**Hypercholesterolaemia-induced oxidative stress on blood brain barrier**

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**Abstract**

Blood cholesterol levels are not consistently elevated in subjects with age-related cognitive decline, although epidemiological studies suggest that Alzheimer’s disease and cardiovascular diseases share common risk factors. These include the presence of an unusual genetic variant, the APOE4 (apolipoprotein E4) allele, which modulates LDL (low-density lipoproteins) metabolism, increases free radical formation and reduces plasma antioxidant concentrations. Together, these risk factors support a mechanism for increased LDL circulation time and free radical modification of LDL. Plasma oxycholesterols, hydroxylated metabolites of cholesterol, are carried by oxidized LDL, and elevated lipids in mid-life are associated with increased long-term risk of dementia. Although brain cholesterol metabolism is segregated from the systemic circulation, during oxidative stress, plasma oxycholesterols could have damaging effects on BBB (blood–brain barrier) function and consequently on neuronal cells. Cholesterol-lowering drugs such as statins may prevent the modifications to LDL in mid-life and might show beneficial effects in later life.

**Background**

Dementia prevalence is estimated at 35.6 million and is projected to nearly double every 20 years, reaching 65.7 million in 2030 and 115.4 million in 2050. The total number of new cases of dementia each year is nearly 7.7 million worldwide[[1](#_ENREF_1)].The most common form of dementia, Alzheimer’s disease (AD) constitutes 60–70% of dementia cases and is characterized by a progression from episodic memory loss to a slow global decline of cognitive function. Age remains the strongest risk factor for its development and late onset AD, i.e. onset of symptoms after age 60 years and has annual incidence rates increasing from 1% at 65–70 years to 6–8% at 85 years and older[[2](#_ENREF_2)]. By 85 years of age and older, prevalence is 10–30% or more[[3](#_ENREF_3)].

The aetiological mechanisms underlying the neuropathological changes in AD remain unknown, but are considered to be affected by both environmental and genetic factors. Only one gene, APOE, has been unequivocally established as a “susceptibility” gene for late onset AD [[4](#_ENREF_4)]; the ε2alleleis associated with decreased risk and the ε4 allele with increased risk in a dose-dependent manner. However, there is incomplete penetrance of APOE genotypes and the fraction of genetic variance for late onset AD risk attributed to APOE is estimated at between 10–20% [[5](#_ENREF_5)]. Since these early studies, several GWAS studies have been undertaken [[6](#_ENREF_6)]and added to knowledge of risk alleles/mutations of *APOE* in late onset and *APP*, *PS1* and *PS2* in early onset AD was reviewed [[7](#_ENREF_7)].

Evidence is emerging that systemic inflammation is involved in Alzheimer’s disease. Risk factors for Alzheimer’s disease including obesity, diabetes, dyslipidemia, stroke and high blood pressure are known to cause inflammation systemically. It is found that the factors which increase systemic inflammation exacerbate the onset or progression of late-onset dementia and the risk of AD increased with the cumulative accumulation of risk factors [[8](#_ENREF_8)]. However, research is still required to recognise clinically defined vascular risk factors that are associated with incidents of AD.

**Plasma cholesterol level in mid-life is associated with later AD development**

Hypercholesterolaemia induced oxidative stress is reported in many studies. High level of radical production over antioxidant defence mechanisms cause irreversible damage to proteins, lipids and DNA. Evidence for oxidation and nitration of proteins to form carbonyl groups and 3-nitrotyrosine is frequent in both hypercholesterolaemic and dementia plasmas [[9](#_ENREF_9), [10](#_ENREF_10)]. Similarly, radical oxidised lipids (e.g. isoprostanes, lipid hydroperoxides) are more prevalent in plasma from AD and hypercholesterolaemic subjects compared to controls [[11](#_ENREF_11)]. Together, these studies suggest that systemic lipid/protein oxidation is common to AD and hypercholesterolaemia.

