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Oxysterols in biological systems: The gastrointestinal tract, liver, vascular wall and central nervous system

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Abstract

Oxysterols are oxygenated derivatives of cholesterol generated from exogenous (food) or endogenous (auto-oxidation and enzymatic conversion) sources. Despite their hepatic capacity to transform into bile acids, oxysterols are present in the blood circulation and central nervous system. This review aims to provide a better understanding of the origins and roles of oxysterols under normal and pathophysiological conditions, such as atherosclerosis and Alzheimer's disease. Oxysterols are metabolites of the cholesterol auto-oxidation pathway present in atherosclerotic plaque and are concomitantly endogenous activators of nuclear receptor liver X receptors known to enhance cholesterol efflux. Despite their honourable role in the gastrointestinal tract and central nervous system, oxysterols have, in general, adverse effects in atherogenesis during which they accumulate and trigger cellular and molecular insults that lead to foam cell formation. This study will discuss the paradox that oxysterols are essential for the normal physiology of the hepatic, central nervous and vascular systems, but that they are also bioactive molecules that lead to adverse effects when they accumulate in the vascular wall.

Keywords: *Oxysterols, atherosclerosis, nuclear receptors, liver, enterocyte, Alzheimer's disease*

Introduction

Under normal conditions, the liver controls plasma cholesterol homeostasis by excreting cholesterol into polar bile acids (BA) or redistributing it towards peripheral tissues via circulating lipoprotein particles. The hydrophobic nature of cholesterol molecules requires the use of blood carriers: lipoproteins. However, lipoprotein-bound cholesterol transport is not the only type of transport. In peripheral cells, cholesterol can be converted into hydrophilic derivatives, such as oxysterols, that are transported into lipoproteins in the blood circulation [1] before being trapped and removed by the liver. Oxysterols are derived from cholesterol hydroxylation of the side chain and they exist in several forms. The major circulating oxysterols in humans are 24(S)-hydroxycholesterol (24(S)-OH) and 27-hydroxycholesterol (27-OH) [2,3]. Oxysterols serve important functions: (a) as regulators of the expression of genes involved in lipid and sterol biosynthesis [4,5]; (b) as substrates for the formation of BA [6]; and (c) as mediators of reverse cholesterol transport whereby excess cholesterol is returned to the liver for excretion [2,7–9]. However, the absolute amount of cholesterol returned to the liver through the generation of oxysterol species remains relatively small [10]. Under normal conditions, the systematic hepatic conversion of oxysterols into BA prevents accumulation in extra-hepatic tissues [11,12]. However, in many pathologies [13], such as atherosclerosis, oxysterols are bypassed into the vascular wall where they accumulate into enriched lipid-laden foam cells originating from macrophages or vascular smooth muscle cells [14]. Disturbances in choles-

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terol homeostasis resulting in the accumulation of cerebral oxysterol have also been found in brain disorders, such as Alzheimer's disease (AD). This review discusses the importance of oxysterols in the gastrointestinal tract, as well as their adverse effects in the pathophysiologies of atherosclerosis and brain disorders.

Oxysterols

Oxysterol: Origins, features and effects

Oxysterols originate from dietary [15,16] or endogenous sources [17]. As defined by Björkhem and Diczfalusy [17], the majority of endogenous oxysterol species (Table I) are oxygenated derivatives of cholesterol formed by auto-oxidation (non-enzymatic) through the action of a specific monooxygenase of the cytochrome P450 family or by enzymatic lipid peroxidation (Figure 1). The addition of an oxygen function to the cholesterol chemical frame confers hydrophilic properties to oxysterols and increases their propensity for intracellular esterification, allowing faster transfer from cells to endoplasmic reticulum [18,19] or an alternative strategy to eliminate cholesterol [20]. This modification of the cholesterol property is generally recognized as an extra-hepatic export mechanism of cholesterol [15,17,21]. Due to technical limitations, the detection of oxysterol forms in blood plasma and tissues was low [22]. Indeed, analysing oxysterols in plasma or biological samples is a very tricky procedure, since they are only present in trace amounts (~ 10 000 times less than plasma cholesterol concentrations for several oxysterol species) [3], unstable molecules with a short half-life (1–62 h) [23], sensitive to auto-oxidation during isolation and extraction and require special techniques, such as isotope-dilution gas chromatography-mass spectrometry using deuterium-labelled internal standards [17,22]. With the introduction of new state-of-the-art technologies, researchers are now able to accurately detect micro-quantities of various forms of oxysterols. *In vitro* studies are needed to better understand the role of specific forms of oxysterols present within cell systems and to replicate *in vivo* conditions. Finally, an overview of the current scientific literature shows that the concentrations of various oxysterol species as well as the experimental protocols differed widely among studies, thus rendering the interpretation of all oxysterol effects difficult to integrate within a common physiological context.

Progresses in oxysterol detection technologies and in the understanding of their effects unveiled their relationship with cell cytotoxicity [24–28], inflammation [26,29] and phospholipidosis [25,26,30]. Numerous research groups focused their interests on the oxysterol-induced cytotoxicity [31,32], knowing that the endpoint results on programmed cell death (apoptosis) or oncosis (necrosis) as observed in atherosclerotic lesions [33–35]. However, the complexity of such studies did not reveal all aspects of oxysterol cytotoxic effects observed during atherosclerosis development. The exact mechanisms associated with oxysterol-induced cell death are not clearly defined and seems to be oxysterol- and cell-specific [26]. Knowing that bioactive lipids have the ability to trigger phospholipidosis, a phenomenon occurring in response to the accumulation of xenobiotics usually attributed to drugs with cationic amphiphilic structures [26,36] and associated to cell death [37], a new concept has been unveiled in order to explain oxysterol effects. Thus, the association of oxysterols to inflammation signalling is related to the ability of oxysterols to activate the nuclear receptor LXR [38,39], an important lipid sensor that represses inflammation via the activation of anti-inflammatory genes [40].

Oxysterols generated by auto-oxidation

The first substrate that initiates cholesterol non-enzymatic radical oxidation reactions is 7α-hydroperoxycholesterol (7α-OOH). Since it is very unstable, it progressively epimerizes into 7β-OOH. The 7α-OOH and 7β-OOH rapidly transform themselves into other

Table I. Common and scientific names of principal oxysterols.

Abbreviation	Common name	Scientific name
	Cholesterol	5-Cholesten-3β-ol
7α-OOH	7α-Hydroperoxycholesterol	5-Cholesten-3β-ol-7α-peroxide
7α-OH	7α-Hydroxycholesterol	5-Cholesten-3β, 7α-diol
7β-OH	7β-Hydroxycholesterol	5-Cholesten-3β, 7β-diol
7KC	7-Ketocholesterol	5-Cholesten-3β-ol-7-one
α-Epox	5α,6α-Epoxycholesterol	Cholestan-5α, 6α-epoxy-3β-ol
β-Epox	5β,6β-Epoxycholesterol	Cholestan-5β, 6β-epoxy-3β-ol
22(R)-OH	22(R)-hydroxycholesterol	5-Cholesten-3β, 22R-diol
24(S)-OH	24(S)-hydroxycholesterol (cerebrosterol)	5-Cholesten-3β, 24S-diol
24(S),25-Epox	24(S),25-epoxycholesterol	5-Cholesten-24(S), 25-epoxy-3β-ol
25-OH	25-Hydroxycholesterol	5-Cholesten-3β, 25-diol
27-OH	27-Hydroxycholesterol	5-25R-Cholesten-3β, 26-diol
	Cholestenoic acid	3β-Hydroxy-5-cholestenoic acid

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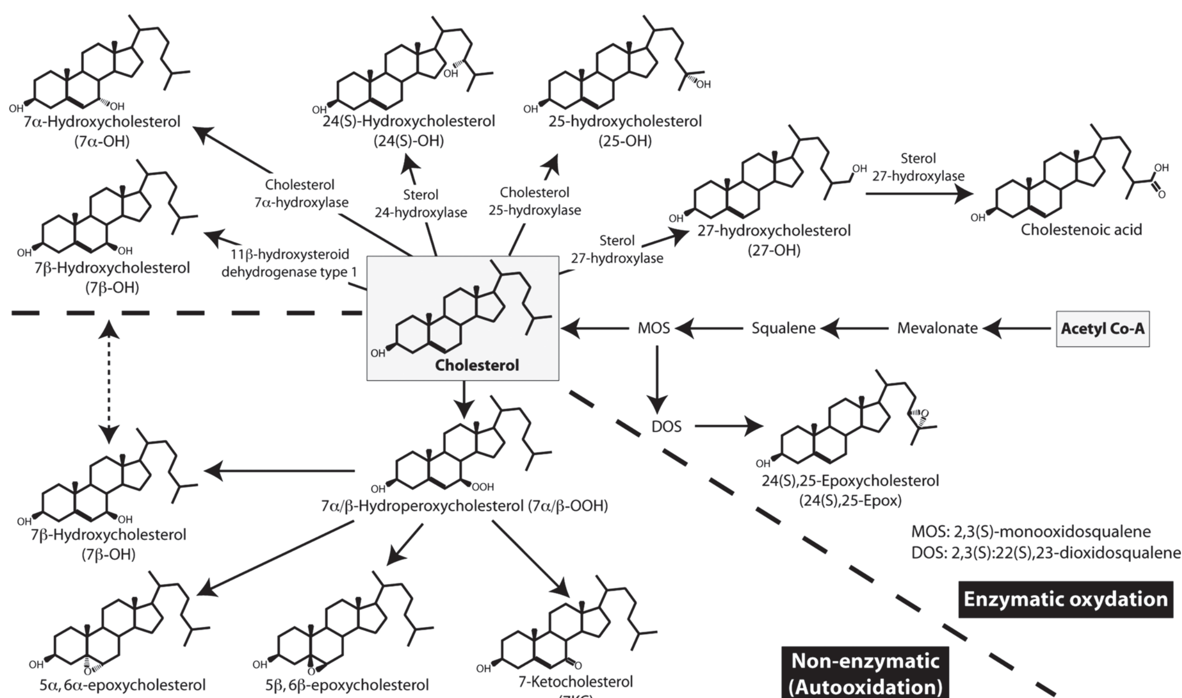

Figure 1. Enzymatic and non-enzymatic pathways of oxysterol formation.

oxysterol species such as 7β-OH, 5α,6α-epoxycholesterol (αEpox), 5β,6β-Epox and 7-ketocholesterol (7KC) [15] (Figure 1). 7β-OH is generated via either a cholesterol auto-oxidation processing or an enzymatic pathway involving the type I 11β-hydroxysteroid dehydrogenase [12] expressed in various tissues including vascular cells [41,42].

