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## DIETARY CHOLESTEROL: FRIEND OR FOE? CHOLESTÉROL ALIMENTAIRE, AMI OU ENNEMI ?

# Cholesterol and ocular pathologies: focus on the role of cholesterol-24S-hydroxylase in cholesterol homeostasis

Cynthia Fourgeux<sup>1,2,3</sup>, Lucy Martine<sup>1,2,3</sup>, Ségolène Gambert-Nicot<sup>1,2,3,4</sup>, Alain Bron<sup>1,2,3,5</sup>, Catherine Creuzot-Garcher<sup>1,2,3,5</sup> and Lionel Bretilon<sup>1,2,3,\*</sup>

<sup>1</sup> INRA, UMR1324 Centre des Sciences du Goût et de l'Alimentation, 21000 Dijon, France

<sup>2</sup> CNRS, UMR6265 Centre des Sciences du Goût et de l'Alimentation, 21000 Dijon, France

<sup>3</sup> Université de Bourgogne, Centre des Sciences du Goût et de l'Alimentation, 21000 Dijon, France

<sup>4</sup> CHU Dijon, Service de Biochimie Clinique, 21000 Dijon, France

<sup>5</sup> CHU Dijon, Service d'Ophtalmologie, 21000 Dijon, France

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**Abstract** – The retina is responsible for coding the light stimulus into a nervous signal that is transferred to the brain *via* the optic nerve. The retina is formed by the association of the neurosensory retina and the retinal pigment epithelium that is supported by Bruch's membrane. Both the physical and metabolic associations between these partners are crucial for the functioning of the retina, by means of nutrient intake and removal of the cell and metabolic debris from the retina. Dysequilibrium are involved in the aging processes and pathologies such as age-related macular degeneration, the leading cause of visual loss after the age of 50 years in Western countries. The retina is composed of several populations of cells including glia that is involved in cholesterol biosynthesis. Cholesterol is the main sterol in the retina. It is present as free form in cells and as esters in Bruch's membrane. Accumulation of cholesteryl esters has been associated with aging of the retina and impairment of the retinal function. Under dietary influence and *in situ* synthesized, the metabolism of cholesterol is regulated by cell interactions, including neurons and glia *via* cholesterol-24S-hydroxylase. Several pathophysiological associations with cholesterol and its metabolism can be suggested, especially in relation to glaucoma and age-related macular degeneration.

**Keywords:** Retina / lipid / cholesterol / glaucoma / age-related macular degeneration / aging

**Résumé** – **Cholestérol et pathologies oculaires : focus sur le rôle de la cholestérol-24S-hydroxylase dans l'homéostasie du cholestérol.** La rétine est le tissu neurosensoriel de l'œil qui assure la transduction visuelle, c'est-à-dire le codage de l'information lumineuse en influx nerveux. Le terme de rétine regroupe l'association de la rétine neurale et de l'épithélium pigmentaire rétinien qui repose sur la membrane de Bruch. L'association physique de ces deux partenaires et leurs interactions métaboliques sont des éléments indispensables au bon fonctionnement de la rétine, à la fois en termes d'apport en nutriments et de maintien de l'homéostasie. Des dérèglements de ces équilibres sont associés au vieillissement et à des pathologies comme la dégénérescence maculaire liée à l'âge, première cause de malvoyance après 50 ans dans les populations occidentales. La rétine neurale est composée de plusieurs types cellulaires, incluant la glie dont le rôle est crucial pour la biosynthèse du cholestérol dans la rétine. Le cholestérol est le principal stérol de la rétine. Il y est présent dans la cellule essentiellement en tant que cholestérol libre et d'esters de cholestérol au niveau extracellulaire dans la membrane de Bruch. L'accumulation de ces esters de cholestérol à ce niveau est un marqueur du vieillissement de la rétine et de la diminution de sa fonction. Sous influence alimentaire et synthétisé localement, le cholestérol est également régulé *via* les interactions entre populations cellulaires de la rétine, comme entre neurones et glie, *via* la cholestérol-24S-hydroxylase. Plusieurs liens physiopathologiques sont envisagés avec le cholestérol et son métabolisme en particulier avec les glaucomes ou la dégénérescence maculaire liée à l'âge.

**Mots clés :** Rétine / lipide / cholestérol / glaucome / dégénérescence maculaire liée à l'âge

\* Correspondence: [lionel.bretilon@dijon.inra.fr](mailto:lionel.bretilon@dijon.inra.fr)

## 1 Introduction

The retina is the neurosensory tissue that covers the internal part of the posterior pole of the eye. The retina is formed by the association of the neuroretina and the retinal pigment epithelium (RPE) that is supported by Bruch's membrane (BrM). Beyond the physical vicinity of the neuroretina and RPE, their metabolic links are crucial for the functioning of the neuroretina. It is now widely accepted that dysfunctions of RPE, and alterations of BrM properties participate to the aging process in the neuroretina. Aging is the prominent risk factor for the development of Age-related Macular Degeneration (AMD), the leading cause of visual loss after the age of 50 years in Western populations (Klein and Klein, 2013). AMD is characterized by its early stages called maculopathies, and late forms: geographic atrophy also called dry AMD, and neovascular AMD (or wet AMD). Accumulation of lipids, including cholesterol and cholesteryl esters, in BrM is a hallmark of aging and AMD in humans (Curcio *et al.*, 2011). By modifying the dynamics of BrM, reducing its elasticity and hydraulic permeability, lipids participate in the functional changes associated to aging (Booij *et al.*, 2010). Cholesterol is the main sterol in the retina. It is widely distributed in all cell layers of the neuroretina, including retinal ganglion cells (RGC), the most inner cells of the retina which axons form the optic nerve. Cholesterol is provided to the retina by both endogenous synthesis and circulating lipoproteins (Fliesler and Bretillon, 2010). Cellular cholesterol deficiency and accumulation are hallmarks of Smith-Lemli-Opitz syndrome (Irons *et al.*, 1993) and Niemann Pick type C disease (Vanier, 2014), respectively. Both clinical cases are characterized by neurodegeneration (Vance, 2012) including in the retina (Claudepierre *et al.*, 2010; Fliesler *et al.*, 2004) highlighting the crucial importance of maintaining cholesterol homeostasis in neurons.

