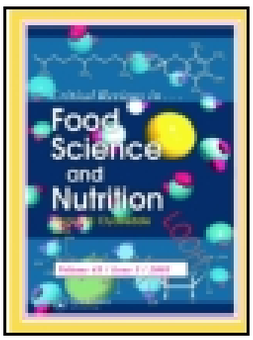

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REVIEW

Linking phytosterols and oxyphytosterols from food to brain health: origins, effects, and underlying mechanisms

Fan Jie[a,b,c], Xuan Yang[d], Lipeng Wu[a,b,c], Mengmeng Wang[a,b,c], and Baiyi Lu[a,b,c]

[a]College of Biosystems Engineering and Food Science, National-Local Joint Engineering Laboratory of Intelligent Food Technology and Equipment, Key Laboratory for Agro-Products Nutritional Evaluation of Ministry of Agriculture and Rural Affairs, Key Laboratory of Agro-Products Postharvest Handling of Ministry of Agriculture and Rural Affairs, Zhejiang Key Laboratory for Agro-Food Processing, Zhejiang International Scientific and Technological Cooperation Base of Health Food Manufacturing and Quality Control, Zhejiang University, Hangzhou, China; [b]Fuli Institute of Food Science, Zhejiang University, Hangzhou, China; [c]Ningbo Research Institute, Zhejiang University, Ningbo, China; [d]Division of Nutritional Sciences, Cornell University, Ithaca, New York, USA

ABSTRACT

Phytosterols and their oxidation products, namely oxyphytosterols, are natural compounds present in plant foods. With increased intake of phytosterol-enriched functional food products, the exposure of both phytosterols and oxyphytosterols is rising. Over the past ten years, researches have been focused on their absorption and metabolism in human body, as well as their biological effects. More importantly, recent studies showed that phytosterols and oxyphytosterols can traverse the blood–brain barrier and accumulate in the brain. As brain health problems resulting from ageing being more serious, attenuating central nervous system (CNS) disorders with active compounds in food are becoming a hot topic. Phytosterols and oxyphytosterols have been shown to implicated in cognition altering and the pathologies of several CNS disorders, including Alzheimer's disease and multiple sclerosis. We will overview these findings with a focus on the contents of phytosterols and oxyphytosterols in food and their dietary intake, as well as their origins in the brain, and illustrate molecular pathways through which they affect brain health, in terms of inflammation, cholesterol homeostasis, oxidative stress, and mitochondria function. The existing scientific gaps of phytosterols and oxyphytosterols to brain health in knowledge are also discussed, highlighting research directions in the future.

KEYWORDS

Cholesterol homeostasis; CNS; inflammation; oxidative stress; oxyphytosterols; phytosterols

Introduction

As structural analogs of cholesterol, phytosterols are a class of tetracyclic triterpenes that regulate the physiochemical function of plant membranes. Plant sterols, plant stanols, and plant sterol esters are common forms of phytosterols (Dutta 2004). Plant sterols differ from cholesterol in the side chain and plant stanols are additionally saturated at C-5, while in plant cells, most phytosterols appear in the esterified form for storage (Piironen et al. 2000). Phytosterols cannot be synthesized by mammals, thus, they can be only obtained from diet, and food such as grains, vegetables, fruits, and edible oils are good sources of these natural molecules (Vanmierlo et al. 2015). Typically, sitosterol, campesterol, and stigmasterol are major phytosterols present in food and human body (Vanmierlo et al. 2011a). They differentiate from cholesterol with additional methyl or ethyl at C-24, or double bond at C-22. Fucosterol, brassicasterol, spinasterol, and schottenol are also common phytosterols being studied in their bioactivities (structures shown in Figure 1).

Both cholesterol and phytosterols are prone to be autoxidized under conditions such as heat and light during food processing, or by reactive oxygen species in tissues (Barriuso, Ansorena, and Astiasarán 2017). And theoretically, sterols can be either oxidized automatically or by endogenous enzymes (Luu et al. 2016; Mutemberezi, Guillemot-Legris, and Muccioli 2016). Oxyphytosterols in food such as 7-keto-, 7-hydroxy (OH)-, and 5,6-epoxy-phytosterol are autoxidized on the sterol-ring (Figure 1), while side-chain oxidation is mediated by specific enzymes (García-Llatas and Rodríguez-Estrada 2011). Compared with cholesterol, it is clear that they can be oxidized endogenously via enzymatic catalysis to form side-chain hydroxylation products. For example, 24-OH-cholesterol (24-OHC) and 27-OHC are hydroxylated by cytochrome P450 oxidase CYP46A1 and CYP27A1, respectively (Vaya and Schipper 2007). However, the enzymatic formation of oxyphytosterols is currently unresolved.

Phytosterols have been supplemented in functional food products for their cholesterol-lowering ability, as well as other beneficial effects, leading to increased dietary exposure of both phytosterols and oxidation products (Katan et al. 2003; Abumweis, Barake, and Jones 2008). As a result, the physiological effects of phytosterols and oxyphytosterols on humans are constantly researched. Except for the cholesterol-lowering ability, phytosterols also possess anti-inflammatory

CONTACT Baiyi Lu bylu@zju.edu.cn College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China.

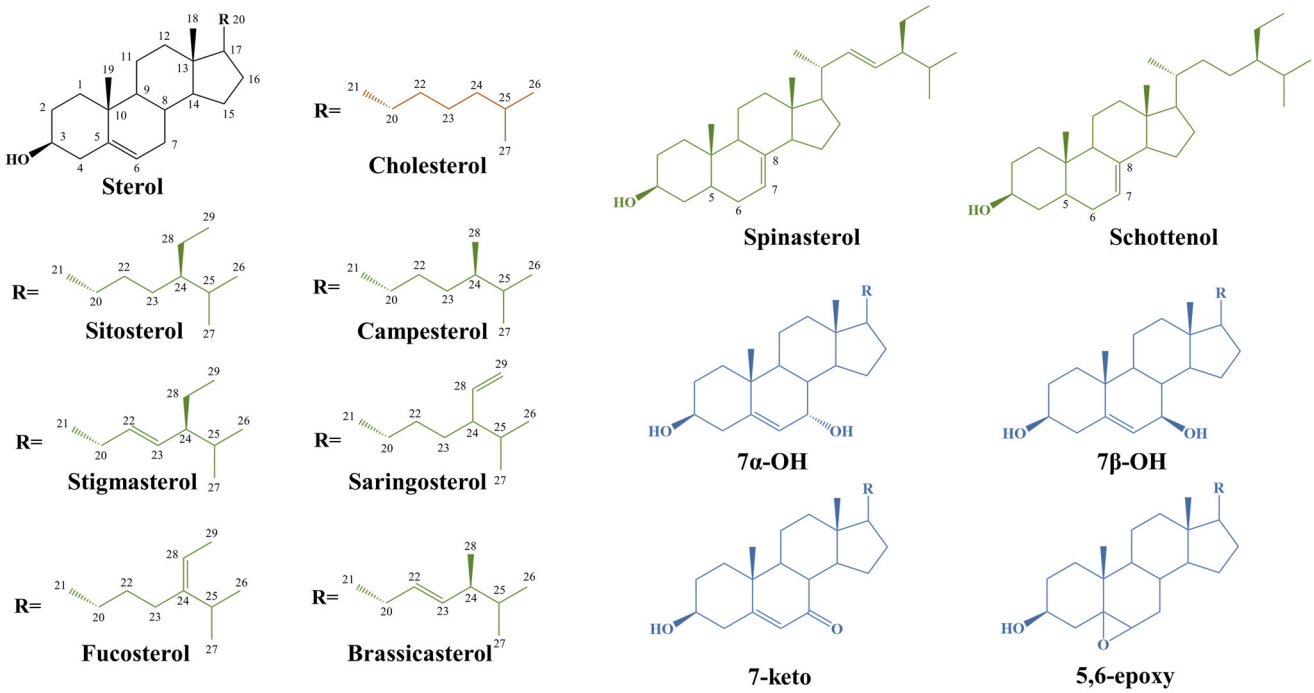

Figure 1. Structure of phytosterols and oxyphytosterols.

