Published in final edited form as:

J Alzheimers Dis. 2013 ; 33(4): 891–911. doi:10.3233/JAD-2012-121585.

Brain Cholesterol Metabolism, Oxysterols, and Dementia

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Abstract

Cholesterol metabolism is implicated in the etiology of Alzheimer's disease (AD) and amyloid production in the brain. While brain cholesterol cannot be measured directly *in vivo*, the oxysterol, 24S-hydroxycholesterol (24-OHC), is the predominant metabolite of brain cholesterol and can be measured in the blood. The aim of this review is to evaluate plasma 24-OHC as a potential biomarker of AD risk and discuss factors related to its levels in the brain and blood. This systematic review examines studies published between 1950 and June 2012 that examined the relationship between plasma 24-OHC, cognition, brain structure, and dementia using the following key words ("24S-hydroxycholesterol" or "24-hydroxycholesterol") and ("Brain" or "Cognitive"). We found a total of 28 studies of plasma 24-OHC and neurodegenerative disease, including a subset of 12 that used dementia as a clinical endpoint. These studies vary in the direction of the observed associations. Results suggest plasma 24-OHC may be higher in the early stages of cognitive impairment and lower in more advanced stages of AD when compared to cognitively normal controls. Measures of 24-OHC in the blood may be an important potential marker for cholesterol metabolism in the brain and risk of AD. Further studies of plasma 24-OHC and dementia must account for the stage of disease, establish the temporal trends in oxysterol concentrations, and employ neuroimaging modalities to assess the structural and metabolic changes occurring in the brain prior to the onset of cognitive impairment.

Keywords

Alzheimer's disease; brain; dementia; 24-hydroxycholesterol; 24S-hydroxycholesterol; oxysterols

INTRODUCTION

Aberrant cholesterol metabolism has been implicated in the development of Alzheimer's disease (AD) since 1906 when Alois Alzheimer first described 'lipoid granules' along with amyloid- β (A β) plaques and phosphorylated tau (p-tau) tangles as the pathological hallmarks of AD [1]. Cholesterol's role in AD remained largely ignored until the discovery of apolipoprotein E (ApoE) as the primary genetic risk factor for AD. ApoE is known to be the

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Authors' disclosures available online (<http://www.j-alz.com/disclosures/view.php?id=1526>).

primary mechanism of cholesterol transport within the brain; however, its direct involvement in AD pathogenesis is unknown and remains an important topic in AD research. Recently, large consortium studies have identified several genes involved in cholesterol transport and metabolism that increase AD susceptibility [2, 3].

The relationship between blood cholesterol and AD is tenuous. Observational studies of blood cholesterol suggest that elevated levels in midlife are associated with an increased risk of developing dementia [4]; however, studies of cholesterol in older adults suggest no elevated risk and statin trials for the treatment and prevention of dementia have failed to show benefit [5, 6]. A compelling explanation is that cholesterol does not cross the blood brain barrier (BBB) directly. The BBB effectively creates two pools of cholesterol in the body. Blood cholesterol is likely to be a poor surrogate marker of cholesterol homeostasis in the brain because it provides little information about cholesterol levels in the brain [7]. 24S-hydroxycholesterol (24-OHC) is the primary metabolite of brain cholesterol and can be measured in the cerebrospinal fluid (CSF) and blood [7]. 24-OHC levels in the CSF and blood are seen to have great potential as being more proximate markers of cholesterol homeostasis in the brain.

This review is organized into three major sections. First, we review cholesterol synthesis and metabolism to 24-OHC in the brain. Then, we review the available literature regarding oxysterols and age-related neurodegeneration, with specific focus given to dementia and cognitive impairment. Finally, we discuss these findings in the context of potential mechanisms linking excess cholesterol in the brain to the development of dementia.

CHOLESTEROL SYNTHESIS AND METABOLISM IN THE BRAIN

Dysregulation of cholesterol homeostasis may lead to states of excess cholesterol levels in the brain. Potential sources of excess cholesterol in the brain are likely to include: excessive synthesis from glial cells, plasma membrane breakdown, myelin breakdown, and neuronal loss. While hypercholesterolemia in the blood can come from *de novo* synthesis and dietary sources, hypercholesterolemia in the brain is likely to be only affected by *de novo* synthesis and metabolism [7]. In the brain, cells must produce cholesterol or import it from neighboring cells. This process ensures brain cholesterol is derived locally and its homeostasis is dependent upon the metabolism of cholesterol to oxysterols.

The oxysterol 24-OHC is produced enzymatically by CYP46A1 (24-hydroxylase) within the neuron. Genetic variation in 24-hydroxylase enzyme not only alters 24-OHC production but it is associated with an increased load of A β [8] and AD (see review of CYP46A1 and AD by Garcia et al. [9]). 24-hydroxylase metabolizes cholesterol with the addition of a hydroxyl group at the 24-position. The additional hydroxyl group makes the 24-OHC molecule more lipophilic and enables it to cross the BBB directly by diffusion. It is estimated that 90% of the 24-OHC in the blood originates in the central nervous system, with less than 10% originating from the lungs, adrenals, bone marrow, and other peripheral sources [7]. Its ability to diffuse directly into the blood makes of 24-OHC a less invasive and direct marker of cerebral cholesterol metabolism [10].

The peripherally-derived oxysterol 27-hydroxycholesterol (27-OHC) also crosses the BBB directly by diffusion in a flow counter to 24-OHC. 27-OHC is an endogenous selective estrogen receptor modulator [11] and is suspected to have impact on estrogen receptor containing tissues of the vasculature, bone, and breast. 27-OHC is used in studies of oxysterols to indicate oxidative cholesterol metabolism in the periphery. Its effects within the brain are unknown. However, it is not expected to revert back to molecular cholesterol and contribute to the pool of cholesterol in the brain.

The vast majority of brain cholesterol is stored in the myelin [12]. Myelin disruption is likely to be an important source of excess cholesterol in the brain during the aging and neurodegenerative disease processes. Myelin breakdown is a common phenomenon of aging [13], and the most vulnerable myelin are produced later in life [13]. Myelin and its constituents are reduced in old age and substantially further reduced in mild cognitive impairment (MCI) and dementia [13]. *In vitro* evidence demonstrates that breakdown of the myelin releases fat, iron, and cholesterol into the CSF and extracellular space leading to excess cholesterol levels and neuronal death (reviewed by Bartzokis [13]). Myelin breakdown would be expected to increase levels of free, unesterified cholesterol in the brain and the production of 24-OHC.

The early stages of myelin breakdown would be expected to lead to the increased production of 24-OHC in the brain and elevated levels of 24-OHC in the CSF and plasma. A progressive course of demyelination—characterized by pervasive synaptic and neuronal loss leading to progressive atrophy—will result net loss of 24-hydroxylase and net shift in 24-OHC production. During this process, plasma levels of 24-OHC are expected to remain elevated until the number of metabolically active neurons in the brain cannot keep pace with excess cholesterol in the brain. With extensive neuronal loss and subsequent loss of 24-hydroxylase, 24-OHC production and diffusion into the blood is expected to decline in step with severity. However, the relationship between neuronal loss and 24-OHC production may not be so simple for AD. Bogdanovic et al. noted an induction of CYP46A1 in astrocytes of AD patients, but not in controls [14], suggesting aberrant and compensatory mechanisms of cholesterol metabolism at work in AD.

Cholesterol metabolism may play a key role in amyloidogenesis in the brain. Experimental animal and cell culture studies suggest that excess cholesterol in the brain increases amyloid production. *In vitro* studies show that plasma membranes containing high levels of cholesterol are more likely to lead to a primary cleavage of the C99 terminal of the A β protein precursor (A β PP) by γ -secretase to release A β polypeptides [15]. Recently, a cholesterol binding site on C99 terminal of A β PP was found by Barrett et al. [16]. While the functional consequences of cholesterol binding to A β PP remains unknown, this mechanism may provide further insight into the role of cholesterol in amyloidogenesis [16]. The deposition and aggregation of A β as neuritic plaques is believed to lead to widespread loss of neurons and synapses [17]. In turn, neuronal and synaptic degeneration destroys myelin, releasing excess cholesterol and other myelin constituents in to the extracellular space [17] (see Fig. 1).