Blood cholesterol levels are not consistently elevated in subjects with age-related cognitive decline. However based on a prospective population study, Kivipelto et al. observed that elevated plasma cholesterol in mid-life, but not in late-life, is an independent risk factor for AD [[12](#_ENREF_12)]. Similarly, subjects aged 70-89 years with diagnosed AD had significantly elevated levels of serum cholesterol from 15-25 years before the disease onset [[13](#_ENREF_13)]. Chronically elevated cholesterol levels in mid-life could potentially trigger a cascade of pathological events in the brain, resulting in AD onset decades later.In support of this hypothesis, a prospective study has shown a reduced risk for AD in users of cholesterol-lowering statin drugs; HMGCoA reductase inhibitors[[14](#_ENREF_14)].

In addition, an association between previous statin use and reduced neurofibrillary tangle burden post-mortem has also been reported [[15](#_ENREF_15)]. In the TgCRND8 mouse model of AD, a reduction in β amyloid accumulation was been observed in the brain after treatment pravastatin, a non-hydrophilic, non-blood brain barrier (BBB) permeant statin [[16](#_ENREF_16)], supporting the hypothesis that peripheral cholesterol metabolism in mid-life is important to brain pathology.

Clinical trials that have been designed to reduce cholesterol or to reduce inflammation in patients with AD have not led to any clinical improvements. This suggests that important changes are happening early before symptoms appear and any interventions in mid-life are more likely to be beneficial.

**BBB integrity is affected by oxidative stress**

In health, the BBB serves as a robust gating mechanism to the brain. However, neurodegenerative conditions including stroke are associated with increased BBB permeability and the extent of damage can be attenuated by inhibitors of the free radical-generating, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system[[17](#_ENREF_17)].

There is controversy over whether ageing alone associates with increased BBB permeability; Pelegri et al[[18](#_ENREF_18)] have reported an early increase in permeability in the senescence-accelerated mouse model SAMP-8 at 12 months which is not present in control animals and is suggested to underlie deficits in learning and memory. SAMP-8 mice are also characterised by early loss of antioxidant enzymes i.e. of altered control of the scavenging of oxidative species [[19](#_ENREF_19)].

In a model of localised excitotoxicity-induced neurodegeneration, BBB breakdown in itself was not sufficient to elicit cell death; a subsequent peroxynitrite-mediated event was required [[20](#_ENREF_20)]**, where peroxynitrite is directly lipophilic and can cross membranes via diffusion in the absence of a transporter**. While the BBB requires the presence of carrier/proteins and transporters for rapid molecular transport of nutrients e.g. fatty acids, direct uptake of lipid hydroperoxides proceeds at an eight times faster rate than diffusion-controlled uptake of parent lipids [[21](#_ENREF_21)]. Thus, clear pathways exist for transport of reactive species and modified lipids from the periphery to the CNS where they are neurotoxic to hippocampal neurones.

**Cholesterol homeostasis in the brain is segregated from the peripheral circulation**

In the adult brain, primary cholesterol synthesis occurs in astrocytes and to a lesser extent in neurons[[22](#_ENREF_22)]; it is transported within the brain by local high density lipoproteins (HDL). Brain cholesterol metabolism appears to be quite distinctive from that of peripheral tissues and the CNS and plasma cholesterol/lipoprotein compartments are strictly segregated by the BBB. The BBB is created by the tight junctions between the endothelial cells of brain vascular tissue and assisted by the astrocyte foot processes surrounding the capillary endothelial cells[[23](#_ENREF_23)]. Transfer across the BBB to the CNS is achieved via ATP-dependent transporters, facilitated diffusion, transmembrane diffusion, and leakage through extracellular pathways.

Low density lipoprotein levels (LDL) are consistently elevated in cardiovascular disease. Increased systemic oxidative modification (oxLDL) and nitration is also observed during hypercholesterolemia. It has been suggested that those with high circulating LDL levels in their mid-life may be susceptible to develop neurodegenerative diseases in their later life. There is no evidence that lipoprotein cholesterol (C) (e.g. HDL-C and LDL-C) originating in the plasma compartment crosses the BBB to be transported into the CNS [[23](#_ENREF_23)]. In support of this, knockout of the LDL receptor gene in mice and rabbits or disruption of peripheral SR-BI or ABCA1 in mice changes neither the cholesterol synthesis rate nor the cholesterol concentration in the brain [[23](#_ENREF_23), [24](#_ENREF_24)]. Taken together, these data are in agreement with the view that plasma cholesterol is not transported to the brain because plasma lipoproteins do not cross the BBB. This raises a question, if plasma lipoproteins are segregated from brain cholesterol, how can chronically elevated plasma cholesterol in mid-life influence brain function?