The purpose of the present review is to summarize the associations between cholesterol, cholesterol metabolism and retinal pathologies. Our paper will especially emphasize the associations between the cholesterol-metabolizing enzyme cholesterol-24S-hydroxylase (CYP46A1), RGC and glaucoma.

## 2 Retinal pathologies

Age-related macular degeneration (AMD) is the leading cause of visual impairment in the Western populations after the age of 50 years. Prevalence of AMD increases dramatically with age, late AMD reached up to 11.8% of the population above 80 years (Klein, 2013). Advanced AMD includes patients with geographic atrophy (GA) or choroidal neovascularization (CNV). Nevertheless, AMD cannot be restricted to its advanced stages. The Wisconsin Age-related Maculopathy Grading System (Klein *et al.*, 1991; Varma *et al.*, 2010) remains the most widely used and recognized grading scale by ophthalmologists. Using this scale, the early stages of the disease are called maculopathies and correspond to the presence of soft, indistinct or reticular drusen or any drusen except hard indistinct, with retinal pigment epithelium degeneration, or increased retinal pigment in the macular area. Drusen are extracellular deposits under the RPE,

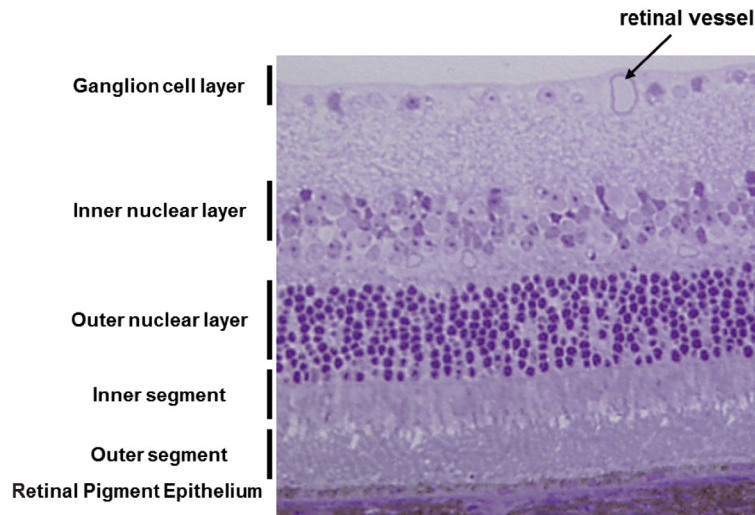
within Bruch's membrane. Only by considering the presence of large drusen ( $>125\ \mu\text{m}$  in diameter), the prevalence of early AMD was 23.6% after the age of 80 years (Klein and Klein, 2013). In 2005, the Age-related eye disease study (AREDS) consortium launched a simplified scoring method for ophthalmologists, based on gradings of fundus photographs, to construct a scale for AMD severity that would evaluate the risk for developing late advanced AMD. As the number of risk factors increased from 0 to 4, the 5-year risk of advanced AMD raised from 0.5 to 50% (Ferris *et al.*, 2005). Drusen are the hallmarks of aging and characteristic to AMD. Drusen contain complement factor-H, -B, -I, -C3, -C5 and -C9, in addition to vitronectin, clusterin, amyloid beta, lipofuscin, C-reactive protein, advanced-glycation end-products, cholesterol and cholesteryl esters (Anderson *et al.*, 2010). The origin of drusen is partly uncertain but there is a large body of evidence that the partial loss of RPE function participates to their deposition in BrM. Indeed, the RPE has a dual function. It first remodels lipoproteins coming from the choroid, and therefore provides nutrients to the photoreceptors. Secondly, the RPE eliminates the debris generated by the retina to the vascular choroid. It is broadly recognized that these processes become less efficient with advanced age, leading to RPE and retinal impairments. Late AMD is characterized by the loss of central vision.

Glaucoma is the second major cause of blindness worldwide. More than 80 million of glaucoma patients are expected in the world in 2020, including 1 million blind people (Quigley and Broman, 2006). There are different types of glaucoma that are all characterized by the loss of RGC, and consequently of the shrinkage of the peripheral visual field. Among risk factors, elevated intra ocular pressure, ethnicity, susceptibility genes are the most prominent.

## 3 The retina and the central nervous system

The retina belongs to the Central nervous system (CNS). Embryonically, the retina derives from the same neural ectoderm as the brain. The retina originates from a pool of proliferating cells that are organized in a pseudostratified neuroepithelium. In vertebrates, the generation of neuronal and non-neural retinal cell types follows a stereotyped order. Retinal ganglion cells are generated first, followed by cones, amacrine and horizontal cells, bipolar cells, rods and lastly glial Müller cells (Centanin and Wittbrodt, 2014). Retinal cells are commonly classified as light-sensitive cells (cones and rods), neurons (amacrine cells, horizontal cells, retinal ganglion cells), and glial cells (Müller cells, astrocytes, microglial cells). Nevertheless, beyond this simplified classification, more than 55 different types of cells have been identified in the mammalian retina. Those include 9–11 types of bipolar cells, 29 amacrine cells, 2 types of horizontal cells, 10–15 RGC (Masland, 2001). In mature retina, the cells are organized in a fundamental plan that comprises the outer segments of the photoreceptors, the outer nuclear layer, the outer interplexiform layer, the inner nuclear layer, the inner interplexiform layer, the RGC nuclear layer and the nerve fiber layer (Fig. 1).