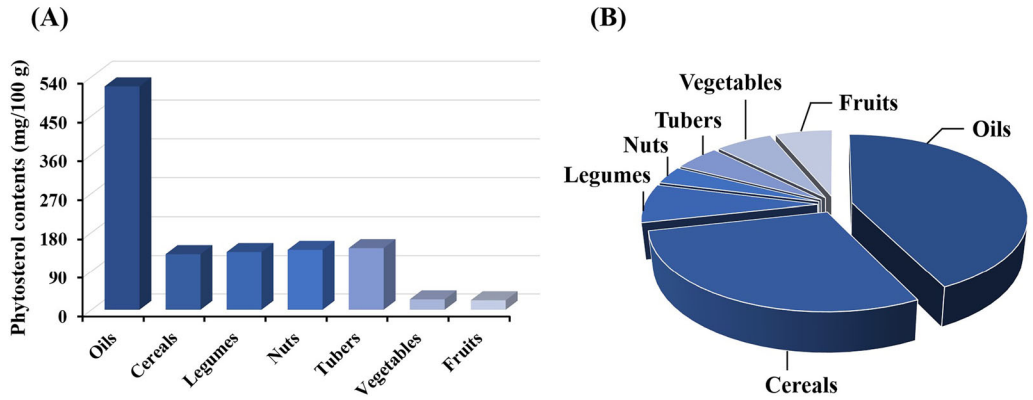

Figure 2. (A) Average total phytosterol contents in common food (mg/100 g). (B) Phytosterol intake from different food groups. Adapted from (Wang et al. 2018) with permission.

and antioxidative effects (Shahzad et al. 2017; Vilahur et al. 2018), and have potential implication in anticancer, antidiabetes, and hepatoprotection (Miras-Moreno et al. 2016; Alvarez-Sala et al. 2019; Sánchez-Crisóstomo et al. 2019). Also, studies found that phytosterols may modify the microbial profiles (Martínez et al. 2013; Cuevas-Tena et al. 2019; Song et al. 2020), thus, might be effective against nonalcoholic fatty liver disease and hypercholesterolemia, which are associated with gut microbiota (Hao et al. 2019; Song et al. 2020). Intake of oxyphytosterols influences human health as well, from the perspectives of cholesterol metabolism and atherosclerosis (O'Callaghan, McCarthy, and O'Brien 2014; Wang and Lu 2018). Furthermore, recent studies have suggested both phytosterols and oxyphytosterols can traverse the blood–brain barrier (BBB) and accumulate in the brain (Schött et al. 2015; Vanmierlo et al. 2015; Shuang, Rui, and Wenfang 2016; Dierckx, Bogie, and Hendriks 2019). Although effects of phytosterols on CNS diseases have been studied and reviewed, there is still need of summarizing possible mechanisms to exert the effects. As neuroinflammation, cholesterol homeostasis and oxidative status are crucial factors that contributing to the pathology of CNS disorders such as Alzheimer's disease (AD) and multiple sclerosis (MS) (Whitney et al. 2002; Petrov, Kasimov, and Zefirov 2016; Salim 2016; Chitnis and Weiner 2017), it would be essential to know whether/how phytosterols and oxyphytosterols may affect the brain from these aspects.

Origins of phytosterols and oxyphytosterols

Contents in food and dietary intake

Oils, cereals, nuts, legumes, tubers, vegetables, and fruits are common resources of phytosterols (Figure 2). The concentrations of phytosterols in grain vary from 329 to 1780 mg/kg, while those in fruits and vegetables are about 38 to

Table 1. Daily intake of phytosterols worldwide.

Region	Composition of total phytosterols	Intake (mg/d)		Reference
Netherland	campesterol, stigmasterol, sitosterol, campestanol, and sitostanol	$n = 3123$ 285.0 ± 96.7		Normén et al. (2001)
		$n = 1525$ (men) 307.3 ± 103.9	$n = 1598$ (women) 262.9 ± 83.7	
Finland	sitosterol, campesterol, sitostanol, campestanol, stigmasterol, D5- and D7-avenasterols, brassicasterol, and other phytosterols	$n = 1292$ (men) 305	$n = 1446$ (women) 237	Valsta et al. (2004)
Norfolk, UK	campesterol, stigmasterol, sitosterol, campestanol, and sitostanol	$n = 10{,}016$ (men) 310 ± 108	$n = 12{,}240$ (women) 303 ± 100	Andersson et al. (2004)
Spain	campesterol, sitosterol, stigmasterol, and stigmastanol	275.5		Jiménez-Escrig, Santos-Hidalgo, and Saura-Calixto (2006)
Northern Sweden	campesterol, stigmasterol, sitosterol, campestanol, and sitostanol	$n = 96$ (men) 257	$n = 99$ (women) 219	Klingberg et al. (2013)
São Paulo, Brazil	sitosterol, campesterol, stigmasterol, campestanol, and sitostanol	$n = 1609$ 100.6 ± 1.2		Martins et al. (2013)
		$n = 531$ (men) 100.8 ± 1.4	$n = 1076$ (women) 100.4 ± 1.0	
Northern America	sitosterol, stigmasterol, other phytosterols (calculated as the sum of plant stanols and other plant sterols)	$n = 861$ 363 ± 176		Jaceldo-Siegl et al. (2017)
		$n = 370$ (nonvegetarian) 263	$n = 79$ (vegan) 428	
Five cities of China (Beijing, Hangzhou, Wuhan, Chongqing, and Guangzhou)	stigmasterol, sitosterol, campesterol, brassicasterol, ergosterol, campestanol, sitostanol, Δ5-avenasterol, spinasterol, and cycloartnol	473.7, 382.9, 322.2, 305.6, 257.7, and 323.6 for each city, respectively		Wang et al. (2018)
China	sitosterol, campesterol, stigmasterol, sitostanol, and campesterol	392.3		Yang et al. (2019)

439 mg/kg of fresh weight (Moreau, Whitaker, and Hicks 2002). Seed oils contain relatively higher amount of phytosterols. The concentrations in corn, cottonseed and sunflower oils are 11.7, 4.26, and 4.03 g/kg, respectively (Sujith Kumar, Mawlong, and Singh 2017). For supplemented food products, total phytosterol contents vary from 0.2 to 55.2 g/100 g (Srigley and Haile 2015). The recommended intake of phytosterols is 2–3 g/d in most countries, to ensure the established low-density lipoprotein-cholesterol (LDL-C)-lowering effect. A meta-analysis in 2014 revealed consistent dose–response relationship between reduction of LDL-C by 6–12% and daily intake of 0.6–3.3 g phytosterols (Ras, Geleijnse, and Trautwein 2014). Another meta-analysis in 2016 investigating patients treated with statin also concluded significant LDL-C lowering ability of phytosterols (Han et al. 2016). While the reality is that the insufficient intake of phytosterols are common in different regions, which has been estimated to be 100–500 mg/d (Table 1). Consumption of phytosterols worldwide have similar patterns: the most abundant food sources are oils, followed by cereals, legumes, vegetables, and fruits (Figure 2); and sitosterol is the predominant phytosterol (Jiménez-Escrig, Santos-Hidalgo, and Saura-Calixto 2006; Moreau 2015; Wang et al. 2018). Moreover, dietary patterns are associated with the phytosterols intake. For example, phytosterols intake of vegetarians is 428 mg/d, which is 1.67 times higher than that of nonvegetarians (263 mg/d) in Northern America (Jaceldo-Siegl et al. 2017).

For oxyphytosterols, as the oxidation process mainly happens in food heating, high levels of oxyphytosterols were found in baking foods such as spreads, French fries, cookies, and cake. According to Hu et al. (2018b), the oxidation rates of phytosterols in baked food ranged from 1.5% to 23.2%. And the median content of total oxyphytosterols was 6.26 mg/kg, with the highest level appeared in wafer biscuit, which was 27.81 mg/kg. Scholz et al. (2015) reported the intake of oxyphytosterols range from 1.2 to 2.9 mg/d for consuming nonheated food, and 3.5 to 4.2 mg/d for heated food. For foods supplemented with plant sterol esters or free plant sterols, the upper oxyphytosterols intakes are 47.7 mg/d and 78.3 mg/d, respectively (Lin, Knol, and Trautwein 2015).