**Generation and physiological effects of oxysterols**

Cholesterol which is synthesised de novo and absorbed from the diet can be oxidised through enzymatic or non-enzymatic mechanisms. Cholesterol oxidation plays an important role by producing active intermediate molecules that are involved in synthetic pathways for bile acids, hormones and vitamin D. Non-enzymatic oxidation of cholesterol requires transition metals or reactive oxygen species which are inevitably found in biological systems. As reviewed in Luliano 2011[[25](#_ENREF_25)], among many intermediates, commonly reported examples of oxidised cholesterols (oxysterols) include 6-cholesten-5α-hydroperoxide, 7-ketocholesterol, 7-beta hydroxyl cholesterol (7β-OH), 7-dehydrocholesterol and 25-hydroxycholesterol.

The brain, as an organ which regulates de novo synthesis of cholesterol, maintains its cholesterol homeostasis by conversion of cholesterol into the oxysterol 24-hydroxycholesterol (24-OHC) by the action of cytochrome-P450 related enzyme CYP46A1, which is almost exclusively found in brain neurones [[26](#_ENREF_26)]. 24-OHC is released from the brain through the BBB, so regulating brain cholesterol. In the periphery, sterol 27-hydroxylase (CYP27A1) which is the enzyme responsible of converting cholesterol to 27- hydroxyl cholesterol (27-OHC) exists in almost all cells. Studies have shown that 27-OHC can readily cross the BBB into brain compartment [[27](#_ENREF_27)]. This led to a number of investigations of the concentrations of circulating (24S)-hydroxycholesterol (24-OHC) and 27-OHC in relation to AD (table 1). Introduction of 27-OHC to an in vitro systems resulted altered cellular signalling, lipid raft formation [[28](#_ENREF_28)] and Aβ formation by neuronal cell lines [[29](#_ENREF_29)]. Even though 7β-hydroxycholesterol enhanced the binding of amyloid-beta (Aβ) in vitro studies, it did not potentiate the pro-apoptotic and pro-necrotic effects on neuronal cell lines [[29](#_ENREF_29)], showing differential effects of oxysterols.

Due to the low abundance of oxysterols different chromatographic and mass spectrometry approaches are taken to separate and identify various species in complex mixtures such as plasma or cerebrospinal fluid. However, the absence of a comprehensive standards and the low abundance of oxysterols make the quantification process challenging.

The identification of oxysterols in biological systems flagged their importance and steered research into understanding their receptor interactions and cellular uptake mechanisms over the last few decades[[30](#_ENREF_30)]. Two liver X receptors found in mammals (LXRα/NR1H3) and (LXRβ/NR1H2) were identified as oxysterol-activated nuclear receptors which play a pivotal role in the control of whole body cholesterol homeostasis[[31](#_ENREF_31)]. These receptors are expressed in metabolically active tissues like liver, intestine, macrophages, lungs, adrenal glands and found in cultured neurons, glia, and astrocytes [[32](#_ENREF_32)]. As reviewed by De Boussac et al [[33](#_ENREF_33)] other proteins that received attention include cytoplasmic oxysterol-binding protein (OSBP), oestrogen receptors and sterol regulating binding element. In recent years these receptors have been considered as therapeutic targets for AD [[31](#_ENREF_31)].