Previous studies of oxysterols in various neurodegenerative diseases suggest that 24-OHC is a marker of the number of metabolically active neurons containing 24-hydroxylase and may be associated with various neurodegenerative diseases [10]. Early reports indicate 24-OHC is associated with the diagnosis and severity of dementia; however, the direction of associations between 24-OHC and dementia are inconsistent. This review details these studies of plasma 24-OHC and age-related cognitive impairment, giving specific focus to whether 24-OHC is a potential marker of the changes in cholesterol homeostasis occurring in the brain early in the dementia process and its usefulness as a potential biomarker of dementia. We provide a detailed overview of the issues regarding the measurement of 24-OHC as a marker of cholesterol metabolism in relation to the natural history of dementia, specifically AD. Finally, we discuss these findings in the context of potential mechanisms linking excess cholesterol in the brain to the development of dementia.

METHODS

Search strategy

Original articles and reviews were identified by search of Ovid MEDLINE® for articles published between 1950 and the third week of June 2012. Searches utilized MeSH terms (“24S-hydroxycholesterol” or “24-hydroxycholesterol”) and (“Brain” or “Cognitive”). The relative sensitivity of this search strategy was evaluated by reconditioning the search to include addition of the keyword “oxysterol”. The search was repeated using alternative keywords (“oxysterols” and (“Brain” or “Cognitive”)) not (“24S-hydroxycholesterol” or “24-hydroxycholesterol”). This latter search provided no additional articles relating 24-OHC levels to neurodegenerative disease.

Selection criteria

All observational and experimental studies in humans investigating cholesterol metabolism in the brain and its relationship to neurodegeneration and cognition were considered. We chose to limit our review to studies evaluating 24-hydroxycholesterol in human subjects and cell lines ($n = 93$). Only original articles involving human subjects were considered for this review; we eliminated 17 *in vitro* and 20 review articles which left 51 suitable articles. These 51 articles were evaluated and categorized according to the following criteria. Pertinent studies included in this review focused on: 1) oxysterols and neurodegenerative diseases (including AD, multiple sclerosis (MS), Huntington’s disease); 2) population based genetics studies of CYP family hydroxylases; 3) extracellular metabolism of cholesterol in the brain; and 4) interventions modifying oxysterol metabolism. Articles investigating the relationship between oxysterol levels in human subjects with neurodegenerative disease were evaluated for the use of magnetic resonance imaging (MRI) modalities. Only eight studies were found which utilized MRI when evaluating the association between levels of oxysterols and neurodegenerative diseases. The selection of articles for review was then limited to articles pertaining to the dementia process, including: dementia, Alzheimer’s disease, mild cognitive impairment, and studies with cognitively normal participants. All review articles were assessed for references potentially missed by Ovid MEDLINE searches. One reviewer (TMH) prepared searches, applied the selection criteria, and collected articles

relevant to the topic. See Fig. 2 for a diagram of the search strategy used to identify articles for the present review.

Methods used to measure oxysterols

The measures of plasma oxysterols from studies investigating the relationship between oxysterols and dementia and cognitive decline are listed in Table 1. Nearly all of the studies of neurodegenerative disease measuring plasma or CSF levels of oxysterols use a gas chromatography-mass spectroscopy (GC/MS) and isotope dilution mass spectroscopy (IDMS) method introduced by Dzeletovic et al. [18]. It is important to understand this technique is not standardized and actual values obtained from this method may differ by study and laboratory. Therefore, we advise caution when comparing the levels of oxysterols between studies and laboratories. Briefly, this technique involves the addition of deuterated internal standards for each oxysterol and butylated hydroxytoluene as an antioxidant. The samples then undergo saponification to release unesterified oxysterols. Solid-phase extraction employing a silica column removes cholesterol with a hexane wash and oxysterols are eluted with propanol/hexane mixtures. The samples are then converted into trimethylsilyl ester derivatives prior to GC/MS. The identification of ions for oxysterols is done in the single ion monitoring mode. The limit of detection for 24-OHC using this assay is 2 ng/mL at a signal to noise ratio of 3: 1 (not reported for 27-OHC). Intra- and inter-assay coefficients of variation were 3.7% and 3.9% for 24-OH, respectively and 4.6% and 3.9% for 27-OH, respectively [19, 20].

Two studies of oxysterols and cognitive change [21, 22] utilized flame ion detection instead of mass spectroscopy. In general, this technique is less sensitive than GC/MS and is entirely dependent on retention time for identification of analytes. Despite these limitations, van den Kommer et al. reported lower inter- and intra-assay coefficient of variation for 24-OHC than IDMS of 4% and 3%, respectively [22].

Recently, alternative techniques for oxysterol assay have been developed using liquid chromatography-mass spectroscopy [23–25]. These methods do not require derivatization of oxysterol analytes prior to analysis. These techniques still utilize saponification and solid-phase extraction. This is then followed by HPLC separation under reversed-phase column conditions and detection by mass spectroscopy using atmospheric pressure chemical ionization in the select ion monitoring mode. This method has yet to be adopted for oxysterol and neurodegenerative disease studies.

OXYSTEROLS AND NEURODEGENERATIVE DISEASE

The use of 24-OHC as a biomarker of neurodegeneration has been investigated in the context of various neurodegenerative diseases. Early reports relied on clinical definitions of disease [19, 20, 26–33]. Of the 21 studies identified using the keywords mentioned above (summarized in Tables 2–5), nine studies correlated 24-OHC with structural measures of brain disease. More recent publications aimed to correlate levels of 24-OHC with structural or biochemical imaging of the brain [34–39].

Studies of 24-OHC in non-dementia neurodegenerative diseases

Demyelinating diseases degrade the myelin, where the vast majority of brain cholesterol is stored. They provide unique insight into cholesterol metabolism during the neurodegenerative disease process (detailed in Table 2). In studies of Guillain-Barre syndrome and inflammatory demyelinating polyradiculoneuropathy, the absolute levels of 24-OHC in the CSF and the ratio of 24-OHC/27-OHC in the plasma were higher in patients with compared to controls [19]. Plasma and CSF levels of 24-OHC depend upon severity and duration of MS [10]. In patients with MS, levels of 24-OHC in the plasma and CSF increase during remitting-relapsing and early stages of disease [19]. Later stages of disease result in consistently lower levels of 24-OHC in the plasma [19, 34, 35]. Among MS patients, levels of 24-OHC are higher in younger patients and decrease with increasing age and disability scores [19, 35]. This hypothesized trajectory of plasma 24-OHC across the stages of MS progression can provide an important framework for evaluating plasma oxysterol concentrations in other progressive neurodegenerative disease, including dementia and cognitive impairment.

Case-control studies of 24-OHC and dementia using diagnostic outcomes

Dementia and absolute plasma levels of 24-OHC—Case-control studies found significant associations between 24-OHC and dementia sub-types as well as the severity of AD. However, these studies (presented in Table 3) also indicate inconsistencies in the direction of association. Studies of newly diagnosed patients report that the absolute levels of plasma 24-OHC were higher in AD, vascular disease (VaD), and MCI patients compared to both cognitively normal controls [27, 28]. A smaller study of 14 AD and 10 healthy controls reported the absolute plasma 24-OHC levels were higher in AD cases compared to controls (60 ± 19 versus 53 ± 14 ng/mL, respectively); however, the differences were not statistically significant at $p < 0.05$ [29]. Interestingly, the levels of plasma 24-OHC levels may not differ between patients with MCI and dementia sub-types (AD and VaD) [28].

When AD is stratified by early versus late stage of disease, the levels of 24-OHC were found to be slightly higher in the early stages compared to later stages of AD [27]. Plasma 24-OHC levels were reported to be highest in recently diagnosed AD patients and lowest in patients with longer durations of VaD in a recent study by Zuliani et al. [40]. Additionally, patients with cognitive impairment that did not meet standard criteria for dementia had plasma levels of 24-OHC similar to those of cognitively normal controls [40]. This initial increase in plasma 24-OHC has also been reported in studies of Huntington's disease and MS [10, 19, 37]. Lower plasma levels of 24-OHC correlate with lower global cognitive scores, using The Mini Mental State Exam (MMSE) [28]. Further, severe AD and having the ApoE4 allele were independently associated with both lower absolute levels of 24-OHC and a lower ratio of 24-OHC relative to cholesterol in the plasma [27]. These results suggest plasma 24-OHC levels decline with longer duration of disease and poorer memory performance.

