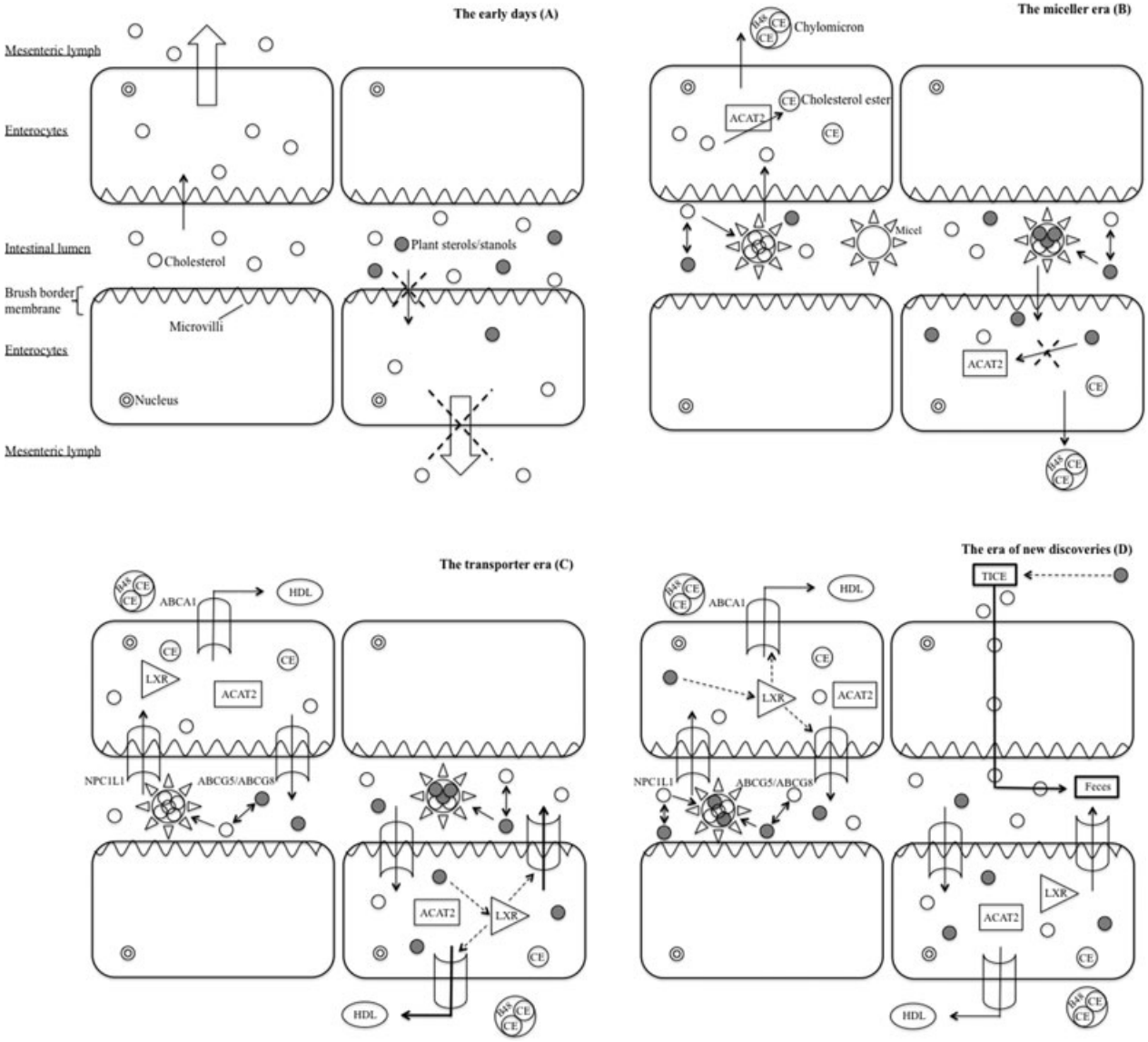

Figure 1. An overview of the paradigms explaining the cholesterol-lowering activity of plant sterols and stanols over the past decades. (A) *The era of the observation*: cholesterol, plant sterols, and stanols are taken up into the enterocyte. It was already suggested in 1951 that plant sterols suppress intestinal cholesterol absorption, resulting in decreased serum cholesterol concentrations. (B) *The micellar era*: there is a competition between cholesterol and the plant sterols/stanols for incorporation into mixed micelles, which is a crucial step for cholesterol absorption. If plant sterols/stanols replace micellar cholesterol, less cholesterol will be taken up into the enterocyte. After uptake, cholesterol is normally esterified by intestinal ACAT-2. The so-formed cholesteryl esters are incorporated into chylomicrons and secreted into the lymph. In contrast, plant sterols/stanols are poor substrates for ACAT-2 and remain in their free form inside the enterocyte. (C) *The transporter era*: different sterol transporters such as ABCG5/ABCG8 and NPC1L1 and their regulatory mechanisms are discovered. It is questioned whether plant sterols and stanols interact with intracellular cholesterol sensors such as LXR, leading to an increased expression of ABCG5/ABCG8 and ABCA1. The latter transports sterols to a nascent HDL particle, whereas ABCG5/ABCG8 promotes the efflux of sterols back into the intestinal lumen, resulting in decreased cholesterol absorption. At the same time, possible regulation of NPC1L1 by plant sterols/stanols is proposed. (D) *The era of new discoveries*: recently, transintestinal cholesterol excretion (TICE) has been suggested as a possible target for the plant sterol/stanol mediated cholesterol-lowering effect. Stimulation of TICE increases fecal neutral sterol loss. However, further research is needed to explore the effects of the plant sterols/stanols on the intestinal cholesterol absorption into more detail. For example, the transporters responsible for basolateral and apical cholesterol secretion need to be identified. It is also debated whether TICE alone or possibly together with other mechanisms described in panels B and C explain the full cholesterol lowering effect of plant sterols and stanols.