Oxysterols generated by enzymatic conversion

In the liver, the conversion of cholesterol into 7α-hydroxycholesterol (7α-OH) before its transformation into BA is the first step for cholesterol disposal in mammals [43]. The regulation of BA synthesis is under strict metabolic control using multiple mechanisms such as the classic neutral pathway involving microsomal 7α-hydroxylase (CYP7A1) largely expressed in hepatocytes and ovary cells [26]. An alternative pathway of BA synthesis, called the acidic pathway, involving a microsomal oxysterol 7α-hydroxylase 1 (CYP7B1) expressed in extra-hepatic tissues [44], converts the 27-OH into another oxysterol intermediate before hepatic BA conversion.

Cerebrosterol, also called 24(S)-OH, is a brain-specific oxysterol and the product of 24-hydroxylase (CYP46A1) activity predominantly expressed in neurons and neural retina [26]. This enzyme, which belongs to the P450 cytochrome family, is located almost exclusively (90%) in the endoplasmic reticulum (ER) of brain cells, principally in neurons [45]. Cerebrosterol is a key component involved in the maintenance of brain cholesterol homeostasis [45–47].

The microsomal enzyme 25-hydroxylase [26], which is not a P-450 cytochrome, but belongs to a smaller family of non-heme iron-containing proteins expressed in most tissues, forms 25-hydroxycholesterol (25-OH) enzymatically [48]. This oxysterol is the most efficient inhibitor of cholesterol synthesis in isolated cultured cells by suppressing the synthesis of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) that is the rate-limiting enzyme responsible for cholesterol synthesis [49]. This suppression of HMG-CoA synthesis may results from the binding of oxysterols (27-OH, 25-OH, 22(R)-OH, 24(S)-OH and 24-25-Epoxycholesterol) to the retention protein Insig that, in turn, impairs the exportation of sterol regulatory element-binding protein (SREBP) from the endoplasmic reticulum to the Golgi [50,51]. Despite its role in modulating cellular cholesterol homeostasis, it has been proposed that 25-OH is of minor importance, since only a small proportion of this oxysterol species is observed in human plasma and atherosclerotic lesions [21].

The sterol 27-hydroxylase (Cyp27A1), also called 26-hydroxylase in older reports, is responsible for the one-step conversion of cholesterol into 27-OH, previously referred to as the 26-OH [52]. This mitochondrial cytochrome P450 enzyme is widely expressed in the liver, intestine, lung, adrenals and kidneys [53], as well as macrophages [7,54] and endothelial cells [55]. In macrophages, intracellular accumulation of cholesterol induces the excretion of 27-OH, whereas the specific inhibition of 27-hydroxylase causes intracellular cholesterol accumulation. These observations suggest that 27-hydroxylase plays an important role in the

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elimination of extra-hepatic cholesterol [56], through the production of 27-OH at a rate of 17 mg/day, of which ~ 9% is converted into BA [57]. In the blood circulation, 27-OH is mostly found in high-density lipoprotein (HDL), representing 0.01% of total cholesterol molecules transported by these particles [3]. Mainly expressed in the liver but also in peripheral tissues [58] and vascular cells [55], sterol 27-hydroxylase catalyses, via the acidic pathway involved in BA biosynthesis, a three-step conversion of cholesterol into cholestenoic acid (3*β*-hydroxy-5-cholestenoic acid) with 27-OH as intermediate. This oxysterol hydroxylated at position C27 provides an alternative mechanism to prevent intracellular cholesterol accumulation, as compared with the classical ATP-binding cassette family members-mediated cholesterol efflux [52,59]. Indeed, subjects with high plasma concentrations of 27-OH exhibited significant decreases in HDL-cholesterol levels compared to subjects with low plasma concentrations of 27-OH [60]. Furthermore, in this same recent study, the plasma level of 27-OH was an indication of the whole body saturation in cholesterol and predicted responsiveness to dietary cholesterol [60]. Cholestenoic acid was shown to be exported largely from macrophages rather than endothelial cells [52]. However, its formation is inversely correlated with plasma HDL levels [52]. This suggests that the sterol 27-hydroxylase system plays an important role in the removal of intracellular cholesterol from macrophages exposed to low levels of HDL [1], as it is the case in most patients suffering from atherosclerosis. In contrast to other oxysterols, cholestenoic acid is mainly transported into the plasma fraction bound to albumin rather than lipoproteins [3].

Under natural circumstances, shunting the cholesterol synthesis pathway via squalen epoxidase to form the 24(S),25-epoxycholesterol (24(S),25-Epox) may unveil some beneficial effects, since it impairs foam cell formation [61]. In addition to its presence in the human liver [62], 24(S),25-Epox is formed in human macrophages [63], but it is shut down in non-lipid-loaded human macrophages treated by statins [63]. Indeed, in mammalian systems, 24(S),25-Epox has been demonstrated to be a better Liver X receptor (LXR) activator (IC_{50} = 4 μM for LXR*α* and IC_{50} = 3 μM for LXR*β*) [38,64] than the partial endogenous activator 27-OH (IC_{50} = 0.2 μM for LXR*α* and IC_{50} = 0.3 μM for LXR*β*) [65], playing an important role in LXR-mediated cholesterol efflux [66]. It is worth noting that endogenous levels of 24(S),25-Epox are very difficult to assess, since this compound may not support the temperature required for its detection using GC-MS analysis [67].

The gastrointestinal tract

The enterohepatic cycle

With increasing recognition of the pivotal role of postprandial lipids in the progression of atherosclerosis, intestinal fat absorption has emerged as an important contributing process. The dietary behaviour, which characterizes individuals who spend a large proportion of the day in the postprandial state, not only contributes to dyslipidemia, but also affects vascular reactivity. Recently, the atherogenic nature of chylomicrons (CM) and CM-remnants has been evaluated in view of their ability to infiltrate the arterial wall, in addition to their influence on the composition and metabolic fate of other lipoprotein classes via neutral lipid exchange. Additionally, increased dietary cholesterol intake and absorption promote coronary artery disease and the elucidation of mechanisms responsible for regulating enterocyte cholesterol uptake is relevant to the prevention of cardiovascular diseases, given the significant positive correlation between plasma low density-lipoprotein (LDL)-cholesterol concentrations and the efficiency of intestinal cholesterol absorption. The focus of this section is to discuss the relationship of intestinal fat transport and cholesterol homeostasis with oxysterols that are absorbed through the intestine and incorporated into CM or are products of cholesterol catabolism by P450 enzymes. Through molecular signalling, oxysterols bind to their respective nuclear hormone receptor sensors so that genetic transcription is accomplished to either enhance or repress cholesterol absorption and transport, thereby achieving normal cholesterol and BA homeostasis. However, this oxysterol nuclear receptor sensing appears to be tissue-specific (see the section 'Oxysterols and nuclear receptors'). Therefore, the major part of this review will address the families of intracellular receptor proteins known to specifically bind oxysterols and the expression of important genes in cholesterol homeostasis. To facilitate the reader's appreciation of this major area, we will first describe the normal digestive and absorptive processes.

Intestinal fat absorption

Triacylglycerols (TG) are pre-digested by gastric activity [68,69], but major TG digestion occurs with pancreatic lipase [70]. Upon hormonal response from cholecystokinin [71], the output of which is stimulated by gastric fatty acids, this lipase is secreted from the exocrine pancreas and bile salts are released from the gallbladder into the duodenum. The action of pancreatic lipase leads to the formation of free fatty acids (FFA) and 2-monoacyl-sn-glycerol. These lipolytic products accumulate on the fat globule surface, transfer into structures made of phospholipids or bile salts, form multi- or unilamellar vesicles and mixed micelles in the aqueous phase [72–76] and are then absorbed by enterocytes. The fat droplets are emulsified by BA and the available surface is thereby increased. The degradation process is region-specific and ideally results in the formation of 2-monoacylglycerol (MG) and FFAs.

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Cholesterol esterase is known for its hydrolytic activity against dietary cholesterol ester, resulting in free cholesterol and FFA products. Several proteins are involved in mediating cholesterol transport, including scavenger receptor class B type I (SR-BI), Nieman Pick type C-Like 1 (NPC1L1), antigen CD36 (CD36), aminopeptidase N, P-glycoprotein and the caveolin-1/annexin-II heterocomplex [77]. Other ATP-binding cassette (ABC) family members (ABCA1 and ABCG5/G8) act as efflux pumps favouring cholesterol export out of absorptive cells into the lumen or basolateral compartment. MG, fatty acids and cholesterol are transported into enterocytes and reconstitute TG by monoacylglycerol O-acyltransferase, acyl-CoA:diacylglycerol acyltransferase and acetyl-CoA acetyltransferase activities. Re-synthesized lipids in intestinal enterocytes are assembled into CM by microsomal triglyceride transfer protein [78,79]. CMs are quickly transported to other tissues through the lymphatic system and blood stream.

The expression of ABCG5 and ABCG8 transporters seems to be controlled by dietary cholesterol via LXRs. LXRα and LXRβ are expressed in most tissues, including the intestine, and are activated by endogenous oxysterols such as 22(R)-hydroxycholesterol (22(R)-OH), 24(S),25-Epox, 24(S)-hydroxycholesterol (24(S)-OH) and 27-OH [65,80–82]. LXRs regulate their target genes in the form of heterodimers with the 9-cis-retinoic acid receptors (RXRs) which bind to LXR response elements in the regulatory regions of target genes. Experiments with LXRα und LXRβ as well as ABCG5 and ABCG8 knockout mice confirmed that activation of LXRs is associated with an increase in biliary cholesterol secretion and a decrease in intestinal cholesterol absorption [83,84], which may reduce the risks of cardiovascular diseases. Besides the regulation of ABCG5 and ABCG8 by oxysterol-mediated LXR, the target genes also include the ABCA1 and ABCG1 transporters [85–87]. LXR agonists increase the expression of ABCA1 and ABCG1 by binding to the LXRs within their genes, which is accompanied by enhanced cholesterol efflux from various cell types both *in vitro* and *in vivo* [88–91]. LXR agonists not only stimulate the expression of the ABC transporters, but they can also induce their redistribution from intracellular stores to the plasma membrane, further facilitating cholesterol efflux [92]. By stimulating ABCA1 in the enterocytes, LXR agonists enhance apolipoprotein (apo)AI-mediated cholesterol efflux from the basolateral membrane and increases intestinal HDL formation. Indeed, intestine-specific deletion of ABCA1 results in an ~ 30% reduction of plasma HDL [93].

Intestinal NPC1L1 gene expression has also been demonstrated to be under the control of LXR [94]. Recently, Duval et al. [94] demonstrated that LXR agonists decrease the expression of NPC1L1 protein in mouse intestine *in vivo* and in human enterocyte cell culture *in vitro*, thus completing a complex mechanism through which LXRs reduce cholesterol absorption. Studies have also reported that SR-BI and CD36 are shared targets of LXR and peroxisome proliferator-activated receptors [77,95–98]. Overall, intestinal LXRs operate as oxysterols sensors to prevent intracellular overload via the inhibition of cholesterol transporters, as well as via stimulation of cholesterol efflux to HDL (through ABCA1) and cholesterol export out of absorptive cells into the lumen (through ABCG5/G8). (Figure 2).

