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Review

Plant-based sterols and stanols in health & disease: "Consequences of human development in a plant-based environment?"

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ABSTRACT

Dietary plant sterols and stanols as present in our diet and in functional foods are well-known for their inhibitory effects on intestinal cholesterol absorption, which translates into lower low-density lipoprotein cholesterol concentrations. However, emerging evidence suggests that plant sterols and stanols have numerous additional health effects, which are largely unnoticed in the current scientific literature. Therefore, in this review we pose the intriguing question "What would have occurred if plant sterols and stanols had been discovered and embraced by disciplines such as immunology, hepatology, pulmonology or gastroenterology before being positioned as cholesterol-lowering molecules?" What would then have been the main benefits and fields of application of plant sterols and stanols today? We here discuss potential effects ranging from its presence and function intrauterine and in breast milk towards a potential role in the development of non-alcoholic steatohepatitis (NASH), cardiovascular disease (CVD), inflammatory bowel diseases (IBD) and allergic asthma. Interestingly, effects clearly depend on the route of entrance as observed in intestinal-failure associated liver disease (IFALD)

Abbreviations: ABCG5/G8, ATP-binding cassette subfamily G member 5/8; ACAT, acyl-CoA cholesterol acyltransferase; APC, antigen presenting cell; BSEP, bile salt export pump; CAD, cardiovascular atherosclerotic disease; CHD, coronary heart disease; CNS, central nervous system; CVD, cardiovascular disease; EAE, experimental autoimmune encephalitis; EAS, European Atherosclerosis Society; FOLE, fish oil-based lipid emulsions; FXR, farnesoid X receptor; HDL, high-density lipoprotein; HFHCD, high-fat high-cholesterol diet; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; IBD, inflammatory bowel disease; IFALD, intestinal failure associated liver disease; IKKβ, NF-κB activation; IPA, ingenuity pathway analysis; LAL-D, lysosomal acid lipase deficiency; LDL, low-density lipoprotein; LDLr, low-density lipoprotein receptor; MS, multiple sclerosis; MUFA, mono-unsaturated fatty acids; NASH, non-alcoholic steatohepatitis; NEC, necrotizing enterocolitis; NF-κB, nuclear factor kappa B; NPC, Niemann-Pick C; NPC1L1, Niemann-Pick C1 Like 1; PBMC, peripheral blood mononuclear cell; PN, parenteral nutrition; PNALD, parenteral nutrition associated liver disease; PUFA, poly-unsaturated fatty acids; ROS, reactive oxygen species; SAFA, saturated fatty acids; SOLE, soy-based lipid emulsions; TICE, trans intestinal cholesterol excretion; TLR, toll-like receptor; TNBS, 2,4,6-trinitrovenzene acid; VLDL, very low-density lipoprotein

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during parenteral nutrition regimens. It is only until recently that effects beyond lowering of cholesterol concentrations are being explored systematically. Thus, there is a clear need to understand the full health effects of plant sterols and stanols.

1. Introduction

Nowadays, most humans are typical omnivores. However, from fossil characteristics, extensive evidence clearly indicates that a plant-based diet was the traditional eating pattern of our distant ancestors [1]. In those days, meat was only used when available, but the majority of calories and concomitant nutrients were plant-derived. Moreover, cholesterol levels in the diet were typically very low, while at the same time many foods enhanced cholesterol elimination through the intestine. Still, also in those days, cholesterol was an essential component for human life and the body developed mechanisms such as endogenous cholesterol synthesis and reuptake of bile acids in the lower parts of the small intestine in order to preserve cholesterol homeostasis. In this balanced condition, serum cholesterol concentrations remained low, whereas nowadays a higher cholesterol intake adds to the fact that high intakes of plant based foods which should maintain low intestinal cholesterol absorption are no longer present [1]. Typical nutrients of these original ancient plant-based diets are soluble fibers, poly- and monounsaturated fatty acids (PUFA and MUFA), vegetable proteins, fat- and water-soluble vitamins and minerals, and other phytonutrients including plant sterols and stanols, a dietary composition that is now generally considered as being healthy. Indeed, many dietary guidelines stress the advantages of plant-based diets providing large amounts of fruits, vegetables, soy, nuts and pulses [2–5]. In addition, it can be envisaged that intakes of plant sterols, which are structural components of plants, analogue to cholesterol in animals, in these ancient diets were 2.5 to 5 times higher as compared to levels in our current diets [1]. An intriguing question therefore is whether these plant sterols are desired or unwanted by the human body, since plant sterol concentrations are tightly regulated by absorption and secretion via intestinal and hepatic ATP-binding cassette subfamily G member 5/8 (ABCG5/G8) transporter activity. Plant sterols were immediately considered to be harmful after the identification of two sisters suffering from phytosterolemia, also known as sitosterolemia, back in 1974 by Bhattacharyya and Connor [6]. Phytosterolemia is a rare autosomal recessively inheritable sterol storage disease characterized by mutations in either one of the ABCG5/G8 heterodimer parts, which translates into intestinal hyper absorption and decreased biliary secretion of plant sterols [7–11]. It is estimated that worldwide about 100 cases of homozygous sitosterolemia exist, which have clearly distinct pathological phenotypes [12]. The first sitosterolemic patients as described by Bhattacharyya and Connor [6] were characterized by tendon and tuberous xanthomas, while no other physical, mental, or biochemical abnormalities were detected. The link between sitosterolemia and atherosclerosis was shown for the first time by Shulman et al. [13]. They therefore postulated that plant sterols were highly atherogenic. Interestingly, more recently, sitosterolemic patients have been diagnosed without any signs of premature cardiovascular disease (CVD), despite having highly elevated serum plant sterol concentrations [14]. This observation challenged the original concept of plant sterols being the only culprit of high CVD risk in phytosterolemia. The question is what can be learned from these apparently “non-symptomatic” phytosterolemic subjects in terms of resilience against elevated serum plant sterol concentrations. Alternatively, it might not be resilience as such, but the lack or presence of another characteristic that needs to be present before elevated plant sterol concentrations translate into cardiovascular complaints. Finally, it might also be that elevated plant sterol concentrations are not involved in the pathophysiology of CVD at all, but are only a so-called flag or surrogate marker for a yet unknown risk factor in sitosterolemic patients. Notably, individuals with heterozygous sitosterolemia, which is far more prevalent, have either normal or only modestly elevated serum plant sterol concentrations, indicating that regulation of one functional allele of the ABCG5 or ABCG8 monomer is almost sufficient to maintain normal intestinal absorption and/or biliary secretion plant sterols. More importantly, individuals with heterozygous phytosterolemia did not show any clinical abnormalities or premature atherosclerosis [11].

Several years before the discovery of the defect in the ABCG5/G8 genes in sitosterolemic patients, Peterson and colleagues [15,16] as well as the research group of Pollak [17] were exploring the applicability of sitosterol-rich soy bean plant sterols as a tool to lower intestinal cholesterol absorption with the ultimate aim to lower circulating serum cholesterol concentrations. This was also the era in which Ancel Keys, in the famous Seven Countries Study, suggested that increased serum total cholesterol concentrations resulting from high saturated fat (SAFA) intake were the culprit of atherosclerosis and coronary heart diseases [18–20]. The advice was to eat more healthy diets rich in MUFA and PUFA, and low in SAFA as typically consumed on Crete in those days to maintain healthy serum cholesterol concentrations. Later studies showed that in particular cholesterol carried in LDL particles related to coronary heart disease (CHD) risk and occurring events. These observations caused an enormous interest in identifying effective pharmacological as well as dietary lifestyle interventions to lower serum LDL-cholesterol concentrations. In this context, the success of pharmacological approaches such as the different types of statins, inhibitors of the rate limiting enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), following the identification of the LDL-receptor (LDLr) regulatory pathways by Goldstein and Brown [21], can be explained. Next, drugs like ezetimibe that lower intestinal cholesterol uptake by blocking the Niemann-Pick C1 Like 1 (NPC1L1) receptor were introduced to further lower serum LDL-cholesterol through combination therapies [22,23]. More recently, PCSK9 inhibitors have been developed that even further enhance LDLr-mediated clearance of LDL particles from the circulation in hypercholesterolemic patients [24].

After the initial studies by Peterson and Pollak and the apparent absence of any toxicity of plant sterols, these components became of interest for cholesterol-lowering therapies [25]. Commercial products based on sitosterol preparations such as Cytellin® and Sito-Lande® have been used as pharmacological treatments of hypercholesterolemia and have been marketed until the 1980s and 1990s respectively. Their efficacy was however rather low and doses of 3 to up to 30 g per day were needed to lower cholesterol [26]. The pharmacological use of plant sterols further remained limited to cases of hyperlipidemia, mainly due to the high costs associated to produce pharmaceutical grade sitosterol.