Abbreviations: ABCA1: adenosine triphosphate (ATP) binding cassette A1 transporter; ABCG5/ABCG8: ATP-binding cassette G5 and G8 transporter; ACAT-2: acylcoenzyme A cholesterol acyltransferase 2; CE: cholesteryl esters; CM: chylomicron; HDL: high-density lipoprotein; LXR: liver X receptor; NPC1L1: Nieman-pick C1 like 1; TICE: transintestinal cholesterol excretion.

to cholic acid micelles, and as a consequence a more effective displacement of cholesterol from the micelles and a more pronounced reduction in the cholesterol absorption. In their in vivo studies, they compared the intestinal cholesterol absorption in humans after infusion of a high dose of sitosterol or sitostanol dissolved in monoleate. Sitosterol significantly reduced the intestinal cholesterol absorption by almost 50%, and sitostanol by almost 85%. Thus, there is a vast majority of evidence showing that plant sterols and stanols lower the incorporation of cholesterol in mixed micelles and as such the amount of cholesterol available for absorption (Table 1 and Fig. 2B–2E).

This proposed mechanism–i.e. interfering with micellar cholesterol incorporation–suggests that plant sterols and stanols have to be consumed simultaneously with dietary cholesterol to achieve a maximal cholesterol lowering effect. However, in 2000, Plat and colleagues [19] showed that a daily consumption of 2.5 g plant stanols as their fatty acid esters once per day at lunch was as effective as an equal total dose of 2.5 g but now divided over three meals. They hypothesized that the plant stanols remained in the intestinal lumen or even within the enterocyte after consumption. It should be realized that this hypothesis was proposed before identification of transporters such as Niemann-Pick C1 like 1 protein (NPC1L1). In agreement, Weststrate and Meijer [34] found that consumption of plant sterols at lunch and dinner only decreased LDL-C to the same extent as in studies that provided the plant sterols three times daily. Later, many more studies using the "once a day" protocol indeed found serum LDL-C reductions in line with predicted changes [35, 36]. This finding of "once a day efficacy" clearly questioned the mechanisms underlying the reduced intestinal cholesterol absorption. Effects could no longer solely be explained by a reduced incorporation of cholesterol into mixed micelles. However, not all studies using the "**once a day" approach were successful. It should be noticed that the oil phase is crucial for the formation of mixed micelles, which subsequently transport the emulsified food components toward the enterocytes via the aqueous micellar phase (Fig. 2). Therefore, it is of utmost importance that the ingested foods induce bile flow and release of pancreatic lipases. This could explain why Abumweis et al. [16] did not find a reduction in serum LDL-C concentrations after a single consumption of plant sterols/stanols before or with breakfast. However, results of this subgroup analysis should be interpreted with caution since the number of subjects included was small. Doornbos and colleagues [37] included 186 subjects to evaluate the impact of time of intake of plant sterol-enriched (±3 g/day) single-dose yoghurt drinks. The drinks, which were different in total fat content (2.2 versus 3.3%), were consumed at least half an hour before breakfast or after lunch. They concluded that the total cholesterol and LDL-C concentrations were significantly reduced in both conditions, independent of the fat content of the drinks. A significantly larger reduction, however, was observed when the drinks were consumed with or immediately after lunch, suggesting that a fed state is necessary for an optimal cholesterol-lowering activity. As suggested by Doornbos et al. [37], not only the amount of fat, but also the protein content of a meal could be important, since both trigger the release of cholecystokinin after a meal, thereby causing secretion of bile, a necessary step in the formation of mixed micelles.

A crucial factor that has been addressed in only a few studies in the mixed micelle era, is the physical state of the plant sterols and stanols. The physical state may influence the partitioning of plant sterols and cholesterol over the different phases in the intestinal lumen (Fig. 2). Grundy and colleagues [38] showed that the inhibition of intestinal cholesterol absorption by plant sterols was augmented if the plant sterols were administrated as a micellar solution (as used in perfusion studies) as compared to administration of plant sterols in suspension (as in almost all dietary studies). The importance of the physical state was further substantiated by Lees et al. [28], who fed hypercholesterolemic patients 3 g/day of two different sitosterol preparations (either suspension or powder) from tall oil. Serum cholesterol concentrations were reduced in both conditions, but the decrease was more pronounced after administration of the powdered tall oil sterols (12%) as compared to the tall oil in suspension (7%). Ostlund and colleagues [39] agreed that the efficacy of plant sterols/stanols depends on the form in which they are presented. Administration of 1 g pure sitostanol powder had no significant effect on cholesterol absorption, whereas 700, 300, and even 100 mg sitostanol packaged in lecithin vesicles reduced intestinal cholesterol absorption as compared with a placebo by 37, 35, and 6%, respectively (Fig. 2E). These findings can be explained by the fact that sterols form stable crystals, which are solid solutions characterized by an extremely low bioaccessibility. Therefore, a powder forms hardly any micelle making this form almost ineffective.

Besides the physical state, it was also considered important whether plant sterols and stanols were provided as free sterols/stanols or as sterol/stanol esters. Mattson et al. [40] reported a 9% larger reduction in intestinal cholesterol absorption when the subjects ingested plant sterols in the free form as compared with the sterol esters. It was suggested that the ester bond was not completely hydrolyzed by the bile acid-activated pancreatic cholesterol hydrolase in the intestinal lumen. Since plant sterol and stanol esters solubilize poorly into the micellar phase, the major part accumulates in the oil phase. This agrees with the earlier mentioned observations that plant sterol esters, and also cholesterol esters in the oil phase, are less effectively absorbed into the enterocytes [41]. More recently, Kobayashi and co-workers [42] compared the cholesterol-lowering activity of free and esterified plant sterols side-by-side in Sprague-Dawley male rats. After feeding a commercial chow for 1 week, a catheter was placed in the stomach for administration of the test emulsions containing cholesterol without plant sterols, cholesterol with unesterified plant sterols or with plant sterol oleates. The lymphatic 24 h recovery of radiolabeled cholesterol was significantly lower in rats fed the free plant sterols than in those receiving the