Bile acid enterohepatic circulation and cholesterol homeostasis

BA are synthesized from cholesterol exclusively in the liver. After conjugation with glycine or taurine, these major cholesterol metabolites, with detergent-like properties, are secreted within the bile, via the bile canalicular lumen, and stored in the gallbladder. Upon ingestion of a meal, they are expelled into the intestinal lumen where they actively participate in the solubilization of alimentary cholesterol, dietary fat and lipid-soluble vitamins during the digestion and absorption processes. In healthy men, more than 90% of BA are reabsorbed throughout the ileum by active mechanisms involving specific transporters and then returned by the portal vein to the liver where they are secreted again into bile. This enterohepatic circulation is essential for the maintenance of a BA balance and, hence, for various important physiological processes [99–101] and especially cholesterol homeostasis [102,103]. BA biosynthesis represents a catabolic process by which cholesterol is converted into the soluble molecules of BA that are easily secreted. Indeed, *de novo* synthesis, which is negatively regulated by its BA end-products and the faecal excretion of BA, represents the principal physiological pathway for the elimination of hydrophobic and insoluble cholesterol and in which oxysterols participate. Sequestration of BA by resins lowers circulating cholesterol via the up-regulation of LDL-receptors [104,105]. Therefore, the liver and small intestine appear to play an essential role in the maintenance of cholesterol balance, which is exquisitely maintained by transcription factors. It is important to note that for BA to accomplish their enterohepatic circulation, they must cross plasma membrane barriers in the intestine and liver.

Oxysterols are naturally occurring intermediates in the conversion of cholesterol to BA, the major route for elimination of cholesterol. Enzymes with capacity to form and metabolize oxysterols are present in the liver, thereby contributing to significant concentrations of oxysterols in biliary tract [106]. The initial and rate-limiting step in the synthesis of BA from cholesterol is the liver specific enzyme 7α-hydroxylase (CYP7A1), which converts cholesterol into 7α-OH.

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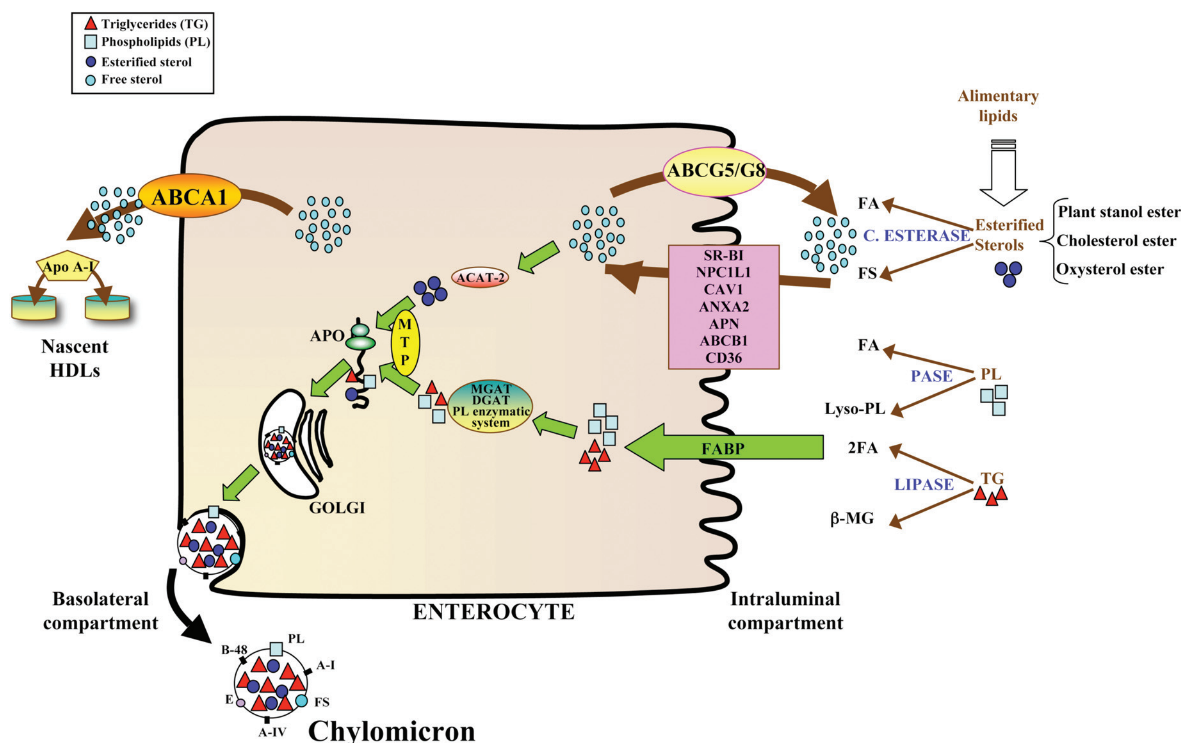

Figure 2. Enterocyte absorption of cholesterol and delivery into chylomicrons. Intestinal lipid absorption is a complex process, which involves a tight coordination between luminal and cellular function. The assimilation of dietary fats requires an efficient digestive system where lipolysis and emulsification take place. Following this digestive phase by various enzymes (pancreatic lipase for triglycerides (TG), phosphilipase A2 for phospholipids (PL), cholesterol esterase for sterol esters (SE: cholesterol ester, oxysterol ester and plant stanolester)) and biliary bile acids, the lipolytic products are absorbed by the epithelial cells where a series of synthetic events occur, resulting in the formation of chylomicrons. The assembly of chylomicrons within the enterocyte is a multistep pathway that includes the uptake of lipolytic products, lipid esterification in the endoplasmic reticulum, synthesis and post-translational modification of different apolipoproteins (apos) and the packaging of lipid and apo components into a lipoprotein particle. The key process in chylomicron formation consists of its intracellular association to apos, particularly apo B-48, the structural protein that maintains the solubility of the particle in plasma. The assembly of apo-B-containing lipoproteins has an absolute requirement for a microsomal transfer protein (MTP). Growing evidence supports the concept that several proteins are involved in mediating intestinal cholesterol transport, including SR-BI, NPC1L1, CD36, aminopeptidase N, P-glycoprotein and the caveolin-1/annexin-2 heterocomplex, while other ABC family members (ABCA1 and ABCG5/ABCG8) act as efflux pumps favouring cholesterol export out of absorptive cells into the lumen or basolateral compartment. Several of these cholesterol carriers influence intracellular cholesterol homeostasis and are controlled by transcription factors, including RXR, LXR, SREBP-2 and PPAR-*α*. Finally, chylomicrons product of digestive and absorptive products consist of an oily core of triglyceride, sterols esters (including oxysterols) surrounded by a membrane of phospholipids, free cholesterol and apos.

It has been known for several years that the expression of CYP7A1 is transcriptionally up-regulated in rats fed a high cholesterol diet, thus enabling the conversion of excess cholesterol into BA for removal from the body. This effect appears to be mediated by LXR, since a functional LXRE has been identified in the proximal promoter region of the rat *CYP7A1* gene, and shown to be activated by RXR/LXR heterodimers in an oxysterol and retinoid-dependent manner [38]. Importantly, the liver compensates for an increase of dietary cholesterol by secreting more cholesterol into the bile [107]. Cholesterol intake enhanced 7*α*-hydroxylase in rat liver, which increased the pool size of 7*α*-OH in the plasma of hypercholesterolemic rats [108,109].

In addition to being important intermediates in the early steps of cholesterol conversion into BA, oxysterols have been ascribed a role in connection with the pathogenesis of biliary tract disease, such as chronic inflammation and gallstone formation [110]. Human gallbladder bile and gallstones contain the oxysterols cholesta-4,6-dien-3-one and cholest-4-en-3-one, with the highest concentrations observed in brown pigment stones [110]. The cytotoxic and apoptotic potential of biliary oxysterols might have important implications in the pathogenesis of biliary tract disorders, including cancers [111]. Besides, there is a continuous flux of 27-OH, the first product of the alternative pathway of BA biosynthesis [112] and the most important oxysterol involved in transport mechanisms from extra-hepatic tissues to the liver [1], where it is taken up and converted into BA [113]. Therefore, 27-OH may be particularly important for the excretion of extra-hepatic cholesterol [56]. In sum, when investigating the adverse and beneficial impact of individual compounds on the biliary

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epithelium, their possible combined effects should be Oxysterols taken into consideration (Figure 3).

Intestinal BA transport and basolateral efflux of bile acids

Efficient intestinal reabsorption of BA and delivery to portal blood represents the second major component of enterohepatic circulation. It has been established that BA transport in the enterocytes basically consists of three components: (i) the passive diffusion of unconjugated BA; (ii) the uptake of conjugated BA in the terminal ileum via Apical Sodium dependent Bile Acid Transporter (ASBT, SLC10A2) [99,114,115]; and (iii) an Na-independent anion exchange mechanism via the Na independent BA organic anion transporting polypeptides (Oatp1a5) [116,117].

The heteromeric organic solute transporter Ostα-Ostβ has been identified as a new basolateral BA carrier candidate in ileal enterocytes [118]. It is transactivated by farnesoid X receptor (FXR). More recent studies demonstrated the coordinated regulation of both mouse Ostα and Ostβ by FXR/RXRα and LXRα/RXRα heterodimers via functional FXREs/LXREs (IR-1 elements) in their promoter region [119].

