UNIVERSITÀ DEGLI STUDI DI TORINO

Gamba P, Testa G, Sottero B, Gargiulo S, Poli G, Leonarduzzi G. The link between altered cholesterol metabolism and Alzheimer's disease. Ann N Y Acad Sci. 2012;1259:54-64. DOI: 10.1111/j.1749-6632.2012.06513.x.

Short title: Oxysterols and Alzheimer's disease

The link between altered cholesterol metabolism and Alzheimer's disease

Paola Gamba, Gabriella Testa, Barbara Sottero, Simona Gargiulo, Giuseppe Poli and Gabriella Leonarduzzi

Department of Clinical and Biological Sciences, Faculty of Medicine San Luigi Gonzaga, University of Turin, Italy

Address for correspondence: Giuseppe Poli, MD, Department of Clinical and Biological Sciences, University of Torino, San Luigi Gonzaga Hospital, Regione Gonzole 10, 10043 Orbassano, Torino, Italy. giuseppe.poli@unito.it

Keywords: Alzheimer's disease; cholesterol; oxysterols; amyloid-β; oxidative stress; neurotoxicity

Alzheimer's disease (AD), the most common form of dementia, is characterized by the progressive loss of neurons and synapses, and by extracellular deposits of amyloid-β (Aβ) as senile plaques, Aβ deposits in the cerebral blood vessels, and intracellular inclusions of hyperphosphorylated tau in the form of neurofibrillary tangles. Several mechanisms contribute to AD development and progression, and increasing epidemiological and molecular evidence suggests a key role of cholesterol in its initiation and progression. Altered cholesterol metabolism and hypercholesterolemia appear to play fundamental roles in amyloid plaque formation and tau hyperphosphorylation. Over the last decade, growing evidence supports the idea that cholesterol oxidation products, known as oxysterols, may be the missing link

between altered brain cholesterol metabolism and AD pathogenesis, as their involvement in neurotoxicity, mainly by interacting with Aβ peptides, is reported.

Alzheimer's disease (AD), a neurodegenerative disorder, is the most common form of dementia in developed countries. It is a complex and genetically heterogeneous disease, characterized by progressive memory deficit, cognitive impairment and personality changes, accompanied by specific structural abnormalities in the brain. The main histological features of AD are extracellular deposits of amyloid-β (Aβ) in the form of senile plaques, Aβ deposits in the cerebral blood vessels, and intracellular inclusions of hyperphosphorylated tau in the form of neurofibrillary tangles (NFT). The loss of neurons and synapses in the neocortex, hippocampus and other subcortical regions of the brain is also a common feature of AD.[1,2]

AD begins with the abnormal processing of amyloid precursor protein (APP) by the sequential enzymatic actions of two enzymes of the amyloidogenic pathway: beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1), a β-secretase, and γ-secretase; these actions lead to excess of production and/or reduced clearance of Aβ peptides, which comprise 39-43 amino acids. An imbalance between production and clearance of Aβ in the brain, and their aggregation, causes Aβ to accumulate, and this excess may be the initiating factor in AD. Monomers of $A\beta_{40}$ are usually much more prevalent than the aggregation-prone and damaging $A\beta_{42}$ species, but an increased proportion of $A\beta_{42}$ appears sufficient to cause early onset of AD. Additionally, insoluble oligomers and intermediate amyloids are the most neurotoxic forms of $A\beta_{42}$.[3]

Several mechanisms (e.g. perturbation of brain metabolism, oxidative stress, inflammation, presence of the apolipoprotein E (ApoE) ε4 allele, impaired cholesterol metabolism) contribute to the development and progression of AD. Among these, a growing body of epidemiological and molecular evidence suggests a mechanistic link between cholesterol and AD progression. A number of genes involved in cholesterol homeostasis have been identified as susceptibility loci for sporadic or late-onset AD,[4-6] and altered cholesterol metabolism seems to play a fundamental role in the formation of amyloid plaques and in tau hyperphosphorylation.[7,8] In addition, hypercholesterolemia

is unanimously recognized to be a risk factor for sporadic AD, a form that accounts for the great majority of cases.[4,9-11] Finally, this evidence is supported by epidemiological studies indicating that cholesterol-lowering agents belonging to the family of statins reduce the prevalence of AD,[12-14] a conclusion not yet fully accepted because of the contradictory results reported by prospective clinical studies.[15-17]

Apolipoprotein E and its receptors in AD

ApoE is the brain's principal cholesterol-carrier protein, mainly transporting it from astrocytes to neurons. The association between ApoE polymorphism and AD is presumably related to the disturbance of cholesterol transport. Of note, subjects who are homozygous for the ApoE ε4 genotype express an increased AD risk, versus those carrying ε2 or ε3, evidence that is consistently confirmed by numerous independent studies.[18-21] In addition, receptors recognizing ApoE are also widely expressed in the AD brain.[4,20,22]

Although ApoE mediates Aβ clearance by binding Aβ and forming a stable complex, ApoE may also stimulate Aβ aggregation and amyloid deposition, as well as tau hypersphorylation.[20,22-25] Moreover, among the mechanisms that might explain the effects of ApoE on the brain of AD subjects, ApoE ε4 and its receptors are also reported to be involved in APP trafficking and its processing to Aβ. Additionally, ApoE may mediate Aβ cell internalization, by binding to the LDL receptor-related protein (LRP).[26] ApoE might also modulate the distribution and metabolism of cholesterol in neuronal membranes, and regulate the role of cholesterol in synapse formation and function, through ApoE receptors.[20,25,27] Moreover, γ-secretase cleavage of APP could thus regulate ApoE metabolism through the LRP1 receptor.[27]

A number of epidemiological studies also report that individuals with high levels of blood cholesterol have an increased susceptibility to AD, apparently influenced by the ApoE ε4 genotype,

which may influence cholesterol metabolism and the formation of cholesterol oxidation products, known as oxysterols.[28]

Role of cholesterol in AD

The brain is the organ with the highest concentration of cholesterol, which is essential for its normal function, being a major component of neuronal cell membranes and a determinant of membrane fluidity.[29] In the brain, cholesterol is mostly present in the free form and is derived from *de novo* biosynthesis from acetyl-coenzyme A mediated by 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, rather than from plasma lipoproteins, which are prevented by the blood-brain barrier (BBB) from crossing from the peripheral circulation into the brain. Further, the astrocytic compartment meets neuronal cholesterol demands by secreting ApoE-cholesterol complexes, which are transported to the neurons.[30,31]

The mechanism by which cholesterol affects Aβ production and metabolism is not fully understood; however, a change in membrane properties has been suggested.[9] Cholesterol is mainly concentrated in membrane microdomains termed "lipid rafts", where considerable evidence indicates that the amyloidogenic pathway takes place.[32,33] In this connection, it has been reported that cellular cholesterol, especially when it is elevated in the membrane, binds directly to APP and thus promotes APP's insertion into the phospholipid monolayers of the "lipid rafts" and other organelles where β- and γ-secretases reside, and favors the amyloidogenic pathway.[34-36] Amyloidogenic activity is thus linked to cholesterol levels: β- and γ-secretase activities are positively regulated by high and inhibited by low levels of cholesterol.[37,38] Since APP processing and Aβ generation are associated with cholesterol-rich microdomains, and both are present in "lipid rafts", Aβ production may require raft integrity and a lipid component as optimal conditions. An alteration in raft components could thus change the configuration of either the enzymes or the substrate associated with the rafts, leading to an alteration in Aβ generation. Conversely, in the non-

amyloidogenic pathway, APP is processed by α-secretase in non-raft domains, and this event is promoted by a decreased cellular cholesterol level.[39] Moreover, the amyloidogenic pathway is inactivated when α-secretase is forced to associate with "lipid rafts".[40]

Cholesterol also enhances Aβ to form neurotoxic aggregates.[41] In addition, fibrillogenesis of Aβ has been proposed to take place in "lipid rafts" at ganglioside clusters, where Aβ displays a specific affinity to cholesterol which binds avidly to Aβ protofibrils. [42,43] Moreover, it has been shown that increased cholesterol levels in the lipid bilayers facilitate binding of Aβ to the membranes, promoting Aβ conformational change from a helix-rich to a β-sheet-rich structure, and thus becoming an endogenous seed for amyloid formation.[44] The conversion of soluble and nontoxic monomeric Aβ to insoluble and toxic oligomeric and aggregate Aβ is thus the critical step in AD development.