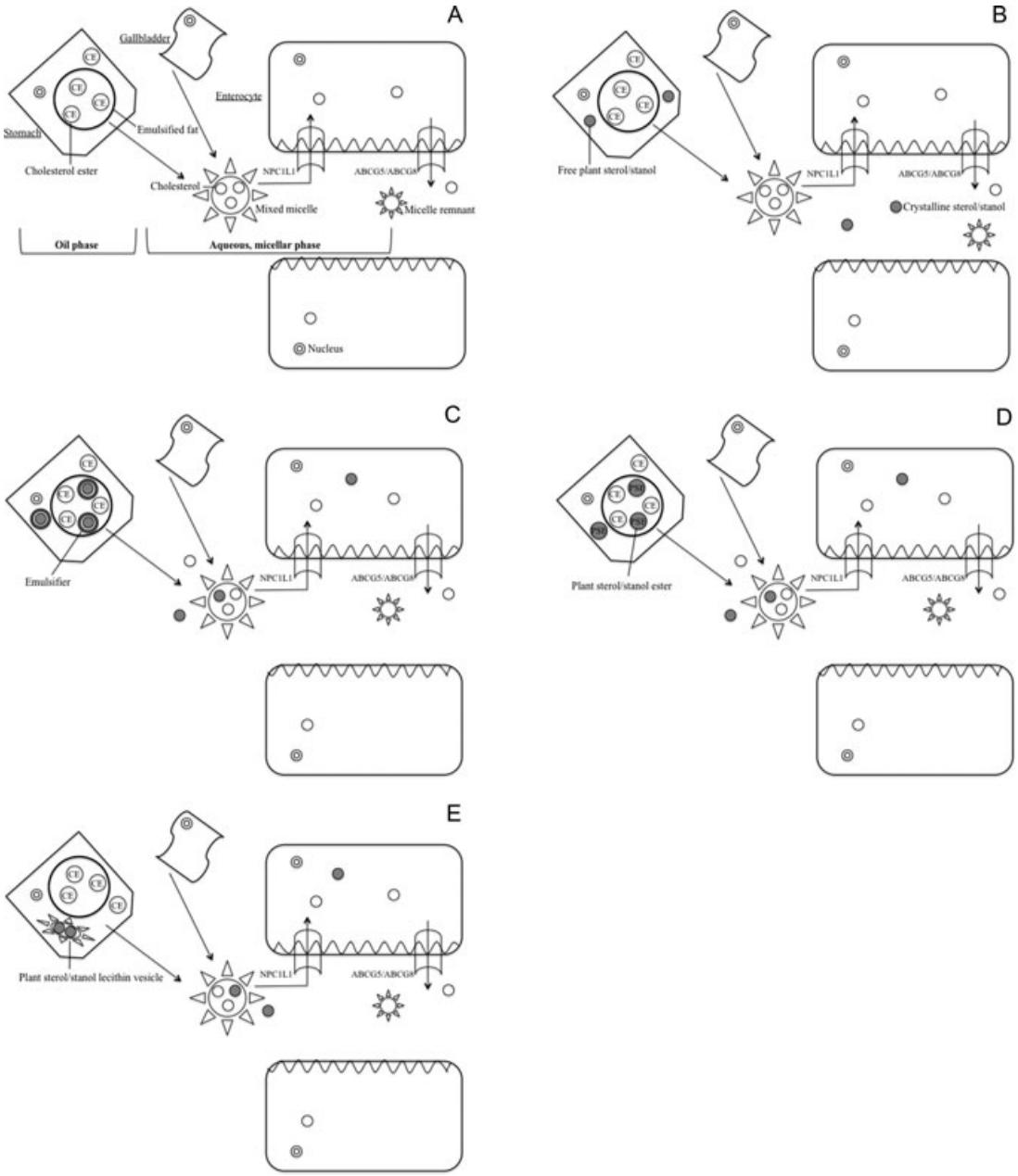

Figure 2. A representation of the crucial role of micelles in the process of cholesterol absorption.
The digestion of food-derived fats is initiated in the stomach by gastric lipase, leading to the formation of crude emulsions, which are further hydrolyzed by pancreatic lipase and cholesterol esterase in the small intestine. Cholesterol as well as plant sterols and stanols have to be incorporated into mixed micelles before absorption can occur.
(A) Mixed micelles are formed on the surface of the emulsified fat droplets as a combined action of bile acids and pancreatic enzymes. They transport free cholesterol through the micellar phase into the enterocyte. (B) Free plant sterols/stanols are not solubilized in the emulsified fat of the food digesta and pass the small intestine as crystalline sterols. In other words, they are unable to compete with cholesterol for incorporation into mixed micelles. (C) Therefore, free plant sterols/stanols need to be made "bioavailable" to the oil phase before competition can occur. This can be realized by the use of an emulsifier. (D) After consumption of plant sterol/stanol esters, the esters are hydrolyzed by pancreatic cholesterol esterase in the small intestine. Again, the free form will compete with cholesterol for incorporation into mixed micelles, thereby reducing intestinal cholesterol absorption. (E) Another possibility to increase the solubility of plant sterols/stanols is the formation of micellar solutions with lecithin. In contrast to the esterified form, which first has to dissolve in dietary fat for entry into the oil phase and next equilibrate with the micellar phase, the use of lecithin micelles allows a direct delivery of plant sterols/stanols into the intestinal micellar phase.
Abbreviations: ABCG5/ABCG8: adenosine-triphosphate (ATP) binding cassette G5 and G8 transporter; CE: cholesteryl esters; NPC1L1: Nieman-pick C1 like 1.

control or the plant sterol oleates at 3 h after administration. However, when it was repeated after incorporating the different sterols into the feed, no significant differences were observed. They suggested that administration of cholesterol and plant sterol oleates as an emulsion into the stomach resulted in a rapid accumulation of plant sterol oleates in the duodenum. The presence of a large amount of the esters in the intestinal lumen might induce a delay in the hydrolysis of the plant sterol oleates, causing a less effective reduction in the cholesterol absorption. Addition of plant sterol oleates to the diet did not lead to an excessive accumulation of the compounds into the duodenum. These studies illustrate the importance of optimal cleavage of the ester bound, thereby releasing free sterols or stanols for the micellar phase.

