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Plant sterols: friend or foe in CNS disorders?

Tim Vanmierlo^{1*}, Jeroen J.F. Bogie^{1,2*}, Jo Mailleux¹, Jasmine Vanmol¹, Dieter Lütjohann³,
Monique Mulder² and Jerome J.A. Hendriks^{1#}

¹Biomedisch Onderzoeksinstituut and transnationale Universiteit Limburg, School of Life Sciences, Hasselt University, Martelarenlaan 42, 3500 Hasselt, Belgium.

² Division of Pharmacology, Vascular and Metabolic Diseases, Department of Internal Medicine, Rotterdam University, Wytemaweg 80, 3015 CN Rotterdam, The Netherlands.

³ Institute for Clinical Chemistry and Clinical Pharmacology, University Clinics Bonn, Sigmund-Freud.-Str. 25, 53127 Bonn, Germany.

*equal contribution

#corresponding author

Biomedisch Onderzoeksinstituut and transnationale Universiteit Limburg, School of Life Sciences, Hasselt University, Martelarenlaan 42, 3500 Hasselt, Belgium. +32(0)11269207
jerome.hendriks@uhasselt.be

In mammals, the central nervous system (CNS) is the most cholesterol rich organ by weight. Cholesterol metabolism is tightly regulated in the CNS and all cholesterol available is synthesized in situ. Deficits in cholesterol homeostasis at the level of synthesis, transport, or catabolism result in severe disorders featured by neurological disability. Recent studies indicate that a disturbed cholesterol metabolism is involved in CNS disorders, such as Alzheimer's disease (AD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS). In contrast to circulating cholesterol, dietary plant sterols, can cross the blood-brain barrier and accumulate in the membranes of CNS cells. Plant sterols are well-known for their ability to lower circulating cholesterol levels. The finding that they gain access to the CNS has fueled research focusing on the physiological roles of plant sterols in the healthy and diseased CNS. To date, both beneficial and detrimental effects of plant sterols on CNS disorders are defined. In this review, we discuss recent findings regarding the impact of plant sterols on homeostatic and pathogenic processes in the CNS, and elaborate on the therapeutic potential of plant sterols in CNS disorders.

Author information

Tim Vanmierlo: tim.vanmierlo@uhasselt.be

Jeroen Bogie: jeroen.bogie@uhasselt.be

Jo Mailleux: jo.mailleux@uhasselt.be

Jasmine Vanmol: jasmine.vanmol@uhasselt.be

Dieter Lütjohann: dieter.luetjohann@ukb.uni-bonn.de

Monique Mulder: m.t.mulder@erasmusmc.nl

Jerome Hendriks: jerome.hendriks@uhasselt.be

Introduction

Plant sterols and plant stanols, collectively known as phytosterols, are naturally occurring compounds that structurally and functionally resemble cholesterol in mammals. Over the last decades, more than 260 different phytosterols (derivatives) have been described [1]. The chemical structure of phytosterols consists of a sterol-derived core that is decorated with divergent ring and/or C17 side-chain modifications. Plant stanols are saturated plant sterols, without double bonds in the sterol ring moiety (figure 1). Mammals are unable to synthesize phytosterols and therefore can only obtain them from their diet. Food rich in phytosterols include vegetables, fruits, nuts, cereals, vegetable oils, and phytosterol-enriched dairy spreads. Typically, the daily intake of plant sterols and stanols in humans is estimated on 300 mg and 20 mg, respectively [2, 3]. Interestingly, whilst the daily intake of cholesterol approaches that of phytosterols, plasma levels of plant sterols (7-24 $\mu\text{mol/L}$) and stanols (0.05-0.3 $\mu\text{mol/L}$) are markedly lower than those of cholesterol (~ 5 mmol/L) [4, 5]. In the jejunum, both phytosterols and cholesterol are incorporated into mixed micelles and are subsequently absorbed at the apical site of enterocytes via the Niemann Pick C1 Like 1 (NPC1L1) transporter [6]. However, in contrast to cholesterol, phytosterols are poor substrates for the esterifying enzyme acetyl-sterol O-acyltransferase 2 (SOAT2/ACAT2) in enterocytes [7]. Due to incomplete or inefficient esterification, the bulk of phytosterols is transported back into the intestinal lumen. Moreover, phytosterols that enter the circulation are quickly excreted into the bile by hepatocytes [7]. The obligatory heterodimeric complex ATP-binding cassette co-transporter G5 and G8 (ABCG5/G8) exerts a crucial role in the selective excretion of phytosterols by enterocytes and hepatocytes, and thereby contributes to the relatively low plasma and tissue levels of phytosterols [8-10].

Elevated levels of total cholesterol, and in particular low density lipoprotein (LDL)-cholesterol, are important risk factors for cardiovascular diseases (CVD), such as coronary heart disease (CHD) [11, 12]. Phytosterols are well-known for their ability to lower plasma cholesterol levels and the FDA has approved this health claim of phytosterols as cholesterol lowering agent. A recent meta-analysis of 44 studies and 2084 individuals defined that phytosterol intake (~ 1.6 g/d) decreases total- and LDL-cholesterol by 0.36 mmol/L (5.9%) and 0.33 mmol/L (8.5%), respectively [13]. This drop in circulating cholesterol levels is achieved at the expense of higher serum phytosterol levels [14]. In spite of the cholesterol-lowering impact, the use of phytosterols as functional food in the prevention of CVD is a current topic of debate [5, 15]. Patients homozygous for phytosterolemia, a rare autosomal inherited disorder characterized by dramatically heightened circulating phytosterols, demonstrate premature tissue deposition of phytosterols and signs of accelerated atherosclerosis [15-17]. The latter suggests that in excess phytosterols or phytosterol metabolites may promote CVD. Moreover, no relevant clinical endpoint studies showing a survival benefit of phytosterols in CVD have been published to date [15, 18, 19]. Consequently, in July 2014, the 2013 American College of Cardiology/American Heart Association (ACC/AHA) Task Force on practice guidelines removed the recommendation of phytosterol use to prevent atherosclerotic CVD events from their guidelines [20]. Preceding this retraction, an international consensus panel of basic researchers and clinical investigators with expertise in the cholesterol metabolism and phytosterol biology already limited the recommendation of phytosterols consumption to (1) patients with elevated cholesterol levels that have a low to intermediate risk on CVD and do not qualify for pharmacotherapy, (2) statin-intolerant patients with high cholesterol levels, and (3) familiar hypercholesterolemic patients receiving a combination therapy with statins

[5]. Collectively, multiple studies show that phytosterols lower plasma cholesterol levels, however, the underlying mechanisms and long-term perspectives of phytosterol use remain poorly understood. Therefore, prospective studies are mandatory to validate the therapeutic applicability of phytosterols to treat hypercholesterolemic patients.