Absorption and distribution in the body

The absorption mechanisms of phytosterols and cholesterol are alike. In the small intestine, sterols are primarily incorporated into mixed micelles and sensed by Niemann–Pick C1-like 1 (NPC1L1), a protein which mediates the endocytosis of enterocytes (Altmann et al. 2004). Free sterols are then esterified by acetyl-CoA acetyltransferase (ACAT) 2 before being packed into chylomicrons (Lee et al. 2001). The unesterified sterols will return to the lumen of the intestine via the ATP-binding cassettes (ABC) G5 and G8. Sterols in the circulation finally reach the liver, where they are converted into bile acids and excreted through hepatocyte ABCG5/G8 (Ling and Jones 1995). There are studies concerning the bioaccessibility of bioactive compounds in foods, which is the amount released from food matrix in the gastrointestinal tract and becomes accessible for absorption (Santos et al. 2019). Generally, the bioaccessibility of phytosterols is closely related with the type of matrix in which they are added (Tolve et al. 2020). For example, after an in vitro gastrointestinal digestion, the bioaccessibility

percentage of total phytosterols (8.90–16.84%) differed among four types of fermented milk, and the percentage decreased with higher carbohydrates and fiber contents in samples (Vaghini et al. 2016). Meanwhile, differences in structure affect bioaccessibility as well. Alemany et al. (2013a) discovered that campesterol (3.75–8.31%) had higher bioaccessibility than sitosterol (2.51–6.42%) and stigmasterol (2.95–5.96%) in simulated gastrointestinal digestion, despite of the type of beverage. This may be resulted from the higher solubility in the aqueous phase of campesterol, while increased number of C-24 carbon bond reduce the solubility (Matsuoka et al. 2010). In line with it, the bioaccessibility of cholesterol (53.34–98.96%) is much higher than that of phytosterols (2.62–6.48%) (Alemany et al. 2013a). Similarly, phytosterols possess relatively low absorption rates in human body compared to cholesterol: 4.2% for sitosterol, 4.8% for stigmasterol, and 9.6% for campesterol (Heinemann, Axtmann, and Bergmann 1993). The low bioaccessibility, poor absorption, and quick excretion out of body lead to low contents of phytosterols in circulation. In human plasma, concentrations of phytosterols vary from 7 to 24 μmol/L, which are 500-fold lower than those of cholesterol (Gylling et al. 2014), and the half-life of campesterol and sitosterol in human plasma were measured to be 4.06 days and 2.94 days, respectively (Ostlund et al. 2002). Long-term consumption of phytosterols added foods may lead to higher circulating levels. According to Ras et al. (2016), before intervention, the plasma concentration of sitosterol and campesterol were 7.2 μmol/L and 11.4 μmol/L, and after consuming low-fat spreads with added phytosterols (3 g/d) for 4 weeks, plasma concentration increased by 69% and 28%, respectively (healthy men and women, $n = 240$).

Meanwhile, the main oxyphytosterols in human plasma are 7-oxygenated products of sitosterol and campesterol, with concentrations ranging from 1 to 10 ng/mL (Wang and Lu 2018). Unlike phytosterols, the origin of oxyphytosterols can be either from absorption in the diet, or from the oxidation of plant sterols in vivo (Baumgartner et al. 2013a). In simulated gastrointestinal digestion, total oxyphytosterol bioaccessibility of different samples ranged from 19.08% to 49.29%, which is higher than that of phytosterols (Alemany et al. 2013a). However, research about absorption rates of oxyphytosterols in humans *is* lacking. In thoracic duct-cannulated rats, Tomoyori et al. (2004) reported that the lymphatic recoveries of campesterol oxidation products (15.9 ± 2.8%) and sitosterol oxidation products (9.12 ± 1.77%) were higher than those of campesterol (5.47 ± 1.02%, $p < .05$) and sitosterol (2.16 ± 0.37%, $p < .05$). Nonetheless, several studies did suggest an endogenous synthesis of oxyphytosterols in human body (Salen et al. 1985; Baumgartner et al. 2013a, 2015). For example, the plasma concentrations of 7β-OH-campesterol and 7β-OH-sitosterol were significantly higher after consumption of a mixed meal enriched with plant sterol esters ($n = 10$, $p < .01$) (Baumgartner et al. 2015). However, in a study performed within 43 healthy subjects consumed plant sterol-enriched (3.0 g/day) margarine for 4 weeks, plasma sitosterol and campesterol concentrations did not correlate with plasma concentrations of oxysitosterol and oxycampesterol, and the oxyphytosterols levels remained consistent over the intervention period (Baumgartner et al. 2013b). Contradictory results appeared when considering the endogenous synthesis of oxyphytosterols, and information regarding the oxidation process in tissues is not as enough as that of cholesterol, either. It has been assumed that the enzymatic formation of side-chain oxyphytosterols may also be mediated by cytochrome P450 oxidase, but the hypothesis has not been confirmed (Wang and Lu 2018). Conversely, for the nonenzymatic formation of oxyphytosterols, a study performed in 104 severe aortic stenosis patients has shown the ratio of oxidized-sitosterol-to-sitosterol exceeded the ratio for oxidized-campesterol-to-campesterol in plasma and tissue, indicating a preferred in vivo autoxidation of sitosterol than campesterol (Schött et al. 2016). Moreover, Baumgartner et al. (2013b) reported that unlike oxycholesterol, oxidation of phytosterols might not be iron mediated. Considering the various origins of oxyphytosterol, including diet, autoxidation, and possibly enzymatic oxidation, it would be essential to know the amount of phytosterols oxidized during food processing, digestion and then in the tissues, thus, they can be utilized more effectively in the body.

Phytosterols and oxyphytosterols across the BBB

The BBB, which is located at brain capillary network level, regulates the sterol metabolism between peripheral and cerebral compartments. It is composed of brain capillary endothelial cells surrounded by brain pericytes, and brain capillaries are wrapped by end-foot of astrocytes, which connect neurons to the blood circulation. This structure permits a continuous supply of nutrients directly to the brain, maintaining the homeostasis of CNS (Gosselet, Saint-Pol, and Fenart 2014; Prat and Daneman 2015).

Sitosterol, campesterol, and stigmasterol have been reported to appear in murine brain, as well as the cortex and cerebrospinal fluid (CSF) of human brain (Table 2). Long-term consumption of phytosterols added foods may lead to higher brain concentrations in mice. For example, after being fed a plant sterol ester-enriched diet for 6 weeks, C57BL/6N mice displayed significantly increasing concentrations of phytosterols in serum, liver, and brain by twofold to threefold (Vanmierlo et al. 2012). Besides, levels of phytosterols are higher in normal brain than those of AD patients (Vanmierlo et al. 2011a). Moreover, several studies have revealed that campesterol traversed the BBB more efficiently than sitosterol (Jansen et al. 2002; Vanmierlo et al. 2011b; Smiljanic et al. 2013; Saeed et al. 2014, 2015). In male Wistar rats, the concentration of sitosterol in hippocampus was lower than that of campesterol, despite of its predominance in serum and food provided (Smiljanic et al. 2013). However, the predominance of campesterol in brain seems not appear in humans, as shown in Table 2, so there is need of more data revealing the phytosterol levels in different parts of human brains.

There are few studies investigating the mechanism of phytosterols traversing the BBB. Phytosterols were found to accumulate in serum and brain of both ABCG5- and

Table 2. Concentrations of phytosterols in the brain.