With the introduction of statins as potent cholesterol-lowering medication, interest in plant sterols decreased and there was a relatively “silent” plant sterol period, also due to the sub-optimal physicochemical characteristics of the available preparations hampering practical applicability to enrich for instance common foods with plant sterols. The use of plant sterol- or stanol-enriched diets celebrated its renaissance in the early 1990s when food industries focused on the development of functional foods based on new insights showing that physical properties and handling of plant sterols and stanols could be improved by esterification with fatty acids [27]. Initially, plant stanols (the saturated counterpart of plant sterols) were esterified with dietary fatty acids, which increased their fat-solubility and therefore their applicability to be incorporated into fat-rich foods, like margarines [28]. A few years later also margarines and spreads with added plant sterol esters, and mixtures of sterol and stanol esters became available as functional foods. The cholesterol-lowering efficacy of foods with added

plant sterols or stanols or their mixtures in esterified as well as in their free forms has been demonstrated in > 120 randomized, placebo controlled intervention studies [29]. In a recent consensus panel paper of the European Atherosclerosis Society (EAS), it was concluded that a daily intake of 2–3 g plant sterols or stanols lowers serum LDL-cholesterol concentrations up to 12% [30]. Regarding the mechanism underlying the LDL-cholesterol lowering effects, there is no clear consensus. The common idea is that plant sterols decrease cholesterol incorporation into mixed micelles thereby lowering cholesterol bioavailability and absorption [31]. However, recently more challenging mechanisms such as plant sterol induced elevation in trans intestinal cholesterol excretion (TICE), i.e. stimulated efflux of cholesterol from the brush border membrane into the intestinal lumen, have been proposed [32]. Irrespective of the underlying mechanism, advising plant sterols or stanols as part of a healthy diet to lower serum LDL-cholesterol concentrations is nowadays mentioned in international guidelines for the management of hyper- or dyslipidemia [33,34]. In terms of societal impact, two recent reports assessing the cost-effectiveness of foods with added plant sterols or stanols as a primary prevention strategy for people with CVD in the UK [35] and the potential healthcare cost savings that could be derived from plant sterol/stanol intake in the European Union [36] have shown their potential benefits on public health including health economics. The report written by the non-profit organization Frost & Sullivan on request of Food Supplements Europe (http://www.foodsupplementseurope.org/value-of-supplementation) indicated that the total avoidable healthcare cost savings from plant sterol/stanol supplements in Europe is €5.3 billion per year. After subtracting implementation costs of €1.2 billion, the annual saving in Europe is €4.1 billion. In other words, the return from healthcare savings for each €1 spent on supplementation is €4.37. Moreover, Yang et al. [35] have described that daily intake of spreads with added plant sterols or stanols could reduce CVD risk, especially in men and older age groups. Assuming a 50% compliance rate, over a period of 20 years, 69 CVD events per 10,000 men and 40 CVD events per 10,000 women aged 45–85 years could be saved. The intriguing thought is that - if the additional potential benefits of consuming plant sterols or stanols, as described in this review are consistent- health care costs savings may increase even further.

2. Plant sterol physiology

Our current Western-type habitual diets provide on average 300 mg plant sterols and 17–24 mg plant stanols per day with somewhat higher intakes (up to 600 mg sterols) in vegetarians [37]. Plant sterols and stanols are present mainly as fatty acid esters, hydroxycinnamic acid esters, and glycosides [38]. Within the gastro-intestinal tract, all ester bonds are cleaved by specific enzymes, resulting in the formation of free plant sterols and stanols. The predominant plant sterols and stanols in our habitual diets are sitosterol (66%), campesterol (22%), stigmasterol (8%), and sitostanol plus campestanol (4%) which are present in bread, cereals, vegetables, fruits and (vegetable) oils and products based on these oils [37]. The free plant sterols and stanols are subsequently incorporated into mixed micelles, a process in which they interfere with cholesterol incorporation into these micelles. This effect at least partly contributes to their lowering effect on intestinal cholesterol absorption. Like cholesterol, plant sterols are taken up from the mixed micelles into enterocytes via the NPC1L1 receptor, located at the apical membrane [39]. NPC1L1 is the pharmacological target for ezetimibe, which efficiently lowers both intestinal absorption of cholesterol as well as of plant sterols and stanols [40]. After uptake into the enterocytes, metabolism of plant sterols is different from that of cholesterol. The plant sterols and stanols are poor substrates for intestinal acyl-CoA cholesterol acyltransferase (ACAT), which explains why plant sterols are not easily esterified and remain in the free form within the cell. As a consequence, the free plant sterols, in contrast to cholesterol esters, are not efficiently incorporated into chylomicrons. Instead, the majority of the free intracellular plant sterols and stanols are excreted back into the intestinal lumen via the ATP-driven transporter heterodimer ABCG5/G8. This explains why net intestinal absorption of plant sterols is very low, varying from 0.5% for sitosterol to 1.9% for campesterol [41], which is considerably lower compared to cholesterol absorption rates which varies between subjects from 19 and 77% [42]. The small amounts of plant sterols and stanols that enter the circulation are rapidly taken up by the liver and secreted into bile via hepatic ABCG5/G8, further explaining the very low plasma plant sterol and stanol concentrations. Plant sterols are found in all lipoproteins with the highest concentrations in LDL and high-density lipoproteins (HDL)

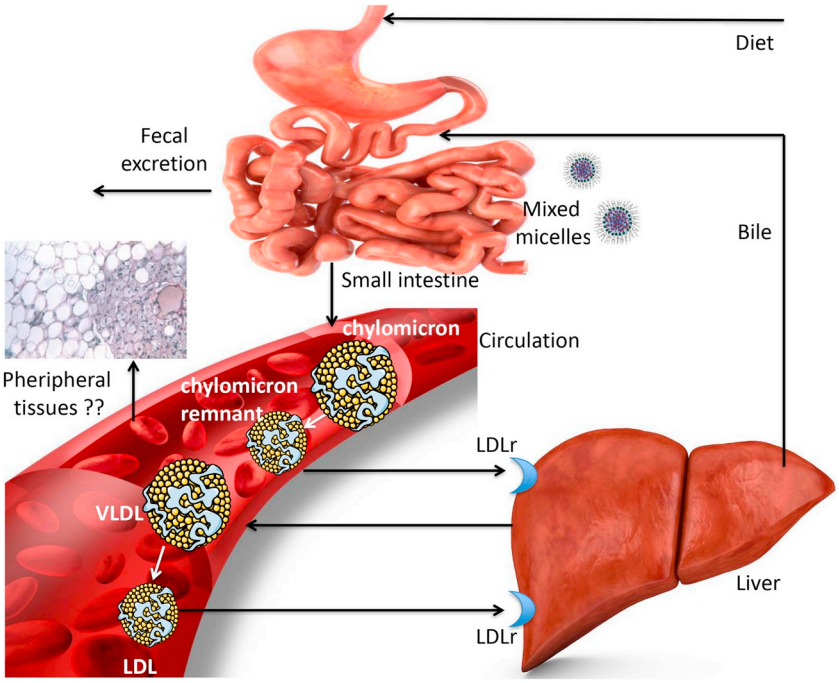

Fig. 1. Plant sterol and stanol physiology in the classical paradigm.

[30]. In contrast to cholesterol, plant sterols are not synthesized endogenously so each plant sterol molecule found in the circulation is derived from the diet. Together with the observation that plant sterols are not further metabolized into for example bile acids, but excreted in bile as such, makes the classical paradigm of plant sterol physiology rather straightforward (Fig. 1). Moreover, in the early days of plant sterol and stanol research, these compounds were considered as molecules that entered the intestinal lumen with only minimal spill over into the circulation. Nowadays, it becomes increasingly appreciated that plant sterols and stanols do enter the circulation. Moreover, although circulating concentrations are very low as compared to cholesterol, they are taken up by different tissues [30] and may affect various (patho) physiological processes (Fig. 2).

Until recently, plant sterols and stanols were seen as inert molecules that enter the gastro-intestinal tract either via diet or via biliary secretion, lower cholesterol absorption via disturbing luminal mixed micelle composition, are hardly absorbed and leave the body unmodified via the feces. The small amounts of plant sterols and stanols that are absorbed are rapidly taken up by the liver and excreted via bile into the gastro-intestinal tract. According to the classical paradigm, plant sterols and stanols hardly appear in the circulation, while knowledge on the effects – beyond LDL-cholesterol lowering - of absorbed plant sterols and stanols on metabolic and functional markers related to health or disease is scarce.

In the new paradigm, plant sterols and stanols enter the gastro-intestinal tract either via diet or via biliary secretion, lower cholesterol absorption via different mechanisms including a decrease in micellar incorporation of cholesterol. Plant sterol and stanol absorption is low, but plasma concentrations increase after higher intakes. The small amounts of plant sterols and stanols that are absorbed are rapidly taken up by the liver and transported by lipoproteins to other tissues where they can have (patho)physiological effects beyond lowering intestinal cholesterol absorption. 1: Plant sterols interfere with mixed micelle composition in the intestinal lumen thereby lowering cholesterol bioavailability and fractional cholesterol absorption. 2: In contrast to cholesterol, which absorption varies between subjects from 19 and 77%, absorption of plant sterols and stanols is much lower varying between 0.5 and 1.9%. Thus, chylomicrons contain more cholesterol than plant sterols although dietary intakes are comparable, around 300 mg/day. 3: During transport from the intestinal lumen into the circulation, plant sterols and stanols can easily interfere with all types of immune cells as present in the Peyer's patches. 4: Plant sterols and stanols are taken up by immune cells in the circulation and are also incorporated into red blood cells and platelets. Hepatic plant sterol levels can easily increase (5) and interact with different cell types such as the Kupffer cells. 6: Plant sterols are also incorporated into other peripheral tissues including the vascular wall, the lungs, the brain and into breast milk. 7: While on a parenteral nutrition (PN) feeding protocol, plant sterol concentrations can be greatly increased, since these formulae like, for example, Intralipid are based on soy bean oil which is rich in plant sterols. Infusing these formulae directly into the circulation circumvents the selective intestinal wall.