Intestinal fat absorption and oxysterols

Oxysterols have been quantitated in cholesterol-rich processed foods and may contribute to arterial lesions [120,121]. Consistent with this possibility are the findings that CM can transport dietary cholesterol oxidation products (oxysterols) to the blood circulation via the lymph. Dietary oxysterols, including 7-ketocholesterol, have been shown to be absorbed and incorporated into CM in rats [120] and humans [122,123]. Interestingly, 7-KC that is incorporated into CM may be cleared by the liver

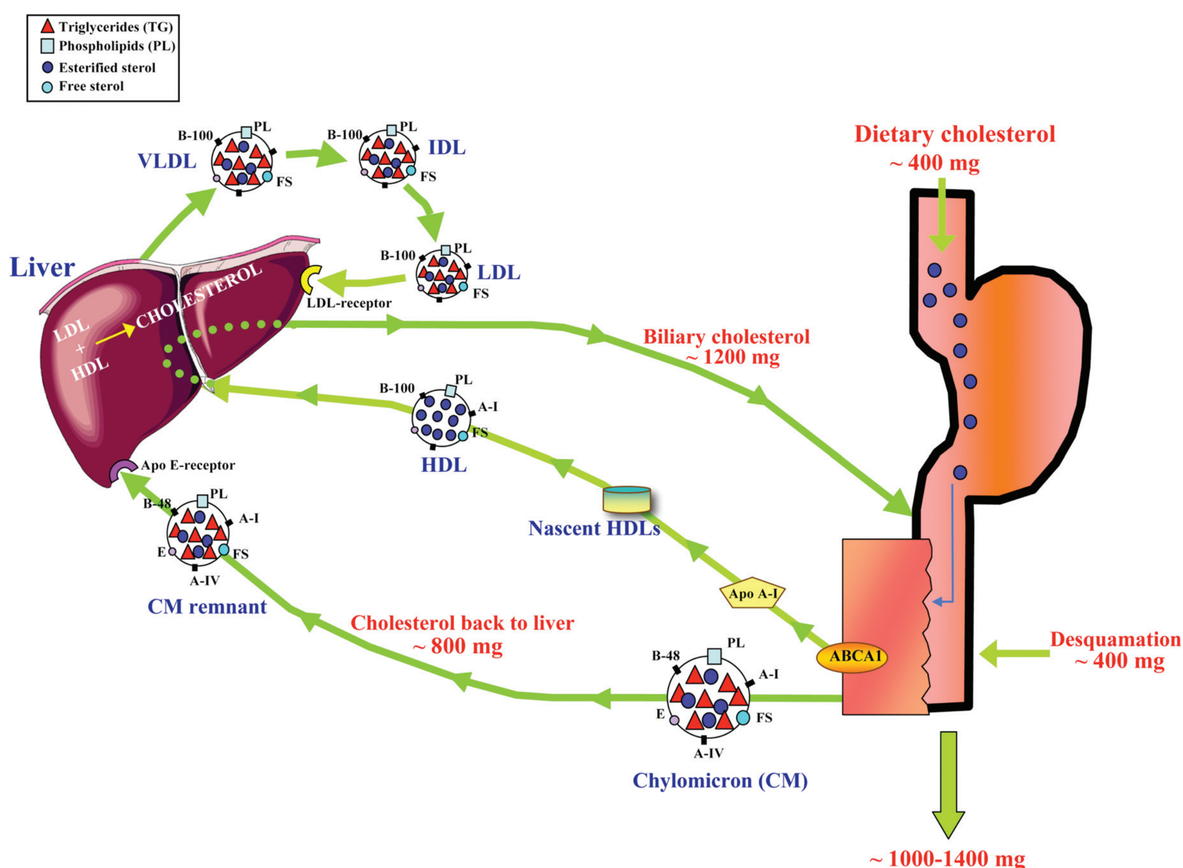

Figure 3. Intestinal sterol absorption, basolateral sterol efflux from enterocytes and hepatic bile acid transport. The intestine must challenge the alimentary triglyceride and phospholipid intake, as well as profuse daily flux of cholesterol from dietary (~ 400 mg), biliary (~1200 mg) and sloughed intestinal cell (~ 400 mg) sources. Cholesterol and oxysterols are solubilized in the lumen of the small intestine by bile acids, which are produced by the liver and secreted into the intestinal lumen. Uptake from the brush border of the enterocyte involves both micelles and protein transporters. Following intestinal uptake, cholesterol and oxysterol molecules are transported by chylomicrons or effluxed by ABCA1 to extracellular apo-AI at the basolateral membrane. Then, Cholesterol and oxysterol molecules transferred by LDL and HDL could be converted into bile acids at the hepatic level.

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as CM-remnants and rapidly metabolized by the liver for excretion via the bile duct into the intestine [11]. However, dietary oxysterols have been suggested to exacerbate the atherogenicity of postprandial lipoproteins by delaying their clearance from plasma and enhancing arterial uptake [124]. Oxysterols could also influence the kinetics and arterial retention of hepatic-derived lipoproteins after transfer from CM [124]. Moreover, other laboratories have suggested that ingestion of lipid oxidation products may increase metabolic oxidative stress *in vivo* [125,126]. Collectively, most of these studies suggest that dietary oxysterols are pro-atherogenic and that CM and CM-remnants enriched with dietary oxysterols may be a key atherogenic component.

Hepatocellular BA transport

The main source of biliary cholesterol appears to be derived from HDL particles that bind to SR-BI. The selective uptake of HDL-cholesterol is stimulated in mice over-expressing hepatic SR-BI [127]. These mice undergo a substantial increase in biliary cholesterol, indicating that SR-BI regulates not only plasma HDL but also biliary cholesterol concentrations [128].

BA flux through the hepatocytes occurs against a concentration gradient and requires an active transport process to maintain high concentrations in the bile [129]. This secretory process is governed by distinct active carrier-mediated transport systems expressed in a polarized fashion in the hepatocytes. The first step in BA clearance from portal blood into the hepatocytes is initiated by several transporters on the basolateral (sinusoidal) membrane of the hepatocytes. Na-dependent taurocholic cotransporting polypeptide (NTCP or SLC10A2) is the main transporter that facilitates the uptake of the BA process by an inwardly Na gradient maintained by the activity of Na/K ATPase. Microsomal epoxide hydrolase (mEH), essential for the metabolism of many xenobiotics, is also capable of mediating 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid (DIDS)-sensitive Na-dependent BA uptake [130], although its physiological significance and accurate contribution to the total basolateral Na-dependent BA transport is still controversial [129].

The other pathway for BA uptake across the basolateral membrane of the hepatocytes occurs in a Na-independent manner [129,131]. This transport is responsible for most of the uptake of unconjugated BA in the portal blood [132]. Several members of the SLC21 (recently renamed as SLCO) OATP superfamily of transporters are involved in Na-independent BA and organic anion uptake [129,131,132]. In particular, the OATP1A2 polypeptide is localized to the basolateral membrane of the hepatocytes and participates in the transport not only of BA, but also of a variety of amphipathic organic

Table II. Effect of different oxysterols on cellular fate and oxidative stress components

Oxysterols	Cellular fate	Oxydative stress (% cell)	Caspase 3 activity	Proliferation	Lysosomal damage	Mitochondrial damage	Loss of δΨm
7α-OH	- [154]	Thiol: ↗↗↗ [32] O2- : ↗ [154]					-% [154]
7β-OH	Mortality: ↗↗ [149,162,163, 168,169] Apoptosis: ↗↗ [32,149,154,162–165,168,170], ↗ (•) [150] Necrosis: ↗ [32,164,165]	SOD: ↗↗ [149,162] GSH: - [168] GSH: ↘↘ [149,162,163] ROS: ↗↗ [32] O2- : ↗ [154]	- [149]	↘ [162] ↘ (•) [150]	↗↗↗ [32, 164,165]	↗↗↗ [32]	↗↗↗ [154]
7KC	Mortality: ↗↗ [163], ↗↗↗ [162] Apoptosis: ↗ [32,164], ↗↗ [153–155], ↗↗ (•) [150] Necrosis: ↗ [32,164]	SOD: - [162] GSH: ↘ [162,163] ROS: ↗↗↗ [32] Thiol: ↗↗↗ [32] O2-: ↗↗↗ [154]		- [155] ↗ [155] ↘ [162] ↘ (•)[150]	↗↗ [164]		↗↗↗ [153] ↗↗ [154]
25-OH	Mortality: ↗ [162,168], - [32,162] Apoptosis: - [168]	SOD: - [162] GSH: - [162,168] ROS: - [32]		- [162]			
27-OH	Mortality: - [32]	ROS: - [32]					
α-Epox	Mortality: - [162] Apoptosis: - [163]	SOD: - [162] GSH: ↘ [162,163]		- [162]			
β-Epox	Mortality: - [162], ↗ [163], ↗↗↗ [168] Apoptosis : ↗ [163,170], ↗↗ [162,168]	SOD: ↘ [162,163,168] GSH: - [162]		- [162]			

All cells used in these studies are human U937 monocytes except for those that are human artery smooth muscle cells (λ). -: tested but without any significant effects. SOD (Superoxide dismutase), GSH (aminothiols glutathione), ROS (Reactive oxygen species). dΨm: mitochondrial trasmembrane potential ; Ú: increasing effect; Ø: decreasing effect.

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compounds, including steroid conjugates, thyroid hormones and prostaglandins [133].

Canalicular BA transport

Canalicular BA transport is a critical component of BA enterohepatic circulation and represents a rate-limiting step in hepatic excretion and bile formation [129]. The major transporters in this pathway are Bile Salt Export Pump (BSEP or ABCB11), which is responsible for the transport of monovalent BA and the Multidrug Resistance Protein, which is the main transporter of divalent BA [129,134,135]. Interestingly, the expression of BSEP was induced by oxysterol 22(R)-OH and the induction was mediated through activation of nuclear receptor [136]. The IR1 element in the BSEP promoter was required for maximal induction of BSEP expression by 22(R)-OH [136]. It seems therefore that 22(R)-OH functions as a dual ligand for LXR and FXR, suggesting that oxysterol/LXR and BA/FXR pathways coordinately regulate their target genes to maintain the cholesterol and BA homeostasis.

Apart from the intestine, ABCG5 and ABCG8 are abundantly expressed in the canalicular membrane of hepatocytes, where they drive cholesterol transport to the bile. Hepatic ABCG5 and ABCG8 are also stimulated by LXR agonists and result in enhanced biliary cholesterol excretion [84]. In contrast to wild-type animals, T0901317 does not stimulate biliary cholesterol excretion and fails to reduce fractional cholesterol absorption in ABCG5/ABCG8 double-knockout mice [84].

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The vascular wall

Oxysterols in human plasma

With the exception of cholestenoic acid that is transported in the plasma lipoprotein-free fraction, oxysterols are mostly esterified [137] by the serum lectin:cholesterol acyltransferase (LCAT) [138] before being transported in the blood circulation into lipoproteins and are distributed among lipoproteins and tissues in the way cholesterol is [3]. The 7α-OH, 25-OH, α Epox, β Epox, 7β-OH and 7KC species are mainly transported into LDL particles supporting the hepatic origin of oxysterols [3]. Indeed, the blood 7α-OH bound to LDL is used as an *in vivo* marker of the hepatic 7α-hydroxylase activity [139]. In contrast, 27-OH generated by the mitochondrial sterol 27-hydroxylase and expressed in many extrahepatic tissues is principally transported through the reverse cholesterol transport system in HDL particles, supporting peripheral tissue production [3].

Recently, using a gas chromatography/mass spectrometry technique, levels of plasma oxysterols have been determined in subjects developing atherosclerosis. Levels of 7KC, 7β-OH and 25-OH did not differ among normal subjects and those with clinical signs of atherosclerosis [140]. Significantly different results have been obtained using a more accurate technique, the GC-MS with isotope dilution-mass spectrometry, able to detect a larger number of oxysterol species, which demonstrates that the three main plasma oxysterols are 27-OH, β–Epox and 7α-OH [3,137,141,142].

Oxysterols in human atherosclerotic plaque

In the pathology of atherosclerosis, 27-OH is 100-times more concentrated in atherosclerotic plaque than in plasma [13]. However, overall oxysterol levels represent only 2% of the total cholesterol level itself [21] and oxysterol molecules are mostly esterified [13,21].