Three cross-sectional studies found that absolute levels of 24-OHC were significantly lower in patients with AD compared to controls [26, 39, 41]. These results were likely a result of longer disease duration and the use of patients with memory complaint as controls subjects. The first study included AD patients with a duration of disease of four years or more and

compared their levels of plasma 24-OHC with cognitively normal controls [26]. They report that the levels of plasma 24-OHC were lower in patients with advanced AD than in controls. Another study found the levels of 24-OHC were also lower in newly diagnosed cases of AD and MCI when compared with patients reporting subjective memory complaint at a university memory clinic [39]. In this study, individuals with subjective cognitive impairment who did not meet objective criteria for cognitive impairment were used as controls. The presence or extent of cerebrovascular disease was not reported for individuals with subjective memory complaint. Therefore, the structural brain integrity and the reason for their subjective memory complaint remain unknown in these individuals and may complicate interpretation. A recent study from the same group [41] using a similar patient population that reported memory complaint to a hospital memory clinic used objective CSF biomarkers to characterize participants with an AD-like CSF profile. The AD-like CSF profile represented individuals with abnormally low CSF A β ₄₂ and abnormally high CSF total tau levels to differentiate these individuals from other patients with more normal CSF levels. Patients identified with an AD-like CSF profile had lower plasma 24-OHC and 27-OHC levels compared with patients without CSF abnormalities and showed no differences in CSF levels of either measured oxysterol. This study also reported that the degree of white matter disease was similar between the AD-like and CSF-normal groups [41]. However, the presence of other MRI defined markers of cerebrovascular disease, such as infarcts and microbleeds, were not discussed. More importantly, all participants in these two studies were referred to a hospital memory clinic with memory complaint. Therefore, these studies made no direct comparisons of the levels of plasma 24-OHC between cognitively impaired individuals and cognitively normal controls without memory complaint.

Collectively, these studies indicate that plasma 24-OHC may be higher in the early stages of a neurodegenerative process and decline with disease severity. Plasma levels of 24-OHC across the dementia process may follow a similar trajectory as hypothesized in MS (Fig. 3). These studies suggest that plasma 24-OHC is, potentially, a useful marker in the early stages of the dementia and cognitive impairment.

24-OHC measures relative to other markers associated AD in the plasma and CSF—In the blood, oxysterols (24-OHC and 27-OHC) are carried through the circulation by high density (HDL) and low density (LDL) lipoproteins [42]. Oxysterols are removed from the circulation through lipoprotein transport to the liver for metabolism to bile acids. Because these are the same lipoproteins that carry cholesterol [42], it is possible that oxysterols and cholesterol compete for space within the lipoproteins. In the absence of preferential binding, the ratio of plasma oxysterols relative to cholesterol may provide more information than the absolute levels of oxysterols alone. This raises two interesting points. First, it is unknown whether binding affinity of HDL and LDL to oxysterols affects their levels in the periphery and brain. Because oxysterols cross the BBB directly by diffusion, binding affinity may have no effect on the rate of 24-OHC entering the blood. On the other hand, free 27-OHC may enter the brain more readily if it is unbound. Second, the potential competitive binding between oxysterols and cholesterol for space within the lipoprotein would be on different scales. Plasma oxysterols constitute a relatively small portion (ng/mL)

of the constituents of lipoproteins while cholesterol contributes a greater proportion of the lipoprotein content (mg/mL).

While the absolute levels of plasma 24-OHC appear to increase in the early stages of dementia, the ratio of 24-OHC to total circulating cholesterol (24-OHC/Chol) may be lower in AD, VaD, and MCI compared to controls [32]. Additionally, lower ratios of plasma 24-OHC/Chol are associated with severity of disease and established biomarkers of AD, including ApoE4 allele [27, 28, 39]. The ratio of 24-OHC/Chol was also negatively correlated with worse performance on cognitive tests of memory recall and positively correlated with tests of frontal/executive function [40]. Together, these findings suggest that the ratio of plasma 24-OHC/Chol decline with AD severity and poor performance on cognitive tests of memory and executive function; however, ratio of 24-OHC/Chol appears be lower in both dementia and MCI compared to controls. Unfortunately, most studies reporting the ratio of 24-OHC/Chol neither report the individual measures of 24-OHC and total cholesterol, nor test the individual markers for their between-group differences. Without the reporting of the individual levels of 24-OHC or total cholesterol and testing their associations with AD, interpretations are limited. For example, a high ratio of 24-OHC/Chol may represent: 1) elevated 24-OHC relative to normal cholesterol levels or 2) average 24-OHC relative to low cholesterol levels. Without this information, the reader is unable to determine whether each measure is independently associated with AD or where the absolute level of 24-OHC elevated within the individual.

The ratio of plasma 24-OHC relative to 27-OHC (24-OHC/27-OHC) is used to represent oxidative cholesterol metabolism in the brain relative to the periphery. The plasma ratio of 24-OHC/27-OHC was shown to be higher in AD, VaD, and MCI relative to cognitively normal controls [32]. In a recent study, we measured the ratio of 24-OHC/27-OHC using retrospective stored plasma samples from cognitively normal individuals who were followed longitudinally with repeated cognitive assessments over 8 years [43]. We found that cognitively normal participants with a higher ratio of 24-OHC/27-OHC had a greater likelihood of developing incident MCI and AD over 8 years of follow-up. The ratios were similar for participants with incident MCI and AD. These two studies suggest that the plasma levels of 24-OHC are higher relative to 27-OHC in individuals with dementia and MCI as well as in cognitively normal individuals who go on to develop cognitive impairment.

The relationship between ApoE genotype and the levels of 24-OHC in the plasma remain unclear. ApoE genotype was an independent predictor of the plasma ratio of 24-OHC/Chol [22, 27, 28], while others [43, 44] have not found these associations between with the absolute levels of plasma 24-OHC and ApoE genotype. The lack of association between ApoE4 genotype and plasma levels of 24-OHC may be inconsequential. *In vitro*, 24-OHC regulates ApoE synthesis and secretion by astrocytes dose dependently, implicating these oxysterols as key players in liver X receptor-mediated cholesterol homeostasis within the CNS [45]. A considerable number of other factors are related to circulating ApoE levels in the plasma.

Measurements of 24-OHC taken from spinal fluid is believed to reflect active neurodegeneration and are more consistently related to dementia than measurements from the plasma [46]. CSF levels of 24-OHC were found to be consistently higher in MCI and AD patients [31, 33, 47] compared to controls. CSF concentrations of 24-OHC were significantly, yet slightly, elevated in MCI patients compared to controls [47]. In participants with normal cholesterol levels, CSF 24-OHC levels were significantly higher in AD patients compared to controls [29]. The levels of 24-OHC in the CSF increased with the number of ApoE4 alleles [47] and levels of total and phosphorylated tau in the CSF [20]; however, the levels of 24-OHC were not correlated with A β in the CSF [33]. Using autopsied brain tissue samples, Heverin and colleagues measured tissue concentrations of oxysterols and cholesterol in the frontal cortex, occipital cortex, basil ganglia, and pons of decedent AD subjects and matched controls. They reported slightly lower concentrations of 24-OHC and higher 27-OHC for AD subjects compared to controls in these regions. This resulted in a ratio of 24-OHC to 27-OHC in the frontal and occipital cortices, basil ganglia, and pons of decedent AD subjects compared to matched controls. These results show that patients who died from AD have slightly lower levels of 24-OHC and increased levels of 27-OHC in the brain, despite little to no differences in cholesterol levels in these same regions. This data suggests that AD patients may have an increased production and infiltration of 27-OHC into the brain from the periphery. Despite having autopsied brain samples, the authors did not control for brain volume or weight and no designation was made for the amount of healthy or diseased brain tissue [31].

The blood levels of 24-OHC and the ratio of 24-OHC/Chol are positively and significantly correlated with established CSF markers of AD, including total tau and phosphorylated tau [39]. The 24-OHC/Chol ratio in plasma is also positively associated with CSF levels of A β ₄₂, total and phosphorylated tau after controlling for age, gender, ApoE4 status, and statin therapy [39]. Interestingly, the levels of oxysterol in the plasma, but not of CSF, were more strongly associated with AD-like CSF biomarkers [41]. These participants with a combination of abnormally low A β ₄₂ and abnormally high total tau CSF profiles had lower levels of plasma 24-OHC and 27-OHC [41].