Phytosterols are biochemically heterogeneous, undergoing diverse structural modifications. For instance, in contrast to plant stanols and similar to cholesterol, plant sterols can undergo steroid ring and side chain oxidation to form plant sterol oxidation products (oxyphytosterols) [21, 22]. Oxyphytosterols are present in plasma of healthy subjects and are markedly elevated in phytosterolemic patients [23, 24]. However, controversy exist as to whether normo-phytosterolemic subjects treated with plant sterol enriched margarine have elevated levels of oxidized phytosterols [25, 26]. The source of oxyphytosterols in the body is dual. Oxyphytosterols are present in phytosterol-enriched dairy products, which indicates that intestinal absorption likely contributes to the presence of oxyphytosterols in serum [27]. However, only trace amounts of oxyphytosterols are found in these spreads [28]. Alternatively, systemic oxyphytosterols can be formed from plant sterols that undergo autoxidation or enzymatic conversion in the body [21, 22]. Of note, plant sterol oxidation products found in human plasma are typically 10-100 times lower than those of cholesterol oxidation products [29]. To date, little is known about the physiological and pathological role of oxyphytosterols. The general impression is that they represent a health problem due to their structural similarity with cholesterol oxidation products. However, other studies indicate that oxyphytosterols have beneficial properties as well, such as counteracting inflammation and anti-tumor activity [21, 22, 30, 31]. Apart from oxidation, phytosterols are found in glycosylated forms, such as phytosterol glycosides and acyl phytosterol glycosides. The hydroxyl group at C3 is linked to a hexose or a 6-fatty-acyl hexose moiety for phytosterol glucosides and acyl glucosides, respectively [32]. Glycosylated phytosterols are present in particular foods and plants, and efficiently lower plasma cholesterol levels [33-37]. It is unclear to what extent phytosterol glucosides are formed from non-modified phytosterols within the body. Irrespective of their origin, low amounts of phytosterol glucosides have been demonstrated in serum of humans [38]. In addition, animals fed β -sitosterol β -D-glucoside have significantly higher serum-sterol glucoside levels [39]. Biologically, studies defined both toxic properties and anti-inflammatory properties of a number of glycosylated phytosterols [36, 39-42]. In summary, phytosterols undergo diverse modifications that may alter their biological impact.

Recent studies indicate that dietary phytosterols stably accumulate in the CNS [43-45]. This finding has prompted research focusing on the physiological roles of phytosterols in the healthy and diseased CNS. In this review, we summarize and discuss findings regarding the impact of phytosterols on homeostatic and pathogenic processes in the CNS, and elaborate on the therapeutic potential of phytosterols in CNS disorders. The scope of our review is limited to the most prevalent phytosterols in the Western diet and to those phytosterols which have been described to modulate CNS metabolism: sitosterol, campesterol, brassicasterol, fucosterol, spinasterol, stigmasterol, 24(S)-saringosterol, and schottenol [4, 5, 46]. Nevertheless, future purification and analyses of other, less prominent dietary phytosterols may reveal novel physiological roles of phytosterols.

Phytosterols in the healthy CNS

The consumption of phytosterol-enriched functional foods over five years roughly doubles circulating plant sterol and stanol levels [47]. Moreover, phytosterols accumulate in peripheral tissues, such as aortic valves, liver, and the CNS [48-50]. Quantitative data on temporal and spatial plant sterol accumulation in the human brain parenchyma is rather scarce. One study measured plant sterols in non-demented and demented brain samples [50]. Herein, Saeed and colleagues found that sitosterol and campesterol are present in the “5 to 10 ng/mg wet tissue”-range in the temporal and parietal cortex. These concentrations were comparable to the oxysterol concentrations in these regions (24(S)OHcholesterol: 15-25ng/mg; 27OHcholesterol: 1-3ng/mg). In contrast, we found that the concentration of the most prominent plant sterol in the cerebrospinal fluid (CSF), sitosterol (2.48 µg/dl), was 10-fold higher than the major CNS cholesterol metabolite in the CSF, 24(S)OHcholesterol (0.264µg/dl) [48, 51]. Moreover, in an animal study, it was shown that a 2% plant sterol-enriched diet over six weeks resulted in a stable doubling of plant sterols in the CNS of mice [45]. Based on the accumulation in the CNS and the structural similarity to cholesterol, phytosterols may interfere with cholesterol metabolism in the CNS. In this section, we elaborate on sterol metabolism in the CNS and the impact of phytosterols hereon, and speculate on mechanisms involved in the transport of sterols across the BBB.

Although the brain only accounts for 2.1% of the total body weight, Dietschy and Turley estimated it to contain about 23% of all free cholesterol in the body [52]. Remarkably, virtually all cholesterol within the CNS is synthesized *in situ* [53, 54]. In the CNS, cholesterol plays a pivotal role in synapse formation, cell-cell interactions, and intracellular signaling [52, 55]. A steady cholesterol turnover is needed to maintain these homeostatic processes. Overall, cholesterol turnover in the brain is rather slow and far more stable than that in the rest of the body [56]. Cholesterol in the brain resides in three compartments. The majority (70-80%, or ~260 mg/g of the total ~330 mg/g dry weight) of lipids is present in the myelin sheaths surrounding the axons and has a slow turnover rate, displaying half-replacement times of 359 days or ~0.3%/day [57-59]. The remaining 70 mg/g cholesterol resides within neurons (~7 mg/g) and glial cells (~63 mg/g) [60-63]. In contrast to the sturdy myelin cholesterol pool, cholesterol turnover in the neuronal membranes is high. To illustrate, pyramidal cells of the cortex and Purkinje cells of the cerebellum have a cholesterol turnover of more than 20%/day, whereas whole body cholesterol turnover is 0.7%/day [52, 64, 65]. The high cholesterol turnover rate in neurons facilitates their ability to adapt efficiently and quickly to dynamic structural changes during synaptic plasticity [62, 66]. Locally in the CNS, cholesterol is metabolized into the more polar CNS-specific 24(S)-hydroxycholesterol, which is more easily released from the CNS than cholesterol [54]. In contrast to the periphery, cholesterol is hardly metabolized into 27-hydroxycholesterol in the CNS. The conversion of cholesterol to 24(S)-hydroxycholesterol in neurons accounts for over 60% of cholesterol efflux from the CNS [62, 67-70]. Interestingly, as opposed to cholesterol, desmosterol, 7 α -hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol, the predominant plant sterol sitosterol cannot be metabolized into 24(S)-hydroxysitosterol [71]. In line herewith, in animals fed a plant sterol-enriched diet over six weeks, the concentration of sitosterol, as well as campesterol, was shown to be unaltered in the CNS upon six months of plasma plant sterol depletion [45]. This finding indicates that phytosterols, such as sitosterol and campesterol, stably accumulate in the CNS and suggests that an inability to be catabolized into 24(S)-hydroxysterol underlies this accumulation. Collectively, these studies imply that sterol turnover is precisely regulated in divergent compartments in the CNS, and differs between cholesterol and phytosterols. However, more research is warranted to validate this hypothesis.