What do we know about the efficacy of the esterase enzymes in vivo? Miettinen et al. [43] quantified the hydrolysis of 2 g/day of plant stanols in 11 colectomized patients fed plant stanol esters for 1 week and observed that 95% of cholesterol and 90% of plant sterols/stanols were in the free form. In agreement, Normen and co-workers [44] performed a study in seven ileostomy subjects receiving 2.5 g/day of plant sterol or stanol esters. The proportion of the esterified forms of the plant sterols and stanols were 12.6 and 15.5%, respectively. This implicates that the major part of the plant sterols/stanols is hydrolyzed in the small intestine. In fact, almost 50% of the esters are hydrolyzed in the lower duodenum. The findings of Kobayashi and co-workers [42] can also be explained by the activity of lingual lipase, present in the serous (von Ebner) glands of the tongue and by the activity of gastric lipase [45]. It could be that these lipases already hydrolyze a part of the plant sterol esters when added to the diet, an effect that may be less when the test emulsion is given intragastrically.

Although free plant sterols and stanols may at least be as effective as the esterified forms, mainly the plant sterol/stanol esters are used for incorporation into the functional foods due to their higher solubility in oils. However, only the free form of the sterols and the stanols participate into the emulsified fat phase, causing a reduction in the intestinal cholesterol absorption. For this, optimal esterase activity is required (Fig. 2). Unfortunately, not many human studies have compared the cholesterol-lowering effects of free and esterified plant sterols and stanols. Richelle and colleagues [46] found no significant differences in the reduction of cholesterol absorption (±60%) in normocholesterolemic subjects receiving 2.2 g plant sterols either free or esterified for seven consecutive days. It should be noted that not solely the sterols were incorporated into the foods, but that sorbitan tristearate was added to the free form as an emulsifier (Fig. 2C). Due to the emulsifier, the free plant sterols/stanols could more easily interact with the emulsified fat phase making them as efficient as the esterified form. Regarding free sitostanol, Ostlund et al. [39] have described another procedure to facilitate partitioning into the emulsified fat phase. Free sitostanol was administered as part of lecithin micelles, which also lowered cholesterol absorption very efficiently (Fig. 2E). They reported that the effective dose of free sitostanol, when incorporated into lecithin micelles, was between 100 and 300 mg. In later studies [47], it was found that 1.8–1.9 g/day plant stanols in lecithin micelles reduced LDL-C to the same extent as has been reported for plant stanol esters at the same daily intake. In a more recent study, Soderholm et al. [48] showed that free plant sterols incorporated into a rye bread significantly lowered serum LDL-C concentrations. The rye bread was enriched with 2 g/day of free plant sterols. Before adding to the dough, the plant sterols were micronized in order to increase the bioavailability in the oil phase.

In summary, if free sterols or stanols are provided without facilitating its solubilization into the oil phase, they will be poorly incorporated into the mixed micelles and have limited cholesterol-lowering activity (Fig. 2B). For the enhancement of free sterols and stanols into the emulsified fat phase, several procedures have been presented (Fig. 2C–E). Ultimately, the free forms–for sterol esters and stanol esters after cleavage by the esterases–will compete with cholesterol for incorporation into mixed micelles, thereby reducing intestinal cholesterol absorption.

2.3 The cellular era

Although displacement of cholesterol from mixed micelles in the intestinal lumen seemed to be an important mechanism of plant sterol- and stanol-induced inhibition of intestinal cholesterol absorption, several other mechanisms involving actively regulated processes have been suggested. Inhibition of cholesterol transport into the brush border membranes is one example, although many of the earlier textbooks mentioned that this uptake was driven by passive diffusion. However, already in 1957, Glover and Green [49] published that the brush border contains a specific binding site for cholesterol, making passive diffusion as the main driver for cholesterol uptake less likely. Similar results were found by Ikeda et al. [50], who confirmed the existence of an independent binding site for cholesterol and sitosterol in an isolated brush border at low micellar concentrations. Cholesterol-binding approached saturation at higher concentrations, which could not be observed for sitosterol [51]. Based on these results, it was concluded that competition at the brush border membrane had almost no influence on the plant sterols and stanols-mediated cholesterol-lowering activity.

It has also been suggested that plant sterols and stanols interfere with the incorporation of cholesterol into chylomicrons. Before incorporation into a chylomicron, free sterols are (re)esterified by ACAT-2. Newly synthesized apoB-48 and triacylglycerol (TAG) accumulate together with cholesterol esters in the smooth endoplasmatic reticulum membrane followed by an MTP protein-dependent formation of chylomicrons [52]. In vitro as well as in vivo studies have clearly indicated that mucosal ACAT is a rate-controlling enzyme in the absorption of cholesterol. Kam et al. [53] incubated colorectal adenocarcinoma (Caco2) cells, a frequently used in vitro model for absorption studies, for 1 h with