Oxysterol and receptor interaction drives down stream process of lipid biosynthesis, metabolism or catabolism. Activated LXR receptors up-regulate its target genes of ABC (ATP Binding Cassette transporter isoforms A1, G1, G5 and G8), ApoE (Apolipoprotein E), CETP (CholEsterylester Transfer Protein), FAS (Fatty Acid Synthase), CYP7A1 (Cytochrome P450 isoform 7A1 - cholesterol 7α-hydroxylase), LPL (Lipoprotein Lipase), SREBP-1c ( Sterol Regulatory Element Binding Protein 1c), ChREBP (Carbohydrate Regulatory Element Binding Protein). As reviewed in Edward et al.,[[34](#_ENREF_34)] these LXR target genes encode proteins that have critical roles in regulating either the catabolism of cholesterol to bile acids (CYP7A1), the synthesis of fatty acids from precursors (SREBP-1c and fatty acid synthase, FAS), the metabolism of plasma lipoproteins (cholesterol ester transfer protein, CETP, and LPL), or the trans membrane transport of phospholipids and/or sterols (ABCA1, ABCG1, ABCG5, ABCG8).

LXR signalling impacts the development of AD pathology through multiple pathways due to its mechanistic link between ABC1, ApoE and amyloid beta clearance. While genetic inactivation of either LXRα or LXRβ reduced the levels of both ApoE and ABCA1 protein in APP/PS1 mice and exacerbated the plaque pathology[[35](#_ENREF_35)]. LXRs ligand activation in Tg2576 mice enhanced, the expression of *APOE,ABCA1* down regulated APP processing and Aβ production with significant improvement in memory functions[[36](#_ENREF_36)].

**Possible implications**

In an inflammatory milieu, where oxidative stress is observed, it is likely that oxysterol concentrations are increased. Elevated levels of oxysterols are reported in vascular lesions, particularly 27-OHC and 7αOHC[[36](#_ENREF_36)].On chronic inflammatory exposure, oxysterols accumulate, release to circulation and incorporated to LDL for removal thereby leading to foam cell formation and overt disease. Relative solubility of oxysterols facilitates cross the BBB and could increase neuroinflammation and Aβ accumulation years before the impairment of memory is diagnosed. Whilst the beneficial effects of statins against cardiovascular disease have been attributed to reduction in cholesterol, others have suggested that benefit is due to oxidised LDL removal [[30](#_ENREF_30)]. Indeed, clinical trials showing protective effects of statins against AD do not show that a reduction in plasma cholesterol correlates with reduced risk for AD, indicating an alternative mechanism of benefit [[31](#_ENREF_31)]. It is not known whether removal of LDL or oxidised lipids accounts for the protective effects of statin-usage in mid-life against later AD development.

**References**

1 WHO. (2012) Dementia: A public health priority. World Health Organisation

2 Mayeux, R. (2003) Epidemiology of neurodegeneration. Annual Review of Neuroscience. **26**, 81-104

3 Fratiglioni, L., De Ronchi, D. and Agüero-Torres, H. (1999) Worldwide Prevalence and Incidence of Dementia. Drugs & Aging. **15**, 365-375

4 Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L. and Pericak-Vance, M. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science. **261**, 868-869

5 Slooter, A. J., Cruts, M., Kalmijn, S., Hofman, A., Breteler, M. M., Van Broeckhoven, C. and Van-Duijn, C. M. (1998) Risk estimates of dementia by apolipoprotein e genotypes from a population-based incidence study: The rotterdam study. Archives of Neurology. **55**, 964-968

6 Wijsman, E. M., Pankratz, N. D., Choi, Y., Rothstein, J. H., Faber, K. M., Cheng, R., Lee, J. H., Bird, T. D., Bennett, D. A., Diaz-Arrastia, R., Goate, A. M., Farlow, M., Ghetti, B., Sweet, R. A., Foroud, T. M. and Mayeux, R. (2011) Genome-wide association of familial late-onset Alzheimer's disease replicates BIN1 and CLU and nominates CUGBP2 in interaction with APOE. PLoS Genet. **7**, e1001308

7 Davinelli, S., Intrieri, M., Russo, C., Di Costanzo, A., Zella, D., Bosco, P. and Scapagnini, G. (2011) The "Alzheimer's disease signature": potential perspectives for novel biomarkers. Immunity & ageing : I & A. **8**, 7