The recruitment of circulating sterols across an intact BBB is very limited [45, 72]. Although discrepancies in the literature exist [73], several *in vivo* studies defined a flux of sterols into the CNS [66, 74, 75]. For instance, terminally ill patients that are daily dosed with 4-C¹⁴-cholesterol (ranging from 1 to 226 days) reveal an average cerebral cholesterol accumulation of 3.2% [66]. However, a treatment- or disease-induced impairment of the BBB integrity in these patients may contribute to cholesterol accumulation in the CNS. Yet, in agreement with these data, administration of deuterium-labeled cholesterol-d6 in different rodent models results in a ~1% cerebral cholesterol accumulation [74, 75]. In parallel to cholesterol, transport of plant sterols towards the CNS is also limited under physiological conditions. However, a dysfunctional BBB may disturb the balanced exchange of sterols between the CNS and the circulation. For instance, *pdgfb^{ret/ret}* mice, which are characterized by an increased permeability of the BBB, show a significant flux of phytosterols into the brain [73]. Additionally, it has been shown in an BBB model and in a dietary mouse study that sterol accumulation in CNS cells depends on the molecular complexity of the sterol side chain [45]. Sterols with a lower molecular side-chain complexity such as cholesterol and campesterol cross an endothelial barrier more easily as compared to phytosterols that contain a more complex hydrophobic side chain such as sitosterol and stigmasterol [45, 73, 76]. Interestingly, sitosterol and campesterol crossed a brain endothelial monolayer **less efficient than** cholesterol. Whether cholesterol and plant sterol use similar transport mechanisms to cross the BBB remains to be elucidated. Compared to wild type mice, *Abcg5^{-/-}* and *ApoE^{-/-}* mice display up to 12-fold increased levels of phytosterols in their circulation. Remarkably, only *Abcg5^{-/-}*, but not *ApoE^{-/-}* mice, show increased phytosterol levels in the CNS [44]. Importantly, the *Abcg5/g8* transporters are not detectable within the brain [44]. Therefore, it is unlikely that the *Abcg5/g8* transporter complex modulates phytosterol transport across the BBB. In addition, whereas in wild type and *Abcg5^{-/-}* mice phytosterols are predominantly located in high density lipoproteins (HDL), phytosterols are mainly incorporated in very low density lipoproteins (VLDL) in *ApoE^{-/-}* mice [44]. Although it remains speculative, the latter supports a role for HDL-mediated transport of sterols across the BBB. Interestingly, the scavenger receptor class B member 1 (SR-BI) is the major receptor for HDL and is expressed at the apical membrane of BBB endothelial cells [72]. Collectively, these studies make us speculate that, although limited in quantity, sterols may be transported across the BBB into the CNS via HDL/SR-BI-dependent mechanism (figure 2).

Once dietary sterols have entered the CNS, in particular upon increased dietary intake, they tend to accumulate within lipid rafts of CNS parenchymal cells [44, 45]. The incorporation of sterols in biological membranes can result in structural and functional changes in membrane properties. The majority of lipid membrane bilayers occur as a homogeneous liquid-disordered (ld) phase. However, transient lateral heterogeneities coexist in a liquid-ordered (lo) phase, so called lipid rafts (figure 3a) [77]. Raft domains are enriched in free sterols, mostly cholesterol, and saturated lipids, including sphingomyelin, glycosphingolipids, cerebrosides, and gangliosides. The main membrane phospholipids – phosphatidylcholine, and phosphatidylethanolamine - are mostly excluded from the lipid rafts [78]. Cholesterol increases the lipid order and rigidity of the lipid rafts at the cytofacial leaflet of the membrane [79]. Moreover, cholesterol is essential to promote the separation between the ld and lo phase, allowing lipid raft-specific cell signaling [80, 81]. Lipid rafts are integral membrane scaffolds, acting as a movable platform for processes involved in membrane trafficking and signaling, and regulation of the activity of membrane proteins [78, 82, 83]. Incorporation of less compact lipids reduces the rigidity of lipids rafts and alters their function. Indeed, phytosterols reduce the molecular order of membranes and therefore alter the membrane fluidity and functionality [84, 85]. It was demonstrated that the magnitude of the lipid order in

membranes depends on the geometry of the side chain (cholesterol >> campesterol > sitosterol > stigmasterol) [84]. Interestingly, we found that feeding mice a 2% w/w plant sterol enriched diet for six weeks, resulted in a two-fold increase in lipid raft-associated plant sterol concentration (~3.5 ng/mg protein to ~7 ng/mg protein), whereas cholesterol concentration in the lipid raft remained stable (~2,300 ng/mg protein) [45]. It remains to be defined whether this 1:300 plant sterol-to-cholesterol ratio is sufficient to functionally modulate membrane and/or raft properties. However, most of the sterols in the CNS are trapped in the sturdy oligodendrocyte myelin pool. The actual, biological active sterol pool in the CNS is limited and consequently more vulnerable for changes in their micro-constitution. Although speculative, the “plant sterol-to-cholesterol balance” is expected to shift more towards the plant sterol side of the balance in those metabolic more active cells. Furthermore, incorporation of 15 μ M sitosterol in HT22 hippocampal cells improved mitochondrial function by lowering the liquid order in mitochondrial membranes [86]. Despite the physiological concentration applied, the use of the methyl- β -cyclodextrin sterol-loading method may confound the neuronal functional outcome [87]. Yet, *in vivo* antioxidant activity of sitosterol is suggested to be mediated via lipid raft related ER-PI3K-GSK3 β signaling, leading to an increased expression of antioxidant proteins such as glutathione-transferase A1/A2, glutathione peroxidase, and γ -glutamyl cysteine ligase [88-90].