3. Plant sterols in breast milk

Cholesterol is an important building block for cell synthesis in newborns, a precursor for several hormones, bile acids and vitamin D, explaining why breast milk is relatively rich in cholesterol. Still, cholesterol represents < 0.5% of the milk lipid fraction, which mainly contains triacylglycerols [43]. Especially during early postnatal life, newborns need large amounts of cholesterol to meet their requirements. Moreover, infants with low serum cholesterol concentrations may be more susceptible for allergic diseases and infections [44]. The importance of dietary cholesterol for infants is further illustrated by the finding that breast milk remains rich in cholesterol, even when a lactating mother is treated with cholesterol-lowering plant stanol therapy [45] Therefore, in breast milk, cholesterol should be considered beneficial instead of potentially harmful. The cholesterol in breast milk originates from uptake from the circulation as well as local synthesis. LDL is the predominant cholesterol donating lipoprotein to the basolateral membrane of the secretory cells in the mammary tissues, where LDLr indeed have been identified [46]. This cholesterol is incorporated into the milk at the apical side of the secretory cells, predominantly via extrusion of fat globules from the endoplasmatic reticulum of the mammary cells [47]. However, also ABCA1 and ABCG1 transporter mediated secretion to the cholesterol acceptor apoA-I has been

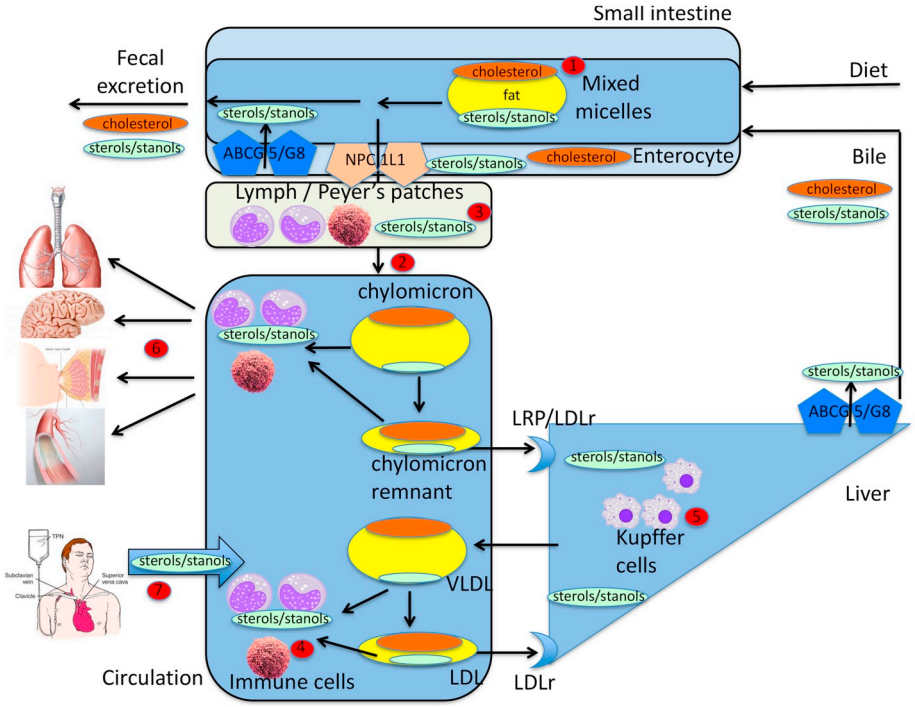

Fig. 2. Plant sterol and stanol physiology after the paradigm shift.

suggested [48].

Besides cholesterol, breast milk also contains plant sterols, although in much lower quantities [49]. Cholesterol is generally present in the millimolar range, whereas plant sterols are present in micromolar ranges. From the perspective that breast milk is thought to be the healthiest product for a newborn, this observation triggers the question whether these plant sterols have any beneficial effects for the breast-fed infant. Moreover, plant sterols are already present in the amniotic fluid and the fetal circulation [50,51]. This further raises the question whether plant sterols are beneficial for development in utero and whether supplementation of plant sterol to the maternal diet has positive consequences for the developing fetus. Of course, one could also argue that plant sterols and stanols might have disadvantages for development and health, which is the reason why nature keeps levels as low as possible.

The hypothesis that plant sterols are important perinatal, is even more intriguing when the plant sterol composition in breast milk is considered (Fig. 3). Apparently, plant sterol composition in breast milk does not simply reflect circulating plant sterol concentrations in the mother. In plasma as well as in cell membranes, campesterol concentrations are generally higher as compared to those of sitosterol. A systemic review showed that human serum concentrations of sitosterol were on average 6.92 μmol/l (95% CI 6.23/7.61 μmol/l) and those of campesterol 13.07 μmol/l (95% CI 11.65/14.48 μmol/l) [52] However, we observed the opposite pattern in human breast milk showing higher sitosterol than campesterol concentrations (Fig. 3). This finding suggests that the presence of plant sterols in milk is not simply extrusion of fat globules as described for cholesterol [47]. It suggests more active regulation of plant sterol uptake into the mammary gland and consequent secretion into the milk. Indeed, ABCG5 and ABCG8 mRNA expression was identified in bovine mammary glands [53]. Since the sterol transporter ABCG5/G8 has a higher affinity for sitosterol [8,54,55] than for campesterol, this could lead to a higher flux of sitosterol into breast milk. Moreover, this observation seems more or less specific for humans, since milk obtained from several animals like cow, camel and goat, except the horse, have different plant sterol compositions (Table 1). Moreover, all these animals are, in contrast to humans, pure herbivores. Considering the plant sterol and cholesterol concentrations in the milk from different mammals, dietary availability of plant sterols does apparently not have a large impact on breast milk sterol composition. Therefore, it would be intriguing to examine the specific benefits for the neonate of the presence of plant sterols in human breast milk. Moreover, differences in plant sterol concentrations between milk samples of various animals is of interest and more particularly the question why the campesterol/sitosterol ratio differs between humans and herbivores. Obviously, the most prominent question is whether these plant sterols have a role for the growing and developing newborn. A next question is whether these effects are dose- or ratio dependent and how easily plant sterol concentrations in breast milk can be regulated through changing maternal plant sterol intake before and/or during the lactating period and what the optimal maternal dietary plant sterol composition is. For cholesterol, it is known that concentrations in breast milk vary during the course of lactation with highest levels in colostrum [56,57]. For plant sterols, it has been suggested that their content is influenced by the composition of the maternal diet. For infant formulas, the sterol content depends on the types and quantity of the oils used for manufacturing. As shown in Table 1, sitosterol levels in infant formulas are higher than those of campesterol [58,59], due to the compositions of the vegetable oils used. It has been estimated that formula-feeding results in plant sterol intakes between 19 and 50 mg/d [49]. While many studies have focused on the role of fatty acids on short and long-term health of the child, no such studies exist for plant sterols. Thus, opportunities may exist to improve the sterol composition of maternal diets or infant formulas [60].

4. Plant sterols and immune 'fitness'

An immune response is the resultant of two interacting and closely collaborating parts, the innate and the adaptive immune systems. It is evident that a resilient immune system is needed to cope with and recover from the numerous challenges it is daily confronted with. An essential challenge is the ability to distinguish between innocent and harmful triggers, and to respond adequately to eliminating bacteria or viruses, while tolerating for example our own cells and foods. Moreover, a weak or hypo-responsive immune response will increase the risk of not adequately responding to infections, whereas a hyper-responsive immune system is thought to induce chronic inflammatory conditions, auto-immune diseases and allergies. There are specific conditions in which the resilience of our immune system is diminished, for example during aging, a phenomenon called immune-senescence. Consequently, elderly with an impaired immune response are more susceptible to infections and have increased morbidity and mortality rates from infections. On the other side of the lifecycle, newborns are characterized by various parts of the immune system that are naive such as T-cell differentiation and the number of antigen-presenting cells, resulting in a higher risk for infections than adults has. It is a challenge to evaluate what changes in diet and lifestyle characteristics can be used to modulate the resilience and fitness of our immune responses.

Evidence is accumulating that several intracellular metabolic pathways, including glycolysis, the Krebs cycle, fatty acid oxidation and synthesis, and cholesterol synthesis tightly regulate immune cell function [61]. Several intermediate metabolites of the cholesterol synthesis pathway may have both pro- and anti-inflammatory regulatory functions. Recently, it has been reported that monocytes and macrophages can build a long-term pro-inflammatory phenotype after short stimulation, e.g. with β-glucan or with oxidized LDL particles, which has been termed trained immunity [62]. Trained macrophages with a pro-inflammatory phenotype display a profound upregulation of the cholesterol synthesis pathway [63]. Pharmacological studies revealed that accumulation of mevalonate is crucial for developing the trained phenotype, which corresponds to the increased cytokine production capacity of monocytes from patients with the Hyper IgD syndrome, who have mevalonate accumulation due to a deficiency of mevalonate kinase [64]. Prolonged exposure of macrophages to high cholesterol levels induces characteristics of foam cell formation [65]. Interestingly, peritoneal foam cells from LDLr−/− mice have a suppressed cholesterol biosynthesis and an LXR-mediated downregulation of inflammatory pathways, which appeared to be dependent on regulated accumulation of desmosterol [65]. Relating these observations to the

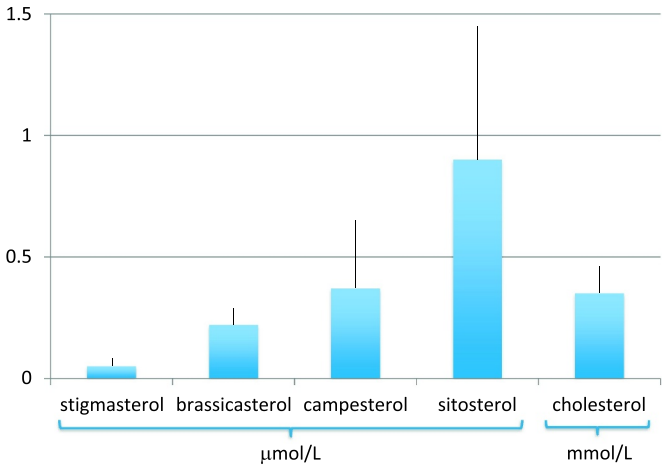

Fig. 3. Plant sterol and total cholesterol concentrations in 311 human breast milk samples (unpublished data).