In normal arteries, sterol 27-hydroxylase, the enzyme responsible for the formation of 27-OH and cholestenoic acid, is mainly expressed in intimal smooth muscle cells. However, in advanced atherosclerotic lesions, this enzyme is largely detected in macrophages [55]. Indeed, in human atherosclerotic plaque, sterol 27-hydroxylase is expressed principally in macrophage-rich core regions of complicated lesions suggesting that the conversion of cholesterol into 27-OH may be used by macrophages as an alternative to cholesterol efflux to prevent the intracellular accumulation of cholesterol [54]. The enhanced expression of sterol 27-hydroxylase during the progression of the atherosclerotic lesion may reflect this adaptive response to prevent an important accumulation of cholesterol [55] that leads to cytotoxic effects [143]. It appears that, at a specific point in time during the progression of the atherosclerotic plaque, these adaptive mechanisms are overwhelmed, leading to an accelerated formation of foam cells. However, the stage at which vascular cells are not able to overcome cholesterol/oxysterol insults is not well defined and needs further investigation.

The amount of oxysterols found in atherosclerotic plaque is also dependent on the location of the lesion. Indeed, 27-OH is the major oxysterol species found in human coronary and carotid lesions, while βEpox, αEpox and 7β-OH are principally found in coronary lesions and, to a lesser extent, in carotid lesions [144]. No region specificity was observed for 7KC, whereas 7α-OH was exclusively found in coronary lesions [144]. These observations suggest that different mechanisms are probably involved in the oxysterol-mediated development of atherosclerosis in carotid and coronary arteries.

Based on the latest technical improvements set up to accurately measure specific forms of oxysterols [22], Hultén et al. [145] studied the oxysterol composition of human macrophages isolated from atherosclerotic plaque. Their data provided evidence for the

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first time that 7KC in human macrophages is the major oxysterol molecule that reaches levels similar to those of the 27-OH. Furthermore, these macrophagic cells exhibited enrichment in 7α-OH, 7β-OH, ~βEpox and 27-OH [13,144,146]. In coronary lesions, we could then speculate that these oxysterols, in addition to 27-OH, are the major oxysterol forms involved in the cellular and molecular damages observed in macrophages (i.e. 7KC, 7β-OH and ~βEpox).

Oxysterol-induced damages in human atherosclerotic plaque

Apoptosis, in contrast to necrosis, occurs without the induction of inflammatory signals. Morphological hallmarks of apoptosis include cellular shrinkage, plasma and nuclear membrane ruffling, nuclear condensation and DNA fragmentation. These changes are usually associated with mitochondrial cytochrome C leakage to the cytosol and other apoptotic activating factors, such as caspase activations. However, in apoptosis, plasma membranes are not disrupted. In contrast, necrosis is characterized by cellular swelling, nuclear disintegration and plasma membrane disruption [147]. Oxidative stress, including oxysterol-induced effects, triggers either cell survival or death depending on the magnitude of the insult. At low levels, oxidative stress induces cell survival [148], at high levels it induces apoptosis [149], while overwhelming oxidative stress leads to necrosis [150]. In general (Table II), 7β-OH, 7KC and β-Epox, which are abundant in macrophages [145], have damaging effects on human U937 macrophages. Recent work from Larsson et al. [32] demonstrated that 7β-OH and 7KC individually induce caspase activation, the génération of reactive oxygen species (ROS) and the permeabilization of lysosomal and mitochondrial membranes leading to human macrophage (U937) death as well as toxic effects. In contrast, 25-OH and 27-OH do not trigger such cytotoxic effects, but rather exhibit quenching effects on both 7β-OH- and 7KC-induced cell death [32].

Oxysterols have been shown to induce cell death via various pathways such as the Fas-induced death pathway and the activation of the mitochondrial death pathway [151] via caspase-3 [31,152] and caspase-9 [31] activations. In human atherosclerotic plaque where oxysterols are abundant [144], the various oxysterols are not present in equivalent quantities and do not trigger the same cytotoxic effects [24]. Among them, the principal oxysterols are 7KC, 7β-OH and β-Epox, while 7α-OH and 27-OH do not demonstrate such effects (Table II). Among the oxysterols generated by auto-oxidation, 7KC, α-Epox, 7β-OH and β-Epox are all of importance but with different relative distributions in the blood stream (β-Epox > 7KC > α-Epox > 7β-OH) [3,137], atherosclerotic plaque (7β-OH > β-Epox > 7KC > α-Epox) [144] and macrophages isolated from atherosclerotic plaque (7KC > 7β-OH > β-Epox > α-Epox) [145]. The following sections provide a systematic description of oxysterol-induced cellular damage to make readers aware that oxysterols effects are dependent on many factors such as concentration, incubation time, cell type, combination with other oxysterols or antioxidant compounds.

7-ketocholesterol

In human U937 macrophages, 7KC triggers a reduction in mitochondrial membrane permeability [153,154], an increase in the relative percentage of apoptotic cells [153,154] and an impairment of cell growth [155]. 7KC-induced U937 macrophage apoptosis was shown to activate simultaneously caspase-3 -dependent and -independent modes of cell death [152,154,156] and a caspase-2 dependant mode of apoptosis [157] associated with an accumulation of neutral and polar lipids (free cholesterol, phosphatidylcholine and sphyngomyelin) [158]. In other cell type such as human vascular smooth muscle cells (VSMC), the 7KC triggered cell death [150,159] in a caspase-independent manner and did not induce irreversible mitochondrial swelling or rupture of the outer membrane, suggesting that the apoptotic cascade could be stopped before a point of no return [160]. In the same cell type, 7KC has been shown to trigger the unfolded protein response (UPR) pathway leading to endoplasmic reticulum stress and apoptosis [161] as well as the formation of multilamellar cytoplasmic structures also called myelin figures [26,154]. Taken together, 7KC triggers the accumulation of polar lipids in myelin figures that are located in acidic compartments, a phenomenon associated with phospholipidosis [26]. In J774 murine macrophages, 20 μM of 7KC activates the caspase-3 as soon as 12 h after treatment and reaches a maximal effect at 21 h [31]. In contrast, other studies showed that treating cells with different concentrations of the 7β-OH relative to the 7KC induce (1:1 ratio) [32] or not (1:1.77 ratio) cell apoptosis [31]. In U937 cells, a 24-h cell treatment with 30 μM of 7KC induces apoptosis [162,163], while others reported that 21 μM of 7KC is sufficient to induce necrosis (~ 32%) rather than apoptosis (~ 14%) [32]. These results contrast with works of Li et al. [164] that did not reveal such 7KC-induced necrosis in the same cells incubated with 28 μM of 7KC. Co-treating U937 cells with a total 21 μM of oxysterols (7KC, 7β-OH and 25-OH or 27-OH) completely impaired oxysterol-induced necrosis [32]. However, setting the 7KC at a concentration of 21 μM in an oxysterol mixture did not completely impair this oxysterol-induced necrosis [32].

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These results suggest that oxysterol-induced necrosis occurs at a concentration point where pro-cell death oxysterol (here 7KC) reaches a concentration at which the cell cannot overcome the oxysterol-induced insult, despite the presence of pro-cell survival oxysterols. Within a 30-min period, murine J774A.1 and human U937 macrophages incubated with 7KC (20 μM) rapidly exhibited a generation of ROS that doubled after 3 h of incubation [31,32]. In U937 cells, the reduction of intracellular thiols was more important in presence of 28 μM than in presence of 21 μM of 7KC [32]. Furthermore, the generation of ROS is impaired and the cellular thiols loss is restored in the presence of 25-OH or 27-OH [32]. These results support the quenching effects of 25-OH and 27-OH when they are incubated in the presence of 7KC [32]. U937 cells treated with 100 μM of 7KC demonstrated a loss in mitochondrial membrane permeability [153,154], an increased production of superoxide anions [154], an increase in propidium iodide permeability characterized by an increase in the percentage of apoptotic cells [153,154] and an impairment of cell growth [155], a phenomenon also observed in human artery smooth muscle cells [150]. These 7KC effects can be circumvented when these cells are co-treated with the two antioxidants such as glutathione or N-acetyl-cystein that do not have any impact on 7KC cellular uptake [155]. However, in U937, 30 μM of 7KC did not affect glutathione concentrations or superoxide dismutase (SOD) activity, despite the induction of apoptosis [162]. Previous work demonstrated that incubation of human vascular endothelial (HUVEC) and artery smooth muscle cells with 7KC (12.5–200 μM) triggers apoptosis, but induces necrosis in fibroblasts (MRC5) instead [150]. Incubating U937 cells with 28 μM 7KC induced lysosomal membrane permeability [164].

7b-hydroxycholesterol

In human U937 macrophages, 7*β*-OH enlarges the intracellular labile iron pool, increases the formation of ROS, induces ferritin, the cytosolic accumulation of lipid droplets, lysosomal membrane destabilization and apoptosis [165]. Ferritin has been shown to be increased in human atherosclerotic lesions [166] and can act as a critical anti-oxidant by sequestering unbound or 'free' iron, thereby limiting its participation in oxidative reactions [167]. However, endocytozed iron compounds dramatically augmented 7*β*-OH-induced cytotoxicity, since ferritin upregulation worked as an inefficient defense mechanism [165]. 7*β*-OH can induce U937 cell apoptosis at a concentration of 30 μM [149,162,163,168,169] and 50 μM [154], but this effect is counteracted by the addition of 25-OH (30 μM), an effect observed only in U937 and not in the HL60 cell line [169]. In U937 macrophages incubated with 21 μM of 7*β*-OH, the generation of ROS is observable after 3 h, but it is less important than in the presence of 7KC [32]. In U937 cells, the reduction of intracellular thiols was observed in the presence of 21 μM of 7*β*-OH [32] associated with an increase in SOD [162] (within 12 h) [149] and a decrease in glutathione (GSH) concentrations [149,162,163]. In the same cells, a concentration of 50 μM was sufficient to increase the number of cells producing superoxide anions [154]. The generation of ROS is impaired and the cellular thiol loss is restored in the presence of 25-OH or 27-OH [32]. After 24 h, 7*β*-OH (28 μM) induces a significant permeabilization of lysosomal and mitochondrial membranes in U937 cells [32,154]. Previous work demonstrated that incubation of HUVEC and artery smooth muscle cells with 7*β*-OH (12.5–200 μM) triggers apoptosis, while on the other hand it induces necrosis in fibroblasts (MRC5) [150]. Incubating U937 cells with 28 μM of 7*β*-OH induces lysosomal membrane permeability measured by the acridine orange test and an increase in necrotic cells (20%) compared with cells incubated with 7KC (4%) [164]. In U937 cells incubated with 30 μM 7*β*-OH, Ryan et al. [170] demonstrated that this oxysterol induces apoptosis via a caspase-3-dependant pathway, but did not induce mitochondrial-dependant death. In U937 cells incubated in the presence of 30 μM of 7*β*-OH, the induction of apoptosis is followed by a decrease in GSH and an increase in SOD (at 6 h), an activation of caspase-9 (at 9 h) and caspase-3 (at 12 h) and a cleavage of PARP (at 24 h) [149].