Given the above considerations, it seems that cholesterol distribution and trafficking within brain cells, rather than total cholesterol levels in the neurons, are the relevant factors in the APP processing and Aβ accumulation in the AD progression.[45]

It has also been observed that high concentrations of free cholesterol alone do not affect APP processing or Aβ production; rather, the conversion of excess free cholesterol into cholesterol ester has a profound effect on APP and Aβ enhancing their upregulation.[46] Consequently, clearance of Aβ from the brain is reduced when an overabundance of esterified cholesterol decreases membrane lipid turnover. Conversely, inhibition of the enzyme acyl-coenzyme A:cholesterol acyl-transferase 1 (ACAT1), which esterifies cholesterol, leads to the reduction of both cholesteryl esters and Aβ.[47] These data suggest that the balance between free cholesterol and cholesterol esters is a key parameter controlling amyloidogenesis, although the molecular mechanisms underlying this relationship are still unclear.

A regulatory role for APP and γ-secretase in cholesterol metabolism, jointly acting to lower cellular cholesterol levels, has also been reported.[27] The effect of Aβ and of the intracellular domain

of APP (AICD) on cellular cholesterol metabolism have also been investigated. Aβ, in particular the oligomeric rather than the monomeric form, alters intracellular trafficking and cholesterol homeostasis, by promoting the release of cholesterol and some other lipids from cells, in the form of Aβ-lipid particles.[48] The fibrillar Aβ then down-regulates cholesterol biosynthesis.[49] The AICD, which is released upon γ-secretase cleavage of APP, down-regulates cellular cholesterol uptake by acting as a transcriptional suppressor of LRP1 gene, a major ApoE receptor in the brain.[27] Furthermore, the peptide products of the amyloidogenic pathway ultimately reduce both cholesterol uptake and its biosynthesis, completing a negative feedback loop. A decrease in cellular cholesterol levels then results in an enhancement of tau phosphorylation[50] and synaptic failure.[51] It has also been demonstrated that extracellular cholesterol accumulates in the senile plaques and neurofibrillary tangles of AD patients, and in transgenic mice expressing the Swedish Alzheimer mutation APP751, as well as ApoE, and that cholesterol, ApoE and Aβ all colocalize in the core of fibrillar plaques.[52,53] This shows that both ApoE and cholesterol may be essential for plaque formation, and that extracellular cholesterol, by binding to aggregated Aβ, may be the seed for its deposition.

Role of oxysterols in AD

Because there is little synthesis of cholesterol in the adult brain, and because the brain cannot degrade cholesterol, it must be excreted from the brain in order to prevent its accumulation. The most important mechanism whereby the brain eliminates excess cholesterol is through the formation and excretion into the circulation of oxysterols, a class of cholesterol oxidation products, which are thus important to balance the local synthesis of sterols.[54]

Cholesterol is primarily converted into the oxysterol 24-hydroxycholesterol (24-OH), also known as cerebrosterol, which is produced almost exclusively in the brain by CYP46A1 (cholesterol 24-hydroxylase) and which, unlike cholesterol itself, can easily cross the BBB.[9,30,54,55]

Following its secretion from the brain, 24-OH enters the circulation and reaches the liver, where it is taken up and metabolized. The oxysterol 24-OH also plays an important role in the regulation of cholesterol homeostasis in the brain. Neuronal cells have a lower rate of cholesterol synthesis than glial cells; for this reason, an increased flux of 24-OH from neurons to glial cells causes an increased flux of cholesterol to the neuronal cells, by means of activation of the nuclear liver X receptor (LXR) and upregulation of ApoE in the glial cells (Fig. 1).[56] Another oxysterol, 27-hydroxycholesterol (27-OH), has been found to be produced *in situ* in the brain by CYP27A1, although in small amounts, and then metabolized by the enzyme CYP7B to 7α-hydroxy-3-oxo-4-cholestenoic acid (7-OH-4-C), which, crossing the BBB, reaches the liver where it is eliminated.[54,55,57] However, it has been observed that most 27-OH flows from the circulation into the brain, since, unlike cholesterol, it can cross the BBB.[58] Summarizing, these considerations indicate that there are fluxes of oxysterols in opposite directions across the BBB: two fluxes out of the brain (24-OH and 7-OH-4-C) and one flux into the brain (27-OH). A further compound, 7β-hydroxycholesterol (7β-OH), may also derive in the brain from oxidation of cholesterol following cholesterol interaction with Aβ and APP (Fig. 2).[59]

During the last decade, the idea that oxysterols might be the missing link between altered brain cholesterol metabolism and AD pathogenesis has been increasingly supported by research pointing to the involvement of 24-OH and 27-OH in neurotoxicity, mainly by interacting with Aβ peptides.

Several studies have found higher levels of 24-OH in the peripheral circulation and cerebrospinal fluid (CSF) of AD patients during the early stages than in unaffected individuals, suggesting that cholesterol turnover in the brain increases during the neurodegenerative changes of AD.[60-62] Conversely, plasma levels of 24-OH were decreased in patients with later stages of AD than in the respective controls, suggesting that the rate of cholesterol transport lowers as the disease progresses.[62,63] These contradictory results might be rationalized by considering that increased plasma levels of 24-OH reflect ongoing neurodegeneration and/or demyelinization, whereas

decreased plasma levels reflect a selective loss of neurons expressing CYP46A1.[30] However, in glial cells of AD brains there is some ectopic induction of CYP46A1, and consequently some 24-OH production, which may overlap with decreased neuronal expression in the presence of increased glial expression,[64,65] although this induction of CYP46A1 cannot compensate for the loss of 24-hydroxylase activity due to the neuronal degeneration. Another study, however, has found that plasma levels of 24-OH in AD patients are not significantly different than in control subjects.[66]

It has also been reported that, in all brain areas of deceased AD patients, as well as in aged mice expressing the Swedish Alzheimer mutation APP751, the amount of 24-OH decreases and 27-OH increases.[67] A marked accumulation of 27-OH was also found in the brain of patients carrying the Swedish APP 670/671 mutation.[68] Whereas the decreased levels of 24-OH in the AD brain are presumably due to the loss of neuronal cells and consequent loss of the enzyme CYP46A1, the increased levels of 27-OH may be due to increased flux of this oxysterol across the BBB, because of hypercholesterolemia[69] or a damage of BBB integrity.[70] An alternative explanation for the high levels of 27-OH is a reduced metabolism of the oxysterol into 7-OH-4-C, by the enzyme CYP7B, which is reduced in the brain of AD patients.[71] However, increased levels of both 24-OH and 27-OH have been observed in the CSF in patients with advanced AD.[72]

From these considerations, the hypothesis has been formulated that the balance between 24-OH and 27-OH is important for amyloidogenesis,[54,69] and the increased ratio of 27-OH to 24-OH in AD brains is consistent with this hypothesis.[67] Thus, the shift in balance between the two oxysterols might lead to increased generation and accumulation of Aβ and regulation of the 24-OH/27-OH ratio could be an important strategy in controlling Aβ levels in AD. However, opinions still differ about the involvement of these two oxysterols in the APP processing and Aβ generation.