Studies of 24-OHC and longitudinal cognitive decline

Two studies assessed the relationship between 24-OHC and cognitive decline [21, 22] using longitudinal changes in cognitive test scores (Table 4). They found that plasma 24-OHC was not related to cognitive decline over multiple years of follow-up. However, a higher ratio of 24-OHC/Chol in the blood was associated with a slower rate of decline in processing speed in a small group of adults ($n = 65$) followed for 6 years and ranging in age between 30 to 80 years [21]. Associations with changes in other cognitive domains were not significant. This study only reported the ratio of 24-OHC/Chol and did not report the absolute plasma levels of 24-OHC for the control and patient groups. Cholesterol levels were lower yet not significantly different between AD patients and controls. The absence of the absolute levels of 24-OHC makes it difficult to evaluate any relationship between 24-OHC and AD. In a larger cohort of more than 1000 community-dwelling older adults age 65 and older, van den Kommer et al. found that the absolute levels of both 24-OHC and 27-OHC were correlated with individual cognitive performance at both baseline and follow-up, but not the change in

cognitive performance over time [22]. While the absolute levels of oxysterols were not associated with changes in any other cognitive domain, a lower ratio of 24-OHC/Chol was associated with declines in speed of processing over 6 years. The ratio 24-OHC/Chol was significantly higher in ApoE4 negative participants. The interaction between the ratio of 24-OHC/Chol and the ApoE4 allele carrier status suggested that ApoE4 is a potential moderator of the association between the ratio 24-OHC/Chol and the speed of processing. In our study of incident cognitive impairment based on cognitive testing and neuropsychiatric batteries, we observed participants that went on to develop incident cognitive impairment had significantly higher plasma levels of 24-OHC and slightly lower 27-OHC concentrations compared to those who were cognitively normal [43]. It is important to note that these differences were evident prior to the development of clinical symptoms and all participants were cognitively normal at the time of blood draw. Nevertheless, the levels of 24-OHC and the ratio of 24-OHC/27-OHC were similar for participants with incident MCI and AD.

Studies of 24-OHC utilizing structural neuroimaging

Research using 24-OHC measures and neuroimaging markers may provide insight into the relationship between biochemical changes in cholesterol metabolism and the structural changes occurring in the brain of aging individuals and those with neurodegenerative diseases. Both computed tomography (CT) and MRI have recently been utilized in the study of 24-OHC and neurodegenerative diseases (reviewed in Table 5). Neuroimaging studies of MS show that levels of plasma 24-OHC are associated with markers of neuronal inflammation, demyelination and axonal loss. Gadolinium-enhanced lesions visible on MRI are used in MS to identify primary progressive and relapsing-remitting disease states and can be used to assess disease severity. Gadolinium-enhanced-positive lesions on MRI are believed to indicate areas of active disease where BBB disruption and ongoing perivascular disease may be occurring [19]. Higher levels of plasma 24-OHC were found only in primary progressive MS patients with active disease confirmed by gadolinium-enhanced-positive MRI scans [19].

The presence of hypointense and hyperintense lesions on T₁ and T₂ weighted MRI have also been examined in relation to levels of plasma 24-OHC in MS patients [35]. The volume of T₂-weighted hyperintense lesions is a marker of cumulative disease extent representing a spectrum of events, ranging from demyelination and edema to gliosis and axonal loss [35] in both types of MS. Decreases in the 24-OHC/Chol ratio were associated with the extent of the disease as indicated by the volume of T₂-weighted hyperintense lesions. The volume of T₁ hypointense lesions are believed to represent axonal loss. Only in relapsing-remitting MS patients was the 24-OHC/Chol ratio positively associated with increased T₁ hypointense lesions [35]. These data suggest that increases in the levels of 24-OHC relative to total cholesterol are associated with axonal loss and a lower ratio of 24-OHC/Chol is associated with severity of disease.

MRI studies of 24-OHC and brain volume show levels may be differentially associated with sub-regions of the brain as well as gray matter, white matter, and CSF segments of the brain [37–39]. The plasma levels of 24-OHC and 27-OHC in the paralleled large decreases in caudate volumes in Huntington's disease patients [37]. Lower levels of plasma 24-OHC

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were also seen in later more advanced stages of the disease [37]. The ratio of 24-OHC/Chol was positively associated with larger grey matter fractions across all subjects with AD, MCI, and subjective cognitive impairment based on segmentation of the brain into CSF, gray matter, and white matter [39]. The ratio of plasma 24-OHC/Chol was only associated with gray matter volume and total brain volume in subjects with subjective cognitive impairment, but not in AD and MCI patients. Among cognitively normal adults, lower plasma levels of 24-OHC were associated with smaller total brain volumes [39]. Both the levels of plasma 24-OHC and 27-OHC were positively associated with a larger volume of the hippocampus in cognitively normal middle-aged participants [38]; however, only 24-OHC remained associated with hippocampal volume after adjustment for age, total brain volume, and ApoE4 carrier status.

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Plasma 24-OHC is likely associated with evidence of cerebrovascular disease. A recent study utilized brain CT in AD patients, VaD, cognitive impairment, and cognitively normal controls to determine direct and indirect evidence of atrophy and of lacunar, cortical, and subcortical infarcts [40]. They reported the ratio of 24-OHC/Chol was higher in participants with evidence of brain atrophy, which ranged from 47% of controls to 80% of AD patients. The ratio of 24-OHC/Chol was lowest in patients with VaD and AD, and was negatively correlated with having multiple lacunar infarcts. In this study, the ratio of 24-OHC/Chol was not associated with having larger subcortical infarcts or only a single lacunar infarct [40]. Unfortunately, this and other prior neuroimaging studies of oxysterols and atrophy did not utilize standardized MRI procedures to distinguish normal white matter from white matter hyperintense areas on MRI.

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White matter hyperintensities (WMH) on MRI are indicative of tissue water content and myelin damage. The myelin damage indicated by WMH may be related to increased cholesterol metabolism in the brain. Recently, Besga and colleagues reported that WMH severity grade was differentially associated with CSF levels of 24-OHC. Among memory clinic patients with CSF-defined AD-like disease, CSF levels of 24-OHC was positively associated with WMH severity; among patients without an AD-like CSF profile, CSF levels 24-OHC negatively correlated with WMH severity. While the authors measured plasma oxysterols, they did report the associations between blood 24-OHC and WMH. Our recent study investigated the relationship between plasma oxysterols and cerebrovascular disease and utilized multiple MRI markers of cerebrovascular disease, including the presence of WMH and cerebral infarcts [43]. Both plasma 24-OHC and 27-OHC were higher in individuals with evidence of moderate to severe WMH and the presence of lacunar infarcts on MRI 3–4 years prior to blood draw. WMH and infarcts represent ongoing cerebrovascular disease which has a direct impact on myelin integrity.

DISCUSSION

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Cholesterol homeostasis in the brain is linked to amyloid production and to the risk of AD. The development of techniques to measure amyloid deposition in the brains of large numbers of individuals across the spectrum of cognition using positron emission tomography (PET) ligands has, for the first time, provided opportunities to determine the relationships between risk factors, host susceptibility (genetic) and amyloid deposition. To

date, most studies show that measurements of CSF biomarkers, such as A β ₁₋₄₂ or tau proteins, are more strongly related to both amyloid deposition and risk of dementia than to blood biomarkers. The blood biomarker studies have not been very productive probably because the BBB prevents most compounds of the brain from entering the peripheral blood. For this reason, 24-OHC remains an interesting surrogate marker of cholesterol metabolism in the brain and may provide insight into the cholesterol homeostasis in the brain.

Benefits and challenges to measuring plasma oxysterols?

Plasma cholesterol is probably a poor surrogate for cholesterol homeostasis in the brain because it is unable to cross the BBB. Plasma 24-OHC is likely a more proximate marker of brain cholesterol. The metabolism of cholesterol to 24-OHC enables the molecule to cross the BBB freely by diffusion. This is the primary means by which excess cholesterol is metabolized and eliminated from the brain [7]. With more than 90% of the 24-OHC in the blood originating from the brain [7], plasma 24-OHC is a less invasive, stable, and specific marker of cholesterol metabolism in the brain. Production of 24-OHC is believed to represent the number of metabolically active neurons containing the enzyme 24-hydroxylase responsible for the conversion of excess cholesterol to 24-OHC [10]. In the absence of neurodegeneration, concentrations of 24-OHC are relatively stable between the third and seventh decades of life. Beyond the sixth decade of life, plasma levels of 24-OHC begin to decline with age [7] which parallels the decline in total brain volume age [48].

Plasma levels of 24-OHC can differentiate AD patients from other neurodegenerative diseases, such as Parkinson's disease and acute neurodegeneration [30]. Observational studies show that plasma 24-OHC levels correlated with total and regional brain volumes as well as global cognition scores. They also indicate that the direction of the association between 24-OHC and dementia appears to depend upon the stage of disease at which the 24-OHC is measured. We propose that the concentrations of 24-OHC in the circulation: may be elevated in the early stages of neurodegeneration, as myelin loss is initiated; become equivalent to non-demented controls again in the middle stage, as an equilibrium is reached through increased conversion of cholesterol to oxysterols and subsequent elimination; and become lower than controls in later and progressive stages where the neuronal loss that accompanies the neurodegenerative process lowers levels of 24-hydroxylase, with subsequent reduced conversion of cholesterol to 24-OHC (Fig. 3). An important component of this proposed model is that plasma levels of 24-OHC appear unable to differentiate recently diagnosed dementia patients (AD and VaD) from MCI patients. It is possible that changes in brain cholesterol metabolism may occur early in the dementia process, long before neuronal death, brain atrophy, and cognitive impairment become apparent. Levels of plasma 24-OHC may even be highest in individuals in the early phases of cognitive impairment until significant brain atrophy occurs. A mechanism responsible for the elevation in 24-OHC in the early stages of the neurodegenerative disease process remains uncertain.