The primary function of the retina is to convert the light stimulus into an electric signal that is transmitted *via* the optic



**Fig. 1.** Structure of the retina.

nerve to the cerebral regions. Rods and cones are the cells that are capable to code the light signal. The initial event of the signal transduction pathway is represented by the activation of rhodopsin in the outer segments of the photoreceptors. Rhodopsin is a G-coupled protein that is enfolded in the membrane bilayer of cones and rods. Its seven transmembrane domains create a pocket which contains 11-cis-retinal (a vitamin A derivative). The energy carried by the light photon is able to isomerize 11-cis-retinal into all-trans-retinal. Activation of rhodopsin triggers the closure of cation channels, leading to hyperpolarization of the membrane. Most mammalian retinas contain 20-fold more rods than cones that form complex networks of postsynaptic cells. Glutamate is the main neurotransmitter in the retina, although most retinal neurons also respond to glycine and GABA that therefore participate to the complexity of the postsynaptic responsiveness.

#### 4 Similarities and differences between brain and retinal cholesterol

Despite brain accounts for only two to three percent of total body weight, brain cholesterol represents approximatively twenty five per cent of total cholesterol (and derivatives) found in humans. Cholesterol can be found as a free form and as esters. Esterified cholesterol is a crucial component of the myelin sheath where it accounts for 70–80% of total cholesterol. Myelin, which is specifically formed by lipids such as sphingomyelin, glycosphingolipids, plasmalogens and cholesterol, is essential to protect nerve fiber. Transmission of the nervous influx along the myelin sheath is facilitated by saltatory conductivity between Ranvier nodes. Myelin sheath improves axonal electric properties and increases conduction speed of the signal to 120 m/s. It has been suggested that evolution has favored plasma membrane cholesterol to form compact myelin that rendered it competent in connecting brain in a very condensed form structure within a narrow axonal diameter (Saher *et al.*, 2011). On the contrary to brain neurons and axons of RGC beyond the optic nerve has left the orbit, axons of retinal

neurons are devoid of myelin. Free cholesterol is the exclusive form of cholesterol in the retina. No cholesteryl esters are found therein on the contrary to the RPE and BrM (Bretillon *et al.*, 2008) in which they accumulate as a function of aging (Curcio *et al.*, 2009).

Cholesterol synthesis is relatively important during CNS development but declines until very low levels are reached at adult age (Dietschy and Turley, 2004). From birth to adulthood, brain cholesterol quantity increases nearly ten-fold: from 1.5 to 10.6 mg in mice and from 2.7 to 32.2 g in humans (Dietschy and Turley, 2004). This rise is due to the post-natal myelinization process. Cholesterol is an important component of normal brain cholesterol development and is a critical element of Sonic Hedgehog (SHH) morphogenic factor activation. SHH enables neuronal expansion and its inhibition leads to abnormal brain development (Vaillant and Monard, 2009). During embryonic development, SHH acts as a factor among others which are recycled by embryo to axonal guidance across longitudinal axis of the spinal cord. SHH regulates nervous system connectivity. SHH signaling pathway is involved in cholesterol metabolism. Cholesterol allows the proteolytic cleavage of SHH which releases its N-terminal part. The latter is implicated in signaling and is covalently coupled to cholesterol (Porter *et al.*, 1996). Two SHH membrane receptors have been identified: Smoothed (Smo) and Patched (Ptc). Caveolin-1 (cav-1) from lipid rich-microdomains is associated to Ptc and regulated by SHH complex (Karpen *et al.*, 2001). Production and purification of Smo and Ptc in yeast systems leads to a better characterization of their interactions between plasma membrane and cholesterol (Joubert *et al.*, 2009, 2010). Smo belongs to G-protein receptors and is responsible for Hedgehog signal transduction to the intracellular effectors of the SHH signaling pathway. Smo is constitutively inhibited by Ptc in absence of SHH and the link of SHH ligand on its Ptc receptor leads to Smo activation which in turn activates zinc-fingers transcription factors also named Gli factors. Ptc is a twelve-times-crossing transmembrane protein which interacts with SHH at the two extracellular large loops floor. Increasing intracellular cholesterol concentration can activate Smo



receptor and SHH pathway. Ptc, also known as a SHH pathway repressor and as a SHH protein receptor, interacts with cholesterol and allows cholesterol efflux. Under Ptc influence, intracellular cholesterol is carried out of the cell. Hence, Ptc is a modulator of intracellular cholesterol concentration. Smo and Ptc can be at the root of SHH signaling pathway dysfunction. Indeed, interaction of the complex formed by SHH and cholesterol with Ptc inhibits the link of Ptc with cholesterol. Increasing intracellular cholesterol concentration is responsible for Smo enrichment in plasma membrane. But Smo sur-expression is an important step for SHH signaling activation (Bidet *et al.*, 2011). Smo dysregulation is implicated in a lot of cancers but also in neurodegenerative disorders such as Gorlin syndrom or holoprocencephaly (Nehme *et al.*, 2010). Interestingly, Smo might also be an oxysterol receptor (Nachtergaele *et al.*, 2012).