According to several studies, induction of CYP46A1 activity has beneficial effects, directly preventing Aβ generation by modulating cholesterol homeostasis and reducing cellular cholesterol. Indeed, astrocytes are sensitive to 24-OH-mediated upregulation of the LXR-responsive genes involved in cholesterol efflux, i.e. ATP-binding cassette transporter A1 and G1 (ABCA1 and

ABCG1) and ApoE.[56] Conversely, the low levels of 27-OH in the brain might also be expected not to affect amyloidogenesis. However, since the flux of 27-OH across the BBB increases under conditions of hypercholesterolemia,[69] or in the case of reduced BBB integrity,[70] the inhibitory effect of 24-OH on Aβ generation is consequently reduced. In this connection, the high flux of 27-OH from the peripheral circulation to the brain, and changes in the brain cholesterol/oxysterol balance, may partially explain the link between hypercholesterolemia and AD.[69] Nevertheless, in murine primary neuronal cells both 24-OH and 27-OH were found to inhibit Aβ formation and secretion, 24-OH being about 1000-fold more potent than 27-OH.[65] The study showed that the distribution of the enzymes CYP46A1 and CYP27A1 is altered in the brain of subjects with AD. CYP46A1, whose expression increases in astrocytes and decreases in neurons, is selectively expressed in degenerating neuritis around senile plaques, whereas CYP27A1 expression, which is mostly expressed in neurons but also to a lesser extent in astrocytes and oligodendrocytes, is increased in white-matter oligodendrocytes.[65] Moreover, it has been shown that 27-OH significantly reduces Aβ peptide generation from primary human neurons, not by affecting α-, β-, or γ-secretase but by upregulating LXR responsive genes (ABCA1, ABCG1 and ApoE).[73] Of note the LXR-mediated gene regulation of cholesterol efflux and metabolism not only modulates neurodegeneration and Aβ peptide transport and clearance but also inflammation in the brain. On the basis of these data it cannot be excluded that 27-OH, as an LXR ligand, might exert anti-amyloidogenic effects by reducing extracellular Aβ and inflammation.[74-77]

By contrast, other studies are consistent with the possibility that 27-OH may accelerate neurodegeneration. In human SH-SY5Y neuroblastoma cells, 24-OH directly increases α-secretase activity as well as elevating the α/β activity ratio, whereas 27-OH counteracts the inhibitory effect of 24-OH on the generation of amyloid.[78] In addition, recent studies underlined the different effects of 24-OH and 27-OH on APP levels and processing in human neuroblastoma SH-SY5Y cells and in the brain tissue: 24-OH may favor the non-amyloidogenic pathway, whereas 27-OH is thought to

enhance production of $A\beta_{42}$ by upregulating APP and BACE1, and tau hyperphosphorylation.[79,80] Another study has found that 27-OH increases Aβ accumulation by reducing insulin-like growth factor 1 (IGF1) levels, a neurotrophic factor that promotes neurogenesis and has a neuroprotective effect, in hippocampal slices from adult rabbits;[81] in addition, 27-OH reduces the production of the "memory protein" activity-regulated cytoskeleton-associated protein (Arc) in mouse brain.[82]

Alongside altered cholesterol metabolism and hypercholesterolemia, inflammatory response and oxidative stress also significantly contribute to neuronal damage in AD.[83,84]

The importance of inflammatory processes has been pointed out during the past decade by the intensive investigation of inflammatory mediators and microglia activation in the brain of AD, although it remains unclear whether inflammation represents a cause or a consequence of AD. It has been reported that intraneuronal Aβ and soluble Aβ oligomers activate microglia in the earliest stages of the disease, even before plaque and tangle formation, in particular when cells are stressed.[85-87] Fibrillar Aβ also can activate microglia by binding to cells via specific receptors, in particular through a multireceptor complex involving CD36, α6β1-integrin and CD47.[88] The induction of a microglia-driven inflammatory response results in the release of various inflammatory mediators, including a whole array of neurotoxic cytokines and free radicals.[89] Once activated, microglia cells may also recruit astrocytes, which actively enhance the inflammatory response to extracellular Aβ deposits that intensify neuronal dysfunction and cell death: inflammatory mediators and other components of the immune system are often found near areas of amyloid plaques.[90]

It has also been postulated that oxidative stress may be either a cause or a consequence of the neuropathology associated with AD[91-94] and, in support of its being a consequence, Aβ can stimulate the production of reactive oxygen species (ROS).[95] Conversely, oxidative stress has been shown to contribute to the formation of amyloid plaques,[96] since Aβ peptide has been found in the oxidized form.[97] Since the brain has a high lipid content, it is extremely vulnerable to free radicals

and ROS, which are responsible for enhancing lipid peroxidation, including cholesterol oxidation and oxysterol formation[98] as well as tau hyperphosphorylation and NFT formation,[99] mitochondrial insufficiency and neuronal cell death.[100]

Oxysterols have been shown to enhance Aβ aggregation and its neurotoxicity, by modifying specific sites of Aβ peptide. Following Aβ modification at Lys-16, peptide aggregates were formed faster than in the case of modification at Lys-28 or Asp-1.[101] Moreover, 7β-OH has been found to be neurotoxic at nanomolar concentrations in cultured rat hippocampal neuronal cells, and may therefore contribute to Aβ-related neurodegeneration in the brain of AD patients.[59] Another oxysterol that might derive from the autooxidation of cellular cholesterol released during neurodegeneration, is 7α-hydroperoxycholesterol, which has also been found to be responsible for necrotic cell death of SH-SY5Y cells,[102] and a further possibility is 7-ketocholesterol.[103] Additionally, 24-OH has been shown to enhance the neurotoxic effect of the $A\beta_{42}$ peptide in the human differentiated neuroblastoma cell line MSN, as well as augmenting ROS generation.[104]

In our recent study, we examined the ways in which the oxysterols 24-OH, 27-OH, and 7β-OH, specifically implicated in brain pathophysiology, may modulate and possibly amplify the expression of AD.[105] All three oxysterols strongly enhanced the binding and concentration of $A\beta_{42}$ on membranes of human differentiated neuronal cell lines (SK-N-BE and NT-2) by markedly upregulating expression and synthesis of CD36 and β1-integrin receptors, two components of the multireceptor complex CD36/β1-integrin/CD47, through which Aβ peptide binds to membrane cells.[88] An interesting finding of the same study is that only 24-OH significantly potentiates both the necrogenic and the apoptotic effects exerted by $A\beta_{42}$ peptide on these cells (Fig. 3A and B). These effects were inhibited when 24-OH-treated neuronal cells were incubated with anti-CD36 and anti-β1-integrin antibodies before $A\beta_{42}$ addition, since Aβ peptide binding to the cell surface was prevented. One significant reason for this selective behavior of 24-OH appears to be its marked pro-oxidant action on neuronal cells, by locally increasing ROS generation, an action not exerted by

either 27-OH or 7β-OH (Fig. 3C). The 24-OH-dependent potentiation of Aβ neurotoxicity was completely inhibited by incubation of differentiated SK-N-BE or NT-2 cells with either the flavonol quercetin or the isoflavone genistein.[105]

However, despite the present knowledge demonstrates that cholesterol and some oxystetrols play a key role in the AD pathogenesis interacting with Aβ (Fig. 4), elucidation of the precise mechanisms will clearly require further study.