Upon incorporation in biological membranes in the CNS, sterols are shuttled by astrocytes into HDL-like particles, supplying neurons with sterols [91, 92]. Remarkably, it was shown that the efflux of sterols from a neuronal cell line is only poorly enhanced by adding external HDL as acceptor [45]. **This finding indicates that dietary plant sterols may accumulate more efficient in neurons. Also, a diet rich in cholesterol and fatty acids (predominantly saturated and monounsaturated fatty acids) ameliorated neurological deficits caused by a defective astrocyte lipid metabolism. In these mice the sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP) was deleted from astrocytes, resulting in astrocytes lacking endogenous cholesterol synthesis [93]. These observations establish a critical role for astrocytes in brain lipid metabolism and demonstrated that dietary lipids, can rescue astrocyte-related lipid deficiency. Although the plant sterol campesterol was elevated in the astrocyte-SCAP knockout brains, reflecting the increased transfer of sterols towards the brain, it remains to be determined whether dietary plant sterol supplementation is beneficial to the reduce neurological deficits in this transgenic mouse model [93]. Plant sterol administration may be an interesting alternative to atherogenic cholesterol supplementation.** However, at this stage, the translational relevance of these findings is lacking. Together, although it remains topic of debate whether physiologic relevant plant sterol concentrations are reached within the lipid rafts, a role for plant sterols in modulating lipid raft functioning is gaining support.

To date, it is largely unclear whether the accumulation of phytosterols in the CNS leads to a functional cognitive phenotype. On one hand, long-term exposure to increased levels of phytosterols in the circulation and CNS in *Abcg5*-deficient mice did not lead to an overt cognitive pattern with respect to memory and anxiety [94]. Similar, a combined phytosterols and high fat/high energy diet, from gestation on, did not reveal a significant influence of phytosterols on spatial memory in mice [95]. In concordance, a randomized double-blind placebo-controlled dietary intervention study defined no negative influence of long-term plant sterol or stanol consumption on neurocognitive functioning and mood in hypercholesteromic patients receiving statin treatment [96]. On the other hand, studies demonstrated that plant extracts have anxiolytic-like effects in animal models after intraperitoneal administration [97, 98]. In these studies, plant

sterols, and in particular sitosterol, was identified as the active anxiolytic compound [97, 98]. Of note, the anxiolytic role of sitosterol could not be confirmed in *Abcg5*^{-/-} mice, which have a 12-fold increase in sitosterol concentration in the limbic system [94]. However, in these animals, plant sterol levels are chronically elevated from birth on. Besides the limited role of phytosterols in cognition, anti-nociceptive properties have been ascribed to the plant sterol α -spinosterol after oral administration [99]. In contrast, in a scopolamine-induced memory impairment model, acute administration of stigmasterol to adult rats alleviated memory impairment by enhancing the cholinergic neurotransmission system [100]. Stigmasterol levels in brain were not measured directly but did affect the cerebral expression of memory-related proteins. It should be noted that the role of stigmasterol on acetylcholine esterase activity *in vitro* was found to be rather limited [101]. Together, phytosterols do not enhance cognition in normo-cognitive settings, whereas they do show a therapeutic potential during chemically induced and disease related cognitive impairment.

Phytosterols and the endothelium in CNS disorders

The BBB is one of the three CNS barriers and constitutes the largest interface for the exchange of constituents between the blood and the CNS [102]. Brain endothelial cells tightly regulate BBB function and are regarded as the gatekeepers of the CNS [102-104]. Dysfunction of brain endothelial cells may turn them into culprits for neurological disorders. For instance, endothelial dysfunction fuels the expression of adhesion molecules, chemotactic proteins, angiogenic factors, and reactive oxygen species (ROS), thereby promoting immune cell infiltration and neuroinflammation in CNS disorders such as MS and AD [105-109].

In the last decade, both beneficial and detrimental effects of phytosterols on endothelial function have been reported. Weingärtner and colleagues demonstrated that a 2% plant sterol ester-enriched diet impaired endothelium-dependent vasorelaxation in wild type mice. Moreover, cerebral lesion size was significantly increased on this diet in a mouse stroke model with induced middle cerebral artery occlusion [49]. *ApoE*^{-/-} mice on a Western-type diet supplemented with plant stanol esters significantly decreased vascular superoxide release from the intact aortic ring. In contrast, a plant sterol-enriched diet did not affect vascular superoxide release in these mice, suggesting that the type of phytosterol or its structural modification defines as to whether it modulates the production of superoxides by endothelial cells [110]. In concordance with this hypothesis, a rather high amount of the oxidized form of sitosterol (72 μ M) boosts ROS production in rat aortic endothelial cells [111]. Furthermore, it was shown that sitosterol-induced ROS in turn elevates the expression of cyclooxygenase-2, resulting in an altered prostanoid profile, and ultimately endothelial dysfunction and angiogenesis [111]. In contrast, another study found stigmasterol to have a strong superoxide anion scavenging capacity. However, in this study, a supra-physiological concentration of stigmasterol was used (242 μ M) [101]. Phytosterols have also been reported to possess anti-apoptotic properties in an endothelial cell line. Both hydroxylated and glycosylated phytosterol analogues inhibit serum deprivation-induced apoptosis of human umbilical venous endothelial cells (HUVECs) [112]. At the level of immune cell migration, mainly anti-inflammatory effects of plant sterols are reported; they reduce the expression of adhesion molecules and thereby prevent the migration of immune cells across the BBB. For instance, sitosterol inhibits the expression of intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) in stimulated HUVECs and human aortic endothelial cells (HAECs) [113, 114].

Consequently, sitosterol blocks the adhesion of neutrophils to an endothelial monolayer [115]. However, supra-physiological concentrations of sitosterol were used in this study (100 μ M). Collectively, these studies indicate that phytosterols can modulate diverse endothelium-dependent processes, such as vasorelaxation, oxidative stress, ischemia-reperfusion, and neuroinflammation. These biological processes are key in the development and progression of CNS disorders. Therefore, depending on the nature, concentration, and target cell of the phytosterol applied, phytosterols may have a crucial impact on neurodegenerative disorders and stroke.

Phytosterols in Alzheimer's disease

AD is the most prevalent progressive neurodegenerative disorder in elderly. It is neuropathologically characterized by extracellular deposition of amyloid- β (A β) in senile plaques, the appearance of intracellular neurofibrillary tangles, neuronal loss, synaptic damage, and cholinergic deficits [116]. In AD, A β peptides are released by sequential proteolytic cleavage of APP, a large neuronal type I transmembrane protein [117]. APP is processed via two alternative pathways, the non-amyloidogenic pathway involving α -secretase mediated cleavage, and the amyloidogenic pathway involving sequential cleavage of APP by β -secretase and γ -secretase [118]. The latter pathway results in the secretion of the insoluble amyloidogenic A β . The amyloidogenic pathway is predominantly associated with lipid rafts, whereas the non-amyloidogenic pathway is correlated with non-lipid rafts [119, 120]. Accumulating evidence suggests a key role for a disturbed CNS cholesterol homeostasis in the development and progression of AD [121-127]. In this section, we elaborate on the impact of sterol metabolism on the development and progression of AD, and discuss how phytosterols may impact AD pathology.