Table 1
Concentrations of campesterol and sitosterol and their ratio, and desmosterol and cholesterol concentrations in (breast) milk of humans and different species, or of infant formula feedings.

	Campesterol	Sitosterol	Ratio	Desmosterol	Cholesterol
	(umol/L)	(umol/L)	Camp/Sit	(umol/L)	(mmol/L)
Human	0.37 ± 0.29	0.90 ± 0.58	0.50 ± 0.36	55.7 ± 25.79	0.35 ± 0.11
Camel	2.17 ± 0.66	1.75 ± 0.46	1.18 ± 0.10	27.34 ± 5.42	0.35 ± 0.06
Cow	0.75 ± 0.21	0.19 ± 0.08	4.00 ± 1.17	0.38 ± 0.32	0.24 ± 0.06
Goat	0.99 ± 0.64	0.30 ± 0.18	4.04 ± 2.36	1.09 ± 0.58	0.31 ± 0.13
Horse	0.05 ± 0.07	0.25 ± 0.20	0.23 ± 0.16	1.44 ± 0.67	0.09 ± 0.03
Formula	10.8–34.5	36.0–73.2		5.5–11.5	0.04–0.13

Unpublished data; N = 311 for human milk samples and N = 5 for each animal species, (means ± SD). Values for formula are given in ranges and derived from ref. Hamdan et al. [58].

sterol composition of breast milk, it is interesting to realize breast milk is rich in desmosterol. Camel milk is also rich in desmosterol, while cow, goat and horse milk are relatively desmosterol-poor. Besides these effects of cholesterol synthesis intermediates on immune cell behavior, there is emerging evidence that also plant sterols and stanols influence our immune system [66]. It remains unclear whether these effects are direct or indirect, since it is well-known that consuming plant sterols or stanols increases endogenous cholesterol synthesis as a response to the inhibition of intestinal cholesterol absorption [67] Therefore, it is also possible that increased concentrations of metabolites from the cholesterol-synthesis cascade play an important role in the observed effects of plant sterols and stanols. Up to now, most data on the effects of plant sterols and stanols on immune cell function originates from in vitro experiments, whereas data from in vivo studies in animal models and/or humans is scarce, but growing steadily.

During the last decades, an increasing number of in vitro studies have supported immune modulation by plant sterols and stanols on both the innate and the acquired immune system. Several studies have examined the effects of plant sterols on the secretion of pro-inflammatory mediators by macrophages. In the mouse RAW264.7 macrophage cell line, sitosterol reduced LPS-induced secretion of IL-6, as well as of TNF-α [68,69]. In agreement, sitosterol suppressed IL-6, TNF-α and INF-γ secretion via a phosphatase SHP-1-mediated inhibition of Stat1-NFκB signaling in LPS-stimulated J774a.1 mouse macrophage-like cells [70], and reduced activation of TLR4 receptors via MyD88 in murine macrophages [71,72]. In addition, sitosterol stimulated anti-oxidant responses and counteracted inflammation-induced prostaglandin synthesis in mouse macrophages [73–76]. Yet, Alappat et al. [77] showed that sitosterol significantly increased IL-6, TNF-α, MCP-1, and INF-γ. Effects on the anti-inflammatory cytokine IL-10 in LPS stimulated J774a.1 cells were not consistent, since both increases as well as decreases have been reported [70,71,78]. Another study suggested a moderate induction of IL-6 and TNF-α secretion by sitosterol and campesterol, but only at supra-physiological concentrations [79]. Thus, the majority of in vitro studies support a suppressive effect of plant sterols on LPS-induced inflammatory NFκB signaling. So far, the plant sterol-induced modulation of the innate immune response has been studied into detail in an endotoxin context, i.e. using LPS, whereas the modulatory effects of plant sterols on endogenous inflammatory stimuli such as IFN-γ, IL-β, or TNF-α remain to be elucidated.

With respect to the adaptive immune system, plant sterols and their derivatives are suggested to skew the acquired immune response towards a Th1 response, in particular in relation to Th2-driven diseases. In vitro, a mixture of sitosterol and its glycoside (100:1) boosted T-cell proliferation and enhanced the secretion of IL-2 and IFN-γ, but inhibited the secretion of IL-4 in human CD4+ T cells [68,80,81]. Other studies showed that sitosterol, but not stigmasterol blocked the secretion of Th2 cytokines (IL-4 and IL-10) [82,83]. Moreover, in human peripheral mononuclear blood cells (PBMCs), sitosterol and sitostanol induced a TLR2-dependent Th1 shift, featured by higher IFN-γ production without changing the production of IL-4, IL-10 and IL-13 [84,85]. Sitosterol and sitostanol did not induce antigen presenting cell (APC)-related IL-12 and IL-18 production in PBMCs or monocyte-derived U937 cells, thereby suggesting that plant sterols and stanols do not modulate APC-induced CD4+ T cell differentiation. Instead, they rather push the Th-1/Th-2 balance towards a Th-1 response by enforcing the production of Th-1 cytokines, which might be beneficial in conditions characterized by decreased Th-1 responses (e.g. HIV) or increased Th-2 activity (e.g. allergy) [84,86]. Regulatory T cells (Tregs) play a crucial role in maintaining the Th1/Th2 balance by inhibiting the activation of the dominant effector T-cells [87]. In PBMCs from Th-2 skewed asthma patients, sitostanol increased the number of Foxp3+ Tregs while stimulating IL-10 production [85]. Moreover, sitosterol boosted stromal Tgf-β secretion, a key driver in Treg differentiation [88]. Although speculative, it is tentative to suggest that plant sterols/stanols enforce their Th1 skewing at least partially via Treg activation. Although there are clear indications, more research is needed to explore the in vitro immune-modulatory actions of plant sterols and stanols which apparently depend on (i) the concentration and timing, (ii) the mode of administration (e.g. vehicle or complex), (iii) the inflammatory status of the target cells, and (iv) the relative content of the individual plant sterols/stanols in the mixtures.

The modulatory role of plant sterols and stanols on the complex interaction between the innate and adaptive immune response has been evaluated in various animal models as well as in humans. Several studies have described a modulatory role for plant sterols on inflammation, in particular related to atherosclerosis and hyperlipidemia. A 2% plant sterol-enriched diet increased IL-2 and IFN-γ production in splenocytes of ApoE−/− mice after induction of acute aseptic turpentine-induced inflammation. Splenocytes of plant sterol-fed normo-lipidemic wild type mice injected with turpentine showed a significant elevation in IL-2 production, but not in IFN-γ, IL-4, or IL-10 production. Moreover, the Th1/Th2 ratio was significantly increased suggesting that plant sterols modulated the T-helper immune response in vivo towards a Th1 response [89]. In line, Nashed et al. showed that plant sterol intake enhanced the capacity to mount a Th1 immune response to a Th2-electing inflammatory stimulus in spleen cells of the hyperlipidemic ApoE−/− mice, featured by an increased production of IFN-γ [90]. Also, a strong increase in the production of the Treg cytokine IL-10 was noted. Elevated serum IL-10 levels are associated with a lower risk of cardiovascular atherosclerotic disease (CAD) in humans [91]. Transplantation of T-cells from IL-10 overexpressing mice into LDLr−/− mice resulted in a severe reduction in atherosclerotic lesion size [92]. In contrast, increased levels of IFN-γ stimulated arterial inflammation and atherogenesis [93]. Although the plant sterol-induced IFN-γ and IL-10 production was blocked by anti-CD4 antibodies, supporting a T cell-dependent effect, a role for antigen-presenting cells cannot be excluded. Interestingly, severe hypercholesterolemia is associated with Th1-type cytokines [94]. The immune-modulatory role of plant sterols in mouse models of severe hyperlipidaemia may be

independent of its cholesterol lowering-effect and might be mediated by counteracting the Th2 inflammatory responses [90,94,95]. However, the role of plant sterols on in vivo immune cell behaviour as related to their accepted anti-atherosclerotic effects remains further study.

During the last decade, an increasing number of studies have demonstrated the accumulation of dietary plant sterols in the central nervous system (CNS) [96–101]. Although ABCG5- and ABCG8-knock out mice have higher levels of sitosterol in the circulation, levels of campesterol are higher in brain. This suggests a controlled transport across the blood-brain barrier. However, to the best of our knowledge, the ABCG5/8 transporter is not detectable in the brain [97]. Recent animal studies have investigated how plant sterols modulate neuroinflammatory disorders such as multiple sclerosis (MS). Experimental autoimmune encephalitis (EAE), an animal model for MS, is a Th1/Th17-driven immune disorders of the CNS [98]. Despite the Th1-supporting trend induced by plant sterols, plant sterols counteracted the evoked Th1 response in EAE. In particular, plant sterols beneficially changed the course of the clinical disease, the degree of demyelination, and the degree of immune cell infiltration into the CNS during EAE [78]. Moreover, brain tissue homogenates and splenocyte cultures of the EAE animals showed a reduced TNF-α release in the plant sterol-treated mice. A potential explanation for these findings might be a plant sterol-induced Treg-regulated restoration of the Th1/Th2 ratio. This hypothesis is supported by the increased secretion of IL-10. Alternatively, a CNS parenchymal-dependent decrease in CCL2 secretion might hamper T cell migration towards the CNS [78].

Taken together, most animal models investigating the immune-modulatory role of plant sterols on inflammatory diseases clearly support an anti-inflammatory or immune suppressive response. Although a plant sterol-induced strengthening of the Th1 response (Th1 skewing) is the most prominent response under inflammatory conditions, the nature of this Th1 skewing remains unclear. Both the modulation of the innate immune system and a suppressive control by the regulatory T cells appear to be involved in restoring the Th1/Th2 balance. Nevertheless, how plant sterols or stanols modulate an inflammatory response appears largely context and disease-dependent.