Factors such as statin use should be assessed in studies of blood cholesterol and its metabolites. The metabolism of cholesterol to oxysterols in the brain is likely dependent on brain cholesterol concentrations and affected by statin use. Small studies of patients with

normal or elevated cholesterol levels have examined the effect of various statins on 24-OHC levels in the plasma and CSF [49–52]. The ability of a statin to alter plasma 24-OHC levels appears to be largely dependent on its ability to cross the BBB. Studies demonstrated that high-dose statin therapy using lipophilic statins able to penetrate the BBB resulted in decreased levels of 24-OHC, cholesterol, and its precursors in the blood [51]. Potent lipophilic statins, such as atorvastatin and simvastatin, can cross the BBB and suppress cholesterol synthesis both in peripheral tissue and brain leading to decreases in the absolute levels of plasma 24-OHC [51]. Conversely, pravastatin is considerably more hydrophilic and less likely to cross the BBB and affect 24-OHC levels. As a result, the ratio of plasma 24-OHC/Chol is expected to be higher in patients treated with pravastatin compared to patients treated with the BBB penetrating statins, atorvastatin and simvastatin.

Summary and future directions

Epidemiological and clinical studies suggest that plasma 24-OHC is a direct and minimally invasive marker of cholesterol homeostasis in the brain. Plasma and CSF levels of 24-OHC have been associated with brain volume, cognitive performance, MCI, and dementia at the cross-sectional level. Recently higher levels of plasma 24-OHC and lower levels of 27-OHC were associated with incident cognitive impairment (MCI and AD). Temporality of changes in oxysterol levels prior to the onset of dementia have yet to be assessed. Associations between 24-OHC and ‘pre-AD’ states (e.g., MCI, and cognitive decline) suggest that higher levels of 24-OHC are related to earlier stages of dementia.

Studies of factors regulating cholesterol homeostasis in the brain (both its synthesis and elimination) are essential to understanding the role of cholesterol metabolism in the brain and its link to AD. Plasma and neuroimaging biomarkers have an important potential relevance to characterizing individuals with subclinical disease and those at risk for developing AD. The inconsistencies of the associations between plasma 24-OHC and AD underscore the importance of examining 24-OHC in the context of the structural abnormalities occurring over the entire dementia process.

Few existing studies of 24-OHC and dementia have considered the underlying pathology and structural changes which could account for states of excess brain cholesterol and its metabolism to 24-OHC. MRI sequences can visualize and quantify structural changes in the brain. Volumetric MRI is used to calculate brain volume and assess areas of relative atrophy. Studies of brain atrophy show that plasma 24-OHC is associated total brain volume [39] and volume of the grey matter in non-demented participants [39]; however, the results for demented patients differ by CT [40] and MRI [39] modalities. The association between 24-OHC and grey matter volume supports the theory that 24-OHC is a marker of the number of metabolically active neurons in the brain. The association between 24-OHC and brain volume is potentially inaccurate if studies do not account for the presence of active white matter disease.

The selection of proper control subjects is critically important to eliminating selection bias in observational, non-randomized studies of oxysterols and cognitive impairment. Not only do future studies need to utilize neuropsychiatric testing to reliably confirm cognitive status and time to conversion, but the nature of cholesterol storage in the brain also necessitates

that researches use brain imaging to assess integrity of brain structure. The myelin model of AD development suggests that white matter disease could be the event leading to AD development. MRI can be used to assess the integrity of the white matter and the extent of white matter disease *in vivo*. Studies of 24-OHC and dementia should consider the importance of white matter disease and myelin breakdown as: 1) a potential source of excess cholesterol in the brain; 2) a potential source of increased levels of 24-OHC in the circulation; and 3) a potential initiating event in the development of neurodegeneration and dementia.

Long standing and recent advances in MRI have enabled visualization of abnormal white matter as well as quantitative means to evaluate the integrity of the brain's white matter. MRI sequences needed to visualize and quantify structural changes in the brain are readily available. Standard sequences (T1, T2, FLAIR, and PD) provide insight into integrity of the white matter. These techniques quantify the macrostructural integrity of the white matter (healthy white matter fraction), the extent of white matter disease (white matter lesion grade or volume), and the relative health of the brain tissue. Initial studies suggest that oxysterols 24-OHC and 27-OHC are associated with these lesions commonly seen in cerebrovascular disease and dementia [43]. Relatively recent advances in MRI, including magnetic transfer imaging and diffusion tensor imaging techniques provide microstructural measures of white matter integrity and axonal diffusion, respectively. Understanding the relationship between 24-OHC and white matter integrity and other structural changes occurring in the aging brain may be essential to our understanding of dementia and its initiating events.

Early in the disease process, excess cholesterol in the brain may also lead to both increases in A β deposition in the brain and increases in 24-OHC production. The associations between 24-OHC and A β deposition also remain unknown. Measures of 24-OHC in blood may reflect brain cholesterol turnover and be a potential blood marker of amyloid production and deposition. Amyloid deposition can now be visualized *in vivo* by PET. This association can be examined by visualizing amyloid deposition *in vivo* using PiB-PET. Early stage studies would be expected to show positive correlations between 24-OHC and PiB-PET. A comprehensive study utilizing longitudinal measures of cholesterol measures (total cholesterol, 27-OHC, and 24-OHC) and longitudinal measures of cognitive function and assessment of white matter integrity using MRI and amyloid deposition using PET ligands is greatly needed. The new tools may provide an important opportunity to determine if AD is a 'brain lipid disorder' that could possibly be prevented or treated by modifying brain lipid metabolism and production of amyloidogenic peptides.

Acknowledgments

This work was supported in part by funds from an NHBLI training grant to the University of Pittsburgh (T32HL083825).

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Fig. 1.
Feed forward loop of myelin breakdown leading to excess cholesterol.

Fig. 2.
Flow diagram demonstrating systematic review process of oxysterols and dementia.

Fig. 3. Hypothesized trajectory of 24-OHC and cognition across stages of dementia. MCI, mild cognitive impairment; MMSE, Mini-Mental Status Exam.

Table 1

Measurement of oxysterols in studies of neurodegenerative disease

Author	Measure	Technique	24-OHC Mean levels	27-OHC Mean levels	Variation
Case control studies using structural brain outcomes					
Hughes et al. (2012) [43]	Plasma 24-OHC and 27-OHC (nM)	Isotope dilution mass spectroscopy	Total = 104.3 ± 29.3 Normal = 97.1 ± 26.1 MCI = 107.8 ± 32.3 AD = 107.6 ± 28.6	Total = 591.2 ± 201.4 Normal = 667.4 ± 284.9 MCI = 583.5 ± 164.2 AD = 564.9 ± 158.5	Between Run CoV: 8.9% for 24-OHC 13.6% for 27-OHC Within Run CoV: 5.6% for 24-OHC 7.6% for 27-OHC
Besga et al. (2012) [41]	Plasma and CSF 24-OHC and 27-OHC (nM)	Isotope dilution mass spectroscopy	Based on CSF markers: Normal = 143.4 ± 25 Probable AD = 121.7 ± 25	Based on CSF markers: Normal = 559.6 ± 134.7 Probable AD = 458.6 ± 109.2	NA
Solomon et al. (2009) [39]	Plasma 24-OHC and 27-OHC (nM)	Isotope dilution mass spectroscopy	SCI = 144.1 ± 24.3 MCI = 126.7 ± 19.4 AD = 116.7 ± 27.3	SCI = 561.4 ± 129.7 MCI = 511.7 ± 128.4 AD = 437.2 ± 108.3	NA
Koschack et al. (2009) [38]	Serum 24-OHC & 27-OHC (nM)	Isotope dilution mass spectroscopy	Cognitively normal = 154 ± 47.2	Total = 586.2 ± 96.9	NA
Leoni et al. (2008) [37]	Plasma 24-OHC (nM)	Isotope dilution mass spectroscopy	Controls = 142.8 ± 26.8	NA	NA
Karrenbauer (2006) [35]	Plasma 24-OHC (nM)	Isotope dilution mass spectroscopy	RRMS = 157.2 (134.9–181.3) PPMS = 158.7 (127.9–172.6) Controls = 185.1 (153.3–222.6)	NA	NA
Leoni et al. (2002) [34]	24-OHC Plasma (nM) and CSF	Isotope dilution mass spectroscopy	Controls aged: 21–30 = 193.8 ± 7.45 31–40 = 188.8 ± 9.94 41–50 = 188.8 ± 7.45 51–60 = 211.1 ± 7.45 61–70 = 268.3 ± 14.9	NA	NA
Autopsy study using brain weight					
Thelen et al. (2006) [36]	Dried hippocampal sections (nanomol/mg)	Isotope dilution mass spectroscopy	Younger = 0.154 ± 0.0224 Older = 0.143 ± 0.0495	Younger = 0.385 ± 0.0675 Older = 0.329 ± 0.0787	NA
Heverin et al. (2004) [31]	Brain tissue NA	Isotope dilution mass spectroscopy	NA	NA	NA
Case control studies using diagnostic outcomes					