Diverse studies point towards a role for cholesterol metabolism in the pathophysiology of AD. First, case-control studies defined that AD patients display an impaired cholesterol turnover [50, 125, 126, 128]. Correspondingly, suppression of cholesterol biosynthesis was demonstrated to reduce the production of A β species both *in vitro* and in an AD mouse model [129]. Second, a diet high in cholesterol was found to accelerate A β generation in APP transgenic mice [130, 131]. Third, stimulation of cholesterol turnover in the CNS, through the activation of liver X receptors (LXRs), significantly improved cognitive performance in animal models of AD [132-134]. Notably, discrepancy exist as to whether a reduced A β plaque load underlies the improved cognitive performance in an AD mouse model upon LXR agonist [132-134]. Differences in the treatment regime and animal models used may explain discrepancies between the studies. On the neuronal level, a cholesterol-mediated relocation of APP from the non-lipid raft part of the membranes to the lipid rafts may underlie the stimulatory impact of cholesterol on A β generation [135]. This relocation increases the accessibility of β -secretase to its substrate APP and thereby promotes the amyloidogenic cleavage of APP. These studies indicate that sterol metabolism plays a key role in the progression of AD [136].

Phytosterols have been shown to beneficially modulate molecular processes involved in AD, such as APP processing and amyloid beta plaque formation in several *in vitro* and *in vivo* experimental setups [137-139]. In the next paragraphs, we discuss three theoretical models on how plant sterols may modulate AD on the molecular level.

It has been shown that plant sterols reduce A β generation *in vitro* by affecting the expression, activity, and availability of secretases involved in the amyloigenic processing of the toxic A β peptide (10 μ M) [137]. In particular stigmasterol decreased β -secretase activity, reduced the expression of all γ -secretase components, and decreased BACE1 internalization into endosomal compartments. In parallel, γ -secretase activity was significantly and dose-dependently decreased in the CNS of mice fed a 0.39% stigmasterol-enriched diet, as compared to vehicle treated mice [137]. However, at this stage, it cannot be concluded whether stigmasterol treatment is a translational therapeutic option since the transfer of stigmasterol to the CNS is limited.

LXR are ubiquitously expressed sensors for cholesterol metabolites, such as oxysterols [140]. Upon activation, LXRs regulate cholesterol turnover and suppress the severity of diverse neurodegenerative diseases [110, 132, 133, 141]. In line herewith, LXR activation has recently been shown to be essential for motor neuron survival [142]. With respect to AD, LXR-induced expression of ApoE mediates the proteolytic degradation of A β by microglia [132]. In addition, LXRs transrepress inflammatory pathways in CNS infiltrating and resident immune cells via SUMOylation-dependent pathways (figure 3) [143]. SUMOylation is required for the suppression of STAT1-dependent inflammatory responses by LXRs in IFN- γ -stimulated brain astrocytes [144-146]. Based on these studies, activation of LXRs represents an interesting therapeutic option for neurodegenerative and neuroinflammatory disorders, such as AD. However, synthetic LXR agonists show severe side-effects such as hepatic hypertriglyceridemia, resulting in liver steatosis [147]. By using cell-free and cell-based assays, multiple studies defined that plant sterols, such as sitosterol, fucosterol, stigmasterol, schottenol, 24(S)-saringosterol, and spinasterol, bind and activate LXR α and/or LXR β [46, 53, 148-151]. Interestingly, in contrast to synthetic LXR agonists such as T0901317 and GW3965 [152-154], phytosterols do not induce hypertriglyceridemia and hepatic steatosis [152-158]. A reduced concentration of phytosterols in the liver, likely due to ABCG5/G8-mediated excretion into the bile, may explain why phytosterols do not induce the hepatic side effects as seen upon treatment with synthetic LXR agonists. It should be noted that plant sterols only mildly activate LXRs. Whereas highly specific synthetic agonists increased LXR activation up to 80-fold, a maximum 15-fold increase in LXR activation was observed when divergent plant sterols were used [46]. The rate in which plant sterols activate LXRs is likely dependent on (1) the concentration of plant sterols applied, (2) the type of plant sterol tested, (3) the purity of the plant sterol, and (4) the cell type transfected with the reporter-construct. For instance, Yang et al. published a 5-fold increase in LXR α activation by 60 μ M stigmasterol, using a luciferase reporter assay in CHO-7 cells [53]. However, the concentration stigmasterol applied (60 μ M) is supra-physiologic for non-phytosterolemic patients (\pm 0.3 μ M) [48]. Plat et al. applied a cell-free ligand-sensing assay, initially developed to analyze the structural requirements of activating LXR α and LXR β , to evaluate the potential LXR-activating capacity of various plant stanols/sterols [148]. In this study, fucosterol, sitostanol, and campestanol potently activated LXR α and LXR β at concentrations ranging from 10nM to 10mM. By using a luciferase reporter assay (100-200 μ M) and a TR-FRET assay (1nM to 100 μ M), Hoang and colleagues confirmed the LXR-agonizing capacity of fucosterol [149]. Although fucosterol appears a strong LXR activator, it remains speculative whether concentrations of fucosterol in this range can be reached via nutritional supplementation. A recent study of Chen et al. showed a 4 to 15-fold LXR β activation upon administration of 24(S)saringosterol (10 and 30 μ M) [46]. The 10 μ M concentration is near the range of targetable circulating concentrations and may offer future nutritional perspectives. Strangely, although oxidized cholesterol forms are natural LXR agonists, oxyphytosterols are not

superior LXR agonists as compared to non-modified phytosterols [21]. Collectively, a phytosterol-mediated activation might prove to be an interesting therapeutic option for AD in the future. However, more research is warranted to define if plant sterols mediate their neuroprotective effects exclusively via LXRs, and whether the capacity of plant sterols to activate LXRs is sufficient to control neuroinflammation and neurodegeneration. Moreover, to date, translational studies verifying the exclusive LXR activating potential of plant sterols are lacking. Finally, despite the large number of studies supporting an anti-inflammatory role for LXR activation [159], few studies point towards a pro-inflammatory role of LXRs [160-162]. Consequently, caution should be taken when extrapolating LXR as target in AD to the clinic.

Finally, phytosterols may disturb APP processing in lipid rafts. Similar to plant cell membranes, we demonstrated that phytosterols accumulate in lipid rafts of neuronal membranes upon dietary supplementation [45, 163-165]. As such, phytosterols may reduce the molecular order in membranes. Phytosterols interact less efficiently with saturated phospholipids compared to cholesterol and, therefore, may alter membrane fluidity [84, 85]. Consequently, substitution of membrane cholesterol with sitosterol disrupts lipid raft integrity and promotes the relocalization of APP to non-raft regions, thereby suppressing amyloidogenic processing of APP (figure 3a/c) [166]. Moreover, in a platelet-model for A β production, sitosterol inhibits high cholesterol-induced A β release, likely through maintenance of membrane cholesterol homeostasis [139]. Similarly, it was found that stigmasterol significantly reduces cholesterol and presenilin distribution in lipid rafts. This redistribution has been implicated in amyloidogenic APP cleavage and subsequent A β production (figure 3a) [137]. However, as stated above, it remains to be determined *in vivo* whether the plant sterol-to-cholesterol ratio in the lipid rafts is sufficient to functionally modulate membrane properties in the CNS parenchymal cells.