Biological specimen	Compound	Concentration	Subjects condition	Samples	Reference
ng/mg tissue					
Parietal cortex of human	Sitosterol	5.0 ± 0.8	65- ± 2-year old, no clinical history of psychiatric or neurological disorders	$n = 3$	Shafaati et al. (2011)
	Campesterol	5.0 ± 0.8			
Occipital cortex of human	Sitosterol	6.5 ± 1.6			
	Campesterol	7.5 ± 2.6			
μg/mL					
Cerebrospinal fluid of human	Sitosterol	2.484 ± 2.239	Normal cognition	$n = 29$	Vanmierlo et al. (2011a)
	Campesterol	0.684 ± 0.300			
	Stigmasterol	0.366 ± 0.291			
ng/mg					
Hippocampus of C57BL/6 mice	Sitosterol	35.6 ± 6.2	Fed standard laboratory chow diet	$n = 7$	Vanmierlo et al. (2011b)
	Campesterol	71.3 ± 10.6			
	Stigmasterol	3.7 ± 0.7			
Cortex of C57BL/6 mice	Sitosterol	32.2 ± 3.7			
	Campesterol	60.9 ± 7.4			
	Stigmasterol	4.2 ± 0.5			
Cerebellum of C57BL/6 mice	Sitosterol	61.4 ± 7.4			
	Campesterol	143.6 ± 26.7			
	Stigmasterol	8.4 ± 0.9			
ng/mg dry wight brain					
Brain hemispheres of C57BL/6J male mice	Sitosterol	9.6 ± 2.0	3-month old, fed control diet containing 0.03% (w/w) phytosterols for 4 weeks	$n = 12$	Jansen et al. (2002)
	Campesterol	64 ± 9			
ng/mg protein					
Brain hemispheres of C57BL/6NCrl male mice	Sitosterol	42[a]	12-week old, fed control diet containing 0.015% (w/w) plant sterol esters for 6 weeks	$n = 15$	Vanmierlo et al. (2012)
	Campesterol	129[a]			
ng/mg					
Hippocampus of male Wistar rats	Sitosterol	175[a]	18-month old, fed chow diet	$n = 5$	Smiljanic et al. (2013)
	Campesterol	283[a]			

[a]Estimated from figures, only kept the integer.

ABCG8-dificient mice, while in the normal mouse brain, ABCG5 or ABCG8 mRNA is not expressed (Jansen et al. 2002; Vanmierlo et al. 2011b). This suggests the accumulation of phytosterols in deficient mice may be resulted from the depleted liver excretion, and ABCG5/G8 are not involved in phytosterols transport into the brain. However, the brain phytosterols levels were not elevated in apolipoprotein E (ApoE)-deficient mice fed a plant sterols-enriched diet, despite the significantly increased serum levels (Jansen et al. 2002), indicating the participation of ApoE in passing phytosterols into the brain. Meanwhile, ABCG5-deficient mice retained approximately 50% phytosterols in high-density lipoprotein (HDL) particles. With the major receptor for HDL, scavenger receptor class B member 1 (SR-BI), expressing at the apical membrane of BBB endothelial cells, the ApoE and SR-BI dependent pathway maybe a possible mechanism of phytosterols entering the brain (Jansen et al. 2002; Panzenboeck 2002; Vanmierlo et al. 2015).

As for oxyphytosterols, after intraperitoneal injecting for 28 days, the brain concentration of 7β-OH-sitosterol was 65.8-fold ($p < .001$) higher in male ApoE-deficient mice (Schött et al. 2015), indicating a different mechanism of oxyphytosterols transporting through the BBB, which is independent of ApoE. Compared with oxidation products of cholesterols, levels of 7-keto-cholesterol and 7β-OH-cholesterol increased in brain lesions of patients with neurodegenerative diseases (Gamba et al. 2015; Kreilaus et al. 2016). For side-chain oxidation products, namely 24-OHC and 27-OHC, which are generated cerebral and peripheral, respectively, can directly pass through the BBB and act as intermedium of cholesterol circulation in and out of brain. So here we assume that the levels of oxyphytosterols may also increase during neurodegeneration, and side-chain oxyphytosterols may traverse the BBB easier than those oxidized on sterol-ring. However, the precise mechanisms of traversing remain unknown.

Effects of phytosterols and oxyphytosterols on brain health

Anxiolytic and cognitive amelioration

Plant extracts containing phytosterols have anxiolytic effects on murine animals (Aguirre-Hernández et al. 2007; Jiménez-Ferrer et al. 2017). For example, in male Swiss albino mice, sitosterol isolated from *Tilia amecicana* var. *Mexicana* exerted an anxiolytic-like action with concentrations ranging from 1 to 10 mg/kg and a sedative response when the dose increased to 30 mg/kg (Aguirre-Hernández et al. 2007). Phytosterols also possess cognitive ameliorative ability (Park et al. 2012; Koivisto et al. 2014; Pérez-Cañamás et al. 2016; Xu et al. 2017; Adebiyi, Olopade, and Olayemi 2018; Yadav et al. 2018; Ye et al. 2020). Feeding senescent-accelerated mice with phytosterols-supplemented diet prevented long-term potentiation impairment and cognitive deficits (Pérez-Cañamás et al. 2016). In another ageing rat model with cognitive deficiency, which was induced by a high cholesterol diet, 2% phytosterol esters supplement for 6 months showed significant neuroprotective effects (Xu et al. 2017). Mateos et al. (2009) suggested that the *N*-methyl-D-aspartate receptor (NMDAR), a key receptor controlling memory

function, may be one of the mechanisms by which the high fat diet affects cognitive ability. In line with the assumption, Yu et al. (2013) reported that phytosterols alleviated the detrimental effects of a high fat high energy diet in C57BL/6J mice, including the decline of NMDAR1 mRNA and protein content. Studies based on animal models of CNS disorders also led to similar results. Ye et al. (2020) reported that sitosterol treatment improved spatial learning and recognition memory ability in APP/PS1 mice, a model of Alzheimer's disease (AD). As for oxyphytosterols, Bogie et al. (2019) discovered dietary supplementation of *Sargassum fusiforme*-derived lipid extract containing 24(S)-saringosterol to APP/PS1 mice for ten weeks significantly improved short-term memory. However, an intake of plant sterols ($n = 18$) or stanols ($n = 18$) at 2.5 g/d for 85 weeks did not affect neurocognitive functioning or mood in hypercholesterolemic individuals (Schiepers et al. 2009). Thus, more clinical studies are needed to investigate cognitive effects on human brain.

CNS disorders

CNS disorders cause by neurodegeneration or autoimmune disorders (Sospedra and Martin 2005; Anchisi et al. 2012), lead to cognitive deficiency such as memory loss and learning impairment. Phytosterols and oxyphytosterols may influence pathologies of CNS diseases, and thus, modulate cognitive function. Several researches have proved that both sitosterol and stigmasterol have the potential to ameliorate amyloid β (Aβ) deposition – a main pathological feature of AD (Shi et al. 2011; Koivisto et al. 2014; Pérez-Cañamás et al. 2016; Ye et al. 2020). In general, formation of insoluble Aβ is defined as the amyloidogenic pathway, which includes proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretase. Conversely, the non-amyloidogenic cleavage by α-secretase produces soluble and nontoxic sAPPα. In high cholesterol treated human platelets, sitosterol effectively prevented the activities of β- and γ-secretase from increasing (Shi et al. 2011). And Burg et al. (2013) also suggested that the reduction of Aβ generation by stigmasterol was probably through decreasing β-secretase activity and reducing expression of all γ-secretase components. Except for regulating activities of amyloidogenic enzymes, modulation of cholesterol metabolism is another way to affect Aβ deposition, either by influencing the accessibility of APP to each secretase or Aβ clearance (Vanmierlo et al. 2015). As structural analogs of cholesterol and oxysterol, phytosterols might alter the brain cholesterol metabolism to some extent (Vanmierlo et al. 2011b; Yu et al. 2013). Additionally, due to the importance of oxysterol homeostasis in the development of CNS disorders, it is crucial to know whether oxyphytosterols play similar roles to oxidized cholesterols. Level of 24-OHC, the enzymatic oxidation product of cholesterol, has been found to decrease in the brain of late AD patients, and one of the functions of 24-OHC is to reduce the generation of insoluble Aβ (Gamba et al. 2015; Testa et al. 2016). Correspondingly, 24(S)-saringosterol, an oxyphytosterol derived from *Sargassum fusiforme*, increased microglial Aβ clearance and reduced neuronal Aβ release (Bogie et al. 2019). On the contrary, the brain concentrations of autooxidation products of cholesterol, such as 7α-OH-cholesterol, 7β-OH-cholesterol, and 7-keto-cholesterol elevated in the progression of AD (Testa et al. 2016). They were suggested to have neurotoxic properties and might be markers of inflammation and oxidative stress in the brain (Hascalovici et al. 2009; Testa et al. 2016; Petrov, Kasimov, and Zefirov 2017). While oxyphytosterols oxidized on the sterol-ring are considered to have pro-inflammatory abilities (Wang and Lu 2018), it could be hypothesized that they will affect cerebral inflammatory responses and oxidative status as well. Meanwhile, phytosterols may affect microglia activation and neuroinflammatory response in the opposite way (Koivisto et al. 2014; Xu et al. 2017; Yadav et al. 2018). In a mice model of multiple sclerosis (MS) – an inflammatory, neurodegenerative disease, daily administration of 2 mg phytosterols (60% sitosterol, 25% campesterol, and 15% stigmasterol) retarded the development of experimental autoimmune encephalomyelitis by reducing inflammatory infiltration into CNS (Valerio et al. 2011). In peripheral blood mononuclear cells derived from MS patients, sitosterol effectively regulated the secretion of both pro-inflammatory and anti-inflammatory cytokines (Desai et al. 2009). Oxidative stress and mitochondrial dysfunction also contribute to the pathogenesis of several neurodegenerative disease, such as AD, Parkinson's disease, and Huntington's disease (Reynolds et al. 2007; Cui, Kong, and Zhang 2011), while schottenol and spinasterol, two natural phytosterols present in argan oil and cactus pear seed oil, were found to impact mitochondrial activities in several eukaryotic cell lines of the CNS (El Kharrassi et al. 2014; Badreddine et al. 2015).