increasing concentrations of 58–035, a specific inhibitor of ACAT. The inhibitor caused a dose-dependent decrease in cholesteryl ester synthesis, reaching a maximal effect at 15 μg/mL. After 24 h, there were no measurable amounts of cholesteryl esters left in the chylomicron and very LDL (VLDL) particles isolated from these Caco2 cells. In agreement, Clark and colleagues [54] observed a reduced ACAT activity, if jejunal microsomes were incubated with 0.6 μg/mL of 58–035. In vivo, they investigated also the absorption of cholesterol in mesenteric lymph fistula of Sprague-Dawley rats after ACAT inhibition and observed a reduction in cholesteryl esters in lymph, lymph chylomicrons, and lymph VLDL, whereas the amount of unesterified cholesterol was increased. These results support a major regulatory role for ACAT in cholesterol absorption. It has been suggested that plant sterols interfere with the esterification inside the enterocyte, the first of the final two crucial steps in the process of cholesterol absorption [28]. Since plant sterols and stanols are poor substrates for ACAT-2, they could bind the available sites, thereby decreasing its activity by competitive inhibition (Fig. 1). Field and colleagues [55] indeed observed a decrease in ACAT activity in rabbits after feeding β-sitosterol. In contrast, if they collected intestinal microsomes from rabbits on chow diet and enriched them with β-sitosterol, they could not observe any effect on ACAT activity during the 4 h of measurement. However, some years later, the same group reported differences in the ACAT activity in Caco2 cells incubated with micelles containing cholesterol alone or cholesterol plus β-sitosterol [56]. Addition of cholesterol to the Caco2 cells in a micellar solution increased the basolateral secretion of cholesteryl esters derived from the plasma membrane cholesterol. In other words, micellar cholesterol displaces the cholesterol from the plasma membrane to the endoplasmatic reticulum, which is than used for chylomicron assembly and secretion. If, however, the same amount of β-sitosterol was added together with cholesterol, the movement of cholesterol from the plasma membrane and the subsequent secretion of cholesteryl esters were significantly reduced. This can be attributed to the displacement of cholesterol from the micelles by β-sitosterol. The reduced ACAT activity could be explained by the diminished trafficking of cholesterol from the plasma membrane to the endoplasmatic reticulum, as ACAT activity may be regulated by substrate supply [57]. Moreover, it has been shown again in Caco2 cells that HMG-CoA reductase activity is decreased when sitosterol was added despite a reduction in intracellular cholesterol concentration [56]. This indicates that HMG-CoA reductase cannot discriminate between cholesterol and plant sterols, which even further lower intracellular cholesterol pools. Thus, direct effects on ACAT activity are not a likely explanation for the plant sterol/stanol mediated effects on cholesterol absorption. After esterification, the cholesteryl esters are packaged into chylomicrons, a process in which MTP plays a crucial role. It was recently shown in male Golden Syrian hamsters that the cholesterol-lowering activity of sitosterol was associated with a decrease in the mRNA level of MTP [58]. This implies that plant sterols/stanols could also have an effect on MTP expression, which has to be further elucidated. In line with this assumption, Rideout et al. [59] recently showed that plant sterol feeding lowered intestinal fat absorption in C57BL/6J mice, which could also be related to a reduced chylomicron formation involving effects on MTP.

2.4 The transporter era

More recently, Davis et al. [60] have described the crucial role of NPC1L1 in the intestinal uptake and absorption of cholesterol and the plant sterols/stanols. NPC1L1-deficient mice were characterized by a reduction in cholesterol absorption of almost 90%. Moreover, also serum campesterol and sitosterol concentrations were reduced by ±90% in these mice as compared with the wild-type mice. These results showed that NPC1L1 plays an important role in the uptake of both cholesterol and plant sterols, indicating that cholesterol and plant sterol absorption was not merely due to passive diffusion (Table 1 and Fig. 1C). The annexin2/caveolin1 (ANXA2/CAV1) complexes can also play a role in the plant sterol/stanol mediated cholesterol-lowering activity. ANXA2 forms a lipid–protein complex with CAV1 and cholesteryl esters, which may be involved in the internalization/endocytic transport of cholesterol esters from caveolae to internal membranes in lipid rafts of the intestinal brush border [61]. Smart et al. [62] have demonstrated that ANXA2 could be down regulated by plant sterols, thereby reducing cholesterol processing and transport. The significance of this complex for cholesterol absorption however is unclear, since Valasek et al. [63] have shown that the fractional cholesterol absorption and fecal neutral sterol excretion are similar in CAV1 knockout mice and their wild-type littermates. Besides cholesterol influx, there is also active secretion of cholesterol and plant sterols from enterocytes back into the intestinal lumen. In this process, ABCG5 and ABCG8, two half-transporters localized on the apical membrane of the enterocytes play a crucial role [64]. They function together as a heterodimer and mediate the efflux of free sterols from the enterocytes [13]. Theoretically, as a consequence of increased ABCG5/ABCG8 activity, less sterols will be available for esterification and incorporation into chylomicrons and as such intestinal sterol absorption will be reduced. The ABCG5/ABCG8 transporters are regulated via the liver X receptor (LXR) and numerous attempts have been made to use LXR agonists to influence cholesterol metabolism, i.e. elevate reverse cholesterol transport (RCT) pathways. However, systemic LXR activation causes increased hepatic fatty acid synthesis [65] and steatosis [66]. Therefore, tissue-specific approaches have been initiated. Indeed, Lo Sasso et al. [67] recently showed in an elegant series of experiments that intestine-specific LXR activation increased RCT and lowered intestinal cholesterol absorption. As expected, intestinal ABCG5/ABCG8 expression was increased and fecal neutral sterol excretion enhanced. The question now is whether plant sterols and stanols influence the

expression or activity of these crucial transporter proteins within the enterocytes.