Author	Measure	Technique	24-OHC Mean levels	27-OHC Mean levels	Variation
Shafaei et al. (2007) [33]	CSF 24-OHC and 27-OHC (nM)	Isotope dilution mass spectroscopy	Controls = 3.80 [1.71–11.7] AD = 10.4 [3.60–14.2] MCI = 7.72 [5.02–12.2]	Controls = 3.0 [0.248–10.7] AD = 4.37 [2.01–9.39] MCI = 4.47 [2.63–11.2]	NA
Leoni et al. (2006) [20]	CSF 24-OHC and 27-OHC (nM)	Isotope dilution mass spectroscopy	Controls = 4.12 [0.994–9.29] MCI = 7.72 [5.02–12.2] AD = 10.4 [3.60–14.2]	Controls = 2.41 [0.621–6.56] MCI = 4.47 [2.63–11.2] AD = 4.37 [2.01–9.39]	Within run: 3.7% for 24-OHC 4.6% for 27-OHC
Leoni et al. (2004) [19]	Plasma & CSF 24-OHC & 27-OHC (nM)	Isotope dilution mass spectroscopy [18]	CSF: Controls = 3.43 ± 0.17 AD = 6.14 ± 0.30 Plasma: Controls = 182 ± 8.51 AD = 175 ± 6.67 Not explicitly stated	CSF: Controls = 1.27 ± 0.05 AD = 3.76 ± 0.29 Plasma: Controls = 303 (271–354) AD = 343 (244–381)	Within run: 3.7%, 4.6% IAV = 3.9% and 4.5%
Kosch et al. (2004) [32]	Plasma 24/Chol	Isotope dilution mass spectroscopy	Not explicitly stated		
Teunissen et al. (2003) [30]	Serum 24-OHC/Chol 27-OHC/Chol (nanomol/mg)	Isotope dilution mass spectroscopy	Controls = 0.0869 (0.0730) AD = 0.0795 (0.0646, 0.0944) PD = 0.0844 (0.0720, 0.107) OCD = 0.0770 (0.0646, 14.5)	Controls = 0.276 (0.221, 0.330) AD = 0.246 (0.218, 0.278) PD = 0.248 (0.216, 0.300) OCD = 0.238 (0.194, 0.281)	NA
Schonknecht et al. (2002) [29]	Plasma (nM) CSF (nM)	Isotope dilution mass spectroscopy	Controls = 133.1 ± 35.5, 6.46 ± 2.73 AD = 150.3 ± 47.9, 3.97 ± 1.49	NA	
Lutjohann et al. (2000) [28]	Plasma (nM)	Isotope dilution mass spectroscopy	Controls = 149 (59.6–260.8) Depressed = 134.1 (79.5–245.9) VaD = 193.8 (106.8–283.2) AD = 186.3 (104.3–288.1)	NA	NA
Papassotiropoulos et al. (2000) [27]	Plasma 24-OHC/Chol (nanomol/mg)	Isotope dilution mass spectroscopy	Mild AD = 0.0591 ± 0.0124 Moderate/Severe AD = 0.0497 ± 0.0139	NA	NA
Bretillon et al. (2000) [26]	Plasma 24-OHC (nM)	Isotope dilution mass spectroscopy	Not explicitly stated	NA	NA
Longitudinal studies of cognition					
van den Kommer (2009) [22]	Serum (nM)	GC-FID; GC/MS [55]	Total group non-demented Absolute = 239.9 ± 67.1 Molar ratio (10 ⁶) = 40.1 ± 10.6	Total group non-demented Absolute = 627.1 ± 189.9 Molar ratio (10 ⁶) = 104.9 ± 30.9	CoV = 4% IAV = 3%
Teunissen et al. (2003) [21]	Serum 24-OHC & 27-OHC (nM)	GC-FID; GC/MS	Baseline = 146.5 (121.7, 191.3) Follow-up (+6yrs) = 172.6(129.9, 220.6)	Baseline = 399.9 (330.4, 504.2) Follow-up (+6yrs) = 499.3 (402.4, 650.8)	NA

AD, Alzheimer's disease; CoV, coefficient of variation; CSF, cerebrospinal fluid; GC-FID, gas chromatography-flame ionization; GC/MS, gas chromatography/mass spectroscopy; 24-OHC, 24S-hydroxycholesterol; 27-OHC, 27-hydroxycholesterol; IAV, interassay accuracy; MCI, mild cognitive impairment; OCD, other cognitive disorders; PD, Parkinson's disease; PPMS, primary progressive multiple sclerosis; RRMS, relapsing remitting multiple sclerosis; SCI, subjective cognitive impairment; VaD, vascular dementia.

Table 2
Studies relating 24S-hydroxycholesterol with non-dementia neurodegenerative diseases

Author	Study design	Population	Sample size	Results	Measures
Leoni et al. (2008) [37]	Case-control	Recruited cases with HD and controls from one Italian and one British hospital. Controls were spouses of patients	Controls = 67 HD-positive = 96 Pre-HD = 33	Plasma 24-OHC levels were significantly higher in controls than HD patients. Levels of 24-OHC paralleled large decreases in caudate volumes	Oxysterols: 24-OHC (plasma) Outcomes: Case status, volumetric MRI of caudate
Leoni et al. (2002) [34]	Case-control	Recruited MS patients and controls from hospital. Age range: 21–70 years 65% of cases were remitting/relapsing	MS patients = 118 Age-matched controls = 183	Among MS patients, levels of 24-OHC were higher in younger participants and associated with higher disability scores. Increased levels of 24-OHC seen only in lesion confirmed active disease	Oxysterols: 24-OHC (plasma and CSF) Outcomes: Case status
Leoni et al. (2004) [19]	Case-control stored samples	All patients were selected from one hospital with retrospective blood samples taken for routine diagnostic testing. Controls referred for headache of uncertain cause and no sign of neurological disease	Controls = 58 MS = 88 AD = 54 demyelinating = 21 hemorrhage = 8 viral meningitis = 12 borrelia = 14	Patients with active demyelinating diseases had highest levels of 24-OHC in the CSF, followed by viral meningitis, MS, and AD	Oxysterols: 24-OHC and 27-OHC (plasma and CSF) Outcomes: Case status
Holderrieder et al. (2004) [53]	Case-series	Six patients were selected from patients admitted to the hospital within 24 hours after onset of symptoms. Patients underwent venipuncture twice daily during hospital stay; ranging from 4 to 10 days after onset of symptoms	Occlusion of middle cerebral artery = 4 Occlusion of posterior cerebellar artery = 2 No controls	Levels of cholesterol and oxysterols did not vary significantly in the days following admission to hospital for stroke symptoms indicating that oxysterols are of limited value for assessing BBB function in acute ischemic stroke patients	Oxysterols: 24-OHC and 27-OHC (plasma)
Teunissen et al. (2003) [54]	Case-control	All cases of MS were recruited from MS center in Amsterdam. Control recruitment was not described. Age range 24–65 years	Controls = 37 MS = 60 (20 RRMS, 20 SPMS, 20 PPMS)	Serum ratio 24-OHC/Chol and cholesterol precursors were lower in MS subtypes than healthy controls	Oxysterols: 24-OHC and 27-OHC (serum) Outcomes: Case status
Teunissen et al. (2003) [30]	Case-control	All patients over 50 years visiting the outpatient memory clinic diagnosed with AD, PD, and OCD. Healthy controls from the Maastricht Aging Study	AD = 34 PD = 46 OCD = 46 Controls = 61	Serum 24-OHC/Chol found to be a significant predictor of neuro-cognitive disease. Not included in the final model of combined markers predicting neuro-cognitive disease	Oxysterols: 24-OHC and 27-OHC (plasma) Outcomes: Combined case status (AD, PD, and OCD)
Bretillon et al. (2000) [26]	Case-control	205 healthy volunteers matched for age and gender (21–86 years of age) and patients with various neurodegenerative diseases: AD (at least 4 years from diagnosis); MS; Acute stroke; Guillain-Barré; viral and bacterial meningitis; primary gliomas; brain death patients due to brain hemorrhage.	Controls = 205 AD = 40 MS = 20 Guillain-Barré = 4 Meningitis = 11 Primary gliomas = 7 Brain death = 11	Patients with brain death and AD had significantly lower plasma 24-OHC than controls; no differences were seen for MS, ischemic stroke and primary brain tumors.	Oxysterols: 24-OHC (plasma) Outcome: Case status