Acknowledgments

The authors wish to thank the Italian Ministry of University, Prin 2008 and 2009, the Piedmontese Regional Government (Ricerca Sanitaria Finalizzata 2009), the CRT Foundation, Turin, and the University of Turin, Italy, for supporting this work.

References

1. Querfurth, H.W. & F.M. LaFerla. 2010. Alzheimer's disease. *N. Engl. J. Med.* **362:** 329-344.
2. Chopra, K., S. Misra & A. Kuhad. 2011. Neurobiological aspects of Alzheimer's disease. *Expert. Opin. Ther. Targets* **15:** 535-555.
3. Walsh, D.M. & D.J. Selkoe. 2007. A beta oligomers - a decade of discovery. *J. Neurochem.* **101:** 1172-1184.
4. Martins, I.J. *et al.* 2009. Cholesterol metabolism and transport in the pathogenesis of Alzheimer's disease. *J. Neurochem.* **111:** 1275-1308.
5. Carter, C.J. 2007. Convergence of genes implicated in Alzheimer's disease on the cerebral cholesterol shuttle: APP, cholesterol, lipoproteins, and atherosclerosis. *Neurochem. Int.* **50:** 12-38.

6. Wollmer, M.A. 2010. Cholesterol-related genes in Alzheimer's disease. *Biochim. Biophys. Acta.* **1801:** 762-773.
7. Ghribi, O. 2008. Potential mechanisms linking cholesterol to Alzheimer's disease-like pathology in rabbit brain, hippocampal organotypic slices, and skeletal muscle. *J. Alzheimers Dis.* **15:** 673-684.
8. Di Paolo, G. & T.W. Kim. 2011. Linking lipids to Alzheimer's disease: cholesterol and beyond. *Nat. Rev. Neurosci.* **12:** 284-296.
9. Shobab, L.A., G.Y. Hsiung & H.H. Feldman. 2005. Cholesterol in Alzheimer's disease. *Lancet Neurol.* **4:** 841-852.
10. Panza, F. *et al.* 2006. Lipid metabolism in cognitive decline and dementia. *Brain Res. Rev.* **51:** 275-292.
11. Pappolla, M.A. *et al.* 2003. Mild hypercholesterolemia is an early risk factor for the development of Alzheimer amyloid pathology. *Neurology.* **61:** 199-205.
12. Jick, H. *et al.* 2000. Statins and the risk of dementia. *Lancet.* **356:** 1627-1631.
13. Wolozin, B. *et al.* 2000. Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. *Arch. Neurol.* **57:** 1439-1443.
14. Zamrini, E., G. McGwin & J.M. Roseman. 2004. Association between statin use and Alzheimer's disease. *Neuroepidemiology* **23:** 94-98.
15. Kandiah, N. & H.H. Feldman. 2009. Therapeutic potential of statins in Alzheimer's disease. *J. Neurol. Sci.* **283:** 230-234.
16. Fonseca, A.C. *et al.* 2010. Cholesterol and statins in Alzheimer's disease: current controversies. *Exp. Neurol.* **223:** 282-293.
17. Arvanitakis, Z. *et al.* 2008. Statins, incident Alzheimer disease, change in cognitive function, and neuropathology. *Neurology* **70:** 1795-1802.
18. Puglielli, L., R.E. Tanzi & D.M. Kovacs. 2003. Alzheimer's disease: the cholesterol connection. *Nat. Neurosci.* **6:** 345-351.

19. Evans, R.M. *et al.* 2004. Cholesterol and APOE genotype interact to influence Alzheimer disease progression. *Neurology* **62:** 1879-1881.
20. Bu, G. 2009. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat. Rev. Neurosci.* **10:** 333-344.
21. Kim, J., J.M. Basak & D.M. Holtzman. 2009. The role of apolipoprotein E in Alzheimer's disease. *Neuron.* **63:** 287-303.
22. Marzolo, M.P. & G. Bu. 2009. Lipoprotein receptors and cholesterol in APP trafficking and proteolytic processing, implications for Alzheimer's disease. *Semin. Cell. Dev. Biol.* **20:** 191-200.
23. Jiang, Q. *et al.* 2008. ApoE promotes the proteolytic degradation of Abeta. *Neuron.* **58:** 681-693.
24. Sagare, A. *et al.* 2007. Clearance of amyloid-beta by circulating lipoprotein receptors. *Nat. Med.* **13:** 1029-1031.
25. Cam, J.A. & G. Bu. 2006. Modulation of beta-amyloid precursor protein trafficking and processing by the low density lipoprotein receptor family. *Mol. Neurodegener.* **18:** 1-8.
26. Herz, J. & U. Beffert. 2000. Apolipoprotein E receptors: linking brain development and Alzheimer's disease. *Nat. Rev. Neurosci.* **1:** 51-58.
27. Liu, Q. *et al.* 2007. Amyloid precursor protein regulates brain apolipoprotein E and cholesterol metabolism through lipoprotein receptor LRP1. *Neuron.* **56:** 66-78.
28. Jenner, A.M. *et al.* 2010. The effect of APOE genotype on brain levels of oxysterols in young and old human APOE epsilon2, epsilon3 and epsilon4 knock-in mice. *Neuroscience* **169:** 109-115.
29. Pfrieger, F.W. 2003. Cholesterol homeostasis and function in neurons of the central nervous system. *Cell. Mol. Life Sci.* **60:** 1158-1171.
30. Björkhem, I. & S. Meaney. 2004. Brain cholesterol: long secret life behind a barrier. *Arterioscler. Thromb. Vasc. Biol.* **24:** 806-815.

31. Pfrieger, F.W. 2003. Outsourcing in the brain: do neurons depend on cholesterol delivery by astrocytes? *Bioessays* **25:** 72-78.
32. Cordy, J.M., N.M. Hooper & A.J. Turner. 2006. The involvement of lipid rafts in Alzheimer's disease. *Mol. Membr. Biol.* **23:** 111-122.
33. Vetrivel, K.S. & G. Thinakaran. 2010. Membrane rafts in Alzheimer's disease beta-amyloid production. *Biochim. Biophys. Acta* **1801:** 860-867.
34. Beel, A.J. *et al.* 2010. Direct binding of cholesterol to the amyloid precursor protein: An important interaction in lipid-Alzheimer's disease relationships? *Biochim. Biophys. Acta* **1801:** 975-982.
35. Wahrle, S. *et al.* 2002. Cholesterol-dependent gamma-secretase activity in buoyant cholesterol-rich membrane microdomains. *Neurobiol. Dis.* **9:** 11-23.
36. Hur, J.Y. *et al.* 2008. Active gamma-secretase is localized to detergent-resistant membranes in human brain. *FEBS J.* **275:** 1174-1187.
37. Grimm, M.O. *et al.* 2008. Independent inhibition of Alzheimer disease beta- and gamma-secretase cleavage by lowered cholesterol levels. *J. Biol. Chem.* **283:** 11302-11311.
38. Xiong, H. *et al.* 2008. Cholesterol retention in Alzheimer's brain is responsible for high beta- and gamma-secretase activities and Abeta production. *Neurobiol. Dis.* **29:** 422-437.
39. Reid, P.C. *et al.* 2007. Alzheimer's disease: cholesterol, membrane rafts, isoprenoids and statins. *J. Cell. Mol. Med.* **11:** 383-392.
40. Harris, B., I. Pereira & E. Parkin. 2009. Targeting ADAM10 to lipid rafts in neuroblastoma SH-SY5Y cells impairs amyloidogenic processing of the amyloid precursor protein. *Brain Res.* **1296:** 203-215.
41. Yanagisawa, K. 2005. Cholesterol and amyloid beta fibrillogenesis. *Subcell. Biochem.* **38:** 179-202.