Yamanashi et al. [68] studied the role of NPC1L1 using differentiated Caco2 cells as a model for small intestinal epithelial cells. In Caco2 cells overexpressing NPC1L1, the absorption of sitosterol was higher as compared to nontransfected cells. However, sitosterol absorption remained significantly lower as compared to cholesterol absorption. More recently, Zhang et al. [69] showed that cholesterol binds to the luminal N-terminal domain (NTD) of the NPC1L1 protein and that this specific binding is required for the uptake of cholesterol from the intestinal lumen into the enterocyte. Plant sterols cannot bind to NPC1L1-NTD, which may contribute to the selective cholesterol absorption in mammals. So, based on these cell and animal studies, it seems that NPC1L1 is not involved in the decreased cholesterol absorption after plant sterol or stanol intake. It is therefore interesting to know what will happen when ezetimibe will be combined with plant sterols or stanols in the diet. Jakulj and colleagues [70] examined the effects on serum LDL-C concentration in mildly hypercholesterolemic subjects receiving 10 mg/day of ezetimibe with or without 2 g/day of plant sterols for 4 weeks. Combined treatment of plant sterols and ezetimibe did not further reduce the serum LDL-C concentration compared with ezetimibe monotherapy, i.e. serum LDL-C reductions were 25 and 22%, respectively. One can argue that plant sterols and ezetimibe targeted the same transporter (in this case NPC1L1). Alternatively, it can be hypothesized that ezetimibe blocks NPC1L1, which results in a lower cellular uptake of plant sterols into the enterocytes. As indicated in the following sections, there are indications that plant sterols should be available intracellular to activate cellular processes that contribute to the lowered intestinal cholesterol uptake. Therefore, the lack of an additive effect of the plant sterol-ezetimibe combination could be explained by the reduced intracellular plant sterol concentration due to the ezetimibe-mediated NPC1L1 inhibition. Whatever reason, both are suggestive for the fact that NPC1L1 itself is not involved in the working mechanisms of plant sterols. In contrast to the findings of Jakulj et al. [70], Lin and colleagues [71] very recently showed that adding plant sterols to ezetimibe resulted in a further reduction of cholesterol absorption and a significantly increased fecal cholesterol excretion. This outcome was explained by the authors as an indication that the mechanism by which plant sterols lower intestinal cholesterol absorption is independent of that of ezetimibe. However, more specifically for this purpose designed studies are needed to examine whether NPC1L1 plays a role in the underlying mechanism of plant sterols/stanols.

Also regarding changes in the activity of the ABC transporters and other LXR target genes involved in (intestinal) lipid metabolism, several studies can be addressed. The oxysterol-activated receptor LXR regulates the expression of a panel of genes amongst which NPC1L1 [72], ABCA1, ABCG5, and ABCG8 [73]. As mentioned above, LXR activation results in an increase in the fecal neutral sterol loss and a decrease in the intestinal sterol absorption (Fig. 1C). It has been suggested that LXR—in line with the sterol regulatory element binding protein-2 (SREBP-2)-–detects changes in intracellular cholesterol concentrations. In case of high intracellular cholesterol concentrations, LXR is activated to prevent via expression of its target genes a further accumulation of cellular cholesterol [74]. It has been suggested that plant sterols and stanols also activate LXR either directly or indirectly after conversion into yet unkown metabolites. Indeed, using a cell-free ligand-sensing assay (LiSA), Plat et al. [75] showed that nonoxidized plant sterols and stanols were potent activators of LXR. Moreover, they showed an increase in ABCA1 mRNA expression if Caco2 cells were cultured in the presence of mixed micelles enriched with plant sterols and stanols. Unfortunately, it was not possible to measure the expression pattern of ABCG5 and ABCG8, since the mRNA level of both genes was undetectable in these Caco2 cells. It could however be speculated that plant sterols/stanols can be regarded as local LXR agonists acting only in enterocytes. Physiological intracellular plant sterol concentrations in the enterocytes can indeed reach levels above EC50 concentrations necessary for LXR activation, which seems unlikely in hepatocytes due to the low absorption of plant sterols. The fact that plant sterols and stanols could act as local LXR agonists—and not systemically—also fits with recent observations that plant stanols [76–78] and sterols lower serum TAG concentrations instead of increasing TAG concentrations, as observed for systemic LXR agonists [65]. Systemic LXR agonists induce hepatic lipogenesis, which results in elevated serum TAGs. Plösch and colleagues [64], however, have suggested that plant sterols and stanols lower intestinal cholesterol absorption independently of LXR activation. They fed C57BL/6 mice a diet free of sterols, enriched with cholesterol, or enriched with cholesterol and either plant sterols or stanols for 4 weeks. Addition of plant sterols or stanols to the diet resulted in the expected increase in the fecal neutral sterol excretion. However, gene expression profiles of known LXR target genes were not changed. Moreover, Calpe-Berdiel et al. [79] observed no effects on the intestinal expression of LXR target genes in mice fed a western-type diet enriched with or without plant sterols. Finally, plant sterols were still effective in lowering intestinal cholesterol absorption in ABCG5 knockout mice, illustrating that these transporters are not obligatory to show effects [80]. Despite these inconsistent results regarding the role of the ABC transporters, we still cannot exclude a possible role for plant sterols and stanols on the activation of yet unknown LXR target genes. Therefore, it is too early to exclude LXR as a mediator of the plant sterol/stanol induced effects on intestinal cholesterol absorption.

2.5 The era of new discoveries

The most recent and probably most provocative explanation for the effects of plant sterols/stanols on intestinal cholesterol metabolism is related to the process called transintestinal cholesterol excretion (TICE). Until recently, the RCT route,

which is hepatobiliary cholesterol secretion mediated by hepatic ABCG5/ABCG8, was thought to be the most important route responsible for the disposal of cholesterol. However, disruption of biliary cholesterol secretion in mice had no effect on the fecal neutral sterol excretion [81, 82]. This finding suggested that the hepatobiliary cholesterol secretion might not be the only route for cholesterol excretion into the intestinal lumen. In this respect, van der Velde et al. [83] have demonstrated that cholesterol is secreted throughout the entire length of the small intestine, but most actively in the proximal part. They performed intestinal perfusion studies in mice under bile-diverted conditions and reported that intravenously injected radiolabeled cholesterol ends up in the intestinal perfusate. The direct cholesterol flow from blood into the intestinal lumen is further supported by the results of Brown and Goldstein [84]. They observed in mice with a targeted deletion of hepatic ACAT-2 a twofold increase in the fecal neutral sterol excretion. However, this increased fecal sterol loss occurred without an increase in biliary cholesterol secretion. In contrast, a trend toward a reduction in the cholesterol concentration in the gallbladder bile was observed as compared with the controls. In addition, in line with the observations of van der Velde and colleagues [83], they also showed that intestinal cholesterol secretion was most pronounced in the proximal part of the small intestine. Altogether, these findings indicate that there must be a direct transport of cholesterol from the circulation into the intestinal lumen. This so-called transintestinal cholesterol excretion (TICE) pathway could in theory also be an explanation for the cholesterol-lowering activity of the plant sterols and stanols (Table 1 and Fig. 1D). It should be noted that plant sterols/stanols may also compete with TICE-derived cholesterol for incorporation into mixed micelles, thereby decreasing cholesterol absorption. However, the magnitude of this effect may depend on the place where TICE-derived cholesterol enters the intestinal lumen. Based on the mechanisms described in the mixed micelle era, it is expected that the effects diminished the more distal TICE-derived cholesterol enters the intestinal lumen.