In humans, Brull et al. showed that consumption of a plant stanol ester enriched yogurt for several weeks by allergic asthma patients translated into rebalancing of the skewed Th2 dominant immune response towards a more Th1 oriented immune response [102]. In this response, enhancing the numbers and activation of regulatory T-cells seemed to play an essential role [85]. An additional observation was the strengthening of the Th1 response towards vaccination against Hepatitis A, translating into higher antibody titers and reduced total IgE concentrations. Changes in circulating plant stanol concentrations correlated positively with the Th1/Th2 cytokine index and the change in antibody titers suggesting a causal relationship. Interestingly, the immune stimulating effects were particularly evident in overweight and obese individuals, who suffered from a poor Th1-mediated immune response at baseline (Fig. 4). (See Fig. 5.)

If plant sterol and stanol intake indeed induces Th1 skewing, a potential risk for healthy subjects, may be that the immune system gets out of balance, thereby inducing the risk of developing Th1-associated conditions such as immune diseases. Theoretically, this could be a real concern related to the garding long-term safety of increased plant sterol/stanol intake. However, there is currently no evidence for this suggestion, although it has however not been examined into detail. Recently, DeSmet et al. reported in immunologically healthy human volunteers a decrease in T-cell activity (mRNA and Treg immune staining) in intestinal biopsies after consuming plant stanol ester enriched diets for 3 weeks [103]. This finding may be difficult to interpret, since functional effects were not studied. However, these results do indicate that in healthy individuals the (intestinal) immune system is not Th1 over-activated after plant stanol ester consumption. Hence, immune modulating effects may only be evident when a dis-balance exists. As studies with healthy and hypercholesterolemic individuals did not show changes in circulating plasma inflammation markers and cytokines strengthens this suggestion [104]. Clearly, more data from controlled intervention studies in different population groups is warranted.

5. Plant sterols and CVD risk

There is no doubt that consumption of plant sterols or stanols lowers serum LDL-cholesterol concentrations. As LDL-cholesterol is a risk factor for CVD, lowering LDL-cholesterol by plant sterols and stanols is expected to lower CVD [30]. A limited number of observations however suggests that some individuals do not or poorly respond to the intake of plant sterol or stanol enriched diets [105–107]. In addition, a higher plant sterol intake increases circulating plant sterols concentrations. There is an ongoing debate whether plant sterols are atherogenic or not. Increases in absolute serum plant sterol concentrations after consumption of on average 1.6 g plant sterols per day are about 31% for sitosterol and 37% for campesterol. When standardized for plasma cholesterol concentrations, these values are respectively 42% and 61%. In absolute numbers, sitosterol and campesterol concentrations are

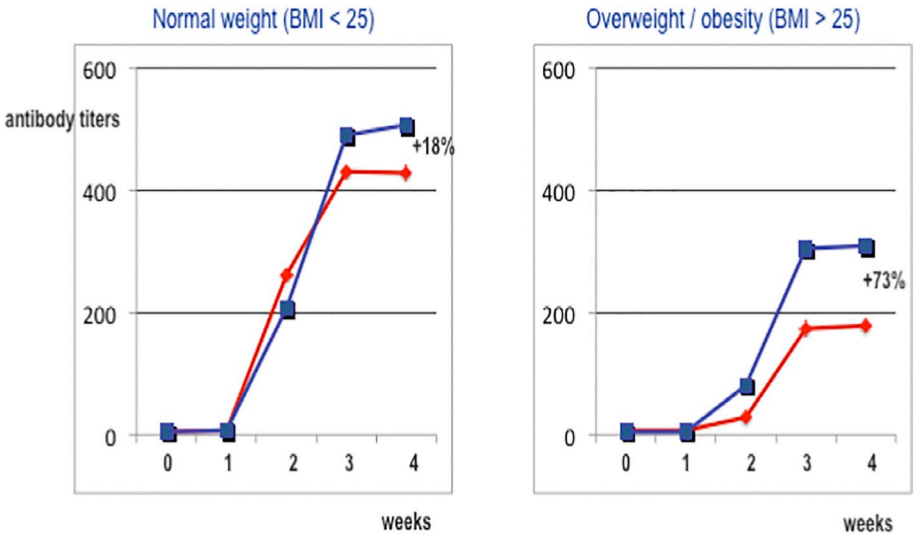

Fig. 4. Changes in antibody titers against Hepatitis A following vaccination after consuming plant sterol enriched vs placebo yogurts in normal weight (left panel) and overweight/obese subjects (right panel) with allergic asthma (unpublished data).

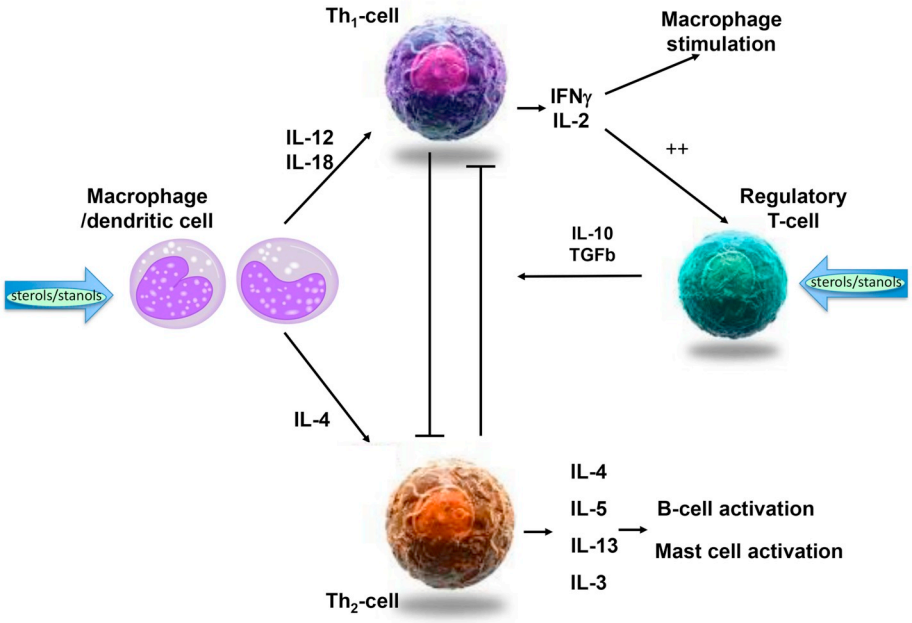

Fig. 5. Potential effects of plant sterols and stanols on different cells and cell-cell interactions of the immune system.

increased on average by 2.2–5.0 μmol/L as compared to an average reduction in LDL- cholesterol of −0.33 mmol/L [52]. It should be emphasized that these plant sterol concentrations are still below those of symptom-free heterozygous sitosterolemia patients [108]. Furthermore, consuming plant stanol esters lowers serum plant sterol concentrations, while serum plant stanol concentrations are increased. However, even after increased plant stanol ester intake, serum plant stanol concentrations are always much lower than those of serum plant sterols (Fig. 6). Finally, plasma plant sterol levels can be used as surrogate markers of fractional intestinal cholesterol absorption, but only under steady-state conditions and when no plant-sterol enriched foods are consumed [109–112].

The current understanding is not that elevated plasma plant sterol concentrations per se increase the risk for CVD, but that individuals characterized by high plant sterol concentrations can actually be classified as subjects with higher cholesterol absorption, which might increase their risk for CVD [113] or stroke [114]. Based on observational findings, the result of the discussion around the 'plant sterol-atherogenicity' hypothesis is the following; CVD risk and its severity are associated with higher cholesterol absorption and reciprocally with lower cholesterol synthesis rates as measured by high levels of surrogate markers for cholesterol absorption (plasma/serum sitosterol, campesterol or cholestanol) and low levels of surrogate markers for synthesis (lathosterol or desmosterol) [113,115–122]. Interestingly, the latest insights suggest that it seems especially the low cholesterol synthesis that is actually driving the elevated CVD risk, an intriguing hypothesis that has not been proven yet. Possibly, certain cholesterol precursors such as desmosterol and mevalonate, which are low in cholesterol-absorbers, are important for maintaining health. For example, as discussed in the previous section, desmosterol was identified as a strong regulator of anti-inflammation in macrophages [65] and mevalonate as a strong inducer of trained immunity in monocytes [64]. Interestingly, cholesterol-lowering effects of plant sterols on lowering LDL-cholesterol were most pronounced in the so-called cholesterol absorbers, who have a low endogenous cholesterol synthesis [105]. As consumption of both plant sterol and stanol esters modestly elevates endogenous cholesterol synthesis [67], their cardio-protective effects may be even further increased by elevation of cholesterol synthesis markers like desmosterol. Unfortunately, there is as yet no human trial with clinical endpoints that has evaluated the health effects of plant sterols or stanols. This question can only be addressed by using results on lesion size development from animal studies with different genetically modified mouse models [123–125]. These effects maybe even larger than can simply be explained by reductions in serum cholesterol concentrations. For example, in female heterozygous LDLr−/− mice fed high fat high cholesterol Western type diet, adding plant sterols, plant stanols or atorvastatin lowered serum cholesterol concentrations to the same extent, i.e. by 26%, 20% and 22% respectively. Thus, cholesterol exposure was more or less reduced to the same extend in these three groups. However, lesion sizes were reduced by 93% and 92% in the plant sterol and stanol groups, but only by 55% in the atorvastatin group [123]. Further, Weingärtner et al. showed that ezetimibe reduced plaque area development even more than plant sterols did, although decreases in LDL-cholesterol were comparable [125]. Possibly, ezetimibe reduced intestinal cholesterol absorption even more, which might then have further enhanced compensatory endogenous cholesterol synthesis. On the other hand, ezetimibe not only lowers cholesterol but also circulating plant sterol concentrations. However, if plant sterols have direct effects on lesion size formation,