Other effects

Recent years, more interest has been paid on bioactivities of stigmasterol as it may have the potential to affect cholinergic neurotransmission system (Xu et al. 2017; Yadav et al. 2018) and neurogenesis (Haque, Bhuiyan, and Moon 2018; Haque and Moon 2018). A transcriptome analysis revealed that, stigmasterol induced many immediate early genes in primary hippocampal neurons, and a major proportion of the upregulated genes were related to CNS development, such as neurogenesis and synaptogenesis, indicating a potential of stigmasterol to promote brain functions (Haque and Moon 2018). Then, Haque, Bhuiyan, and Moon (2018) reported that stigmasterol induced the formations of mushroom-type spines and glutamatergic excitatory synapses in rat hippocampal and cortical cells.

The underlying mechanisms

As aforementioned, neuroinflammation, cholesterol metabolism, and oxidative stress play vital roles in the development of CNS disorders. Thus, in this chapter, the possible mechanisms of how phytosterols and oxyphytosterols influencing brain health will be discussed more detailed based on these three aspects.

Inflammation

The anti-inflammatory activities of phytosterols have been widely researched (Gupta et al. 1980; Bouic 2001). For example, sitosterol can modify the secretion of inflammatory cytokines both in vitro and in vivo (Gupta et al. 1980; Bouic 2001; Ding et al. 2019; Liu et al. 2019). In bone marrow-derived macrophages (BMDMs), 25 μM sitosterol reduced the expression of pro-inflammatory cytokines iNOS, interleukin (IL)-1β, CD86, and MHCII, while increasing the expression of anti-inflammatory cytokines arginase-1, IL-10, CD163, and CD206 (Liu et al. 2019). In dextran sulfate sodium-induced colitis C57BL/6 mice, sitosterol treatment led to reduction of IL-6, tumor necrosis factor α (TNFα), and IL-1β in colonic mucosa (Ding et al. 2019). Meanwhile, in clinical studies, Kurano et al. (2017) reported that there were strong negative relationships between the serum IL-6 and TNFα levels and the serum sitosterol levels. Moreover, in a meta-analysis including 1308 subjects, Rocha et al. (2013) suggested that appropriate dose and duration of phytosterols intake may influence the plasma level of C-reactive protein, which is an inflammatory biomarker associated with potential risk of cardiovascular diseases (CVD). Furthermore, phytosterols have immune modulating effects on asthma patients, by influencing the T helper (Th) response type. In progression of asthma, Th1-type response is impaired, while Th2-type response is stimulated (Woodruff et al. 2009). By treating peripheral blood mononuclear cells from asthma patients with 1.2 μM sitostanol, the inflammatory response was shifted to Th1-type (Brüll et al. 2012). Later, Brüll et al. (2016) revealed in another randomized, double-blind clinical study including 58 asthma patients that, after 8 weeks of plant stanol esters consumption, concentrations of TNFα and IL-1β in plasma significantly decreased. Except for the modulation of Th response, phytosterols can also affect the number of $CD3^+$ and $CD4^+$ T cells (Hu et al. 2017; Smet et al. 2015).

Several studies have suggested phytosterols may mediate inflammation through nuclear factor-κB (NF-κB) signaling pathways (Gabay et al. 2010; Loizou et al. 2010; Valerio and Awad 2011; Rios et al. 2017; Goff et al. 2019). In unstimulated cells, NF-κB dimers are sequestered in the cytoplasm by the inhibitors of κB (IκB). Upon activation, IκB proteins are degraded, and NF-κB is freed to enter the nucleus, leading to the transcription of specific inflammatory cytokines. Sitosterol attenuated the phosphorylation of NF-κB p65 in human aortic endothelial cells (Loizou et al. 2010), and Valerio et al. reported in J774A.1 murine macrophages that, sitosterol decreased the phosphorylation and translocation of NF-κB to the nucleus, by increasing the activity of tyrosine phosphatase SHP-1 (Valerio and Awad 2011). Similarly, stigmasterol tends to counteract the IL-1β-induced NF-κB activation in human osteoarthritis chondrocytes (Gabay et al. 2010). Except for NF-κB, in studies of Liao et al. (2018), sitosterol significantly reduced the expression of NLRP3 (NOD-, LRR-, and pyrin domain-containing 3). NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC), and protease caspase-1 are components of NLRP3 inflammasome. Upon activation, caspase-1 mediates the production of pro-inflammatory cytokines such as IL-1β and IL-18. By decreasing NLRP3 expression, sitosterol inhibited the activation of caspase-1, thus, reduced the conversion of pro-IL-1β into IL-1β. Additionally, the anti-inflammatory activities of phytosterols may correlate with their structures. Yuan et al. (2019) investigated the inflammatory reaction via extracellular signal-regulated protein kinase (ERK) pathway in lipopolysaccharide-induced macrophages and discovered sitosterol had stronger activity than stigmasterol and campesterol, which suggested phytosterols without a double bond on C-22 and with ethyl on C-24 could be more effective. This might be resulted from the stability of phytosterols with different structures, as degradation rates increase with the number of double bond and decrease with the number of C-24 carbon bond (Hu et al. 2018a). Notably, the patterns observed by Yuan et al. were not consistant when considering the cholesterol-lowering effects of sitosterol, stigmasterol, and campesterol (Yuan et al. 2020), suggesting more research should be done on phytosterols' structure–activity relationships. Furthermore, inflammation is associated with several diseases such as CVD and atherosclerosis, and sitosterol can reduce the expression of adhesion molecules in human endothelial cells (Bustos et al. 2008; Loizou et al. 2010), which may attenuate the monocyte–endothelial cell interaction in early atherogenesis.

As for effects of phytosterols on neuroinflammation in the brain, Xu et al. (2017) revealed that plant sterol esters supplement affected the anatomy and morphology of the hippocampal CA1 region in high cholesterol diet-fed rats, suggesting a potential neuroprotective effect. In the hippocampus of APP/PS1 mice, stigmasterol suppressed the microglia activation, which is closely related to inflammatory response in the brain (Koivisto et al. 2014). Stigmasterol also decreased the TNF-α levels in the cortex part of Swiss albino mice (Yadav et al. 2018). More importantly, Shi et al. (2015) reported in GT1–7 neuronal cells that, sitosterol inhibited NF-κB activation via estrogen receptor (ER)-mediated inhibition of IκB. The underlying mechanisms of phytosterols attenuating CNS inflammation have not been completely revealed, nevertheless, it will be worthwhile to investigate their modulation effects through the NF-κB pathway.