AD, Alzheimer's disease; BBB, blood brain barrier; CSF, cerebrospinal fluid; HD, Huntington's disease; 24-OHC, 24S-hydroxycholesterol; MRI, magnetic resonance imaging; MS, multiple sclerosis; OCD, other cognitive disorders; PD, Parkinson's disease; PPMS, primary progressive multiple sclerosis; RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

Table 3

Case-control studies of dementia using diagnostic outcomes

Author	Study design	Population	Sample size	Results	Measures
Zuliani et al. (2011) [40]	Case-control	All cases were referred to out-patient clinic for cognitive decline. Cognitively older normal controls were recruited from the community. LOAD and VaD patients met respective NINCDS criteria for 'probable' dementia. Patients with CIND did not meet dementia criteria classifications. All cases had brain CT scans. Mean age = 76 years	Controls = 40 LOAD = 60 CIND = 25 VaD = 35	Levels of 24-OHC was significantly higher in LOAD and lower in VaD compared to controls. Duration of dementia was longest in VaD and shortest in LOAS patients. The ratio 24-OHC/Chol was significantly correlated with lower serum albumin, higher C-reactive protein levels and atrophy	Oxysterols: 24-OHC (plasma) Outcomes: Case status
Solomon et al. (2009) [39]	Case-control	All participants referred to memory clinic with memory complaint. Classified as SCI, MCI, and AD (NINCDS-ADRA). Mean age = 62 years	SCI = 33 MCI = 36 AD = 27	Ratio 24-OHC/Chol and 27-OHC/Chol were significantly lower in AD patients. Adjusted for age, gender, ApoE, and statins, the significant relationship between ratio 24-OHC/Chol to gray matter volume only seen in controls	Oxysterols: 24-OHC & 27-OHC (Plasma) Outcomes: Volumetric MRI volumes of: CSF, gray matter, and white matter.
Shafaati et al. (2007) [33]	Cross-sectional at diagnosis, Case-control	All participants were referred to university hospital. AD and MCI diagnosed from those referred to the memory clinic. Controls were referred to the neurology clinic for headache of uncertain cause. Ages: 18–85 years	Controls = 43 AD = 17 MCI = 20	Levels of ApoE correspond to levels of 24-OHC in AD cases but not controls. These results are consistent with 24-OHC and ApoE coupling seen in neurodegeneration	Oxysterols: 24-OHC and 27-OH (CSF) Stratification: Case status Outcomes: ApoE levels
Leoni et al. (2006) [20]	Case-control	All participants were referred for cognitive impairment to the memory clinic at Karolinska Univ Hospital (Sweden) over a period of three years. Controls selected as 35 oldest participants from an existing 50 control group used previously (Leoni 2004) Ages: 49 – 85 years	AD = 18 MCI = 20 Controls = 35	CSF levels of 27-OHC and 24-OHC were significantly higher in MCI and AD than in controls. Differences between MCI and AD were not significant	Oxysterols: 24-OHC (plasma and CSF) Outcomes: Case status (AD, MCI, controls)
Kolsch et al. (2004) [32]	Case-controls	AD, VaD, and MCI compared to depressed and controls matched on age and cholesterol levels and recruited from community. Exclusions for lower ratio lathosterol to cholesterol, suggesting treatment with lipid lowering drugs. Mean age = 69 years	AD = 134 VaD = 24 MCI = 36 Depressed = 13 Controls = 43	Plasma ratio of 24-OHC/cholesterol and 27-OHC/cholesterol reduced in AD, VaD, and MCI compared to depressed participants and controls. Higher ratio of 24-OHC/27-OHC seen in AD, VaD, and MCI compared to controls	Oxysterols: Plasma 24-OHC and 27-OH (plasma) Outcomes: Case status
Leoni et al. (2004) [19]	Case Control retrospective samples	All patients were selected from one hospital with retrospective blood samples taken for routine diagnostic testing. Controls referred for headache of uncertain cause and no sign of neurological disease. Mean age: controls = 39	Controls = 58 AD = 54 Other neurodegenerative disease = 143 AD = 72 years	Patients with active demyelinating diseases had highest levels of 24-OHC in the CSF, followed by viral meningitis, MS, and AD	Oxysterols: 24-OHC and 27-OH (plasma and CSF) Outcomes: Case status
Heverin et al. (2004) [31]	Case-control	Formalin-fixed autopsy tissue was obtained from controls and AD patients. Controls from assumed healthy subjects who died in road traffic accidents. AD patients "probable" and "definite". Ages = 61–92 years	Controls = 15 AD = 15	24-OHC was significantly increased in AD patients, specifically in the frontal cortex. The ratio of 24- to 27-OHC was elevated in AD patients	Oxysterols: 24-OHC (CSF from brain samples) Outcomes: Case status
Teunissen et al. (2003) [30]	Case-control	All patients over 50 years visiting the outpatient memory clinic diagnosed with AD,	AD = 34 PD = 46	Serum ratio 24-OHC/Chol is a significant predictor of neuro-cognitive disease. Not	Oxysterols: 24-OHC and 27-OH (plasma)

Author	Study design	Population	Sample size	Results	Measures
Schonknecht et al. (2002) [29]	Case-control	Probable AD patients (NINCDS-ADRA) and 19 controls without cognitive, psychological or neurological deficits	Controls = 19 AD = 25	Elevated levels of 24-OHC found in the CSF, not plasma, of AD treated patients compared to controls	Outcomes: Combined case status (AD, PD, and OCD) Oxysterols: 24-OHC (CSF and plasma) Outcomes: Case status
Lutjohann et al. (2000) [28]	Case-control	Consecutively recruited patients referred to department of psychiatry. Controls age-matched and drawn from an existing epidemiology cohort after exclusions made for dementia and psychological testing. Ages: 48–87 years	Controls = 30 AD = 30 Depressed = 18 Vascular AD = 12	Plasma levels of 24-OHC were elevated in AD and VaD participants compared to normal regardless of ApoE4 genotype	Oxysterols: 24-OHC (plasma) Outcome: Case status severity of disease using MMSE score
Papassotiropoulos et al. (2000) [27]	Case-series, severity of disease	Cases consecutively recruited from outpatient memory clinic. Severity of AD designated by MMSE cut-off < 22. Mean age = 73 years	AD = 53 (18 with mild AD)	Severity of AD and inheritance of the ApoE4 allele were independently associated with reduced plasma 24-OHC	Oxysterols: 24-OHC (plasma) Outcome: Case severity of disease using MMSE score
Bretillon et al. (2000) [26]	Case-control	Healthy volunteers matched for age and gender, 40 AD (at least 4 years from diagnosis), 20 with MS, 19 with acute stroke, 4 with Guillain-Barre, 5 with viral meningitis, 6 with meningitis, 7 with primary gliomas, and 11 brain dead patients due to brain hemorrhage. Ages: 21–86 years	AD = 40 (51–80 years) Controls = 205 (21–86 years) Other brain disease = 68 (28–82 years)	Patients with brain death and AD had significantly lower plasma 24-OHC than controls; no differences were seen for MS, ischemic stroke, and primary brain tumors	Oxysterols: 24-OHC (plasma) Outcome: Case status

AD, Alzheimer's disease; ApoE, apolipoprotein E; CSF, cerebrospinal fluid; CT, computed tomography; CIND, cognitive impairment no dementia; 24-OHC, 24S-hydroxycholesterol; 27-OHC, 27S-hydroxycholesterol; LOAD, late-onset Alzheimer's disease; MCI, mild cognitive impairment; MMSE, Mini-Mental Status Exam; MRI, magnetic resonance imaging; MS, multiple sclerosis; OCD, other cognitive disorders; PD, Parkinson's disease; SCI, subjective cognitive impairment; VaD, vascular dementia.