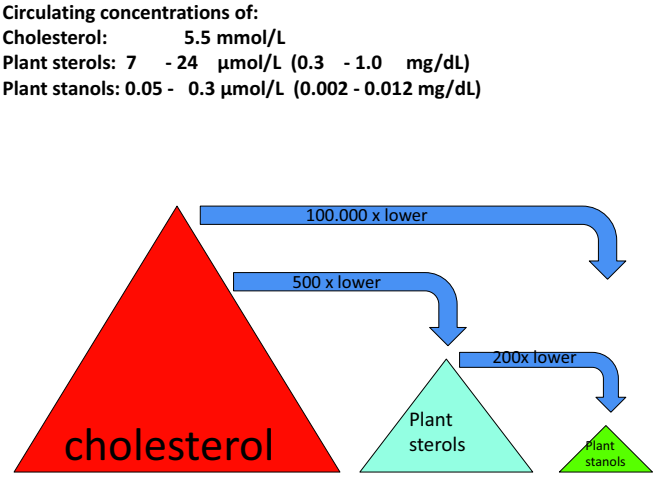

Fig. 6. Average plasma cholesterol, plant sterol and stanol concentrations in the general population.

this would argue against this hypothesis. These considerations also raise the question whether effects of ezetimibe mono-treatment (which lowers intestinal cholesterol uptake and consequently increases endogenous cholesterol synthesis) on CVD morbidity and mortality are different than predicted based on reductions in LDL-cholesterol. Unfortunately, there are no mortality data available from a CVD endpoint trial with ezetimibe monotherapy to address this question. The picture becomes even more complicated when possible effects of plant sterols on the TICE pathway are considered. TICE is an alternative excretion route for cholesterol and activation of TICE may be atheroprotective. It has been shown that absence of ABCG5/G8 strongly inhibits the TICE pathway [126]. Therefore, one could be hypothesized that elevated plant sterols are not directly involved in the pathophysiology of CVD, but are a marker for inhibited activity in the TICE pathway.

6. Plant sterols/stanols and hepatic or intestinal diseases

Lysosomal cholesterol storage is a problem in a number of diseases such as Niemann Pick C (NPC) deficiency, non-alcoholic steatohepatitis (NASH), and lysosomal acid lipase deficiency (LAL)-D. In all these conditions, the liver is affected. This indicates that appropriate cholesterol fluxes and hepatic compartmentalization are essential for normal hepatic cell function. For NASH, a high-fat diet low in dietary cholesterol reduces the development of NASH in LDLr−/− mice [127]. It has been suggested that the flux of cholesterol from the intestine to the liver fuels the hepatic lysosomal cholesterol accumulation thereby triggering hepatic inflammation. This hypothesis was tested in another study using the same NASH model, but animals were now fed a high-fat high-cholesterol diets (HFHCD) enriched with plant sterols or stanols. It was hypothesized that the reduced intestinal cholesterol absorption reduced cholesterol fluxes from the intestine to the liver. Indeed, it was shown that the diets enriched in plant sterols or stanols lowered liver inflammation [128]. Moreover, the effect was probably specific for the inflammatory response, since steatosis was not affected. This raises the question whether these hepato-protective effects of plant sterols or stanols enriched diets on liver inflammation are direct effects or indirect effects due to the reduced cholesterol flux. This would suggest another indirect effect, except for the potential increased amount of regulatory effects of the cholesterol precursors as discussed above. Of interest, in a recent meta-analysis of human intervention studies, ezetimibe treatment did not show consistent effects on NASH pathology [129]. However, ezetimibe not only lowers cholesterol absorption, but also inhibits plant sterol absorption, which results in significantly reduced circulating plant sterol concentrations [23,130]. This triggers the

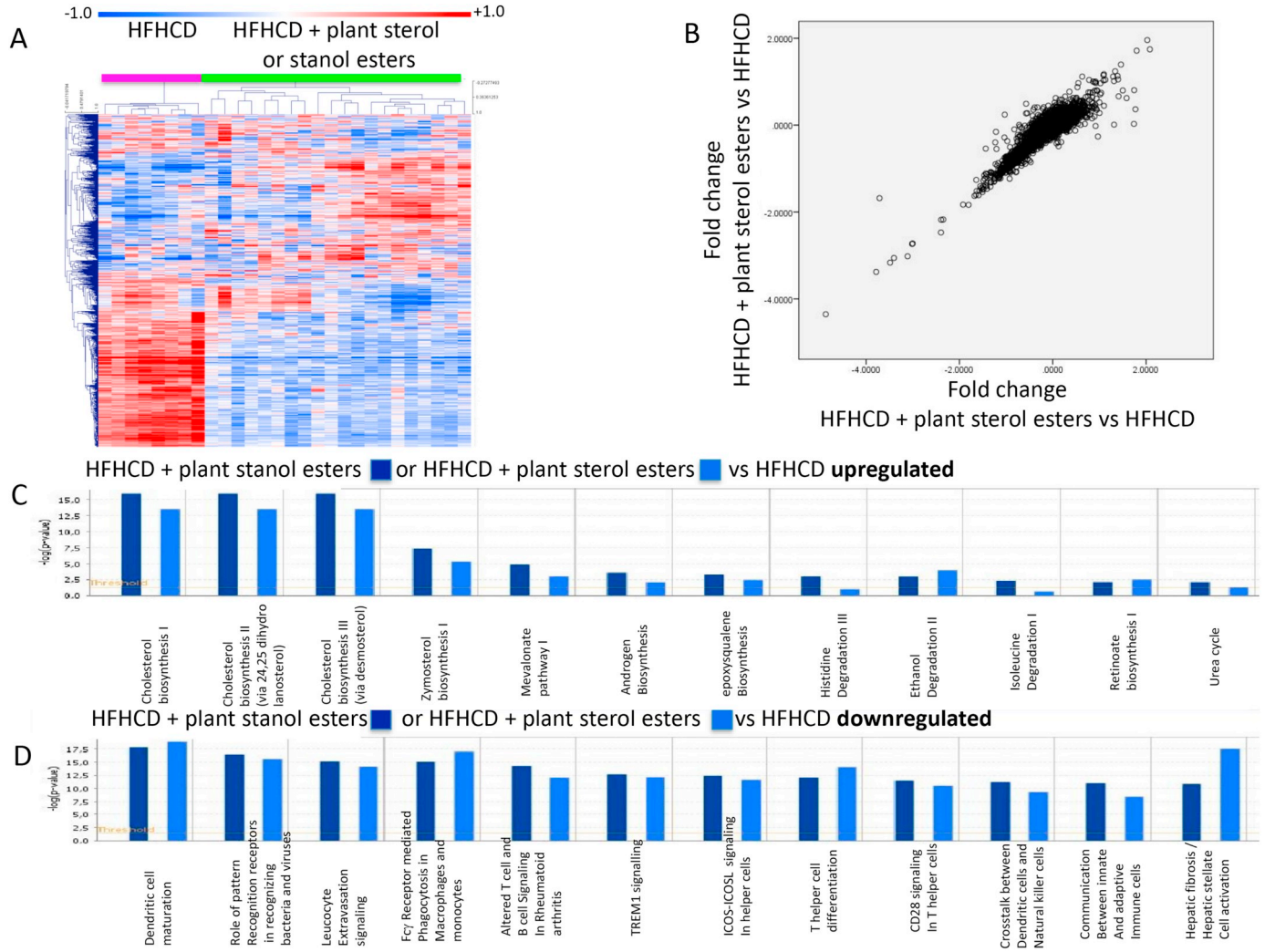

Fig. 7. Microarray analysis in livers of LDL-receptor deficient mice fed a high-fat, high-cholesterol (HFHCD) diet (control) plus plant sterols or stanols. Panel A is the heatmap for gene expression, panel B the correlation plot in gene expression between the HFHCD + plant sterol and HFHCD + plant stanol conditions. Panel C are the results from ingenuity pathway analysis (IPA) for upregulated pathways in the HFHCD + plant sterol and HFHCD + plant stanol condition versus the HFHCD condition and panel D the data from IPA for downregulated pathways in the HFHCD + plant sterol or plant stanol vs HFHCD condition (unpublished data by Plat et al).

question whether these observations suggests that circulating plant sterols may have direct effects on hepatic inflammation, independent from lowering cholesterol fluxes to the liver and of increasing cellular cholesterol precursor concentrations. In line, both sitosterol and sitostanol lowered inflammation in bone marrow derived macrophages in vitro, i.e. independent of cholesterol fluxes [128]. However, what needs to be considered is that plant stanols normally circulate in roughly 10-fold lower concentrations than plant sterols, and should therefore be much more potent than plant sterols on a molar basis.

To address the effects of plant sterols and stanol on liver inflammation further, their effects as part of a HFHCD on hepatic gene expression of LDLr−/− mice were examined. The heatmap of the microarray analyses clearly showed a reduction of inflammatory pathways in the livers of the plant sterol and stanol fed animals (Fig. 7; unpublished data), supporting earlier findings [128]. In agreement, the number of resident immune cells in the livers of the plant sterol and stanol supplemented animals was strongly reduced. The question then arises why the migration of immune cells to the liver was decreased. Unfortunately, this question could not be addressed, since we only had liver material from the end of the study. At that time point there were already significantly less immune cells and inflammation in the sterol and stanol groups, which made it impossible to detangle cause and consequence. However, in an acute human intervention study in which plant stanol esters were provided to healthy human volunteers [103], we observed a clear reduction in the intestinal expression of genes involved in T-cell migration, and chemotaxis. This finding further indicates that migration of immune cells is most is affected by consuming plant sterols and stanols.