Recently, Brufau et al. [85] show a role of plant sterols and stanols in the stimulation of cholesterol excretion via this nonbiliary route. Feeding wild-type mice a plant sterol-enriched diet resulted as expected in an increased fecal neutral sterol excretion, whereas a more moderate increase was observed in ABCG5 knockout mice. Furthermore, the nonbiliary cholesterol excretion was sixfold elevated in the plant sterol group and 3.5-fold in the ABCG5 knockout mice fed plant sterols. It should be mentioned that the transporter protein responsible for cholesterol efflux out of the enterocyte into the lumen—and as such part of the TICE route—is currently unknown. Although it is tempting to speculate that this process is at least partly mediated by ABCG5/ABCG8. Brufau et al. [85] found an unexpected decrease in both the mRNA level and the protein expression of this transporter, while TICE was activated. Although the evidence for a role of ABCG5/ABCG8 in TICE is not strong, LXR is thought to be one of the key players involved in the regulation of TICE. Treating C57BL/6 mice with T0901317, an LXR agonist, caused a significant increase in TICE [86]. Although the evidence for plant sterols and stanols to act as a ligand for LXR is contradictory (see transporter era), this novel pathway does suggest that intestinal LXR activation is needed to explain effects on intestinal cholesterol metabolism. Therefore, intestinal LXR activation in response to plant sterol/stanol consumption needs to be studied into far more detail and especially the search for currently unknown and therefore not yet analyzed LXR target genes needs attention. In this respect, Sehayek et al. [87] reported already in 2002 that specific loci on chromosomes 2 and 14 distinct from ABCG5/ABCG8 regulate plasma plant sterol concentrations in mice.

A remarkable observation that also needs attention was the absence of a dose–response effect of plant sterol consumption on the nonbiliary cholesterol excretion in mice. The lowest supplementation of the plant sterol (1%) resulted already in a maximal stimulation of TICE [85]. This can only be explained by acknowledging that plant sterols and stanols not only activate TICE and as such increase fecal neutral sterol excretion but at the same time must also lower absorption of intestinal cholesterol. The combination of both processes is the observed net effect. Therefore, in future studies, it is a challenge to quantify the postprandial appearance of cholesterol in the chylomicron fraction and at the same time quantify the changes in TICE, to ensure which part of the increase in the fecal neutral sterol loss is due to an increased TICE and to a decreased incorporation of cholesterol into chylomicrons. Up till now, the contribution of TICE in humans has not been described. A better understanding of the process of TICE itself as well as exploring possibilities to activate TICE seems, however, an attractive approach for the prevention and even the treatment of CVDs.

Given the above overview, we conclude that impaired micellar solubilization of intestinal cholesterol is the only unequivocally established effect of the plant sterols/stanols. The so-called cellular and transporter eras have provided many interesting observations, but results are not consistent. However, the fact that both the mixed micelle era, nor the cellular or the transporter eras can fully explain all observations, the exact molecular mechanisms behind the cholesterol-lowering activity of plant sterols/stanols is likely a complex interplay of multiple processes.

2.6 Clinical benefit

Independent from the mechanism underlying the serum LDL-C lowering effects, an important issue often raised is the question "what is the evidence that we benefit from plant sterol or stanol consumption in terms of cardiovascular risk." Several observations suggest that plant sterols and stanols not only lower serum LDL-C concentrations, but also ultimately improve endothelial dysfunction [88, 89]. Up till now, it is unknown whether plant sterols and stanols exert these

effects by a direct or an indirect effect. Direct effects assume a functional effect of the plant sterols/stanols themselves on the vessel wall. For this route, there is hardly any evidence. Indirect effects mean that the reduced CVD risk is explained through effects on LDL-C.

In animals, it is easy to evaluate whether dietary interventions affect lesion development, which is of course in humans more difficult. However, endothelial dysfunction is a reflection of an early, but reversible stage in the development of atherosclerosis, and the presence of endothelial dysfunction is considered to be a preclinical marker of CVD [90]. Here, we will provide a short overview of controlled intervention studies evaluating effects of plant sterols/stanols on endothelial function and/or possible atherosclerotic lesion characteristics in suitable animal models and humans.