Table 4

Studies of 24S-hydroxycholesterol and longitudinal cognitive decline

Author	Study design	Population	Sample size	Results	Measures
Hughes et al. (2012) [43]	Retrospective MRI & prospective assessment of cognition	All participants were cognitively normal at blood draw and underwent prior MRI then followed for incident cognitive impairment. Mean age = 80 years	105 participants: Stayed normal = 26 Immediate conversion = 6 Incident MCI = 36 Incident AD = 37	Evidence of WMH was associated with higher plasma 24-OHC and 27-OHC. Incident cognitive impairment associated with higher 24-OHC and lower 27-OHC	Oxysterols: 24-OHC & 27-OHC (Plasma) Outcomes: MRI WMH and incident cognitive impairment
Teunissen et al. (2003) [21]	Longitudinal	All participants were recruited from the Maastricht Aging Study without comorbidity affecting cognitive performance. Random subset selected for repeated cognitive tests. Ages: 30–50 years	Baseline=92 Follow-up = 116 Both = 65	Higher ratio 24-OHC/Chol was related to slower speed of processing, but not other cognitive tests. Higher ratio of cholesterol precursors associated with poor cognitive performance	Oxysterols: 24-OHC and 27-OH (plasma) Outcomes: Cognitive test for verbal learning and memory
van den Kommer et al. (2009) [22]	Prospective	Recruited from population based Longitudinal Aging Study Amsterdam. Mean age = 76 years, range 55–86 years	Baseline = 1181 Follow-up = 1003	Low Chol in 65 and older is an independent predictor of cognitive declines over 6 years. Ratios of 24-OHC/Chol and 24-OHC/Chol significantly lower in ApoE4 carriers. ApoE4 potential moderator of association between ratio 24-OHC/Chol and speed of processing. ApoE4 status, 24-OHC/Chol and 24-OHC/Chol showed no association to cognitive decline. Only the ratio 27-OHC/Chol inversely associated with performance on MMSE and immediate recall tests in ApoE4 carriers	Oxysterols: 24-OHC & 27-OHC Outcomes: Cognitive function (MMSE, AVLT)

AD, Alzheimer’s disease; ApoE, apolipoprotein E; AVLT, Rey Auditory-Verbal Learning Test; 24-OHC, 24S-hydroxycholesterol; 27-OHC, 27-hydroxycholesterol; MCI, mild cognitive impairment; MMSE, Mini-Mental Status Exam; MRI, magnetic resonance imaging; WMH, white matter hyperintensities

Table 5

Studies of 24S-hydroxycholesterol, brain structure and structural neuroimaging

Author	Study design	Population	Sample size	Results	Measures
Hughes et al. (2012) [43]	Retrospective MRI & prospective assessment of cognition	All participants were cognitively normal at blood draw and underwent prior MRI then followed for incident cognitive impairment. Mean age = 80 years	105 participants: Stayed normal = 26 Immediate conversion = 6 Incident MCI = 36 Incident AD = 37	Evidence of WMH was associated with higher plasma 24-OHC and 27-OHC. Incident cognitive impairment associated with higher 24-OHC and lower 27-OHC	Oxysterols: 24-OHC & 27-OHC (Plasma) Outcomes: MRI WMH and incident cognitive impairment
Besga et al. (2012) [41]	Case-control	All participants referred to memory clinic with memory complaint. Classified as AD-like or controls by CSF ratio of low CSF A β ₄₂ and abnormally high CSF T-tau levels. Mean age: Controls = 58 years and AD-like CSF = 63 years	CSF defined: Controls = 29 AD-like = 30	Plasma 24-OHC and 27-OHC levels were significantly lower in AD-like participants. CSF levels were not significantly different. Correlations between whole brain WMH grade and CSF 24-OHC were negative correlation for controls and positive for AD-like. Correlations with plasma 24-OHC were not given.	Oxysterols: 24-OHC & 27-OHC (Plasma & CSF) Outcomes: MRI WMH
Zuliani et al. (2011) [40]	Case-control	All cases were referred to out-patient clinic for cognitive decline. Cognitively older normal controls were recruited from the community. LOAD and VaD patients met respective NINCDS criteria for 'probable' dementia. Patients with CIND did not meet dementia criteria classifications. Mean age = 76 years	Controls = 40 LOAD = 60 CIND = 25 VaD = 35	Levels of 24-OHC were significantly higher in LOAD and lower in VaD compared to controls. Increases in the ratio 24-OHC/Chol were correlated with lower serum albumin, higher C-reactive protein levels, more brain atrophy and having multiple infarcts.	Oxysterols: 24-OHC (plasma) Outcomes: Case status, brain CT measures (atrophy and infarcts)
Solomon et al. (2009) [39]	Case-control	All participants referred to memory clinic with memory complaint. Classified as: SCI, MCI, and AD (NINCDS-ADRA). Mean age = 62 years	SCI = 33 MCI = 36 AD = 27	Ratio 24-OHC/Chol and 27-OHC/Chol were significantly lower in AD patients. Adjusted for age, gender, ApoE, and statins, the significant relationship between 24-OHC/Chol to gray matter volume only seen in controls.	Oxysterols: 24-OHC & 27-OHC (Plasma) Outcomes: Volumetric MRI volumes of CSF, gray matter, and white matter.
Koschack et al. (2009) [38]	Cross-sectional	Recruited by advertisements in newspapers and one hospital. Excluded cognitive impairment. Mean age = 50 ± 13	Cognitively normal volunteers = 69	Participants with high levels of 24-OHC and 27-OHC also had larger hippocampal volumes. After adjustment for age, total brain volume and ApoE4, only 24-OHC was associated with hippocampal volume	Oxysterols: 24-OHC & 27-OHC (Plasma) Outcomes: Volumetric MRI (total, gray matter, and hippocampal volume)
Leoni et al. (2008) [37]	Case-control	Recruited 67 controls, 96 HD positive, and 33 pre-HD participants between 2005 and 2007 from one Italian and British hospital. Controls are spouses and cognitively normal volunteers. Mean age = 46 years	Controls = 67 HD-positive = 96 pre-HD = 33	Plasma levels 24-OHC significantly higher in controls than HD patients. Levels of 24-OHC paralleled large decreases in caudate volumes.	Oxysterols: 24-OHC (Plasma) Outcomes: MRI of Caudate
Karrenbauer et al. (2005) [35]	Cross-sectional, disease severity	Patients and controls from one hospital. Cases with MS at two stages of disease: RR and PP. Ages: 23–58 years	RRMS = 27 PPMS = 19 Controls = 23	Plasma ratio of 24-OHC/Chol negatively correlated with age (in both RRMS and PPMS patients) and volume of T ₂ lesions in RRMS patients (a marker of disease extent)	Oxysterols: 24-OHC (Plasma) Outcomes: MRI T ₁ and T ₂ lesions and

Author	Study design	Population	Sample size	Results	Measures
Leoni et al. (2002) [34]	Case control	Recruited 118 MS patients and 183 age-matched controls. 65% of cases were remitting/relapsing disease. Ages: 21–70 years	MS = 118 Control = 183	Among MS patients levels of 24-OHC were higher in younger participants and were associated with higher disability scores. Increased levels of 24-OHC seen only in lesion confirmed active disease.	gadolinium positive lesions gadolinium positive lesions Oxysterols: 24-OHC (plasma and CSF) Outcomes: Case status
Thelen et al. (2006) [36]	Cross-sectional	All participants showed no signs of neurological or psychiatric disease prior to sudden or unexpected death. Ages: 18 to 86 years	Decedent = 20	Concentrations of cholesterol precursors were significantly higher in younger versus older participants. Absolute levels of 24-OHC were slightly lower in hippocampus specimens from older participants	Oxysterols: 24-OHC (CSF of brains) Outcomes: post-mortem hippocampal volumes

Aβ, amyloid-β; AD, Alzheimer’s disease; ApoE, apolipoprotein E; CIND, cognitive impairment no dementia; CSF, cerebrospinal fluid; CT, computed tomography; HD, Huntington’s disease; 24-OHC, 24S-hydroxycholesterol; 27-OHC, 27-hydroxycholesterol; LOAD, late-onset Alzheimer’s disease; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; MS, multiple sclerosis; PPMS, primary progressive multiple sclerosis; RRMS, relapsing remitting multiple sclerosis; SCL, subjective cognitive impairment; T-tau; total tau; VaD, vascular dementia; WMH, white matter hyperintensities.