Conversely, although the pro-inflammatory and pro-atherogenic effects of oxidized cholesterols are well observed (Higley et al. 1986; Choi, Sviridov, and Miller 2017), inflammatory abilities of oxyphytosterols still remain unclear. Plat et al. (2014) reported in female LDL receptor-deficient mice that, oxyphytosterols supplement for 35 weeks did not alter the inflammatory markers level, but it increased the proportion of severe atherosclerotic lesions (34%; $p = .011$). Furthermore, increased oxyphytosterol concentrations were found in plasma and aortic valve cusps of patients with concomitant coronary artery disease (CAD) ($n = 104$) (Luister et al. 2015). Fuhrmann et al. (2018) also correlated the absolute plasma levels of 7α-OH-campesterol with cardiovascular events ($n = 376$). However, in Framingham Offspring Study cohort, circulating plasma oxyphytosterols are not associated with CVD risk ($n = 144$) (Baumgartner et al. 2019). Studies of inflammatory effects using different cell lines also lead to

distinct results. Vejux et al. (2012) reported that 7-keto-sitosterol treatment, rather than 7β-OH-sitosterol (20 μM), altered IL-8 secretion in human monocytic U937 cells, and Alemany et al. (2013a) reported that 60 μM 7-keto-stigmasterol incubation increased the production of TNFα and IL-8 in intestinal Caco-2 cells. But in studies of Yvonne et al. (2018), 0.8 ng/mL 7β-OH-sitosterol/campesterol, rather than 3.5 ng/mL 7-keto-sitosterol/campesterol induced modest proinflammatory response in BMDMs derived from C57Bl/6 mice. The type of cells used and oxidation site of oxyphytosterols may result in varying degrees of inflammation. Thus, more in vitro/*vivo* and clinical studies are needed to confirm the pro-inflammatory effects of oxyphytosterols.

Cholesterol homeostasis

Brain is the organ with the highest concentration of cholesterol (Gamba et al. 2012), and failures in cholesterol homeostasis have been recognized as essential factors contributing to the neurodegeneration development (Anchisi et al. 2012). In adult brains, cholesterol is mainly synthesized and secreted by astrocytes. First, 3-hydroxy-3-methylglutary-CoA (HMG-CoA) is catalyzed into mevalonate by HMG-CoA reductase (HMGR) in the endoplasmic reticulum of astrocytes. Mevalonate is then converted into cholesterol and released to the cytoplasm. The efflux of cholesterol is mediated by the ABC transporters ABCG1 and ABCA1 (Shobab, Hsiung, and Feldman 2005). Second, to meet the needs of neurons for cholesterol, ApoE binds cholesterol together with phospholipids to form lipoproteins. Low-density lipoprotein receptor (LDLR) and LDL-receptor-related protein (LRP) regulate the uptake of these lipoproteins, and they are imported into early endosomes in the neurons, where cholesterol is released (Vance 2012). NPC2 and NPC1 in late endosomes then transport cholesterol to oxysterol-binding protein ORP1L (Wijdeven et al. 2016) and StAR-related lipid transfer domain-3 (StARD3) (Wilhelm et al. 2017), through which cholesterol is exported from endosomes to endoplasmic reticulum. Finally, part of the excess cholesterol is esterified by ACAT for storage, while the others is hydroxylated into 24-OHC via enzyme CYP46A1 and eliminated from the brain (Gamba et al. 2012). On contrast, if the cholesterol synthesis is insufficient, sterol regulatory element binding transcription factor 2 (SREBP2) located in the endoplasmic reticulum of astrocytes will be released to the Golgi, where it undergoes cleavage, and the NH_2-segment will enter the nucleus and activate transcription of HMGR (Eberlé et al. 2004).

The plasma concentrations of phytosterols (sitosterol and campesterol) are markers of cholesterol absorption, while lathosterol and desmosterol are measures of cholesterol synthesis (Wu et al. 2014; Andrade, Santos, and Ramos 2015). In neonates supplemented with soy-based, plant sterol-rich lipid for 3 weeks after birth, cholesterol-standardized lathosterol increased by 69% ($n = 5$) (Plat et al. 2019). Accumulated lanosterol was also observed in the cortex of phytosterols fed ABCG5-deficient mice, with desmosterol decreased in the hippocampus (Vanmierlo et al. 2011b). Therefore, phytosterols may affect cholesterol metabolism both peripheral and cerebral.

Liver X Receptors (LXR) α and β are two members of nuclear receptor superfamily which regulate the biosynthesis, efflux, transport, and uptake of brain cholesterol. As natural LXRs ligands, oxysterols in the CNS, especially 24-OHC and 27-OH-cholesterol (27-OHC), are direct link between LXRs and cholesterol metabolism (Hughes et al. 2013). Considering the similar structure of oxysterols and phytosterols, several studies have shown the possibility of phytosterols, such as sitosterol (Plat, Nichols, and Mensink 2005; Kim et al. 2008), campesterol (Plat, Nichols, and Mensink 2005), stigmasterol (Yang et al. 2004; Plat, Nichols, and Mensink 2005), schottenol, and spinasterol (El Kharrassi et al. 2014), to mediate cholesterol metabolism via LXRs. However, although LXRs were activated by phytosterols at concentrations ranging from 10 nM to 10 μM when using a cell-free ligand-sensing assay (Plat, Nichols, and Mensink 2005), results were rather conflicting in cell-based and/or transcription studies. Hutchinson et al. (2019) reported in breast cancer cells that phytosterols (sitosterol, campesterol, brassicasterol, and stigmasterol) with concentrations varying from 1 pM to 50 μM led to modest or no response of LXRα, moreover, 1 μM 24-OHC or 27-OHC induced LXRα activity was impaired similarly by all phytosterols (10 μM). Similarly, Brauner et al. (2012) reported no LXRα response in transfected HEK293 cells, while sitosterol and campesterol (50 μM) additionally reduced 27-OHC formation in Caco-2 cells, and thus, suppressed LXRα activation. Feeding hamsters diet containing 0.1% sitosterol or stigmasterol for 6 weeks did not change mRNA levels of liver LXRα, either (Liang et al. 2011). Contradicting results also appeared when testing the mRNA expression of ABCA1, a target gene of LXRs that controls the efflux of cholesterol (Plat, Nichols, and Mensink 2005; Brauner et al. 2012; Hutchinson et al. 2019). Vanmierlo et al. (2015) summarized four reasons by which phytosterols activate LXRs at different rates: (1) the concentration of phytosterols applied, (2) the type of phytosterol tested, (3) the purity of the phytosterol, and (4) the cell type transfected with the reporter-construct. Yet based on studies aforementioned, the phytosterol-mediated LXRs response should be carefully reconsidered (Table 3). Nonetheless, phytosterols may mediate cholesterol metabolism through pathways other than LXRs. For instance, phytosterols have the potential to affect the expression levels of SREBP2 (Yang et al. 2004; Liang et al. 2011), HMGR and SR-BI (Park and Carr 2013), as well as proliferator-activated receptor γ (PPARγ) (Liland et al. 2019; Shuang, Rui, and Wenfang 2016), which facilitates the transport of cholesterol.

As for oxyphytosterols, the side-chain oxidation products of phytosterols are possible to activate LXRs. Navas et al. (2018) synthesized four C24-hydroxylated stigmastane derivatives, and two of them were LXR positive modulators in U937 monocytic cell line. 24(S)-Saringosterol derived from *Sargassum fusiforme* also activated LXRβ in cultured neurons (Bogie et al. 2019). Additionally, 27-OH-sitosterol and 27-OH-campsterol were proved to be potential LXR agonists in studies of Brauner et al. (2012). However, a mixture of 7-

Table 3. Contradictory results in current studies.