In addition to their impact on the pathophysiology of AD, phytosterols function as biomarkers for AD. Recently, brassicasterol and to a lesser extent sitosterol were identified as clinically relevant cerebrospinal fluid (CSF) biomarkers for early AD (mini-mental state examination score: 21.3 \pm 4.6) [48]. Noteworthy, all plant sterols measured in this study were lowered in AD patients compared to the control population. However, only brassicasterol and to a lesser extent sitosterol reached significance [48]. In parallel to a reduced CSF-to-plasma albumin quotient, the reduced CSF plant sterol concentrations likely reflect an impaired choroid plexus function, resulting in a decreased plant sterol secretion/leakage into the CSF [167]. Despite comparable plasma concentrations, brassicasterol and sitosterol were significantly lower in CSF of AD patients compared to non-demented age matched controls. As stated in the limitation-section of the study, the relatively low sample size (n(AD)=67 and n(ctrl)=29) may explain why the other CSF plant sterols measured did not reach significance between the two groups in this multivariate analysis. Remarkably, the ratio among the different plant sterols differs within the CSF and within the plasma. Yet, the addition of CSF brassicasterol to the panel of established CSF biomarkers, A β ₄₂ and phospho-tau, resulted in significantly increased predictive power[48]. However, more studies are indispensable to confirm the clinical validity and replicability of these findings. The consensus in the field is that the findings reported should be replicated in new, independent studies [168]. The clinical applicability of brassicasterol as biomarker might be hampered by the lack of equipment and experience in central diagnostic laboratories. Additionally, the invasive nature of the required of the lumbar punctures might raise ethical questions concerning routine screening [168].

In conclusion, numerous studies point towards plant sterols being interesting nutritional modulators in the prevention of AD. However, studies focusing on the long term side-effects of high dose plant sterol intake and the subsequent CNS accumulation are warranted. While initial low concentrations of selected plant sterols in the CNS can be beneficial, long-term, extensive accumulation may prove harmful in CNS disorders and peripheral tissues.

Phytosterols in Multiple Sclerosis

MS is an inflammatory, neurodegenerative disease affecting the CNS. It is regarded to be an autoimmune disease as autoaggressive lymphocytes and macrophages are pivotal in orchestrating the immunopathological processes involved in myelin sheath damage and axonal degeneration [169-171]. The impact of cholesterol metabolism on normal brain functioning and the pathophysiology of MS has been extensively scrutinized [140, 172-176]. Even more, cholesterol-lowering statins reduce lesion relapse rate in early MS patients and have recently been found to curtail the annualized rate of whole-brain atrophy in secondary-progressive MS patients [177-179]. Remarkably, despite their immunomodulatory properties, ability to cross the BBB, and capacity to lower blood cholesterol levels, the effect that phytosterols have on the MS disease progression remains largely elusive.

Numerous studies have reported immunomodulatory properties of phytosterols [38, 42, 114, 180-183]. For instance, both non-modified and modified phytosterols suppress an inflammatory transcriptional profile in macrophages [42, 182-188]. Furthermore, plant sterols and plant stanols skew T cells towards a Th1 phenotype independently of their effect of antigen-presenting cells (APCs) and leaving the activity of Th2 cells unaffected [180, 189, 190]. With respect to MS, sitosterol has been reported to decrease the secretion of the inflammatory mediators TNF α and IL-12 by PBMCs from MS patients at physiological relevant concentrations [181]. In line with this finding, daily administration of a mixture of sitosterol (60%), campesterol (25%), and stigmasterol (15%) inhibits inflammatory CNS demyelination in an animal model of MS, the experimental autoimmune encephalomyelitis (EAE) model [191]. Even more, this phytosterol mixture delays the onset and decreases disease severity in EAE. The protective effect of phytosterols on EAE severity was paralleled by a reduced infiltration of lymphocytes and macrophages into the CNS, and a dampened inflammatory activity of these immune cells. These findings indicate that phytosterols modulate the inflammatory and migratory activity of leukocytes in EAE-affected animals, thereby affecting neuroinflammation and neurodegeneration. Of note, the reduced infiltration of leukocytes into the CNS of EAE animals is in line with the fact that phytosterols decrease the chemotactic and docking properties of endothelial cells (see section “*phytosterols and the endothelium in CNS disorders*”). However, EAE only mimics the inflammatory aspects of MS pathology, not taking modifiable and genetic risk factors into account. In conclusion, the role of plant sterols in the prevention and treatment of MS holds promise, but caution should be taken extrapolating findings in animal models to the clinic.

Apart from modulating the autoimmune response in MS, phytosterols may impact the pathophysiology of MS by affecting the viability and activity of parenchymal cells. It has been defined that phytosterols stably accumulate in the CNS, especially in glial cells, such as oligodendrocytes and astrocytes, and to a lesser extent in neurons [43-45]. This finding suggests that phytosterols can directly affect the integrity and functioning of these cells, and thereby MS

disease progression. Notably, incorporation of β -sitosterol in the cell membrane of hippocampal neurons prevents glucose oxidase (GOX)-induced oxidative stress and lipid peroxidation [88]. Thus, phytosterols may protect neurons, and likely also glial cells [43-45], from oxidative stress in MS lesions. On the other hand, oxides of β -sitosterol and campesterol are cytotoxic *in vitro* [192]. Similar, phytosterol β -D-glucosides are neurotoxic when administered to astrocytes, neurons, cortical slices, and mice through glutamate excitotoxicity and their stimulatory effect on the generation of ROS [36, 39, 193]. In addition, microglia and astrocyte activation is apparent in β -sitosterol β -D-glucoside treated mice, supporting a role for glial cells in β -sitosterol β -D-glucoside-induced neurotoxicity [39]. To date, it is unclear if the neurotoxic properties of phytosterol glucosides at some point overwhelm the neuroprotective effects of free and esterified phytosterols in the healthy or diseased CNS. Moreover, considering that phytosterol glucosides also lower cholesterol levels in humans [35, 37], it is unknown whether the neurotoxic impact of glycosylated phytosterol outweighs their protective cholesterol-lowering effect in MS. The above studies indicate that the impact of phytosterols on CNS resident cells is dual and largely depends on structural modifications.