## 5 The pathways of cholesterol intake and excretion

Brain cholesterol level works independently from food intake and hepatic synthesis. Astrocytes are responsible for the larger part of *de novo* cholesterol synthesis required by neurons. In astrocyte cultures, these glial cells release two to three times more cholesterol than neurons (Saito *et al.*, 1987). Neuronal extrinsic cholesterol dependency varies depending on considered cerebral regions and on different neurons types. This cholesterol supply allows neurons growth (Hayashi *et al.*, 2004) and synaptic network development (Mauch *et al.*, 2001). Cerebral cholesterol is *in situ* produced, excluding cholesterol from the blood flow to cross the blood brain barrier (BBB) and enter the brain (Bjorkhem and Meaney, 2004). It was shown that the human brain synthesizes 6 mg of cholesterol per day. The BBB is formed by closely tightened endothelial cells and astrocytes. Less than 1 per cent of radiolabelled cholesterol enters the brain (Bjorkhem, 2006). Several studies show that brain cholesterol does not come from plasma HDL or LDL. Neither the modification of HDL receptor nor ABCA1 expression alters brain cholesterol concentration and synthesis in mice (Osono *et al.*, 1995; Quan *et al.*, 2003). These results show that lipoproteins are highly unlikely to cross the BBB and participate to brain cholesterol. On the contrary, LDL and VLDL are the preferred carriers of cholesterol to the retina (Fliesler and Bretillon, 2010). RPE express a variety of lipoprotein-specific receptors (LDLR, SR-BI, SR-BII) and scavenger receptors (CD36) (Tserentsoodol *et al.*, 2006) that enable the recognition of circulating lipoproteins by RPE and delivery to the retina. One must also point out that the neuroretina expresses numerous lipid-related proteins and receptors, such as ApoE, ApoA1, LRP1, LDLR, CD36, SR-BI, SR-BII, ApoB (Tserentsoodol *et al.*, 2006). LDL-R, SR-BI, ABCA1, ABCG1, ApoE and ApoAI are also expressed in the brain (Pitas *et al.*, 1987). Other molecules are specifically expressed in the brain such as ABCG4 or ABCA2 (Bjorkhem and Meaney, 2004). Until now their exact functions in the CNS remain uncertain. They may participate to the lipid homeostasis between retinal cells, including glia and neurons. But those findings promote the hypothesis for intracerebral and intraretinal lipid homeostasis.

Cerebral lipoproteins are distinct from those found in blood flow (Vance *et al.*, 2005). Similarly, it was demonstrated that the RPE has its own machinery for lipid processing. RPE is able to synthesize its own lipoprotein-like particles that are highly enriched in cholesterol and cholesteryl esters, and contain ApoA1, ApoB100, ApoE, ApoCI, and ApoC-II (Curcio *et al.*, 2010).

Half-life of cholesterol is long in the brain with approximately 6 months (Bjorkhem and Meaney, 2004), and much shorter in the retina with 6–7 days (Fliesler and Bretillon, 2010).

The rate of endogenous synthesis of cholesterol in the brain is balanced by the excretion of cholesterol *via* ApoE-rich lipoproteins (1 mg/day) and *via* its conversion by cholesterol-24S-hydroxylase (CYP46A1) into 24S-hydroxycholesterol (5 mg/day) (Bjorkhem *et al.*, 1998; Lutjohann *et al.*, 1996). 24S-hydroxycholesterol is released from neurons *via* ABCG1 and ABCG4 to ApoE molecules or to the CSF (Abildayeva *et al.*, 2006). This 24S-hydroxycholesterol flux can be recaptured by astrocytes where it regulates cholesterol and ApoE synthesis through LXR-dependent pathways (Abildayeva *et al.*, 2006; Pfrieger, 2003). ApoE is then released from astrocytes by way of ABCA1. 24S-hydroxycholesterol that is excluded from the recapture in astrocytes crosses the BBB and is eliminated in the blood flow (Shobab *et al.*, 2005).

Cholesterol-24S-hydroxylase (CYP46A1) is a cytochrome P450 enzyme that is prominently expressed in neurons (Liu *et al.*, 2010; Lund *et al.*, 1999), but also by retinal ganglion cells (RGC) (Bretillon *et al.*, 2007; Ramirez *et al.*, 2008). It was considered that the addition of one additional hydroxyl group to the molecule of cholesterol renders it more hydrophilic and facilitates its passage through the BBB (Bjorkhem and Meaney, 2004; Meaney *et al.*, 2002). Native cholesterol is oriented alongside to membrane phospholipids in such a way that the 3beta hydroxyl group interacts with the polar head of phospholipids. The 24S-hydroxyl group of 24S-hydroxycholesterol induces lateral chain reorganization of phospholipids which creates a channel whereby 24S-hydroxycholesterol can route through the opposite layer where it can be easily extracted by a lipoproteic acceptor (Bjorkhem and Meaney, 2004). In addition, the organic anion transporting polypeptide 2 (OATP2) was suggested in the rat to be a cargo molecule enabling 24S-hydroxycholesterol to pass through plasma membrane (Ohtsuki *et al.*, 2007).