Uncertainty	Compound	Subject	Treatment	Results	Reference
How do phytosterols modulate LXR?	Sitosterol, campesterol, and fucosterol	A cell-free, coactivator peptide recruitment assay	10 nM to 200 μM	All phytosterols significantly activated LXR	Plat, Nichols, and Mensink (2005)
		Fully differentiated Caco-2 cells	Mixed micelles containing 250 μM phytosterols for 6 h	Phytosterols increased ABCA1 expression by 273%, 213%, and 166%, respectively	
	Sitosterol	LXRβ-deficient mice with a C57BL/6 background	Given 42 mg/kg sitosterol dissolved in olive oil per day for 3 weeks	Sitosterol activated LXRα in the absence of LXRβ	Kim et al. (2008)
	Stigmasterol and sitosterol	CHO-7 cells	25 μM for 16 h	Stigmasterol, instead of sitosterol, activated LXRα	Yang et al. (2004)
	Schottenol and spinasterol	BV2 cells	2.5, 10 μM for 16 h	2.5 or 10 μM schottenol or spinasterol activated LXRα/β 2.5 or 10 μM spinasterol increased ABCA1 mRNA expression	El Kharrassi et al. (2014)
	Sitosterol and campesterol	HEK293 cells transfected with a LXR responsive element-luc construct	10 μM for 24 h	Both phytosteorls did not activate LXR	Brauner et al. (2012)
		Caco-2 cells	50 μM for 24 h	Both phytosteorls did not alter ABCA1 mRNA expression	
	Sitosterol and stigmasterol	Male Golden Syrian hamsters	Fed rodent chow diet containing 0.1% phytosterols for 6 weeks	Both phytosteorls did not change liver LXRα mRNA levels	Liang et al. (2011)
	Sitosterol, campesterol, brassicasterol, and stigmasterol	Breast cancer cells (MDA-MB-468, MDA-MB-231, and MCF7)	1 pM to 50 μM for 16 h	All phytosteorls led to modest or no response of LXRα	Hutchinson et al. (2019)
			10 μM for 16 h	1 μM 24-OHC or 27-OHC induced LXRα activity was impaired similarly by all phytosterols Stigmasterol and sitosterol inhibited 24-OHC or 27-OHC mediated activation of ABCA1	
Do oxyphytosterols correlate with CVD?	Oxycampesterols	104 consecutive patients with severe aortic stenosis	\	Patients with concomitant CAD were characterized by increased campesterol oxides (mainly 7α-OH-campesterol) concentrations in aortic valve cusps	Luister et al. (2015)
	7α-OH-campesterol	376 patients underwent elective coronary angiography	\	7α-OH-campesterol and its cholesterol-corrected ratio were associated with cardiovascular events	Fuhrmann et al. (2018)
	Oxyphytosterols	144 patients with documented CVD and/or more than 50% carotid stenosis	\	The sum of absolute oxyphytosterol concentrations and the sum of TC-standardized oxyphytosterol concentrations were not associated with an increased CVD risk	Baumgartner et al. (2019)
How do oxyphytosterols affect the production of pro-inflammatory cytokines?	7β-OH-sitosterol and 7-keto-sitosterol	U937 cells	20 μM for 24 h	7-keto-sitosterol, instead of 7β-OH-sitosterol decreased IL-8 secretion	Vejux et al. (2012)
	7-keto-stigmasterol	Caco-2 cells	60 μM for 3 h	7-keto-stigmasterol increased the	Alemany et al. (2013b)

(*continued*)

Table 3. Continued.

Uncertainty	Compound	Subject	Treatment	Results	Reference
				production of TNFα and IL-8	
	7β-OH-sito/campesterol, 7keto-sito/ campesterol	Bone marrow-derived macrophages	0.8 ng/mL, 3.5 ng/mL for 24 h	0.8 ng/mL 7β-OH-sitosterol/ campesterol, rather than 3.5 ng/mL 7-keto-sitosterol/ campesterol increased the production of TNFα	Yvonne et al. (2018)
How do oxyphytosterols affect the ROS production?	Oxysitosterols (7-keto: 41.8%, 7-OH: 20.2%, 5,6-epoxy: 25.9%)	Rat aortic endothelial cells	60 μg/mL for 1 h	Oxysitosterols increased NADPH oxidase-derived ROS	Yang et al. (2013)
	7β-OH-sitosterol	ApoE-deficient mice with a C57BL/ 6 background	Fed Western-type diet containing 7-keto-sitosterol for 4 weeks	7-keto-sitosterol increased ROS production in the aortic	Weingätner et al. (2015)
	7β-OH-sitosterol	Caco-2 cells	60 μM for 12, 24 h	7β-OH-sitosterol diminished ROS accumulation	Roussi et al. (2007)

OH-, 7-keto-, and 5,6-epoxy- phytosterols did not alter LXRs activity in the cell-free assays (Plat, Nichols, and Mensink 2005), suggesting oxidation at the sterol-ring to be ineffective. Despite this, 7-keto-stigmasterol was found to inhibit the mRNA expression of HMGR in Caco-2 cells (Alemany et al. 2013a) and similarly, 7-keto-cholesterol has been shown to lower the transcription of HMGR in different cell lines (Brown and Jessup 2009). Thus, Alemany et al. (2013a) hypothesized that 7-keto-phytosterol may influence cholesterol metabolism by interacting with oxysterol binding protein located in the endoplasmic reticulum.

Oxidative stress and mitochondria function

Phytosterols in edible oils have been recognized as the key components that associate with their antioxidative properties (Li et al. 2018). The antioxidative effects of phytosterols were also observed in vitro and in vivo (Vivancos and Moreno 2005, 2007; Shi et al. 2013; Azlina et al. 2016; Ward et al. 2017; Adebiyi, Olopade, and Olayemi 2018; Devaraj et al. 2020). Pre-incubation of 10 μM sitosterol for 3 h decrease H_2O_2 levels, arachidonic acid release and prostaglandin E2 synthesis in ox-LDL stimulated RAW 264.7 macrophages (Vivancos and Moreno 2007). 50 μM stigmasterol treatment for 72 h also inhibited the increase of ROS levels induced by glucolipotoxicity in human INS-1 insulinoma cells (Ward et al. 2017). An in vivo study showed that, sitosterol intake for 4 weeks significantly mitigated the lipid peroxidation and retrieved the activities of intracellular enzymic antioxidants, superoxide dismutase (SOD) and catalase (CAT), in the liver tissue of carbon tetrachloride (CCl4) treated rats (Devaraj et al. 2020). In another CCl4 induced oxidative stress rat model, subcutaneous injections of phytosterols (60% β-sitosterol and 40% stigmasterol and campesterol) at 140 mg/kg rat weight for 5 weeks significantly increased the reduced glutathione (GSH):oxidized glutathione (GSSG) ratio in the heart, liver, kidney, and lung (Azlina et al. 2016). Clinical studies investigating the antioxidative effects of phytosterols in humans are scarce. Ho, Liu, and Loke (2016) reported in a randomized, double blind, placebo-controlled, and crossover study involving 18 healthy participants that, 2 g/d free plant sterols intake for 4 weeks alleviated lipid peroxidation, possibly by modulating the activities of myeloperoxidase, 5-lipoxygenase, and 12-lipoxygenase. Hsu, Kuo, and Huang (2017) designed a two-center, randomized, double-blind, placebo-controlled study which included 50 postmenopausal women, and discovered 24 mg/d intake of *Diascorea alata* extracts containing phytosterols for 12 months improved the plasma antioxidant status. Despite the lack of evidence in whether phytosterols affect the antioxidant status of human brains, there are studies carried out in murine brains. Adebiyi, Olopade, and Olayemi (2018) reported in hippocampal homogenates of BALB/c mice that, stigmasterol increased the activities of antioxidant enzymes SOD and CAT, and decreased lipid peroxidation. The underlying mechanism of phytosterol stimulating antioxidant enzymes has been suggested to be regulation of the GSH redox cycle via ER/phosphatidyl inositol 3-kinase (PI3K)-dependent pathway (Vivancos and Moreno 2005). Culturing HT22 hippocampal cells with 15 μM sitosterol for 2 h prevented the glucose oxidase-induced oxidative stress and further experiments showed that sitosterol incorporated into the cell membrane and recruited PI3K to lipid rafts, which could be inhibited by ER antagonist, supporting the idea of sitosterol modulating ER/PI3K signaling (Shi et al. 2013). However, there is still a need of more in vitro and in vivo studies. As for oxyphytosterols, mixture of sitosterol oxidation products (7-keto: 41.8%, 7-OH: 20.2%, 5,6-epoxy: 25.9%) increased the expression of cyclooxygenase-2 and elevated the ROS levels in rat aortic endothelial cells (Yang et al. 2013). The oxidative stress in the aorta also increased in 7β-OH-sitosterol treated ApoE-deficient mice (Weingätner et al. 2015). However, 60 μM 7β-OH-sitosterol diminished ROS accumulation in Caco-2 cells (Roussi et al. 2007). Thus, the oxidative effects of oxyphytosterols need to be further investigated.