5β,6β-epoxycholesterol

In U937 macrophages, *β*-Epox triggers cell death [162,163,168] in a caspase-3 and mitochondrial-dependent manner [170]. *β*-Epox has been shown to be cytotoxic and induce cell death in U937 cells [162,163,168]. However, in U937, 30 μM of *β*-Epox did not affect GSH concentrations or SOD activity despite the induction of apoptosis [162]. In U937 cells incubated with 30 μM *β*-Epox, Ryan et al. [170] demonstrated that this oxysterol induces apoptosis via a caspase-3-dependent and a mitochondrial-dependent pathway.

Other oxysterols

27-OH is the most abundant oxysterol in the blood stream [3,137], human atherosclerotic lesions [144] and macrophages isolated from atherosclerotic lesions [145], promoting sterol removal [21]. In addition, it is a partial endogenous agonist of the LXR [65], an important nuclear receptor implicated in cholesterol homeostasis [171]. Despite very high concentrations of 27-OH in macrophages derived from atherosclerotic plaque (prob-

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ably through its importance in the elimination of extra hepatic cholesterol [56]) 7KC and 7β-OH followed by β-Epox were found to be the most cytotoxic oxysterols in human macrophages [24]. However, 27-OH did not trigger such cell death effects [32].

Very few studies have tested the effect of 27-OH and α-Epox on U937 survival or death and they have only been shown not to trigger apoptosis [32,162]. Finally, to the best of our knowledge, the effect of 27-OH, α-Epox and β-Epox have not yet been tested on VSMC survival or death. In U937, 7α-OH did not induce apoptosis or superoxide production, lipid peroxidation or the formation of myelin [154]. Many reports demonstrated that 27-OH did not induce cell death [32,162,168]. Using 25-OH in oxysterol mix experimentation, this oxysterol did not alter the toxicity of 7β-OH and β-Epox in U937, but it did offer slight cytotoxic protection in bovine aortic endothelial cells. 25-OH decreases apoptosis in both cell types [168].

The central nervous system

Oxysterols in the central nervous system

The brain must have a continuous supply of cholesterol for its normal function. This is furnished either by *de novo* synthesis or by uptake of extracellular cholesterol through cell membrane receptors. Removal of excess cell cholesterol by metabolically active neurons is mainly controlled by the production of a brain-specific 24(S)-OH, also called cerebrosterol. The cerebrosterol efflux comprises the major pathway for cholesterol elimination from the brain [172] through the blood–brain barrier into the bloodstream that accounts for 99% of the cerebrosterol brain excretion while a minor part (< 1%) conveys via the cerebrospinal fluid [173]. This minor fraction reflects neuronal damage and rate of neuronal loss rather than total number of metabolically active neuronal cells. Thus, cerebrosterol concentration is increased in cerebrospinal fluid while it rather decreases in the circulation in neurodegenerative diseases [173]. Disturbances in cholesterol homeostasis resulting in the altered production of cerebrosterol have been demonstrated to lead to neurodegenerative disorders such as AD [174], Huntington's disease [173,175], Parkinson's [176], demyelinating diseases [177] and multiple sclerosis [178,179]. Axonal damage in acute brain trauma did not change cerebrosterol levels [180]. However, 7 days after a traumatic brain injury, the Cyp46 expression level responsible of cerebrosterol synthesis as well as the LXR target genes, such as ATP-binding cassette transporter A1 and apo-E, are increased [181]. It has been shown that inhibitors of cholesterol synthesis such as statins reduce plasma concentrations of cerebrosterol in AD patients [182]. However, it is unknown whether inhibition of cholesterol synthesis by statins could also have detrimental effects on the establishment and maintenance of new synaptic connections in the brains of patients with neurodegenerative disorders.

The role of oxysterols as signalling molecules between the central nervous system and the periphery

Oxysterols have a broad spectrum of biological and systemic effects, including the modulation of key proteins involved in cholesterol homeostasis and lipoprotein metabolism [15,183,184]. Stimulation of apo-E secretion by cerebrosterol has also been observed in astrocytes, a phenomenon that may limit injury due to AD pathogenesis [185]. Cerebrosterol has a high affinity for the nuclear receptor LXRα [38], an important lipid sensor for cholesterol homeostasis [39,186], and could thus play a critical role in brain-signalling pathways [186].

It has been shown that the secretion of cerebrosterol is developmentally regulated [45]. Based on this observation, it has been proposed that levels of circulating cerebrosterol, compared with 27-OH or total cholesterol, could be a useful marker of pathophysiological events occurring in the brain [187,188]. Indeed, the concentration of plasma cerebrosterol relative to 27-OH or to total cholesterol has been reported to vary depending on normal changes in cholesterol homeostasis occurring in the brain during growth and development [187] or abnormal changes consequent to neuropathologies such as AD [172,189,190], vascular dementia or Smith-Lemli-Opitz syndrome [46,191]. Considering that astrocytes could be the main support cells supplying cholesterol to neurons [192], it has been suggested (Figure 4) that cerebrosterol could favour cholesterol efflux from astrocytes to apo-E through enhanced ABCA1 transporters [193]. A novel abundant neurosterol, 24(S),25-Epox has recently been reported in the brain [194]. This neurosterol is unique in that it is produced through a shunt pathway in cholesterol synthesis, i.e. the mevalonate pathway, thus utilizing the same enzymes. 24(S),25-Epox has been ascribed a function in the regulation of cholesterol homeostasis and is known to be a very good endogen activator of LXR *in vitro* [38]. An accumulation of this oxysterol would thus be expected to trigger the expression of LXR target genes, such as ABCA1 and ABCG1, and consequently of increased cholesterol efflux in macrophages [63] or protecting ovary cells against the accumulation of newly synthesized cholesterol [66,195]. Wong et al. showed that neurons and particularly astrocytes had the capacity to produce 24(S),25-Epox, which modulate, as a ligand, the autoregulated expression of LXR and, as a suppressor, the sterol regulatory element-binding protein-2 (SREBP-2) [194]. Furthermore, 24(S),25-Epox syn-

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thesized by astrocytes can be secreted and taken up by neurons, hence mediating the regulation of genes affecting neuronal cholesterol homeostasis. Alternatively, it has been speculated that 24(S),25-Epox is principally produced by astrocytes, taken-up by neurons and may affect brain cholesterol homeostasis [194]. In rat cortical neurons, SREBP-2 has also been shown to be down-regulated by cerebrosterol in a post-transcriptional manner while apo-E synthesis was up-regulated, leading to restoration of cholesterol balance when cholesterol reaches excess levels [197].

The role of oxysterols in removing excess cholesterol from the central nervous system

Nearly all cholesterol input into the central nervous system (CNS) comes from *in situ* synthesis and not from plasma lipoproteins, even during newborn brain development [198]. Knowing that the blood–brain barrier is equally effective in preventing cholesterol flux in both directions, another more specific mechanism is needed to eliminate excess cholesterol from the brain in order to compensate for the continuous *in situ* synthesis of cholesterol [199]. A number of studies showed that excess cholesterol is removed from the CNS as oxysterol [200], a mechanism by which 4–7 mg of cerebrosterol is continuously eliminated daily from the human brain [2,9,187,199,201]. It is of interest to note that the efflux rate of cholesterol from the brain by this oxysterol mechanism is of the same magnitude as the rate of cholesterol synthesis by the brain [2,187]. Chain-oxidized steroids can transfer through lipophilic membranes at rates of orders of magnitude faster than cholesterol in order to create a rapid equilibrium between the brain and plasma cerebrosterol [202]. The cerebrosterol-to-cholesterol ratio is 20-times higher in the brain than in the circulation and a similar difference is present between the brain and most other organs [9]. Thus, the 'driving force' for cerebrosterol flux from the brain to the blood circulation is likely caused by the concentration gradient [2,174]. In the circulation, cerebrosterol is then mainly transferred from the blood–brain barrier to lipid carriers, such as LDL and HDL particles [3], indicating that the turnover of plasma lipoproteins, from the circulation to the liver for bile excretion, should be crucial for the effective elimination of excess brain cerebrosterol [201]. The balance between cerebral production and hepatic metabolism is therefore a critical determinant for plasma levels of cerebrosterol [188,191,203].

It has thus been concluded that the circulating levels of cerebrosterol are affected not only by the flux of the steroid over the blood–brain barrier, but also by the metabolic capacity of the liver, indicating that the ratio between the size of the brain and the size of the liver is an important determinant for circulating levels of cerebrosterol, i.e. in children vs adults [188] as well as the concentration of hepatic LDL receptors, i.e. the capacity for the liver to effectively remove LDL particles from the bloodstream through specific lipoprotein membrane receptors. It is worth noting that cerebrosterol is a potent inhibitor of cholesterol synthesis that has to be rapidly removed from the CNS in order to permit effective neuronal cell regeneration [199].

Cholesterol homeostasis in AD

AD is a common form of degenerative dementia of the human CNS [204,205]. The neuropathological hallmark of this disease includes amyloid-β (Aβ) peptide deposits, neurofibrillary tangles, astrocytes gliosis and a reduction in the number of neurons and synapses, especially in the cerebral cortex and hippocampus. Epidemiological studies have proposed a number of potential risk factors, such as environmental, sociological, genetic and biological factors including ageing, hypercholesterolaemia and homozygosity for the apo-E4 allele [204,206–218]. Several epidemiological studies have shown that dysfunctional cholesterol metabolism is an important contributing factor in the development and progression of AD [172,219–222] and mild cognitive impairment [223]. Positive correlations between hypercholesterolemia and amyloid load have been found, suggesting that the level of plasma cholesterol could represent an early risk factor for the development of amyloid pathology [211,224,225]. It has been shown that cellular cholesterol level regulates Aβ-protein synthesis through the regulation of a cholesterol-rich domain (lipid raft) [226–228]. Furthermore, abnormal transport and turnover of brain cholesterol has been described in patients with AD whose expression of cholesterol 24-hydroxylase was lower in neuronal cells, but higher in glial cells [229] and who presented a greater brain efflux of cerebrosterol [46,188], characterized by a 27-OH/cerebrosterol ratio that is significantly increased in all brain areas of AD patients [174]. However, discrepancies have been observed in plasma cerebrosterol levels in patients with neurological degeneration compared with less advanced disease with active ongoing neuronal degeneration [230], despite the same technical approach used to measure plasma cerebrosterol levels. As cerebrosterol is recognized to impair the formation of Aβ peptide *in vitro* [231], a phenomenon antagonized by 27-OH [232], it appears that the beneficial effects of cerebrosterol become overwhelmed in the presence of 27-OH. This mechanism may explain how hypercholesterolemia contributes to neurodegenerative diseases [176,233]. Recent knowledge related to oxysterols in the brain unveiled the importance of the balance between the 27-OH and cerebrosterol as an impor-