8 Luchsinger, J. A., Reitz, C., Honig, L. S., Tang, M. X., Shea, S. and Mayeux, R. (2005) Aggregation of vascular risk factors and risk of incident Alzheimer disease. Neurology. **65**, 545-551

9 Polidori, M. C., Mattioli, P., Aldred, S., Cecchetti, R., Stahl, W., Griffiths, H., Senin, U., Sies, H. and Mecocci, P. (2004) Plasma antioxidant status, immunoglobulin g oxidation and lipid peroxidation in demented patients: relevance to Alzheimer disease and vascular dementia. Dementia and geriatric cognitive disorders. **18**, 265-270

9a Dias, I.H.K., Polidori, M.C., Weber, D., Stahl, W., Nelles, G., Grune, T. and Griffiths, H.R. (2014) Plasma levels of HDL and carotenoids are lower in dementia patients with vascular comorbidities. J. Alzheimers Dis. 40,399–408

10 Griffiths, H. R., Aldred, S., Dale, C., Nakano, E., Kitas, G. D., Grant, M. G., Nugent, D., Taiwo, F. A., Li, L. and Powers, H. J. (2006) Homocysteine from endothelial cells promotes LDL nitration and scavenger receptor uptake. Free radical biology & medicine. **40**, 488-500

11 Pratico, D., Clark, C. M., Liun, F., Rokach, J., Lee, V. Y. and Trojanowski, J. Q. (2002) Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Arch Neurol. **59**, 972-976

12 Kivipelto, M., Helkala, E., Laakso, M., Hänninen, T., Hallikainen, M., Alhainen, K., Soininen, H., Tuomilehto, J. and Nissinen, A. (2001) Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. BMJ. **322**, 1447-1451.

13 Notkola, I. L., Sulkava, R., Pekkanen, J., Erkinjuntti, T., Ehnholm, C., Kivinen, P., Tuomilehto, J. and Nissinen, A. (1998) Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. Neuroepidemiology. **17**, 14-20

14 Cramer, C., Haan, M. N., Galea, S., Langa, K. M. and Kalbfleisch, J. D. ( 2008) Use of statins and incidence of dementia and cognitive impairment without dementia in a cohort study. Neurology. **71**, 344-350

15 Li, G., Larson, E. B., Sonnen, J. A., Shofer, J. B., Petrie, E. C., Schantz, A., Peskind, E. R., Raskind, M. A., Breitner, J. C. and Montine, T. J. (2007) Statin therapy is associated with reduced neuropathologic changes of Alzheimer disease. Neurology. **69**, 878-885

16 Chauhan, N. B., Siegel, G. J. and Feinstein, D. L. (2004) Effects of lovastatin and pravastatin on amyloid processing and inflammatory response in TgCRND8 brain. Neurochem Res. **29**, 1897-1911

17 Kahles, T., Luedike, P., Endres, M., Galla, H. J., Steinmetz, H., Busse, R., Neumann-Haefelin, T. and Brandes, R. P. (2007) NADPH oxidase plays a central role in blood-brain barrier damage in experimental stroke. Stroke. **38**, 3000-3006

18 Pelegrí, C., Canudas, A. M. and sel-Valle, J. (2007) Increased permeability of blood-brain barrier on the hippocampus of a murine model of senescence. Mechanisms of Ageing and Development **128**, 522-528

19 Sureda, F. X., Gutierrez-Cuesta, J., Romeu, M., Mulero, M., Canudas, A. M., Camins, A., Mallol, J. and Pallàs, M. ( 2006) Changes in oxidative stress parameters and neurodegeneration markers in the brain of the senescence-accelerated mice SAMP-8. Exp Gerontol. **41**, 360-367

20 Parathath, S. R., Parathath, S. and Tsirka, S. E. (2006) Nitric oxide mediates neurodegeneration and breakdown of the blood-brain barrier in tPA-dependent excitotoxic injury in mice. J. Cell. Sci. **119**, 339-349

21 Vila, A., Levchenko, V. V., Korytowski, W. and Girotti, A. W. (2004) Sterol carrier protein-2-facilitated intermembrane transfer of cholesterol- and phospholipid-derived hydroperoxides. Biochemistry. **43**, 12592-12605