## 6 Cholesterol-24S-hydroxylase, 24S-hydroxycholesterol and neurodegenerative diseases

Since 1995, isotope dilution gas-chromatography mass-spectrometry using deuterium-labelled authentic internal standards was considered as the gold standard method for the measurement of oxysterols including 24S-hydroxycholesterol (Dzeletovic *et al.*, 1995). In humans, plasma 24S-hydroxycholesterol levels reflect the balance between its own cerebral production and hepatic metabolism. Hence, the ratio between brain weight and liver volume was shown to be the best marker for plasma 24S-hydroxycholesterol fluctuations

from birth to adulthood in humans (Bretillon *et al.*, 2000a). Pathological conditions of BBB breakdown or multiple sclerosis have been associated with higher plasma levels of 24S-hydroxycholesterol (Bretillon *et al.*, 2000b; Leoni *et al.*, 2002). One should hypothesize plasma 24S-hydroxycholesterol to be modified in Alzheimer's disease. It was shown that the early stages of neuronal loss in Alzheimer's disease were characterized by elevated plasma 24S-hydroxycholesterol levels (Lutjohann *et al.*, 2000). On the contrary, at late stages while the capacity of the brain to synthesize 24S-hydroxycholesterol was reduced due to the loss of neurons (Heverin *et al.*, 2004), reduced plasma levels of 24S-hydroxycholesterol were observed (Bretillon *et al.*, 2000b).

Accounting that glaucoma is characterized by the loss of RGC, we questioned whether plasma 24S-hydroxycholesterol would be modified in glaucoma patients. Our analysis failed to find any difference compared to controls (Fourgeux *et al.*, 2009). This finding would likely be due to the low capacity of the retina to participate to the plasma levels of 24S-hydroxycholesterol, compared to the brain. In addition, the time-course of RGC loss in glaucoma would likely be too slow to be seen although it was estimated to represent 4% of the RGC population per year in glaucoma compared to 0.4% per year during normal aging (Harwerth *et al.*, 2008; Leung *et al.*, 2011). We published data on gene polymorphism where we reported that one single nucleotide polymorphism in *cyp46a1* gene, called rs754203, was a risk factor for glaucoma (Fourgeux *et al.*, 2009). This finding suggested the association between CYP46A1 expression and glaucoma risk. In parallel, we tested the negative hypothesis that this polymorphism would not be found in AMD, considering that AMD is not characterized by the loss of CYP46A1-expressing cells. Indeed, we did not find higher frequency of the at-risk allele in AMD patients (Fourgeux *et al.*, 2012). In order to add more data on the role of CYP46A1 in glaucoma, we recapitulated a model of glaucoma in the rat (Schnebelen *et al.*, 2009), and tested whether glaucomatous stress induced changes in cholesterol, 24S-hydroxycholesterol and CYP46A1 levels in the retina. We found that elevated intraocular pressure – one of the most prominent risk factor for glaucoma – induced transient changes overexpression of CYP46A1 in the retina and subsequent increase in 24S-hydroxycholesterol levels in plasma and retina (Fourgeux *et al.*, 2012). One possible explanation for this increase might be the induction of neuroinflammation in the retina but also in the brain *via* the secretion of chemokines such as MCP-1. We suggested that circulating factors were produced by laser-injured ocular structures, activated glial cells in the brain which secondarily overexpressed CYP46A1 and produced 24S-hydroxycholesterol (Fourgeux *et al.*, 2012). This finding highlights the cross-talk between neurons and glia in the maintenance of cholesterol homeostasis. One other work confirmed that changes in the level of 24S-hydroxycholesterol are detected by glial cells. Indeed, pharmacological inhibition of CYP46A1 triggered CYP46A1 overexpression in the retina suggesting that retinal glia would be able to sense the ratio between 24S-hydroxycholesterol and cholesterol (Fourgeux *et al.*, 2014). CYP46A1 expression would be an adaptive response of the retina to changes in cholesterol levels. Similar activation of

CYP46A1 in microglia was already reported in the brain in response to injury (Smiljanic *et al.*, 2010).

Within this context, CYP46A1 would be considered as neuroprotective. Spatial, associative and motor learning defects were reported in CYP46A1 knock out mice part (Kotti *et al.*, 2006). Gene therapy experiments with intracerebral injections of adenovirus-associated vector containing CYP46A1 showed that CYP46A1 overexpression reduced by half the accumulation of amyloid-beta peptide in a model of Alzheimer's disease (Hudry *et al.*, 2010). Induction of CYP46A1 expression by astroglial cells has already been demonstrated in Alzheimer's patients, (Bogdanovic *et al.*, 2001; Brown *et al.*, 2004).

24S-hydroxycholesterol formation is not the only mechanism of sterol removal from neurons. Others oxysterols have been identified. Ingoing flux of 27-hydroxycholesterol from the circulation to the brain and outgoing flow of 7-hydroxy-3oxo-4-cholestenoic acid from the brain to the circulation have been measured (Bjorkhem, 2006; Heverin *et al.*, 2005). Cholesterol-27-hydroxylase (CYP27A1) forms primarily 27-hydroxycholesterol and secondarily 7-hydroxy-3oxo-4-cholestenoic acid. The exact roles of 27-hydroxycholesterol in the brain are not well understood yet. 27-hydroxycholesterol might influence cholesterol synthesis by activation of LXR-beta (Gilardi *et al.*, 2009). CYP27A1 is expressed in all cellular types of the brain, especially in microglia (Gilardi *et al.*, 2009). In addition, it was reported that neuronal stem cells eliminate cholesterol by CYP27A1 pathway (Milagre *et al.*, 2012) whereas CYP46A1 pathway is regulated by Sp transcription factors during neuronal differentiation (Milagre *et al.*, 2012). These transcription factors are recruited at the *cyp46a1* promoter gene level. Few data on cholesterol elimination by the inner retina and the RPE are available. No 27-hydroxycholesterol is found in the retina, but its oxidation product, 5-cholestenoic acid, is detected at high level, with major inter-individuals variability (by a factor three). Pregnenolone is a metabolite of CYP11A1 and was detected in the retina but at much lower levels than cholestenoic acid (Mast *et al.*, 2011). Although CYP27A1 and CYP11A1 are expressed in various retinal cellular types, CYP46A1 is specific to RGC (Bretillon *et al.*, 2007).