42. Kakio, A. *et al.* 2002. Interactions of amyloid beta-protein with various gangliosides in raft-like membranes: importance of GM1 ganglioside-bound form as an endogenous seed for Alzheimer amyloid. *Biochemistry* **41:** 7385-7390.
43. Harris, J.R. 2008. Cholesterol binding to amyloid-beta fibrils: a TEM study. *Micron* **9:** 1192-1196.
44. Kakio, A. *et al.* 2001. Cholesterol-dependent formation of GM1 ganglioside-bound amyloid beta-protein, an endogenous seed for Alzheimer amyloid. *J. Biol. Chem.* **276:** 24985-24990.
45. Burns, M.P. *et al.* 2006. Cholesterol distribution, not total levels, correlate with altered amyloid precursor protein processing in statin-treated mice. *Neuromolecular Med.* **8:** 319-328.
46. Puglielli, L. *et al.* 2001. Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid beta-peptide. *Nat. Cell Biol.* **3:** 905-912.
47. Bhattacharyya, R. & D.M. Kovacs. 2010. ACAT inhibition and amyloid beta reduction. *Biochim. Biophys. Acta* **1801:** 960-965.
48. Michikawa, M. *et al.* 2001. A novel action of alzheimer's amyloid beta-protein (Abeta): oligomeric Abeta promotes lipid release. *Neurosci.* **21:** 7226-7235.
49. Gong, J.S. *et al.* 2002. Amyloid beta-protein affects cholesterol metabolism in cultured neurons: implications for pivotal role of cholesterol in the amyloid cascade. *J. Neurosci. Res.* **70:** 438-446.
50. Fan, Q.W. *et al.* 2001. Cholesterol-dependent modulation of tau phosphorylation in cultured neurons. *J. Neurochem.* **76:** 391-400.
51. Koudinov, A.R. & N.V. Koudinova. 2005. Cholesterol homeostasis failure as a unifying cause of synaptic degeneration. *J. Neurol. Sci.* **229-230:** 233-240.
52. Mori, T. *et al.* 2001. Cholesterol accumulates in senile plaques of Alzheimer disease patients and in transgenic APP(SW) mice. *J. Neuropathol. Exp. Neurol.* **60:** 778-785.
53. Burns, M.P. *et al.* 2003. Co-localization of cholesterol, apolipoprotein E and fibrillar Abeta in amyloid plaques. *Brain Res. Mol. Brain Res.* **110:** 119-125.

54. Björkhem, I. *et al.* 2009. Oxysterols and neurodegenerative diseases. *Mol. Aspects Med.* **30:** 171-179.

55. Björkhem I. 2006. Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. *J. Intern. Med.* **260:** 493-508.

56. Abildayeva, K. *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:** 12799-12808.

57. Meaney, S. *et al.* 2007. Novel route for elimination of brain oxysterols across the blood-brain barrier: conversion into 7alpha-hydroxy-3-oxo-4-cholestenoic acid. *J. Lipid Res.* **48:** 944-951.

58. Heverin, M. *et al.* 2005. Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain. *J. Lipid Res.* **46:** 1047-1052.

59. Nelson, T.J. & D.L. Alkon. 2005. Oxidation of cholesterol by amyloid precursor protein and beta-amyloid peptide. *J. Biol. Chem.* **280:** 7377-7387.

60. Lütjohann, D. *et al.* 2000. Plasma 24S-hydroxycholesterol (cerebrosterol) is increased in Alzheimer and vascular demented patients. *J. Lipid Res.* **41:** 195-198.

61. Papassotiropoulos, A. *et al.* 2002. 24S-hydroxycholesterol in cerebrospinal fluid is elevated in early stages of dementia. *J. Psychiatr. Res.* **36:** 27-32.

62. Kölsch, H. *et al.* 2004. Altered levels of plasma 24S- and 27-hydroxycholesterol in demented patients. *Neurosci. Lett.* **368:** 303-308.

63. Bretillon, L. *et al.* 2000. Plasma levels of 24S-hydroxycholesterol in patients with neurological diseases. *Neurosci. Lett.* **293:** 87-90.

64. Bogdanovic, N. *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. Nov.* **314:** 45-48.

65. Brown, J. 3rd *et al.* 2004. Differential expression of cholesterol hydroxylases in Alzheimer's disease. *J. Biol. Chem.* **13:** 34674-34681.

66. Iuliano, L. *et al.* 2010. Vitamin E and enzymatic/oxidative stress-driven oxysterols in amnestic mild cognitive impairment subtypes and Alzheimer's disease. *J. Alzheimers Dis.* **21:** 1383-1392.

67. Heverin, M. *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:** 186-193.

68. Shafaati, M. *et al.* 2011. Marked accumulation of 27-hydroxycholesterol in the brains of Alzheimer's patients with the Swedish APP 670/671 mutation. *J. Lipid Res.* **52:** 1004-1010.

69. Björkhem, I. *et al.* 2006. Oxysterols and Alzheimer's disease. *Acta Neurol. Scand. Suppl.* **185:** 43-49.

70. Leoni, V. *et al.* 2003. Side chain oxidized oxysterols in cerebrospinal fluid and the integrity of blood-brain and blood-cerebrospinal fluid barriers. *J. Lipid Res.* **44:** 793-799.

71. Yau, J.L. *et al.* 2003. Dehydroepiandrosterone 7-hydroxylase CYP7B: predominant expression in primate hippocampus and reduced expression in Alzheimer's disease. *Neuroscience* **121:** 307-314.

72. Leoni, V. *et al.* 2004. Diagnostic use of cerebral and extracerebral oxysterols. *Clin. Chem. Lab. Med.* **42:** 186-191.

73. Kim, W.S. *et al.* 2009. Impact of 27-hydroxycholesterol on amyloid-beta peptide production and ATP-binding cassette transporter expression in primary human neurons. *J. Alzheimers Dis.* **16:** 121-131.

74. Wang, L. *et al.* 2002. Liver X receptors in the central nervous system: from lipid homeostasis to neuronal degeneration. *Proc. Natl. Acad. Sci. U S A.* **15:** 13878-13883.

75. Riddell, D.R. *et al.* 2007 The LXR agonist TO901317 selectively lowers hippocampal Abeta42 and improves memory in the Tg2576 mouse model of Alzheimer's disease. *Mol. Cell. Neurosci.* **34:** 621-628.

76. Zelcer, N. *et al.* 2007. Attenuation of neuroinflammation and Alzheimer's disease pathology by liver x receptors. *Proc. Natl. Acad. Sci. U S A.* **19**: 10601-10606.

77. Cao, G. *et al.* 2007. Liver X receptor-mediated gene regulation and cholesterol homeostasis in brain: relevance to Alzheimer's disease therapeutics. *Curr. Alzheimer Res.* **4:** 179-184.

78. Famer, D. *et al.* 2007. Regulation of alpha- and beta-secretase activity by oxysterols: cerebrosterol stimulates processing of APP via the alpha-secretase pathway. *Biochem. Biophys. Res. Commun.* **359:** 46-50.

79. Prasanthi, J.R. *et al.* 2009. Differential effects of 24-hydroxycholesterol and 27-hydroxycholesterol on beta-amyloid precursor protein levels and processing in human neuroblastoma SH-SY5Y cells. *Mol. Neurodegener.* **4:** 1-8.