Mitochondria is the major source of ROS, and mitochondrial defects are associated with several neurodegenerative

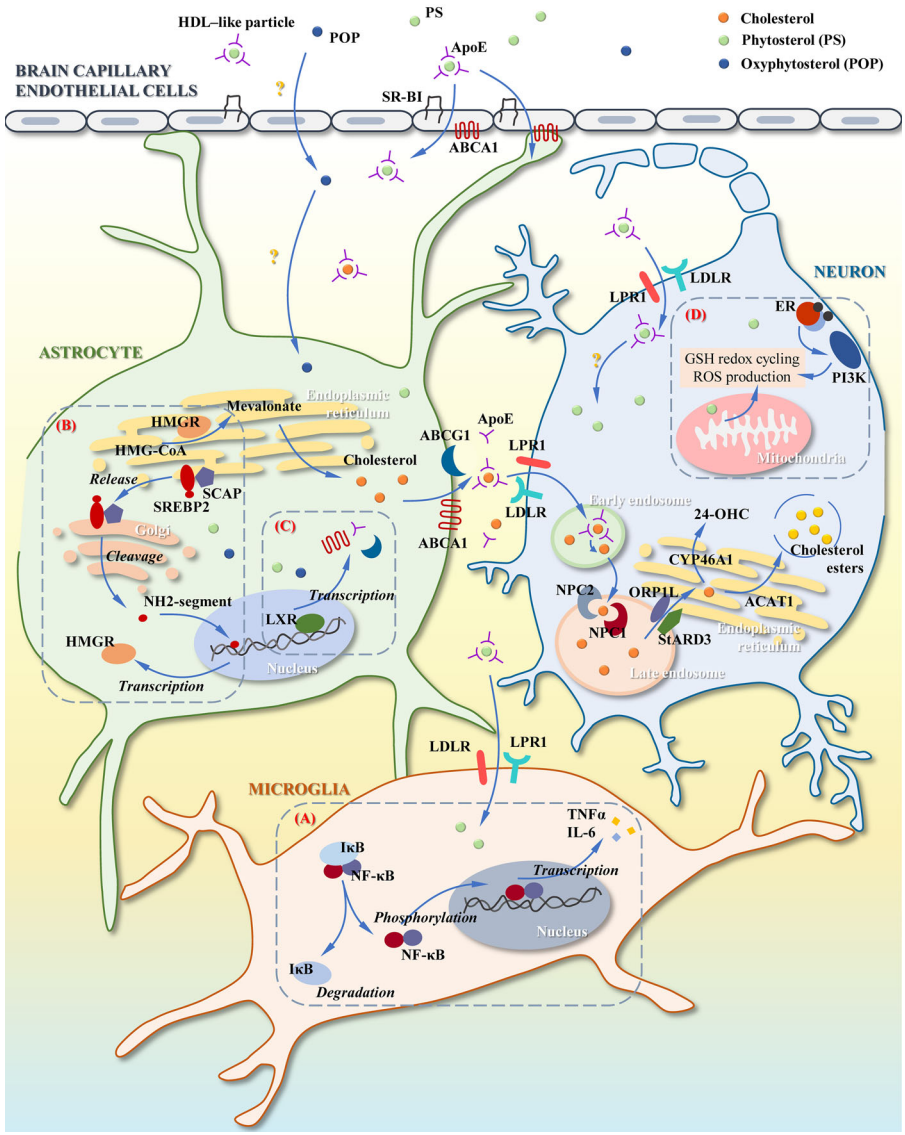

Figure 3. Possible pathways for phytosterols and oxyphytosterols to affect the brain. (A) Phytosterols may modulate inflammatory response in microglia via NF-κB signaling pathway, possibly include inhibition of IκB degradation, NF-κB phosphorylation, and translocation of NF-κB to the nucleus. (B) Phytosterols may downregulate cholesterol synthesis in astrocyte, by decreasing the expression of HMGR and SRBEP2. Oxyphytosterols may also affect the expression of HMGR. (C) LXR controls cholesterol efflux and transport by modulating the expression of ABAC1, ABCG1, and ApoE. However, it is unclear whether phytosterols activate, repress or cause no response of LXR. Side-chain oxyphytosterols may activate LXR, but the origin of side-chain oxyphytosterols in the brain remain unknown. (D) Phytosterols may improve GSH redox cycling and decrease ROS production in neuron, possibly by activating ER/PI3K signaling pathway and modulating mitochondrial function.

diseases (Cui, Kong, and Zhang 2011; Chen, Zhou, and Min 2018), thus, it may be possible for phytosterols to regulate oxidative stress through modulation of mitochondria function. Enhanced oxidative status and mitochondrial biogenesis were observed in phytosterols fed broilers (Cheng et al. 2019). Moreover, antioxidant enzymes, such as glutathione peroxidase and glutathione reductase, are crucial in mitochondrial glutathione redox cycling, and phytosterols could improve the cellular redox status in major organs of healthy or CCl4 treated rats, by mediating these enzymes (Wong et al. 2013; Hoi-Shan et al. 2014; Azlina et al. 2016). Findings also suggested mitochondrial membrane fluidity can affect mitochondrial metabolism. Phytosterols are lipid soluble and may corporate into bio membrane, influencing its structure and properties (Hodzic et al. 2008; Wang, Wu, and Shi 2013; Hąc-Wydro et al. 2015; Mannock et al. 2015). For example, sitosterol and stigmasterol were found to improve the stability of a sphingomyelin/phosphatidylcholine/sterol monolayer by modulating interactions and ordering of lipids, and the effects were correlated with the side chain structures of phytosterols (Hąc-Wydro et al. 2015). Wong et al. (2016) reported that sitosterol fluidized mitochondrial membrane and induced uncoupling, thereby increased mitochondrial electron transport, and enhanced mitochondrial response to ATP production. In studies of Li et al. (2007), sitosterol maintained the mitochondrial stability by decreasing the release of cytochrome c from mitochondrial intermembrane to the cytosol. Interestingly, Shi, Wu, and Xu (2013) observed that incorporation of sitosterol into mitochondrial membrane increased the fluidity of inner membrane without affecting the fluidity of outer membrane, the mitochondrial function was, thus, improved, in terms of

membrane potential and ATP content. Based on existing studies, incorporation of phytosterols into mitochondrial membrane may affect intracellular oxidative status by modulation of mitochondrial function. Besides, oxyphytosterols, such as 7β-OH-sitosterol and 7-keto-stigmasterol, may also affect mitochondrial membrane potential (Roussi et al. 2007; Alemany et al. 2012, 2013a).

Conclusions

Collectively, the origin of phytosterols and oxyphytosterols from diet, and their physiological effects on brain health are summarized in this review. Contradictory topics which needed further investigation are collected in Table 3. Based on exiting research, some possible pathways through which phytosterols and oxyphytosterols may affect the CNS are speculated and presented in Figure 3. Briefly, phytosterols may modulate inflammatory response of via NF-κB signaling pathway and improve oxidative status via ER/PI3K signaling or by mediation of mitochondrial function, while oxyphytosterols may affect the synthesis and efflux of brain cholesterol. There is need to explore further the mechanisms and molecular determinants by which phytosterols may induce their health benefits, as various pathways including NLRP3, ERK, and LXR signaling to be possibly effective. Also, it is worth noting that, the health effects of phytosterols have already been established, but the effects of oxyphytosterols remained largely unknown. Thus, researchers should focus more on the potential harm of oxyphytosterols, and if necessary, carefully access the risk of consuming phytosterol-enriched food products. Understanding the processing characteristics, bioactive forms, absorption characteristics, distribution, and utilization in body of phytosterols and oxyphytosterols could shift to healthier food and condiment choices, and further provide potential strategies to protect or control risk factors linked to brain disorders.

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