## 7 Conclusion

Retinal pathologies are one of the most prevalent pathologies in the Elderly. Cholesterol is the prominent sterol in the retina. Dysregulation of cholesterol in the retina was associated with retinal impairments, surmising its key role in neurons. Glia is an active partner of neurons by maintaining cholesterol synthesis and removal. Cholesterol-24S-hydroxylase (CYP46A1) is central for the efflux of cholesterol from neurons. Several lines of evidence suggest that under pathophysiological conditions and reactive gliosis, CYP46A1 may be induced and may be considered as a neuroprotective mechanism.

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## References

- Abildayeva K, Jansen PJ, Hirsch-Reinshagen V, *et al.* 2006. 24(S)-hydroxycholesterol participates in a liver X receptor-controlled pathway in astrocytes that regulates apolipoprotein E-mediated cholesterol efflux. *J. Biol. Chem.* 281: 12799–808.
- Anderson DH, Radeke MJ, Gallo NB, *et al.* 2010. The pivotal role of the complement system in aging and age-related macular degeneration: Hypothesis re-visited. *Prog. Retin. Eye Res.* 29: 95–112.
- Bidet M, Joubert O, Lacombe B, *et al.* 2011. The hedgehog receptor patched is involved in cholesterol transport. *PLoS One* 6: e23834.
- Björkhem I. 2006. Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. *J. Int. Med.* 260: 493–508.
- Björkhem I, Meaney S. 2004. Brain cholesterol: long secret life behind a barrier. *Arterioscler Thromb Vasc. Biol.* 24: 806–15.
- Björkhem I, Lütjohann D, Diczfalussy U, Stahle L, Ahlborg G, Wahren J. 1998. Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation. *J. Lipid. Res.* 39: 1594–600.
- Bogdanovic N, Bretillon L, Lund EG, *et al.* 2001. On the turnover of brain cholesterol in patients with Alzheimer’s disease. Abnormal induction of the cholesterol-catabolic enzyme CYP46 in glial cells. *Neurosci. Lett.* 314: 45–8.
- Booij JC, Baas DC, Beisekeeva J, Gorgels TGMF, Bergen AAB. 2010. The dynamic nature of Bruch’s membrane. *Progr. Retin. Eye Res.* 29: 1–18.
- Bretillon L, Diczfalussy U, Björkhem I, *et al.* 2007. Cholesterol-24S-hydroxylase (CYP46A1) is specifically expressed in neurons of the neural retina. *Curr. Eye Res.* 32: 361–6.
- Bretillon L, Lutjohann D, Stahle L, *et al.* 2000a. Plasma levels of 24S-hydroxycholesterol reflect the balance between cerebral production and hepatic metabolism and are inversely related to body surface. *J. Lipid. Res.* 41: 840–5.
- Bretillon L, Sidén A, Wahlund LO, *et al.* 2000b. Plasma levels of 24S-hydroxycholesterol in patients with neurological diseases. *Neurosci. Lett.* 293: 87–90.
- Bretillon L, Thuret G, Grégoire S, *et al.* 2008. Lipid and fatty acid profile of the retina, retinal pigment epithelium/choroid, and lacrimal gland, and associations with adipose tissue fatty acids in human subjects. *Exp. Eye Res.* 87: 521–8.
- Brown J, 3rd, Theisler C, Silberman S, *et al.* 2004. Differential expression of cholesterol hydroxylases in Alzheimer’s disease. *J. Biol. Chem.* 279: 34674–81.
- Centanin L, Wittbrodt J. 2014. Retinal neurogenesis. *Development* 141: 241–4.
- Claudepierre T, Paques M, Simonutti M, *et al.* 2010. Lack of Niemann-Pick type C1 induces age-related degeneration in the mouse retina. *Mol. Cell Neurosci.* 43: 164–76.
- Curcio CA, Johnson M, Huang JD, Rudolf M. 2009. Aging, Age-related Macular Degeneration, and the Response-to-Retention of Apolipoprotein B-Containing Lipoproteins. *Prog. Retin. Eye Res.* 28: 393–422.
- Curcio CA, Johnson M, Huang JD, Rudolf M. 2010. Apolipoprotein B-containing lipoproteins in retinal aging and age-related macular degeneration. *J. Lipid. Res.* 51: 451–67.
- Curcio C, Johnson M, Rudolf M, Huang J. 2011. The oil spill in ageing Bruch membrane. *Br. J. Ophthalmol.* 95: 1638–45.
- Dietschy JM, Turley SD. 2004. Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J. Lipid. Res.* 45: 1375–97.
- Dzeletovic S, Breuer O, Lund E, Diczfalussy U. 1995. Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. *Anal. Biochem.* 225: 73–80.
- Ferris FL, Davis MD, Clemons TE, *et al.* 2005. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. *Arch. Ophthalmol.* 123: 1570–4.
- Fliesler SJ, Bretillon L. 2010. The ins and outs of cholesterol in the vertebrate retina. *J. Lipid. Res.* 51: 3399–413.
- Fliesler SJ, Peachey NS, Richards MJ, Nagel BA, Vaughan DK. 2004. Retinal degeneration in a rodent model of Smith-Lemli-Opitz syndrome: electrophysiologic, biochemical, and morphologic features. *Arch. Ophthalmol.* 122: 1190–200.
- Fourgeux C, Martine L, Björkhem I, *et al.* 2009. Primary open-angle glaucoma: association with cholesterol 24S-hydroxylase (CYP46A1) gene polymorphism and plasma 24-hydroxycholesterol levels. *Invest. Ophthalmol. Vis. Sci.* 50: 5712–7.
- Fourgeux C, Dugas B, Richard F, *et al.* 2012. Single nucleotide polymorphism in the cholesterol-24S-hydroxylase (CYP46A1) gene and its association with CFH and LOC387715 gene polymorphisms in age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* 53: 7026–33.
- Fourgeux C, Martine L, Pasquis B, *et al.* 2012. Steady-state levels of 24S-hydroxycholesterol are maintained by glial cells intervention after elevation of intraocular pressure in the rat. *Acta Ophthalmol.* 90: e560–e4.
- Fourgeux C, Martine L, Acar N, Bron AM, Creuzot-Garcher CP, Bretillon L. 2014. *In vivo* consequences of cholesterol-24S-hydroxylase (CYP46A1) inhibition by voriconazole on cholesterol homeostasis and function in the rat retina. *Biochem. Biophys. Res. Commun.* 446: 775–81.
- Gilardi F, Viviani B, Galmozzi A, *et al.* 2009. Expression of sterol 27-hydroxylase in glial cells and its regulation by liver X receptor signaling. *Neuroscience* 164: 530–40.
- Harwerth RS, Wheat JL, Rangaswamy NV. 2008. Age-Related Losses of Retinal Ganglion Cells and Axons. *Invest. Ophthalmol. Vis. Sci.* 49: 4437–43.
- Hayashi H, Campenot RB, Vance DE, Vance JE. 2004. Glial lipoproteins stimulate axon growth of central nervous system neurons in compartmented cultures. *J. Biol. Chem.* 279: 14009–15.
- Heverin M, Bogdanovic N, Lutjohann D, *et al.* 2004. Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer’s disease. *J. Lipid. Res.* 45: 186–93.
- Heverin M, Meaney S, Lutjohann D, *et al.* 2005. Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain. *J. Lipid. Res.* 46: 1047–52.