80. Marwarha, G. *et al.* 2010. Leptin reduces the accumulation of Abeta and phosphorylated tau induced by 27-hydroxycholesterol in rabbit organotypic slices. *J. Alzheimers Dis.* **19:** 1007-1019.

81. Sharma, S. *et al.* 2008. Hypercholesterolemia-induced Abeta accumulation in rabbit brain is associated with alteration in IGF-1 signaling. *Neurobiol. Dis.* **32:** 426-432.

82. Mateos, L. *et al.* 2009. Activity-regulated cytoskeleton-associated protein in rodent brain is down-regulated by high fat diet in vivo and by 27-hydroxycholesterol in vitro. *Brain Pathol.* **19:** 69-80.

83. Akiyama, H. *et al.* 2000 Inflammation and Alzheimer's disease. *Neurobiol. Aging* **21:** 383-421.

84. Guglielmotto, M. *et al.* 2010. Oxidative stress mediates the pathogenic effect of different Alzheimer's disease risk factors. *Front. Aging Neurosci.* **2:** 1-8.

85. Khandelwal, P.J., A.M. Herman & C.E. Moussa. 2011. Inflammation in the early stages of neurodegenerative pathology. *J. Neuroimmunol.* **15:** 1-11.

86. Sastre, M. *et al.* 2011. Inflammatory risk factors and pathologies associated with Alzheimer's disease. Curr. Alzheimer Res. **8:** 132-141.

87. Ferretti, M.T. & A.C. Cuello 2011. Does a pro-inflammatory process precede Alzheimer's disease and mild cognitive impairment? Curr. Alzheimer Res. **8:** 164-174.

88. Verdier, Y., M. Zarándi & B. Penke. 2004. Amyloid beta-peptide interactions with neuronal and glial cell plasma membrane: binding sites and implications for Alzheimer's disease. *J. Pept. Sci.* **10:** 229-248.

89. Schwab, C. & P.L. McGeer. 2008. Inflammatory aspects of Alzheimer disease and other neurodegenerative disorders. *J. Alzheimers Dis.* **13:** 359-369.

90. Abbas, N. *et al.* 2002. Up-regulation of the inflammatory cytokines IFN-gamma and IL-12 and down-regulation of IL-4 in cerebral cortex regions of APP(SWE) transgenic mice. *J. Neuroimmunol.* **126:** 50-57.

91. Nunomura, A. *et al.* 2001. Oxidative damage is the earliest event in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **60:** 759-767.

92. Zhu, X. *et al.* 2007. Causes of oxidative stress in Alzheimer disease. *Cell. Mol. Life Sci.* **64:** 2202-2210.

93. Smith, M.A. *et al.* 2010. Increased iron and free radical generation in preclinical Alzheimer disease and mild cognitive impairment. *J. Alzheimers Dis.* **19:** 363-372.

94. Bonda, D.J. *et al*. 2010. Oxidative stress in Alzheimer disease: a possibility for prevention. *Neuropharmacology* **59:** 290-294.

95. Ding, Q., E. Dimayuga & J.N. Keller. 2007. Oxidative damage, protein synthesis, and protein degradation in Alzheimer's disease. *Curr. Alzheimer Res.* **4:** 73-79.

96. Praticò, D. *et al.* 2001. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *J. Neurosci.* **21:** 4183-4187.

97. Naylor, R., A.F. Hill & K.J. Barnham. 2008. Neurotoxicity in Alzheimer's disease: is covalently crosslinked A beta responsible? *Eur. Biophys. J.* **37:** 265-268.

98. Arca, M. *et al.* 2007. Increased plasma levels of oxysterols, in vivo markers of oxidative stress, in patients with familial combined hyperlipidemia: reduction during atorvastatin and fenofibrate therapy. *Free Radic. Biol. Med.* **42:** 698-705.

99. Melov, S. *et al.* 2007. Mitochondrial oxidative stress causes hyperphosphorylation of tau. *PLoS ONE* **2:** e536.

100. Cassano, T. *et al.* 2011. Glutamatergic alterations and mitochondrial impairment in a murine model of Alzheimer disease. *Neurobiol. Aging.* **27**, Doi:10.1016!j.neurobiolaging.2011.09.021.

101.Usui, K. *et al.* 2009. Site-specific modification of Alzheimer's peptides by cholesterol oxidation products enhances aggregation energetics and neurotoxicity. *Proc. Natl. Acad. Sci. U S A.* **106:** 18563-18568.

102.Kolsch, H. *et al.* 2000. 7alpha-Hydroperoxycholesterol causes CNS neuronal cell death. *Neurochem. Int.* **36:** 507-512.

103.Ong, W.Y. *et al.* 2010. Changes in brain cholesterol metabolome after excitotoxicity. *Mol. Neurobiol.* **41:** 299-313.

104.Ferrera, P. *et al.* 2008. Cholesterol potentiates beta-amyloid-induced toxicity in human neuroblastoma cells: involvement of oxidative stress. *Neurochem. Res.* **33:** 1509-1517.

105.Gamba, P. *et al.* 2011. Interaction between 24-hydroxycholesterol, oxidative stress, and amyloid-β in amplifying neuronal damage in Alzheimer's disease: three partners in crime. *Aging Cell* **10:** 403-417.

Figure legends

Figure 1. Cholesterol homeostasis in the mature brain. Excess cholesterol is converted into 24-hydroxycholesterol (24-OH) in neuronal cells by CYP46A1. Most of the 24-OH goes directly from the brain into the blood circulation, a small quantity entering the cerebrospinal fluid (CSF). 24-OH may also be caught by astrocytes, where it upregulates the nuclear receptor liver X receptor (LXR)-responsive genes involved in cholesterol efflux, i.e. ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1) and apolipoprotein E (ApoE). Synthesized cholesterol is loaded by astrocytes onto ApoE, and the ApoE/cholesterol complex is then internalized by neurons via low-density lipoprotein receptors (LDLR).

Figure 2. Fluxes of 24-hydroxycholesterol (24-OH) and 27-hydroxycholesterol (27-OH) through the blood brain barrier (BBB). The enzymes CYP46A1 and CYP27A1, located in the neuronal cells, are respectively responsible for generating 24-OH and 27-OH in the brain. However, most of the 27-OH flows from the circulation into the brain since, unlike cholesterol, it can cross the BBB, as can 24-OH. In the brain, 27-OH is also metabolized to 7α-hydroxy-3-oxo-4-cholestenoic acid (7-OH-4-C), which crosses the BBB to reach the liver. In conclusion, there is a complex of fluxes of oxysterols in opposite directions at the BBB: two fluxes out of the brain (24-OH and 7-OH-4-C) and one flux into the brain (27-OH). Of note, the shift in balance between 24-OH and 27-OH is important for amyloidogenesis since it might lead to increased generation and accumulation of Aβ in AD brain.

Figure 3. (A) Necrotic effects of 27-hydroxycholesterol (27-OH), 7β-hydroxycholesterol (7β-OH), or 24-hydroxycholesterol (24-OH) evaluated in terms of lactate dehydrogenase (LDH) release. SK-N-BE cells were treated with the oxysterol (1 µM) for 48 h, and then for 24 h with $A\beta_{42}$ (1 µM). Histograms represent the mean values ± SD of 3 experiments. **P < 0.01 vs. control (untreated cells). (B) Apoptotic effects of 27-OH, 7β-OH, or 24-OH on SK-N-BE cells observed by DAPI

staining to determine apoptotic nuclei formation. Cells were treated with the oxysterol for 48 h and then for 24 h with $A\beta_{42}$. (C) Pro-oxidant effects of 27-OH, 7β-OH, or 24-OH. Intracellular generation of reactive oxygen species (ROS) was examined in SK-N-BE cells using 2',7'-dichlorodihydrofluorescein (DCFH-DA) as an intracellular probe. Cells were incubated with oxysterol for 1 h, or simultaneously with oxysterol plus $A\beta_{42}$ for 1 h.