Nuclear receptors play a key role in CNS repair processes and neuroinflammation. Activation of LXRs ameliorates EAE and signaling through retinoid X receptor gamma (RXR γ), a heterodimeric partner of LXRs, accelerates CNS remyelination [194-196]. Hence, a phytosterol-mediated activation of LXRs may suppress neuroinflammation and promote CNS repair processes in MS patients. Similar, sitosterol binds and activates the estrogen receptor (ER), particularly the ER β subtype [88, 89, 197]. Activation of both ER subtypes provides disease protection in the EAE model and estrogens are currently being evaluated in clinical trials of MS [198, 199]. ER β specific ligands have been reported to enhance endogenous remyelination by increasing the number of myelinating oligodendrocytes [200, 201]. It should be noted that, similar to LXR activation, phytosterols only mildly activate ERs as compared to synthetic compounds such as 17- β -Estradiol. Future studies should determine if phytosterols affect CNS repair processes and whether a phytosterol-mediated activation of ERs and LXR represents the biological foundation for their impact on CNS repair (figure 3d).

Phytosterols in ALS-PDC

The amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS-PDC) is a neurodegenerative syndrome that shows a remarkable high prevalence in Guam, West New Guinea, and the Kii peninsula of Japan. It is a heterogeneous syndrome that manifests itself as parkinsonism, dementia, classical ALS, or a combination of these phenotypes. The high prevalence of ALS-PDC in populations in the pacific and its familial clustering point towards a genetic predisposition. However, the occurrence of ALS-PDC in immigrants in these areas and the consistent drop in the incidence over the last decades suggests that environmental risk factors are also imperative for disease development. Moreover, causative genetic and molecular markers that explain the isolated manifestation of ALS-PDC in these areas are yet to be determined [202, 203].

Many studies have attempted to define the environmental trigger that causes ALS-PDC. Disturbances in calcium, vitamin D, heavy metal metabolism [204], and neurotoxins present in cycad seeds, such as β -N-methylamino-L-alanine (BMAA) [205, 206], have been proposed as causal factors but were later on challenged [203, 207-209]. With respect to cycad seeds, few *in*

in vitro studies defined that sterol- β -D-glucosides present in cycad seeds are also potential neurotoxins [36]. First, β -sitosterol- β -D-glucoside induces cell death in human fetal astrocytes and motor neuron cells [36, 39]. Second, in cortical slices and primary cortical cultures β -sitosterol- β -D-glucoside, stigmasterol- β -D-glucoside, and campesterol/dihydrobrassicasterol- β -D-glucoside stimulate rapid depolarization and induce the release of lactate dehydrogenase, which is a marker for cellular apoptosis. Considering that sterol- β -D-glucosides promote the release of glutamate in cortical slices, glutamate excitotoxicity likely underlies the neurotoxic potential of the sterol- β -D-glucosides [36]. In line with this hypothesis, glutamate excitotoxicity is related to the generation of free radicals and sterol- β -D-glucosides stimulate the generation of ROS by isolated brain mitochondria [193, 210]. Finally, acetylated sterol- β -D-glucosides accelerate the aggregation of α -synuclein and promote α -synuclein-induced cytotoxicity *in vitro* [41]. Many α -synuclein positive intraneuronal inclusions are found in the CNS of ALS-PDC patients, primarily in the amygdala, and α -synuclein aggregation has been suggested to be involved in the process of tauopathy in CNS disorders [211, 212]. Collectively, these studies demonstrate that phytosterols-glucosides have neurotoxic properties *in vitro*.

In agreement with the above-mentioned *in vitro* results, mice fed cycad flour (0.5 g/day up to 30 days) containing the various sterol glucosides develop ALS-PDC-resembling motor and cognitive problems, and display neurodegeneration in CNS regions similar to those seen in ALS-PDC patients [36, 213]. In these studies, the cycad flour was washed several times and contained negligible amounts of the previously suspected cycad toxins, such as BMAA. Interestingly, cycad-fed mice show a loss of glutamate transporters and an ionotropic glutamate receptor expression profile that suggests that glutamate excitotoxicity ensues and underlies neurodegenerative events in these mice [214]. In concordance with a neurotoxic role of glycosylated phytosterols in cycad seeds, β -sitosterol- β -D-glucoside feeding results in behavioral deficits, motor neuron degeneration, and activation of astrocytes and microglia. Moreover, similar to cycad-fed mice, β -sitosterol- β -D-glucoside treated CD-1 mice have a decreased glutamate transporter expression in the CNS [39]. Conversely, Wilson and Shaw demonstrated that, although β -sitosterol- β -D-glucoside-treated mice develop ALS-PDC, the glutamate transporter density remained unchanged [215]. Experimental differences, like the mouse strain used and/or purity of glycosylated β -sitosterol, might explain this discrepancy in the expression of glutamate transporters. These studies indicate that phytosterol glucosides likely account for the neurotoxicity of cycad flour. To what extent glutamate excitotoxicity is involved remains to be clarified.

Interestingly, as compared to wild type mice, cycad-fed ApoE^{-/-} mice do not develop progressive behavioral deficits, whilst showing an increase in the number of apoptotic cells in the CNS [216]. This finding suggests that the ApoE isoform could possibly be a genetic susceptibility factor for cycad flour neurotoxicity. As ApoE deficiency also decreases the flux of phytosterols towards the CNS [44], the reduced neurological deterioration in cycad-fed ApoE^{-/-} mice might merely represent a reduced presence and, as a consequence, cytotoxicity of glycosylated phytosterols in the CNS. Of note, specific ApoE isoforms have been associated with several neurodegenerative disorders including AD, ALS, and ALS-PDC [215, 217]. With respect to the latter, a follow-up study using transgenic mice with murine *ApoE* replaced by either human APOE2, APOE3, or APOE4 defined that the APOE2 isoform confers protection against motor deficits induced by cycad flour consumption [215]. On the other hand, mice expressing human APOE4 displayed an increased disease severity. If and how phytosterol glucosides in cycad seeds are associated with APOE

isoform specific neurotoxicity remains unclear. Differences in the capacity of divergent APOE isoforms to transport phytosterols into the CNS might explain the findings [218].

The obvious question that remains is: why and how does glycosylation render phytosterols neurotoxic? A possible explanation may be that glycosylation hampers the binding and activation of nuclear receptors by phytosterols. In line with this hypothesis, β -sitosterol accelerates neuron degeneration in mice that are deficient for LXR β [219], suggesting that LXR β activation by non-glycosylated sitosterol is neuroprotective. Furthermore, glycosylation may suppress the beneficial role that β -sitosterol has on mitochondrial function. Specifically, incorporation of β -sitosterol into the mitochondrial membrane enhances mitochondrial energy metabolism, possibly by promoting inner membrane fluidity [88]. An increase in mitochondrial energy metabolism has been proposed to be beneficial for neurodegenerative diseases. Interestingly, in contrast to non-modified cholesterol, cholesterol β -D-glucoside reduces the membrane fluidity by promoting the packing of the bulk membrane lipids [220]. This finding may underlie the negative role of glycosylation on the impact of cholesterol, and likely phytosterols, on mitochondrial function. On a structural level, molecular characteristics, such as the chiral orientation and the number of glucose ring structures attached to the sterols, have also been proposed to underlie the neurotoxic properties of sterol glucosides as compared to their non-modified counterparts [36]. Whereas it is clear that phytosterol glucosides are neurotoxic, further research is required to define the exact mechanism of action.