- Hudry E, Van Dam D, Kulik W, *et al.* 2010. Adeno-associated virus gene therapy with cholesterol 24-hydroxylase reduces the amyloid pathology before or after the onset of amyloid plaques in mouse models of Alzheimer's disease. *Mol. Ther.* 18: 44–53.
- Irons M, Elias ER, Salen G, Tint GS, Batta AK. 1993. Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome. *Lancet.* 341: 1414.
- Joubert O, Nehme R, Fleury D, *et al.* 2009. Functional studies of membrane-bound and purified human Hedgehog receptor Patched expressed in yeast. *Biochim. Biophys. Acta* 1788: 1813–21.
- Joubert O, Nehme R, Bidet M, Mus-Veteau I. 2010. Heterologous expression of human membrane receptors in the yeast *Saccharomyces cerevisiae*. *Methods Mol. Biol.* 601: 87–103.
- Karpen HE, Bukowski JT, Hughes T, Gratton JP, Sessa WC, Gailani MR. 2001. The sonic hedgehog receptor patched associates with caveolin-1 in cholesterol-rich microdomains of the plasma membrane. *J. Biol. Chem.* 276: 19503–11.
- Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. 1991. The Wisconsin age-related maculopathy grading system. *Ophthalmology* 98: 1128–34.
- Klein R, Klein BEK. 2013. The Prevalence of Age-Related Eye Diseases and Visual Impairment in Aging: Current Estimates. *Invest. Ophthalmol. Vis. Sci.* 54: ORSF5–ORSF13.
- Kotti TJ, Ramirez DM, Pfeiffer BE, Huber KM, Russell DW. 2006. Brain cholesterol turnover required for geranylgeraniol production and learning in mice. *Proc. Natl. Acad. Sci. USA* 103: 3869–74.
- Leoni V, Masterman T, Diczfalusy U, De Luca G, Hillert J, Bjorkhem I. 2002. Changes in human plasma levels of the brain specific oxysterol 24S-hydroxycholesterol during progression of multiple sclerosis. *Neurosci. Lett.* 331: 163–6.
- Leung CK-S, Cheung CY-L, Weinreb RN, *et al.* 2011. Evaluation of retinal nerve fiber layer progression in glaucoma: A comparison between the fast and the regular retinal nerve fiber layer scans. *Ophthalmology* 118: 763–7.
- Liu JP, Tang Y, Zhou S, Toh BH, McLean C, Li H. 2010. Cholesterol involvement in the pathogenesis of neurodegenerative diseases. *Mol. Cell. Neurosci.* 43: 33–42.
- Lund EG, Guileyardo JM, Russell DW. 1999. cDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. *Proc. Natl. Acad. Sci. USA* 96: 7238–43.
- Lutjohann D, Breuer O, Ahlborg G, *et al.* 1996. Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation. *Proc. Natl. Acad. Sci. USA* 93: 9799–804.
- Lutjohann D, Papassotiropoulos A, Bjorkhem I, *et al.* 2000. Plasma 24S-hydroxycholesterol (cerebrosterol) is increased in Alzheimer and vascular demented patients. *J. Lipid. Res.* 41: 195–8.
- Masland RH. 2001. The fundamental plan of the retina. *Nat. Neurosci.* 4: 877–86.
- Mast N, Reem R, Bederman I, *et al.* 2011. Cholestenic Acid is an important elimination product of cholesterol in the retina: comparison of retinal cholesterol metabolism with that in the brain. *Invest. Ophthalmol. Vis. Sci.* 52: 594–603.
- Mauch DH, Nagler K, Schumacher S, *et al.* 2001. CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 294: 1354–7.
- Meaney S, Bodin K, Diczfalusy U, Bjorkhem I. 2002. On the rate of translocation in vitro and kinetics in vivo of the major oxysterols in human circulation: critical importance of the position of the oxygen function. *J. Lipid Res.* 43: 2130–5.
- Milagre I, Nunes MJ, Castro-Caldas M, Moutinho M, Gama MJ, Rodrigues E. 2012. Neuronal differentiation alters the ratio of Sp transcription factors recruited to the CYP46A1 promoter. *J. Neurochem.* 120: 220–9.
- Milagre I, Olin M, Nunes MJ, *et al.* 2012. Marked change in the balance between CYP27A1 and CYP46A1 mediated elimination of cholesterol during differentiation of human neuronal cells. *Neurochem. Int.* 60: 192–8.