Figure 4. Interaction between lipid metabolism and amyloid-β (Aβ) processing. The major source of cerebral cholesterol is *de novo* synthesis from acetyl-coenzyme A mediated by 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. Excess cholesterol is converted into cholesteryl esters by acyl-coenzyme A:cholesterol acyl-transferase (ACAT) and into the oxysterols 24- and 27-hydroxycholesterol (24-OH, 27-OH) by CYP47A1 and CYP27A1, respectively; the oxysterol 7β-hydroxycholesterol (7β-OH) may derives from cholesterol oxidation by Aβ and amyloid protein precursor (APP). Aβ production is increased by HMG-CoA reductase and ACAT activity and by cholesterol which regulates β- and γ-secretase. LDL receptor-related protein (LRP) mediates the internalization of astrocyte-produced ApoE-cholesterol complexes into neurons. The intracellular domain of APP down-regulates cellular cholesterol uptake by acting as a transcriptional suppressor of LRP1 gene. 24-OH and 27-OH upregulate ATP-binding cassette transporter A1 (ABCA1), involved in cholesterol efflux, which modulates Aβ levels in neurons; these oxysterols increase ApoE levels and induce Aβ aggregation which is also promoted by free ApoE and cholesterol.

Fig. 1

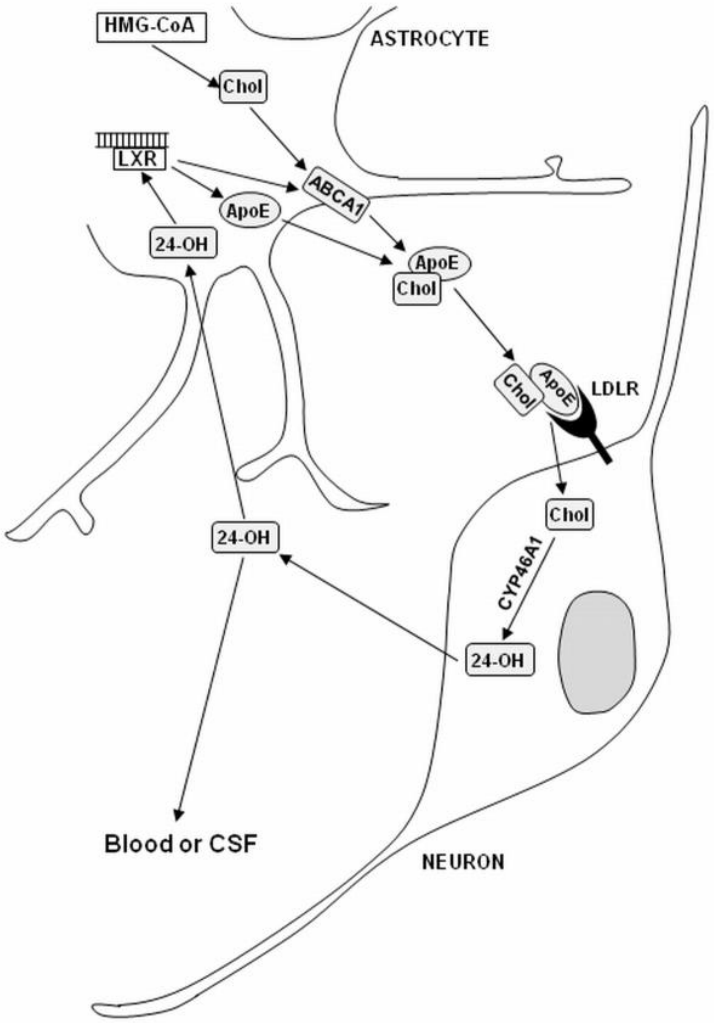

HMG-CoA
ASTROCYTE
Chol
LXR
ABCA1
ApoE
24-OH
ApoE
Chol
Chol
ApoE
LDLR
Chol
CYP46A1
24-OH
24-OH
Blood or CSF
NEURON

Fig. 2

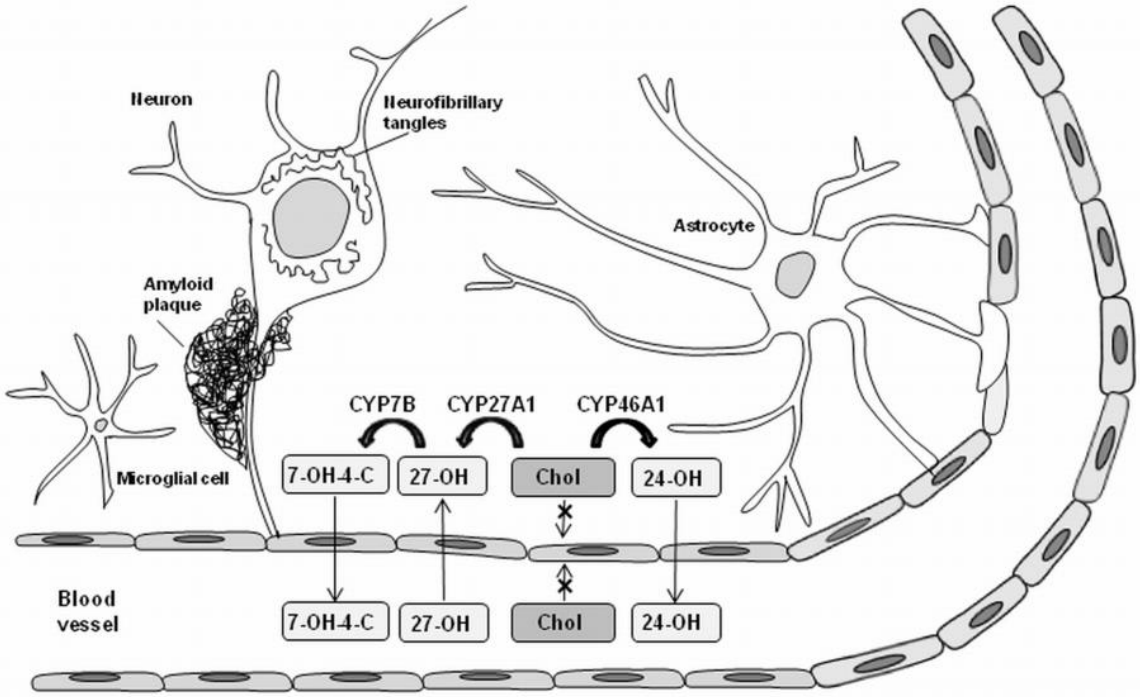

Neuron
Neurofibrillary tangles
Astrocyte
Amyloid plaque
Microglial cell
CYP7B
CYP27A1
CYP46A1
7-OH-4-C
27-OH
Chol
24-OH
Blood vessel
7-OH-4-C
27-OH
Chol
24-OH