Ntanios et al. [91] fed 24 male New Zealand White rabbits a diet rich in cholesterol or in cholesterol with one of the three 1% (w/w) plant sterol mixtures derived from soybean containing 0.01% (w/w) plant stanols, tall oil containing 0.2% (w/w) plant stanols, or tall oil containing 0.8% (w/w) plant stanols. In rabbits fed, the 0.8% (w/w) plant stanols, serum total cholesterol, LDL-C, and VLDL cholesterol (VLDL-C) concentrations were reduced by 49, 37, and 63%, respectively, as compared to the control group. Moreover, lesion developments in the ascending aorta and coronary arteries were substantially reduced as compared to the control group. There was no significant difference in plaque formation between the 0.01, 0.2% (w/w) plant stanol and the control group. The observation that plant stanols can indeed lower lesion formation agreed with a study by Plat et al. [92], who demonstrated that plant sterol or stanol consumption lowered atherosclerotic lesion development in heterozygous LDL receptor$^{+/-}$ mice to the same extent despite opposite changes in serum plant sterol and stanol concentrations. These findings suggest that changes in serum plant sterols or stanols themselves do not directly contribute to plaque development in these mice. Volger and colleagues [93] also evaluated the association between the reduction in serum cholesterol concentration and atherosclerotic lesions. They fed apoE*3-Leiden transgenic mice a control diet or the same diet enriched with plant stanol esters for 38 weeks. The cholesterol-lowering activity of the plant stanol esters was more pronounced in the VLDL and intermediate-density lipoprotein fractions than in the LDL fraction (70, 77, and 20% reductions, respectively). As compared to the control group, plant stanol ester feeding significantly reduced the atherosclerotic lesion area and severity. The control mice showed type 2–3 lesions, characterized by regular intimal fatty streaks/mild plaques, whereas the mice receiving plant stanol esters predominantly had type 1 lesions, which consist of individual foam cells. In contrast, Weingartner et al. [94] suggested that administration of plant sterol esters caused a negative vascular effect, independent of the plasma cholesterol concentrations. They fed C57BL/6 wild-type mice a normal chow enriched with 2% (w/w) plant sterol esters for 4 weeks. These mice developed an impaired endothelium-dependent vasorelaxation compared with the wild-type mice on normal chow. A significant larger lesion size after cerebral ischemia was observed in wild-type SV/129 mice treated for 4 weeks with normal chow and 2% (w/w) plant sterol esters compared with the control group. Finally, Weingartner et al. also used ApoE$^{-/-}$ mice as a model of lipid-driven atherogenesis. Mice were fed a Western-type diet or normal chow for 6 months, enriched with 2% (w/w) plant sterol esters, 0.005% (w/w) ezetimibe, a combination of both or without any supplementation. The reduction in atherosclerotic plaque formation was most pronounced in mice treated with ezetimibe and significantly larger than in mice fed the plant sterol esters. The mice treated with ezetimibe and plant sterol ester showed a trend toward greater lesion formation as compared to mice treated with ezetimibe alone. Despite the equal reduction in serum cholesterol concentration, plant sterol ester consumption was associated with twice the amount of plaque formation compared with ezetimibe. However, further studies are needed to confirm the potentially negative effect of plant sterols on atherogenesis in mice. It should also be noticed that the amount of plant sterol ester supplementation in the animal studies, calculated as milligram per day times kilogram body weight, was approximately 100 times higher as the amount incorporated into margarine used in human studies.

One of the most frequently used surrogate markers for measuring endothelium function in humans is flow-mediated vasodilatation (FMD) [90]. Celermajer et al. [95] have clearly shown that FMD is a valuable predictor for future cardiovascular risk. There are only a few studies investigating the effects of plant sterol or stanol consumption on endothelial function. De Jongh et al. [96] evaluated the short-term effect of plant sterols on endothelial dysfunction in heterozygous familial hypercholesterolemic children. Forty-one children between 5 and 12 years of age received 2.3 g plant sterols per day for 4 weeks. As expected, administration of plant sterols resulted in a 14% decrease in serum LDL-C concentrations. However, this was not associated with an improvement of the impaired FMD. Hallikainen and colleagues [97] also showed that a daily intake of 2 g plant sterol or stanol esters for 10 weeks had no effect on the endothelial function as measured by FMD in 76 hypercholesterolemic adults, although serum LDL-C concentrations were reduced by 9–12% as compared to the controls. Also Jakulj et al. [98] evaluated the effect of plant stanols (2 g/day for 4 weeks) on FMD in 42 heterozygous FH children between 7 and 12 years. Serum total cholesterol and LDL-C concentrations were reduced by 7.5 and 9.2%, respectively, and again, improvement on the endothelial function was not observed. Finally, Raitakari et al. [99] evaluated the effect of plant stanol esters on endothelial function and arterial elasticity. The 150 hypercholesterolemic adults received 2 g/day of plant stanol esters for 3 months. Despite the significant 9.3% reduction in the LDL-C concentration between the treated and the control group, they observed again no significant change in FMD or carotid artery compliance. However, a subgroup analysis demonstrated that arterial elasticity and endothelial function improved in subjects with below

average baseline values for these parameters. This is in line with the observation of De Jong et al. [100] who evaluated the long-term effect (85 weeks) of plant sterol or stanol esters on vascular function in patients on statin treatment. No effect in the whole population was observed, but endothelial dysfunction and arterial stiffness were improved in a subgroup of patients at risk for cardiovascular events [99]. This implies that plant sterols and stanols might improve vascular function in subjects with a suboptimal vessel condition [100] and probably more important that a long follow-up period is needed to see protective effects. Although these observations are of great relevance, it does not prove that cardiovascular events are actually reduced. For this, future studies especially designed for this purpose are needed.

2.7 Mechanisms in relation to the clinical benefit

Reducing intestinal cholesterol absorption and/or stimulating TICE, two processes that both result in elevated fecal neutral sterol excretion are currently the two paradigms explaining the LDL-C lowering activity of plant sterols and stanols. Based on all observations described, it seems most likely that—at least in animals—both mechanisms are effective.

In this respect, a relevant question that remains is whether the long-term clinical benefit will depend on the pathways underlying the well-established LDL-C lowering effects. If the target of the plant sterol/stanol treatment is to lower intestinal cholesterol absorption, the cholesterol concentration inside the body will decrease, while more cholesterol is excreted in the feces as fecal neutral sterols. If, on the other hand, the main mechanism is to stimulate TICE, which also results in an increased fecal neutral sterol excretion, possibly more cholesterol will be secreted directly from the vessel wall to the intestinal lumen and the feces. It can be speculated that this latter route of cholesterol reshuffling throughout the body might be preferable in terms of the most promising long-term clinical outcome. However, one can also argue that reducing intestinal cholesterol absorption lowers the amount of available cholesterol reaching the vessel wall. Activation of the process that has the largest net effect on inhibiting lesion formation remains to be evaluated. This conclusion illustrates that it is of utmost importance to better understand the underlying mechanisms of the cholesterol-lowering activity of plant sterols/stanols—and of other food components as well—to be able to predict the long-term clinical benefits.

The authors have declared no conflict of interest.

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