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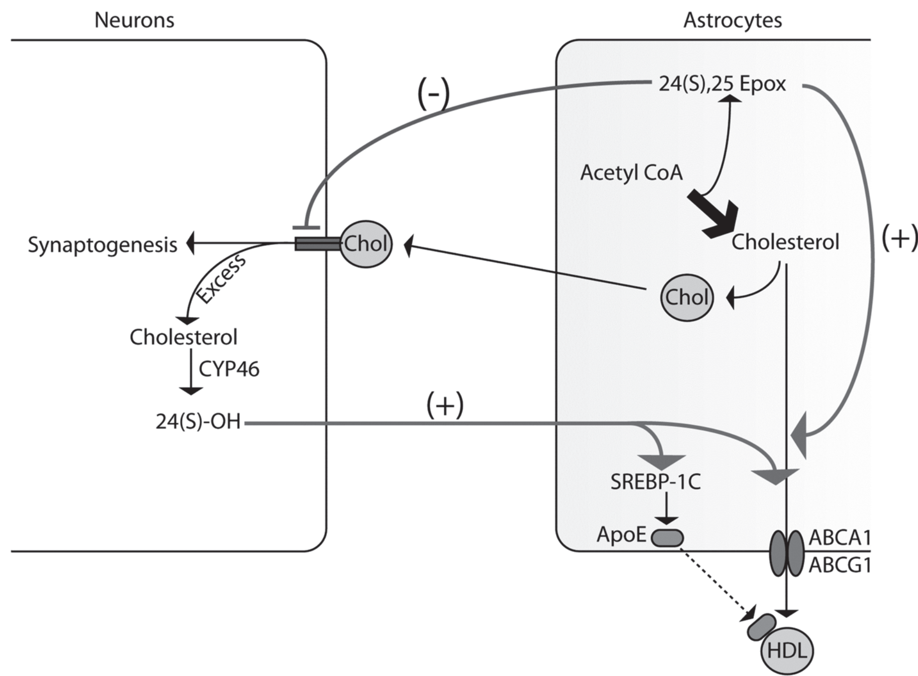

Figure 4. Oxysterol formation in the central nervous system. Astrocytes are support cells supplying cholesterol to neurons. Excess cholesterol imported into neurons is transformed into 24(S)-hydroxycholesterol (24(S)-OH) that in turn favours cholesterol efflux from astrocytes to HDL via ABCA1 and ABCG1 transporters. The 24(S),25-epoxycholesterol (24(S),25-OH) triggers the cholesterol efflux from astrocytes and impairs the cholesterol transfer to neurons.

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tant factor contributing to amyloidogenesis and neurodegeneration [176]. The peripherally-produced 27-OH enters brain tissues through the blood–brain barrier and accumulates with a concentration gradient increasing from the white to the gray matter in an opposite manner to the cerebrosterol [234]. In primary human neurons, this 27-OH brain influx estimated at 5 mg per day [234] has been shown to reduce the production of amyloid-β peptide and to regulate the expression levels of ABCA1, ABCG1 and APOE via LXR-dependant transduction [235]. However, this observation contrasts with the fact that hypercholesterolaemia is recognized to enhance amyloid-β peptide accumulation in association with altered IGF-1 signalling [236] and decreased expression of activity-regulated cytoskeleton-associated protein (Arc) [237] recognized as a key molecule for the maintenance of synaptic potentiation and long-term consolidation of memory [238–240]. As 27-OH is recognized as an endogenous LXR ligand [65], the competition between 27-OH and other brain oxysterols, such as cerebrosterol (neurons) and 24(S),25-Epox (astrocytes), for LXR activation is not very well understood and needs further investigation.

Knowing that the oxidative modification of molecules, including cholesterol, seems to be involved in neurodegenerative mechanisms, including AD [241,242], that excess brain cholesterol is removed by oxidative mechanisms [1], that cerebrosterol is almost exclusively produced by brain tissues [45,47,187,188] and can easily cross the lipophilic blood–brain barrier for removal and excretion by the liver, this oxysterol has been suggested as a potent peripheral indicator of brain cholesterol turnover and neuronal degeneration [243]. Since human plasma cerebrosterol levels are lower at an increasing age compared with children [188], that increasing plasma levels are observed in early AD stage [46,243] and that cerebrosterol concentrations decrease thereafter with the severity of the disease [243], it has been proposed that cerebrosterol could be a state marker of neurodegenerative processes [243,244]. Thus, cholesterol and cerebrosterol appear to be intrinsic modulators of neurotoxic species of Aβ-peptides that characterize AD neuropathology [172,245,246]. Indeed, cerebrosterol has been shown to induce neurotoxic effects, such as the generation of reactive oxygen species (ROS) [247], and to rise calcium levels leading to increase in cell viability in a cerebrosterol level dependant manner [248], reflecting an increase in the brain 'oxidative insult'. This oxidative stress has recently been presented as an important mechanism that should be considered during the production of Aβ–peptide [249], since this peptide has been found to be oxidized in the amyloid plaque [250–252]. AD is associated with progressive neural degeneration and the release of degraded and oxidized cholesterol from degenerating nerve terminals [253], providing an environment favourable to enhanced β-amyloid precursor protein (βAPP) and the pathogenic forms of Aβ-peptides [254].

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Taken together, these observations indicate that cholesterol accumulation in amyloid plaque may result in cholesterol deficiency in other cell structures, resulting in a repartitioning of cholesterol from areas in which it plays a normal physiologic role [255]. The importance of cholesterol in maintaining synapse formation has been studied [256,257]. In the CNS, astrocytes, through the release of apo-E lipid particles, act as suppliers of cholesterol to neural cells via apo-E lipoprotein membrane receptors [203]. Furthermore, it has been shown that apo-E3 has a greater ability to promote cholesterol release from neurons than does apo-E4 [255], resulting in a higher cholesterol level in CNS neurons of apo-E4 carriers and leading to an increased production of Aβ-peptide [258]. However, it has also been shown that apo-E3 particles are richer in cholesterol and could thus supply more cholesterol to neighbouring neurons for neural cell regeneration [259]. It has also been suggested that apo-E4, one of the strongest genetic risk factors for AD, could contribute to disturbances in lipid metabolism, leading to low cholesterol redistribution in the AD brain [260,261]. These findings indicate that cholesterol homeostasis plays an important role in normal physiological mechanisms in the CNS [262] and that it is central to AD pathophysiology, indicating a potential therapeutic target for disease stabilization and primary disease prevention [260].

Statins in AD treatment: Positive and negative effects on the brain

Statins belongs to a family of compounds that inhibit the activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. A number of studies have also shown that statin therapy can effectively reduce strokes in patients with cardiovascular disease and hypercholesterolemia. Furthermore, statins appear to have a number of pleiotropic effects that contribute to the observed neuroprotection [263], including improvement or restoration of endothelial function [264,265], an increase in the stability of atherosclerotic plaque and a decrease in oxidative stress and vascular inflammation [266,267]. Epidemiological studies have shown a strong reduction in the incidence of AD in patients treated with statins that efficiently cross the blood–brain barrier [268–274]. Statins apparently reduce the incidence of AD by reducing the levels of Aβ-peptides present in neurons and in cerebro-spinal fluid, thus preventing the formation of amyloid plaques [268,273,275–278] and βAPP [279]. The association between the use of cholesterol-lowering drugs (statins) and human cerebral cholesterol metabolism is currently being studied intensively. Lipophilic statins, such as Simvastatin, can effectively cross the blood–brain barrier and reduce neuronal cholesterol levels [268,280]. A significant reduction in the levels of cerebrosterol has been reported in normocholesterolemic and hypercholesterolemic subjects treated with the lipophilic Simvastatin [281]. HMG-CoA reductase inhibitors have been associated with a 70% reduction in the incidence of AD. Fassbender et al. [276] have also shown evidence that statins, in clinically relevant dosages (10–40 mg/day), affect cerebral cholesterol levels. Recently, it has been shown that statins given to patients with familial hypercholesterolaemia can significantly reduce plasma cerebrosterol, suggesting that statin therapy could reduce the incidence of AD [182,281–283]. In support of this theory, it has been shown that the prevalence of AD in patients taking statins is significantly reduced compared with the total patient population [271], thereby also reducing the risk of dementia [274].

On the negative side, considerable attention has been given to the possibility that statins may also inhibit the synthesis of various other important products of the mevalonate pathway, such as coenzyme Q10, an essential redox component of the mitochondrial electron transport chain, that acts as an efficient and specific antioxidant [284,285] and/or the novel neurosterol, 24(S),25-Epox, thus reducing cholesterol efflux from astrocytes to neurons and affecting neural cell regeneration. Furthermore, an important question has arisen as to whether lipophilic statins crossing the blood–brain barrier could have beneficial and negative effects on brain cholesterol synthesis by simultaneously preventing cholesterol accumulation in amyloid plaques and inhibiting critical neuronal cell regeneration [286,287] or by acting on oxidative stress [247] or by reducing inflammation rather than decreasing Aβ-peptides [288,289]. In a recent double-blind randomized trial with Atorvastatin in AD patients, Sparks et al. [290] found that Atorvastatin positively influenced clinical outcome measures compared with placebo. However, in men, Pravastatin, a hydrophilic statin, did not affect brain cholesterol metabolism based on the measurement of plasma levels of cerebrosterol [291].

Even though hyperlipidemia may be a risk factor for AD, once AD is established the brain may be lacking in cholesterol for cell regeneration as a result of defective cholesterol homeostasis induced by oxysterol and/or the defective bioavailability of cholesterol [292]. Even when peripheral cholesterol is markedly reduced, CNS cholesterol balance could be maintained by *de novo* synthesis in an apparent effort to maintain the physiological levels of cholesterol [293]. Alternative therapeutic strategies for cholesterol management should be developed in order to reduce the levels of plasma cholesterol without affecting brain *de novo* cholesterol synthesis or to up-regulate cholesterol 24S-hydroxylase to prevent the formation of Aβ-peptides, especially in AD patients.