There are not many other studies that specifically evaluated the effects of plant sterol and stanols on NASH-related outcomes. However, in one study CardioAid™, a plant sterol extract, was administered for 25 weeks to a hyperlipidemic mouse model for NASH. Treatment with CardioAid™ was associated with an anti-inflammatory profile resulting in a significant decrease in the CD4/CD8 ratio and an increase in CD4 + CD25+ Treg cell numbers in plasma. Moreover, a decrease in serum IL-1α and TGFβ levels was noted [131]. In line, a study supplementing plant sterols to a rat hyperlipidemic model for NASH demonstrated a reduction in hepatic inflammatory cytokines TGF-β, IL-6, IL-10, and CRP [132]. Together, the limited number of animal studies in NASH models, support a plant sterol-mediated suppression of hepatic inflammation.

Inflammatory bowel disease (IBD) is a group of inflammatory disorders of the small intestine and colon, characterized by dysfunctional mucosal T cells [133]. Chronic T cell resistance against apoptosis contributes to an inappropriate T cell accumulation and the perpetuation of chronic mucosal inflammation. Several studies have shown an anti-inflammatory role of plant sterols on different aspects of IBD [134–137]. In a T cell transfer mouse model for chronic IBD, a beneficial effect of plant sterols was attributed to an increase in Foxp3+ Tregs in the colon, but only when the animal consumed a low-fat diet [136]. In agreement, another plant derived steroid, guggulsterone, was protective against inflammation in a Th2-mediated IBD mouse model by attenuating neutrophil infiltration and reducing the expression of the pro-inflammatory genes IL-2, IL-4, IL-6 and TNF-α in the colon [135,138]. In an acute dextran sodium sulfate-induced model for IBD, plant sterols attenuated colonic inflammation by decreasing infiltration of inflammatory leukocytes and accelerating mucosal healing [134]. These findings, however, could not be confirmed in the study of Te Velde et al. [136]. Except for effects on T cells, plant sterols decreased the interaction between LPS and toll-like receptor 4 (TLR4) in intestinal macrophages, which may have contributed to the reduced inflammation [72]. Kim et al. have investigated the effect of sitosterol on the innate immune response in an HFD-induced colitis model in mice. Oral sitosterol administration reduced the HFD-induced colonic inflammation and the corresponding activation of nuclear factor kappa B (NF-κB) [72]. Sitosterol treatment in a murine 2,4,6-trinitrovenzene acid (TNBS)-induced colitis model inhibited the production of proinflammatory cytokines (IL-1β, TNF-α, and IL-6) and inflammatory enzymes (iNos and Cox2) in the colon, indicative of NF-κB activation [139]. Comparable results as well as NF-κB activation were found in plant sterol-treated LPS-stimulated intestinal macrophages [72]. To conclude, different mouse models for IBD point towards an immune-suppressive effect by modulating both innate and adaptive immune responses.

Uncontrolled immune activation of the gut can also occur around birth. An infection of the amniotic fluid may provoke inflammation of

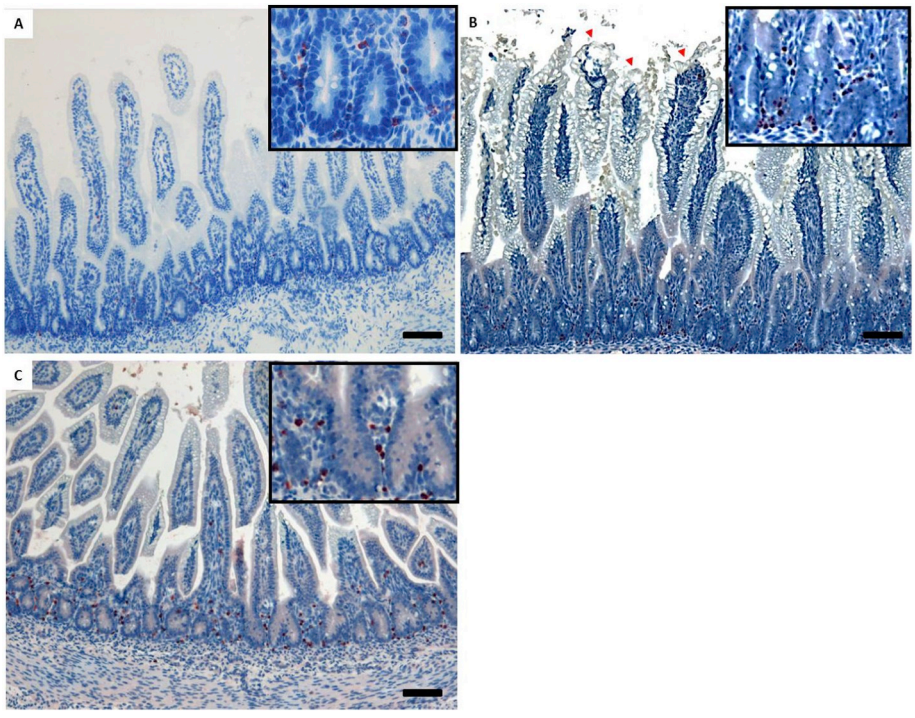

Fig. 8. Prophylactic plant sterol administration prevented influx and neutrophil migration, and mucosal damage in the ileum of preterm lambs that were intra-amniotically exposed to LPS. Panel A: MPO-positive cells visualized by AEC (red) in the ileum of saline-exposed control animals. Panel B: Intra-amniotic LPS exposure for 7 days resulted in increased numbers of MPO-positive cells, injured villus tips (arrow heads) and accumulation of luminal debris. Panel C: MPO-positive cell numbers and villus integrity were normalized by prophylactic plant sterol treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the foetal gut, which is associated with a higher risk of adverse intestinal outcomes including necrotizing enterocolitis (NEC) [140,141]. NEC is an inflammatory bowel disease of neonates and one of the most common gastrointestinal emergencies in newborn infants with high morbidity and mortality rates [142]. Although the exact etiology and pathophysiology of NEC is largely unknown, it is well accepted that NEC is a complex, multi-factorial disease with currently no available cure. Risk markers associated with NEC include prematurity, lack of breast-feeding, and chorioamnionitis [142,143]. Considering that the disease might have at least in part an antenatal origin, we recently studied the effects of enrichment of the amniotic fluid with plant sterols in a pre-clinical chorioamnionitis sheep model. In that study we showed that foetal gut inflammation and mucosal damage following intra-amniotic LPS exposure was prevented by prophylactic plant sterol supplementation of the amniotic fluid (unpublished data; Fig. 8). These findings indicate that enrichment of the amniotic fluid with plant sterols may potentially be used to prevent adverse outcomes of the foetal gut in conditions of antenatal inflammatory stress.

7. Plant sterols in Intestinal Failure Associated Liver Disease

Parenteral nutrition (PN) is a complete macronutrient mixture that is often combined with an intravenous lipid emulsions to supply fatty acids and energy to support growth and development in infants and children that cannot tolerate enteral feeding. In premature, low birth weight infants, and infants with congenital gastrointestinal disorders, PN support is an essential, life-saving component of clinical care. Typically, during short-term PN treatments, complications are limited. However, infants given longer-term PN support, particularly when combined with a soy-based lipid emulsions (SOLE), increase the risk for cholestasis, which is diagnosed by elevated serum total and conjugated bilirubin, and bile acid concentrations. This type of hepatobiliary injury is clinically identified as intestinal failure associated liver disease (IFALD) [144]. Reports show that IFALD develops in about 20% of infants after two weeks of parenteral SOLE feeding, and after three months the incidence increases to > 80% [145]. Clayton et al. were the first to observe that infants that developed IFALD had increased serum plant sterols concentrations that correlated with disease severity [146,147]. The underlying cause of IFALD is not due to the administration of intravenous lipid emulsions per se, since replacement of SOLE with fish oil-based lipid emulsions (FOLE) resulted in resolution of IFALD [148,149]. Importantly, because FOLE contains negligible amounts of plant sterols, these findings suggests that lack of plant sterols in FOLE is primarily responsible for IFALD resolution [150,151]. Interestingly, patients with phytosterolemia do not develop cholestasis or any other hepatic disease [151]. This raises the question why particularly intravenously administered (free) plant sterols cause cholestasis, whereas (esterified) dietary plant sterols that are absorbed from the gut, incorporated into chylomicrons and transported to the liver do not cause hepatic injury. It is tempting to speculate that free plant sterols administered through PN are metabolized by different hepatic compartments than chylomicron-derived plant sterols. Also, feeding dietary plant sterols and stanols as part of a HFHC diet to LDLr−/− mice improved liver function characterized by low hepatic cholesterol levels and no inflammation [128]. It is possible that like plant sterols, plant stanols are also involved in the PN-induced liver pathology, since stanol concentrations increase substantially during PN regimens and reach values above those seen during normal dietary intake. The pharmacokinetics, organ disposition, and metabolism of plant sterols when given orally and intravenously therefore warrants further study. For cholesterol, it has already been shown that the metabolic effects are different when incorporated in the liver into the cytoplasm or into the lysosomes [152]. Another intriguing question is what would happen if one would enrich the diet with plant sterols during partly PN and soy-based lipid emulsion regimens? Would it be possible to counterbalance the harmful effects of the parenterally-delivered plant sterols by increasing the amount of diet-derived plant sterols? To summarize, when plant sterols bypass the gut and its subsequent metabolism, i.e. bypassing what nature had in mind when developing our metabolic system with the gut as barrier, one should be aware of potential unwanted effects.