Therapeutic perspectives and conclusion

Due to the cholesterol lowering properties, dietary phytosterols are steadily gaining both public and scientific attention. Interestingly, apart from their cholesterol-lowering properties, recent evidence indicates that phytosterols also modulate other biological processes and may play a role in a diverse set of disorders. For instance, functional foods enriched with phytosterols are chemopreventive and might hold promise for add-on treatment for particular cancers [221-223]. However, to date, no clinical endpoint studies are available to support plant sterols as functional food. Moreover, the mechanism underlying the severe atherosclerosis in phytosterolemic patients remains unclear.

Considering phytosterols can cross the BBB and virtually irreversibly accumulate in the CNS, an increasing amount of studies is being published on the impact of phytosterols on the healthy and diseased CNS. In this review, we summarized and discussed the role of phytosterols and their metabolites in the growing field of nutritional neurosciences. Phytosterols have the potential affect neuroinflammation, neurodegeneration, and disease progression in experimental animal models for different CNS disorders. Even more, few studies points toward a role for phytosterols in CNS repair. Notably, phytosterols may also indirectly modulate CNS disease progression. For instance, diabetes is a risk factor for AD and cognitive decline in general [224]. Phytosterols, and in particular fucosterol, have been shown to be anti-diabetic and might therefore modulate cognition indirectly [225-227]. However, despite recent advances, longer-term prospective translational studies are mandatory to elucidate the applicability of phytosterols in CNS disorders. At the same time, future studies should determine whether the neuroprotective and neurodegenerative properties of phytosterols are disease-specific and which modifications render a particular phytosterol neuroprotective or neurotoxic. With respect to the latter, care should be taken to minimize the presence of neurotoxic phytosterol metabolites in functional foods, as well as to suppress the neurotoxic conversion of non-modified phytosterols in the body. Also, the role of

phytosterols in other CNS disorder related to a disturbed cholesterol homeostasis, such as Huntington disease, spinal cord injury, and Parkinson disease, remains to be investigated [228-230]. We conclude that CNS disorders that are correlated with an altered cholesterol metabolism, such as AD, MS, and ALS-PDC, are especially interesting for further applied and mechanistic phytosterol research. Yet, the raised contra-indications, such as premature atherosclerosis in phytosterolemic patients and harmful side-effects of phytosterol metabolites underscore the caution with which plant sterol should be dealt with in practice.

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Figure legends

Figure 1. Chemical structures of sterol and its modifications. (A) On the left, an overview of the chemical structure of cholesterol with its numbered carbon atoms and side chain R. Sterols can be modified by hydrogenating the double bond between carbon 5 and 6 to obtain stanols, or sterols can be glycosylated or esterified to form sterol- β -D-glucosides and sterol esters, respectively. (B) On the left, an overview of the chemical structure of phytosterols and stanols. On the right, the chemical structures of all the phytosterols discussed in this review. Of note, all phytosterols depicted here have a stanol counterpart, with the exception of schottenol and spinasterol. (C) Sterol/stanol can be oxidized to form an oxysterol/stanol, such as 24(S)- or 27(S)-hydroxycholesterol, depending on which carbon is oxidized (as showed by the oxidation points).

Figure 2. Transport model of phytosterols across the intact BBB. (A) In the brain, cholesterol is almost entirely synthesized *in situ*. To maintain homeostasis, cerebral cholesterol is metabolized into the more polar 24(S)-hydroxycholesterol by CYP46A1. 24(S)-Hydroxycholesterol can cross the BBB resulting in cholesterol efflux from the brain. In contrast, most phytosterols cannot be converted to 24(S)-hydroxyphytosterol. Hence, there is no efflux of phytosterols to the blood resulting in a steady accumulation in the brain. (B) We suggest that the HDL-SR-B1 axis is playing a crucial role in the flux of phytosterols across the BBB into the CNS. Phytosterols are mainly located in HDL in the circulation and SR-B1, the main HDL receptor, is present at the apical side of cerebral endothelial cells. The release of phytosterols in the CNS is likely mediated by ATP-binding cassette transporters, which are present on astrocytes and the basolateral side of the cerebral endothelium. CNS-HDL-like particles may function as acceptors for phytosterols in the brain.

Figure 3. The impact of phytosterols on neurodegeneration, neuroinflammation, and CNS repair. (A) APP can be processed by the non-amyloidogenic and amyloidogenic pathway. In the non-amyloidogenic pathway, APP is cleaved by α - and γ -secretase in non-lipid raft regions. In contrast, in the amyloidogenic pathway, β - and γ -secretase cleave APP in lipid rafts, leading to the formation of neurotoxic A β peptides. Phytosterols promote the relocalization of APP from lipid rafts to non-lipid raft regions and can directly inhibit β -secretase activity. Thus, phytosterols likely reduce the amyloidogenic cleavage of APP and the consequent formation of neurotoxic A β peptides, and may attenuate AD pathology upon accumulation in the CNS. (B) Apart from having neuroprotective properties, glycosylated forms of phytosterols are neurotoxic. Glutamate excitotoxicity and an increase in oxidative stress (ROS) likely underlie the neurotoxic properties of glycosylated phytosterols. (C) In neuroinflammatory disorders such as MS and AD, leukocytes infiltrate the CNS and release a plethora of inflammatory and toxic mediators. Phytosterols can reduce neuroinflammation by suppressing the inflammatory properties of immune cells, potentially through the ligation and activation of LXRs (SUMOylation-dependent pathway). Moreover, phytosterols can decrease leukocyte infiltration into the inflamed CNS, either by directly affecting the migratory properties of leukocytes and/or by altering the adhesive and chemoattractive properties of BBB endothelial cells. (D) Phytosterols ligate and activate nuclear receptors (LXRs and ERs) that play a crucial role in CNS repair processes. Cholesterol is an essential component of myelin and LXRs control cholesterol homeostasis in oligodendrocytes. Hence, a phytosterol-mediated LXR activation may affect cholesterol homeostasis and remyelination. With respect to

the capacity of phytosterols to stimulate ERs, ER β signaling increases the number of myelinating oligodendrocytes.