Fig. 3

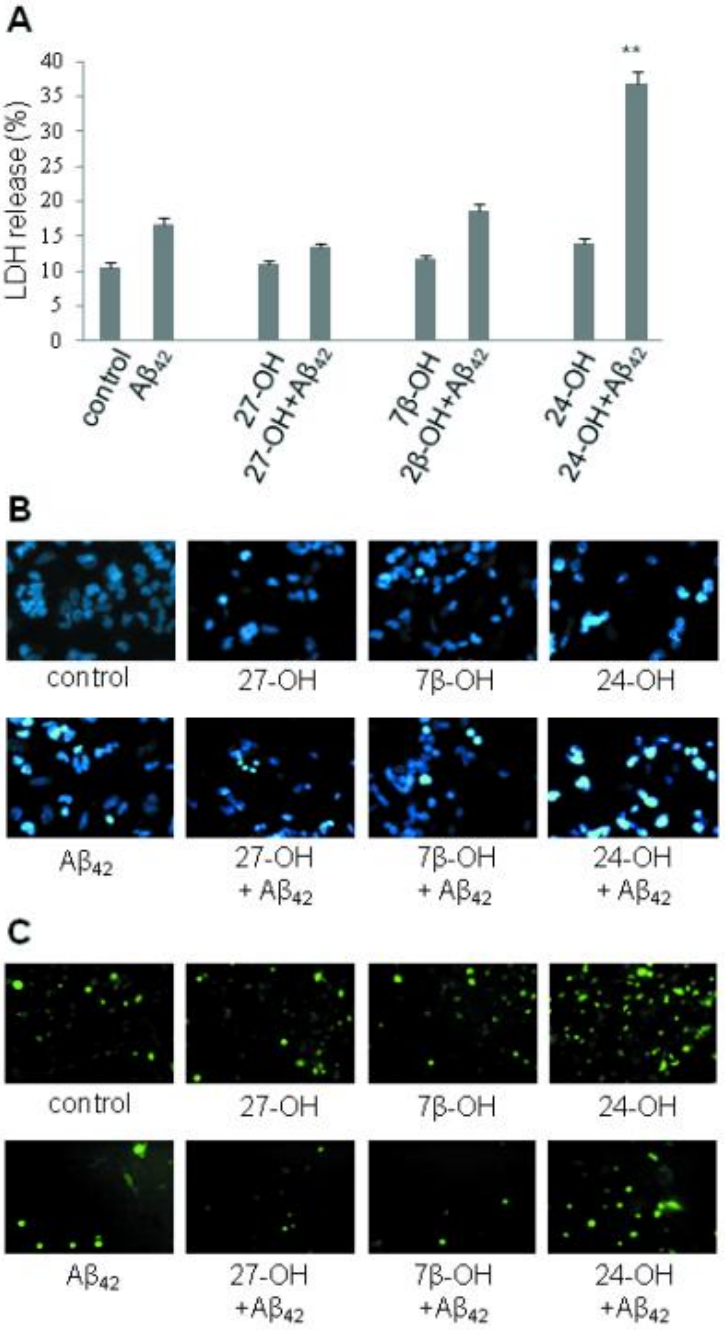
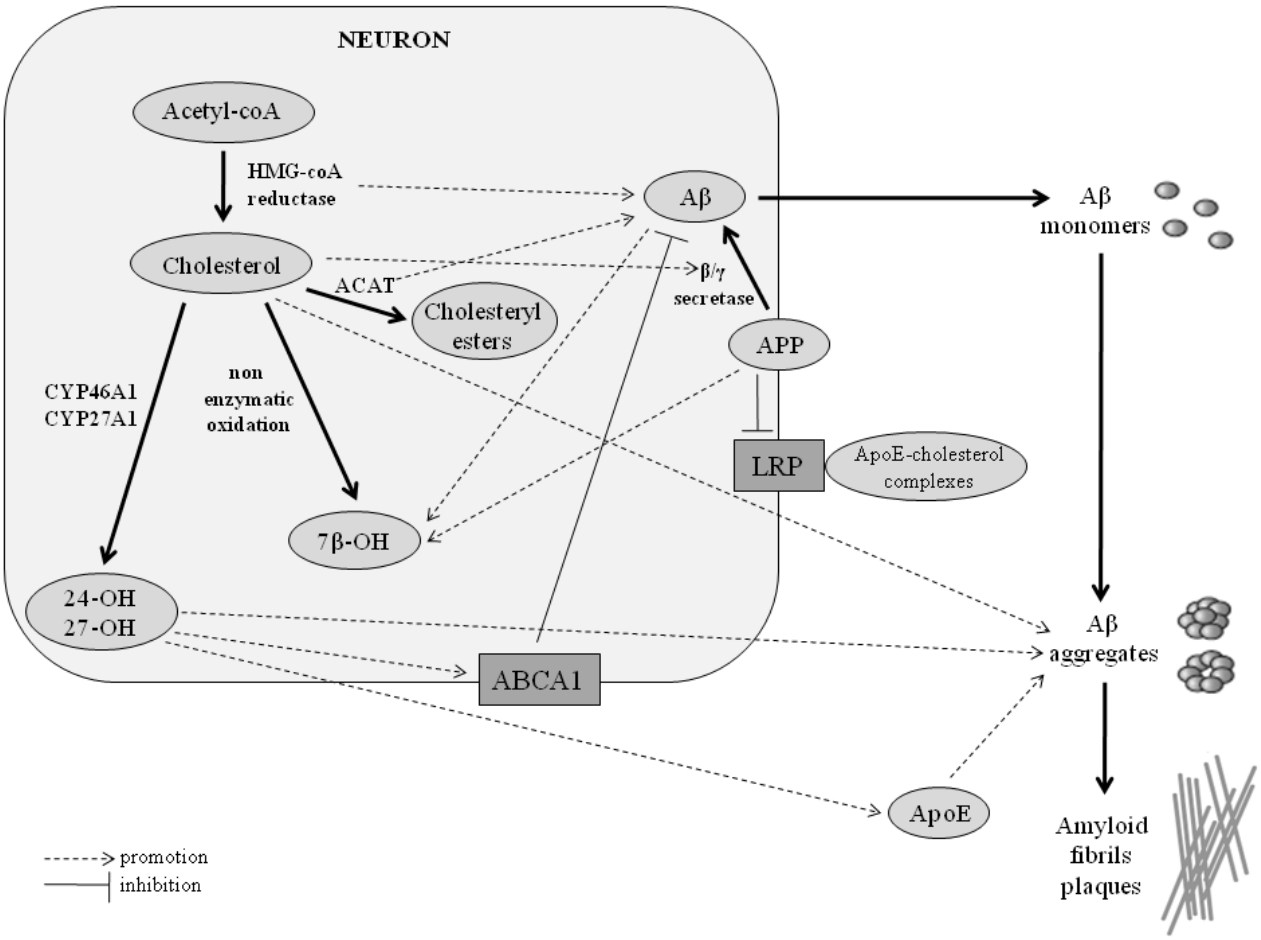

A
LDH release (%)
40
35
30
25
20
15
10
5
0
**
control
Aβ42
27-OH
27-OH+Aβ42
7β-OH
2β-OH+Aβ42
24-OH
24-OH+Aβ42
B
control
27-OH
7β-OH
24-OH
Aβ42
27-OH + Aβ42
7β-OH + Aβ42
24-OH + Aβ42
C
control
27-OH
7β-OH
24-OH
Aβ42
27-OH +Aβ42
7β-OH +Aβ42
24-OH +Aβ42

Fig. 4


NEURON
Acetyl-coA
HMG-coA reductase
Aβ
Aβ monomers
Cholesterol
ACAT
β/γ secretase
Cholesteryl esters
APP
CYP46A1
CYP27A1
non enzymatic oxidation
LRP
ApoE-cholesterol complexes
7β-OH
24-OH
27-OH
Aβ aggregates
ABCA1
ApoE
Amyloid fibrils plaques
promotion
inhibition