- Nachtergaele S, Mydock LK, Krishnan K, *et al.* 2012. Oxysterols are allosteric activators of the oncoprotein Smoothed. *Nat. Chem. Biol.* 8: 211–20.
- Nehme R, Joubert O, Bidet M, Lacombe B, Polidori A, Pucci B, Mus-Veteau I. 2010. Stability study of the human G-protein coupled receptor, Smoothed. *Biochim. Biophys. Acta* 1798: 1100–10.
- Ohtsuki S, Ito S, Matsuda A, Hori S, Abe T, Terasaki T. 2007. Brain-to-blood elimination of 24S-hydroxycholesterol from rat brain is mediated by organic anion transporting polypeptide 2 (oatp2) at the blood-brain barrier. *J. Neurochem.* 103: 1430–8.
- Osono Y, Woollett LA, Herz J, Dietschy JM. 1995. Role of the low density lipoprotein receptor in the flux of cholesterol through the plasma and across the tissues of the mouse. *J. Clin. Invest.* 95: 1124–32.
- Pfrieger FW. 2003. Cholesterol homeostasis and function in neurons of the central nervous system. *Cell Mol. Life Sci.* 60: 1158–71.
- Pitas RE, Boyles JK, Lee SH, Hui D, Weisgraber KH. 1987. Lipoproteins and their receptors in the central nervous system. Characterization of the lipoproteins in cerebrospinal fluid and identification of apolipoprotein B,E(LDL) receptors in the brain. *J. Biol. Chem.* 262: 14352–60.
- Porter JA, Young KE, Beachy PA. 1996. Cholesterol modification of hedgehog signaling proteins in animal development. *Science* 274: 255–9.
- Quan G, Xie C, Dietschy JM, Turley SD. 2003. Ontogenesis and regulation of cholesterol metabolism in the central nervous system of the mouse. *Brain. Res. Dev. Brain. Res.* 146: 87–98.
- Quigley HA, Broman AT. 2006. The number of people with glaucoma worldwide in 2010 and 2020. *Br. J. Ophthalmol.* 90: 262–7.
- Ramirez DM, Andersson S, Russell DW. 2008. Neuronal expression and subcellular localization of cholesterol 24-hydroxylase in the mouse brain. *J. Comp. Neurol.* 507: 1676–93.
- Saher G, Quintes S, Nave KA. 2011. Cholesterol: a novel regulatory role in myelin formation. *Neuroscientist.* 17: 79–93.
- Saito M, Benson EP, Rosenberg A. 1987. Metabolism of cholesterol and triacylglycerol in cultured chick neuronal cells, glial cells, and fibroblasts: accumulation of esterified cholesterol in serum-free culture. *J. Neurosci. Res.* 18: 319–25.
- Schnebelen C, Pasquis B, Salinas-Navarro M, *et al.* 2009. A dietary combination of omega-3 and omega-6 polyunsaturated fatty acids is more efficient than single supplementations in the prevention of retinal damage induced by elevation of intraocular pressure in rats. *Graefes. Arch. Clin. Exp. Ophthalmol.* 247: 1191–203.
- Shobab LA, Hsiung GY, Feldman HH. 2005. Cholesterol in Alzheimer's disease. *Lancet Neurol.* 4: 841–52.
- Smiljanic K, Lavnja I, Mladenovic Djordjevic A, *et al.* 2010. Brain injury induces cholesterol 24-hydroxylase (Cyp46) expression in glial cells in a time-dependent manner. *Histochem. Cell. Biol.* 134: 159–69.



- Tserentsoodol N, Gordiyenko NV, Pascual I, Lee JW, Fliesler SJ, Rodriguez IR. 2006. Intraretinal lipid transport is dependent on high density lipoprotein-like particles and class B scavenger receptors. *Mol. Vis.* 12: 1319–33.
- Vaillant C, Monard D. 2009. SHH pathway and cerebellar development. *Cerebellum* 8: 291–301.
- Vance JE. 2012. Dysregulation of cholesterol balance in the brain: contribution to neurodegenerative diseases. *Dis. Model Mech.* 5: 746–55.
- Vance JE, Hayashi H, Karten B. 2005. Cholesterol homeostasis in neurons and glial cells. *Semin. Cell Dev. Biol.* 16: 193–212.
- Vanier MT. 2014. Complex lipid trafficking in Niemann-Pick disease type C. *J. Inherit. Metab. Dis.*
- Varma R, Foong AWP, Lai M-Y, Choudhury F, Klein R, Azen SP. 2010. Four-Year Incidence and Progression of Age-Related Macular Degeneration: The Los Angeles Latino Eye Study. *Am. J. Ophthalmol.* 149: 741–51.

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