22 Vance, J. E., Hayashi, H. and Karten, B. (2005) Cholesterol homeostasis in neurons and glial cells. Semin Cell Dev Biol. **16**, 193-212

23 Abbott, N. (2002) Astrocyte-endothelial interactions and blood-brain barrier permeability. J Anat. **200**, 629-638

24 Yu, L., von Bergmann, K., Lutjohann, D., Hobbs, H. H. and Cohen, J. C. (2004) Selective sterol accumulation in ABCG5/ABCG8-deficient mice. J Lipid Res. **45**, 301-307

25 Iuliano, L. (2011) Pathways of cholesterol oxidation via non-enzymatic mechanisms. Chemistry and Physics of Lipids. **164**, 457-468

26 Lund, E. G., Guileyardo, J. M. and Russell, D. W. (1999) CDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. Proc. Natl. Acad. Sci. USA,. **96**, 7238-7243

27 Heverin, M., Meaney, S., Lütjohann, D., Diczfalusy, U., Wahren, J. and I, B. (2005) Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain and possible consequences for cerebral cholesterol homeostasis. J. Lipid Res. **46**, 1047-1052

28 Dias, I. H. K., Mistry, J., Fell, S., Reis, A., Spickett, C. M., Polidori, M. C., Lip, G. Y. H. and Griffiths, H. R. (2014) Oxidised LDL-lipids increase beta amyloid production by SH-SY5Y cells through glutathione depletion and lipid raft formation. FRBM. **under review**

29 Gamba, P., Guglielmotto, M., Testa, G., Monteleone, D., Zerbinati, C., Gargiulo, S., Biasi, F., Iuliano, L., Giaccone, G., Mauro, A., Poli, G., Tamagno, E. and Leonarduzzi, G. (2014) Up-regulation of b-amyloidogenesis in neuron-like human cells by both 24- and 27-hydroxycholesterol: protective effect of N-acetyl-cysteine. Aging Cell **13**, 561-572

30 Olkkonen, V. M. and Hynynen, R. (2009) Interactions of oxysterols with membranes and proteins. Mol. Aspects Med. **30**, 123-133

31 Jakobsson, T., Treuter, E., Gustafsson, J. Å. and Steffensen, K. R. (2012) Liver X receptor biology and pharmacology: new pathways, challenges and opportunities. Trends in Pharmacological Sciences. **33**, 394-404

32 Mandrekar-Colucci, S. and Landreth, G. E. (2011) Nuclear receptors as therapeutic targets for Alzheimer's disease. Expert Opinion on Therapeutic Targets. **15**, 1085-1097

33 De Boussac, H., Alioui, A., Viennois, E., Dufour, J., Trousson, A., Vega, A., Guy, L., Volle, D. H., Lobaccaro, J. M. and Baron, S. (2013) Oxysterol receptors and their therapeutic applications in cancer conditions. Expert Opin Ther Targets., 1029-1038

34 Edwards, P. A., Kennedy, M. A. and Mak, P. A. ( 2002) LXRs; oxysterol-activated nuclear receptors that regulate genes controlling lipid homeostasis. Vascul Pharmacol. **38**, 249-256

35 Zelcer, N., Khanlou, N., Clare, R., Jiang, Q., Reed-Geaghan, E. G., Landreth, G. E., Vinters, H. V. and Tontonoz, P. (2007) Attenuation of neuroinflammation and Alzheimer's disease pathology by liver x receptors. Proc Natl Acad Sci USA. **104**, 10601-10606

36 Donkin, J. J., Stukas, S., Hirsch-Reinshagen, V., Namjoshi, D., Wilkinson, A., May, S., Chan, J., Fan, J., Collins, J. and Wellington, C. L. ( 2010) ATP-binding cassette transporter A1 mediates the beneficial effects of the liver X receptor agonist GW3965 on object recognition memory and amyloid burden in amyloid precursor protein/presenilin 1 mice. J Biol Chem. **285**, 34144-34154