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Oxysterols and nuclear receptors

Oxysterol-sensing of LXR nuclear receptors in human macrophages isolated from atherosclerotic plaque

In 1978, Kandutsch et al. [294] proposed the hypothesis that negative feedback regulation of sterol biosynthesis is brought about by oxygenated sterols rather than by cholesterol itself. A good candidate among oxysterol-activated nuclear receptors is LXR, that is represented by two isoforms, LXRα and LXRβ. LXRs are recognized for their anti-inflammatory effects in atherosclerotic lesions [295], have been associated with cell survival [296] and are central in a regulatory network associated to lipid metabolism, cholesterol efflux and fatty acid synthesis [29]. Among a large panel of oxysterols tested, the brain-specific cerebrosterol and 24(S),25-Epox, the 24(R),25-Epox mainly produced by the liver, the 22(R)-OH and the 20(S)-OH have been found to have higher affinities for LXRβ than LXRα [38,80]. Nevertheless, these oxysterols demonstrated the greatest response among many other oxysterols that have been tested [64]. In cholesterol-loaded skin fibroblasts and monocyte-derived macrophages, 27-OH activates LXRs leading to the increased expression of ABCA1 and ABCG1 [65]. In contrast, another study that tested several oxysterols demonstrated that 27-OH is only a partial agonist [65]. Among a large panel of oxysterols, another group demonstrated that the best natural activator of LXRs is the 22(R)-OH [39].

Even though 27-OH is considered to be a partial activator of LXRs, in atherosclerosis, where its concentration is elevated [144], it is likely to become the principal endogenous activator of LXRs [65]. In turn, LXRs, more specifically LXRα [297], promote the expression of the inhibitor of macrophage apoptosis, AIM/Spα/Api6, allowing macrophages to be protected from the apoptotic effects of oxidized LDL [296]. In VSMC, LXRs have been shown to inhibit cell proliferation [298]. This observation highlights the importance of 27-OH in reversing the adverse effects of incoming oxysterols generated by auto-oxidation, while lesions become more advanced. This interesting 27-OH induction of cell survival and the described *in vivo* anti-atherosclerotic effect of LXRs [299] should impair atherogenesis. However, this has not been confirmed *in vivo*, suggesting more complex molecular interactions with other cytotoxic oxysterols. Indeed, why do macrophages and VSMC accumulate so many oxysterol species during the development of fatty streak to complicated lesions, producing more 27-OH [300], but are unable to impair cell apoptosis that occurs throughout atherogenesis [301]? During the different stages of atherogenesis, the presence of 27-OH is lower than that of 7KC in VSMC isolated from normal lesions or fatty streaks, while the reverse is observed in VSMC isolated from fibrous plaque or complicated lesions [300]. In other respects, 25-OH and 27-OH [20,302,303] have been shown to inhibit SREBP that is responsible for major regulation in lipid metabolism [304].

Oxysterol-sensing of LXR nuclear receptors and biliary secretion

While membrane-bound SREBP directly activate the expression of genes involved in the synthesis and cholesterol uptake, which ensure cellular cholesterol requirements [305], LXRs and FXRs prevent uncontrolled cholesterol accumulation and promote transport and catabolism, thus avoiding cholesterol-induced cytotoxicity [306,307]. The 24(S),25-Epox observed in the liver via a shunt in the cholesterol pathway [38,39,308] can activate LXRα, resulting in the induction of gene expression controlling BA synthesis and the excretion of cholesterol into bile. As an example, in rodents, LXRα stimulates the transcription of the rate-limiting enzyme (CYP7A1) in the BA synthesis pathway [308]. Accordingly, LXR-deficient mice fed a high-cholesterol diet developed a massive hepatic cholesterol accumulation, whereas wild-type mice were highly resistant to cholesterol feeding [308]. On the other hand, with bile salts as ligands [309], FXR acts as a sensor of intracellular bile salt concentrations and functions in an opposite direction to that of LXR by repressing cholesterol 7α-hydroxylation (CYP7A1) [310], thereby limiting BA biogenesis.

Two adenosine triphosphate binding cassette half-transporters, ABCG5 and ABCG8, are located in the apical membrane of hepatocytes [311] and participate in the transport of sterols, especially biliary cholesterol excretion [312], avoiding accumulation in tissues [313]. Undoubtedly, the ABCG5/G8 sterol transporters in the liver appear to be the major route for cholesterol excretion into bile. The expression of ABCG5 and ABCG8 is induced following LXRα activation [83]. Mice deficient in ABCG5/G8 have drastically (80–90%) reduced biliary cholesterol concentrations [312,314]. Conversely, biliary cholesterol supersaturation and excretion are enhanced (6–8-fold) in transgenic mice over-expressing human *ABCG5* and *ABCG8* genes [315], which highlight their important role in canalicular cholesterol efflux. LXRα activation by cholesterol feeding or by agonist administration enhances ABCG5 and ABCG8 mRNA as well as biliary cholesterol concentrations [83], whereas these effects are absent in mice lacking LXRα/β, suggesting that LXR is the principal regulator of ABCG5 and ABCG8 expression in response to dietary cholesterol [84].

Role of LXR in intestinal sterol absorption

ABCG5 and ABCG8 encoding sterolin-1 and sterolin-2, respectively, also regulate the excretion of

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sterols from the intestine. Mutations in either of these transporters lead to β-sitosterolemia, an autosomal recessive disease characterized by intestinal hyper absorption of all sterols, impaired ability to excrete sterols into bile, premature coronary atherosclerosis and elevated levels of phytosterols in plasma [316–319]. *ABCG5* and *ABCG8* genes are located in a head-to-head orientation on chromosome 2 and are coordinately expressed in the liver and small intestine [313,320,321]. Both proteins dimerize in the ER before their migration to the apical surface where they promote the excretion of sterols from polarized enterocytes to the gut lumen [83].

Synthetic LXR or RXR ligands modulate intestinal sterol transport by acting on ABCA1 that has shown great affinity for 25-OH and other oxysterols [322]. Even though earlier studies showed the ability of these ligands to suppress cholesterol absorption, an effect that was accounted for by ABCA1 down-regulation, conflicting findings in ABCA1-deficient mice called this conclusion into question. The concept suggesting ABCA1-mediated inhibition of cholesterol absorption via cholesterol efflux from the enterocyte into the gut lumen did not hold the road [323–325], since ABCA1 was repeatedly found to participate in cholesterol efflux from the basolateral membrane [326,327]. In contrast, a growing body of evidence has emphasized the LXR agonist effects on cholesterol absorption through the up-regulation of ABCG5 and ABCG8 [311]. These transporters seem crucial to the elimination of dietary and endogenously synthesized sterols in humans and mice [312–314,320,328]. As previously mentioned, ABCG5 and ABCG8 are ATP binding cassette half-transporters that dimerize in the ER before trafficking the functional ABCG5/G8 sterol transporter to the apical surface where it promotes the excretion of sterols from hepatocytes and enterocytes [311,312,315,329]. ABCA1, which is located on the basolateral surface of enterocytes and other cell types, facilitates cholesterol efflux and so could potentially be involved in delivering unesterified cholesterol from enterocytes into the circulation.

Conclusion

As oxysterols originate from different sources, their levels in the gastrointestinal tract, CNS or vascular wall may reflect either healthy cholesterol homeostasis or a sign of pathologies. Oxysterols are important hepatic intermediates involved in BA synthesis and consequently in the absorption of cholesterol by enterocytes. Furthermore, BA-excreted oxysterols and dietary oxysterol can be absorbed in the intestinal tract and transported to the liver via the CM pathway. Thereby, their presence in CM and remnants can participate in oxysterol deposition in atherosclerotic plaque.

Oxysterols released into the blood circulation by the liver (7α-OH, 25-OH, $\tilde{\alpha}$ Epox, $\tilde{\beta}$Epox, 7β-OH and 7KC) are transported by very low-density lipoprotein (VLDL) and LDL. Thus, small and dense LDL particles found in various lipid disorders are more prone to the deposition of cytotoxic oxysterols (mainly 7KC and 7β-Epox) in the vascular intima leading to the development of atherosclerotic plaque. Intimal oxysterols taken up by macrophages could then trigger the formation of additional oxysterols by auto-oxidation. Macrophages probably try to overcome this important accumulation of cytotoxic oxysterols by synthesizing the 27-OH that controls the level of intracellular cholesterol. This production of 27-OH correlates with the large amount of 27-OH observed in atherosclerotic plaque. Knowing that 27-OH is a partial agonist of the nuclear receptor LXRs largely expressed in macrophages and is controlling genes related to cholesterol efflux, a simple paradox is observed in atherosclerotic plaque in the sense that a large amount of 27-OH is produced and is prone to activate LXRs in macrophages, whereas massive amounts of cholesterol still accumulate in foam cells. This observation suggests that the oxysterol-LXR-cholesterol efflux pathway in macrophages may be shunted down, reflecting the incapacity of macrophages to control properly the overwhelming accumulation of cytotoxic oxysterols. Among oxysterols found in atherosclerotic plaque, 7β-OH- and 7KC appear to be the most cytotoxic, while 27-OH has been shown to be non-cytotoxic.

In contrast to the cytotoxic effects of oxysterols in atherosclerosis, the CNS takes advantage of oxysterols. An efficient cross-talk of oxysterols across the blood–brain barrier may regulate a number of key enzymes within the brain, regulating the generation of β-amyloid peptides. Cerebrosterol is not only used as a mechanism to eliminate excess cholesterol from the CNS, but it is also used to favour cholesterol efflux from astrocytes to neurons. Astrocytes thus become major suppliers of cholesterol to neurons. Furthermore, a second oxysterol, 24(S),25-Epox produced in astrocytes, has the ability to shunt the cholesterol synthesis pathway, thus controlling cholesterol homeostasis. In general, cerebrosterol produced by neurons and 24(S),25-Epox produced by astrocytes control the arrival of cholesterol synthesized by astrocytes to neurons and drain excess cholesterol in blood circulation via HDL particles. In pathologies such as AD, greater cerebrosterol brain production can be seen as a mechanism to counterbalance the lower expression of cholesterol 24-hydroxylase in neuronal cells and thus lower cerebrosterol neuronal production, providing an environment favourable to the production of Aβ-peptides.

A very interesting property of oxysterols is their ability to bind to and activate nuclear receptors recognized to control cholesterol homeostasis. Among nuclear

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receptors of interest, the LXRs (LXRα and LXRβ) that can be activated by numerous endogenous oxysterols are important actors in the control of gene expression associated with cholesterol efflux and thus should impair atherogenesis. However, during atherogenesis, the reality appears different, suggesting that molecular or cellular factors may interfere with the oxysterol-LXR-cholesterol efflux pathway that confers to macrophages the inability to overcome the massive and overwhelming accumulation of cholesterol and oxysterols. In the liver, the LXR controls the expression of genes related to BA synthesis and the excretion of cholesterol into bile via ABC transporters. Hepatic BA synthesis is in turn repressed by another nuclear receptor, the FXR that is activated by BA.

Overall, the presence of oxysterols in the system is praiseworthy, since they actively participate in cholesterol homeostasis in the brain and enterohepatic tract. However, in the case of atherogenesis, these bioactive molecules try to counterbalance the oxidative insults operating in macrophages localized in atherosclerotic plaque, but the cells are surpassed, which results in cellular damage rather than controlled cholesterol homeostasis.

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