Molecular studies on the role of specific plant sterols have increased our understanding on how these compounds may contribute to the development of IFALD. The nuclear hormone receptor Farnesoid X Receptor (FXR) is the primary regulator of hepatic bile acid homeostasis through upregulating the bile salt export pump (BSEP), which facilitates clearance of bile acids from the liver to the bile canaliculi. Cell culture studies in the hepatic carcinoma cell line HepG2 has shown that administration of stigmasterol, but not sitosterol or campesterol, suppressed the transactivation of luciferase reporter plasmids containing response elements for FXR that are found in the BSEP promoter [150,153]. Primary endogenous ligands for FXR are bile acids, with the greatest binding affinity for chenodeoxycholic acid. Therefore, it is possible that some plant sterols may directly interact with the binding domain of FXR, as bile acids are derived from cholesterol and therefore share a strong structural similarity to plant sterols. A direct role of stigmasterol in the development of IFALD is supported in a mouse model of PN, when mice were exposed to intestinal injury and developed a chemically-induced colitis [154]. This study also observed that macrophages isolated from stigmasterol-supplemented mice released more IL-6. Indeed, in isolated Kupffer cells, co-incubation with SOLE, LPS, and exogenous plant sterols markedly increased IL-1β protein compared to LPS alone [155]. This data suggests that plant sterols are also potent mediators of the inflammatory response in macrophages that have accumulated large amounts of lipids. However, it needs to be studied into more detail whether this is true for all plant sterols or only for stigmasterol.

Another possibility is that plant sterols in PN-regimens cause tissue damage, because they are administered in a free form - instead of being absorbed from the intestine and incorporated into chylomicrons, which also contain diet-derived fat-soluble vitamins and antioxidants like tocopherols and carotenoids. Plant sterols injected in their free form may be more prone to oxidation. Plant sterols, as cholesterol, possess a double bond in their ring structure and are therefore susceptible to oxidation by non-enzymatic processes, such as reactions with reactive oxygen species (ROS) [156]. The question is whether elevated oxyphytosterol concentrations in plasma translate to beneficial or undesirable health consequences. Studies in female LDLr+/− mice showed that increased plasma oxyphytosterol concentrations are potentially atherogenic [157]. Moreover, in humans, oxyphytosterols have been found in aortic valve cusps [158] and circulating oxyphytosterol concentrations increased postprandially after a plant sterol enriched meal [159]. The intraperitoneal administration of non-oxidized cholesterol or sitosterol did not increase of plasma oxysterol or oxyphytosterol concentrations. Oxidative stress in the aorta was increased in 7β-OH-sitosterol treated mice, but not in mice treated with cholesterol, sitosterol, or 7β-OH-cholesterol. Moreover, cholesterol, sitosterol, 7β-OH-cholesterol, and 7β-OH-sitosterol did not affect endothelial-dependent vasodilation or early atherosclerosis development [160]. If oxysterol products are injurious in PN, addition of antioxidants could be beneficial. Indeed, mixed oil lipid emulsions containing SOLE, FOLE, olive oil, and medium chain triacylglycerols supplemented with vitamin E suppressed the development of IFALD in piglet studies [153,161] with comparable results in one human trial [162]. However, the administration of supplemental vitamin E to SOLE has produced mixed results. Addition of vitamin E to SOLE prevented the development of PNALD in preterm piglets [163], but not in term piglets [164]. As preterm infants have impaired antioxidant defenses, these results may suggest that plant sterol oxidation may play a role in IFALD development. Clearly, the understand the role of oxyphytosterols in PN conditions on IFALD warrants further study.

8. Role of plant sterol oxidation in excretion

It is well acknowledged that cellular free cholesterol concentrations are tightly regulated for which the cell has an arsenal of options, i.e. esterification (ACAT), reduced synthesis (HMG-CoA reductase) and uptake (LDLr), or enhanced secretion (ABCA1). For cellular plant sterols, fewer options are available. It is even not known whether cells can sense its intracellular or membrane (free) plant sterol concentration. Plant sterols are very poor substrates for ACAT making esterification [165] for intracellular storage not likely. Cellular secretion might be possible, although it is not well established to what extent plant sterols are secreted via ABCA1. While ABCG5/G8 are of course highly specific plant sterol transporters in enterocytes and hepatocytes, these transporters are not ubiquitously expressed in other cell types. As humans do not produce plant sterols, endogenous synthesis is not regulated and the uptake is linked to uptake of lipoproteins, and related to regulatory mechanisms for cholesterol uptake. Does this suggest that a cell cannot actively regulate its plant sterol homeostasis? This is not plausible, as it would mean that any cell without ABCG5/G8 would be severely loaded with plant sterols. Therefore, we recently hypothesized that a cell oxidizes plant sterols to form oxyphytosterols as a mechanism to excrete plant sterols from tissues into the circulation [166]. A comparable mechanism is known for the brain, which secretes excess cholesterol as 24-OH cholesterol across the blood-brain barrier into the circulation [167,168].

9. Integration

The original paradigm that plant sterols and stanols are relatively inert molecules that remain within the intestinal lumen, hardly enter the circulation and are therefore without systemic or local effects, needs revision. We now begin to understand that the plant sterols and stanols that are taken up into the circulation may have systemic and cellular effects, even if circulating and tissue concentrations are low. However, it is also still possible that systemic effects are caused by signaling molecules originating for example from cells in the small intestines after plant sterol intake. This novel area of plant sterol and stanol research is only in its infancy, simply because it has not received much attention over the past decades. These new research areas, as depicted in Fig. 9, will undoubtedly provide new insights on the health effects of plant sterols and stanols in the very near future.

10. Conclusion

There is a need to better understand the health effects of plant sterols and stanols, as evidence is accumulating that these compounds have effects beyond the competition with intestinal cholesterol uptake, resulting in decreased LDL-cholesterol concentrations. One area of interest that needs further fundamental research are effects of plant sterols and stanols on immune fitness, since there are at present hardly any dietary approaches to intervene with immune functioning. Also, potential effects on liver inflammation are of great interest. In this respect, one needs to realize that the route of administration can be important based on findings from studies on PN and IFALD.

11. Goal of this review

The current success foods rich in plant sterols and stanols to improve cholesterol metabolism is understandable and has many health benefits. However, we here pose the intriguing question “What would have happened if plant sterols and stanols had been discovered and embraced by disciplines such as immunology, hepatology or rheumatology before being positioned as cholesterol-lowering molecules?” What would have been the main benefits and fields of application of plant sterols and stanols today? There are ample emerging indications that plant sterols and stanols have numerous effects beyond lowering serum low-density lipoprotein (LDL) cholesterol concentrations. It might well be that these currently largely unnoticed “additional effects” are as important - if not even more important - compared with the established cholesterol-lowering effects. Since their use as LDL-cholesterol lowering dietary ingredients, it has become even more challenging to identify such “additional effects”, because study participants in which these potential effects might be identified are often excluded from clinical trials that evaluate changes in cholesterol metabolism, making unexpected findings less likely. Therefore, in this review we discuss the effects of dietary plant sterols and stanols from a different

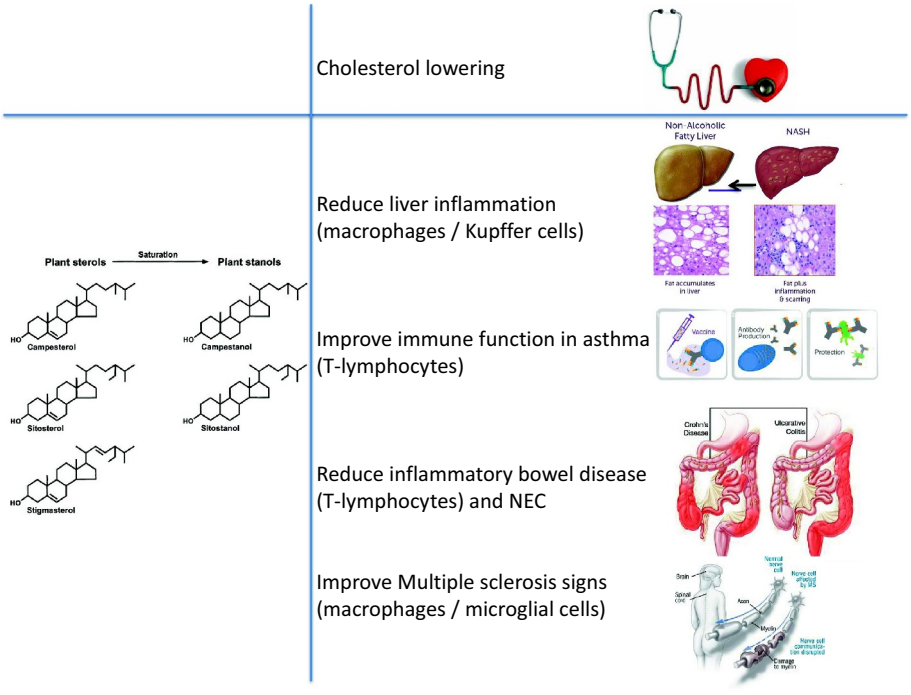

Fig. 9. Overview of the potential effects of plant sterols and stanols beyond lowering LDL-cholesterol concentrations that are currently studied.

perspective with the aim to understand whether consuming plant sterol- and stanol-rich diets could be beneficial to improve other disturbed metabolic conditions or even health, and whether effects depends on the health status of an individual at the time of plant sterol or stanol consumption. Notably, from an evolutionary perspective, as humans have also evolved in a plant-based sterol-rich environment, it seems cholesterol-centric and not rational to think that only a process like intestinal cholesterol absorption was affected by dietary plant sterols and stanols. It is more likely that plant sterols and stanols played a more imperative role in human physiology, since plant sterols and stanols were important dietary constituents at ancient times. Therefore, in this review we summarize the available evidence for potential effects of plant sterols and stanols beyond lowering LDL-cholesterol. Only if we fully understand the complete spectrum of (patho)physiological effects of plant sterols and stanols, we can provide recommendations regarding daily intakes to achieve optimal health benefits.

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