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REVIEW

Circulating oxysterols in Alzheimer’s disease: a systematic review and meta-analysis

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Abstract

Despite decades of research, the cause and series of events underlying the advancement of Alzheimer’s disease (AD) have not yet been established. Lipid, especially cholesterol, levels have been proposed to be implicated in AD. Several studies have been undertaken and many are ongoing in different directions looking at the importance of circulating cholesterols and oxidised cholesterols in AD with inconsistent methods and results. This meta-analysis aims to systematically analyse available data describing the involvement of oxidised cholesterols in mild cognitive impairment (MCI) and AD. We conducted a systematic literature search of six databases MEDLINE (PubMed), BIOSIS (Web of Science), EMBASE (Elsevier), PsycNET, Scopus and Cochrane library for studies measuring oxysterols (24-hydroxycholesterol (24OHC); 26-hydroxycholesterol (26OHC) and 7-oxycholesterols) in serum or plasma from MCI/AD patients compared to age- and gender-matched cognitively normal controls. Data were analysed using the inverse variance and standard mean difference with random-effect analysis model at 95% CI for association between oxysterols and MCI/AD in Review Manager (RevMan) software version 5.4.1. Between January 2000 and April 2022, 175 studies were identified by two independent researchers out of which 14 met the inclusion criteria and were analysed with a total of 957 controls, 469 MCI cases and 509 AD cases. The standard mean differences between MCI/AD participants and controls did not show any difference in the oxysterol levels except for 26OHC level which was higher in AD but not statistically significant.

Key Words

- oxysterols
- Alzheimer’s disease
- serum
- plasma
- meta-analysis

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Introduction

Alzheimer’s disease (AD) is the most common type of dementia where affected individuals suffer progressive cognitive decline and cognitive impairment. It has been estimated that AD sufferers worldwide will reach 81 million by 2040 with most of the sufferers being old-age adults (Ferri *et al.* 2005). Over 9.9 million new cases of dementia are predicted each year worldwide and is forecast to be the major societal health challenge of the 21st century (World Alzheimer Report 2021). It is widely recognised that AD has an extended preclinical stage before presenting cognitive decline and neuropathological changes in middle to older adults. Therefore, it is vital to understand preclinical biomarkers and underlying mechanisms of its progression to predict and apply to successful prevention trials.

The discovery of the genetic association of apolipoprotein E (ApoE) ε4 allele with sporadic and familial late-onset AD suggested the possibility of dysfunction in the lipid metabolism and lipid transport in the brains of AD subjects (Minagawa *et al.* 2009). ApoE is produced by astrocytes and glial cells, and it is a primary

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transporter of cholesterol involving lipoprotein particles in the brain. The key role of ApoE is to redistribute lipids through receptor-mediated uptake for neurite growth (Minagawa *et al.* 2009). In addition to the identification of genetic risk factors for vascular dementia and familial and sporadic AD, multiple lines of evidence suggested a strong relationship between lipid homeostasis and vascular changes in the brain of AD subjects (Papassotiropoulos *et al.* 2000, Vaya & Schipper 2007, Mutemberezi *et al.* 2016). These include epidemiologic studies that link environmental vascular risk factors to dementia; evidence from stroke studies (Leduc *et al.* 2010, Hughes *et al.* 2013), interaction with amyloid deposition (Loera-Valencia *et al.* 2019) and Tau phosphorylation (Papassotiropoulos *et al.* 2003) in a cholesterol-rich environment, microvascular abnormalities in AD and changes to blood–brain barrier (BBB) functions in hypercholesterolemic subjects (Loera-Valencia *et al.* 2019). Lipids are at the core of AD pathology due to their involvement in various cellular functions in the brain including the energy balance, myelination, membrane remodelling, signalling cascades, inflammation and resolution of inflammation. Based on these observations, the relationship between lipids, especially cholesterol, and AD has been extensively investigated in epidemiological studies in the past decade (Ferri *et al.* 2005, Barnes & Yaffe 2011, Dias *et al.* 2015). Previous meta-analysis compared the levels of lipids or lipoproteins in the blood, which include low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and triglycerides (TG) (Sáiz-Vazquez *et al.* 2020). This meta-analysis showed that there is an association between the effect of cholesterol in AD where LDL-C has a significant impact on the development of AD pathologies.

Oxidative stress and oxidative modifications to macromolecules are one of the earliest events in AD pathology (Nunomura *et al.* 2001, Bai *et al.* 2022). Among the lipid oxidation observed in AD, cholesterol can be oxidised via non-enzymatic reactions and enzymatic oxidation, thus resulting in oxysterols. While peripheral cholesterol metabolism is kept independent of the brain, there are evidence to suggest that oxysterols can transfer to and from the brain via BBB. Polar oxysterols that can proficiently pass the lipophilic BBB have obtained substantial recognition as a potential connection between serum cholesterol, modified brain cholesterol metabolism and brain pathophysiology (Iuliano *et al.* 2011, Hughes *et al.* 2013, Dias *et al.* 2014*b*). Non-enzymatic oxidation to cholesterol can occur through reactions initiated by free radical species arising from the superoxide, hydrogen peroxide and hydroxyl radical system. These reactions oxidise the seventh position of the cholesterol main ring deriving 7-oxycholesterols (7α-hydroxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol) and epoxy cholesterols (Iuliano *et al.* 2011). The susceptibility of cholesterol to non-enzymatic oxidation and increased oxidative stress observed in AD has raised considerable interest in the function of these oxysterols as biological effectors and potential biomarkers in AD (Mahalakshmi *et al.* 2021).

Conversion of excess brain cholesterol to 24-hydroxycholesterol (24OHC) by the neuron-specific enzyme CYP46A1 is one of the main routes by which excess cholesterol is removed from the brain (Gamba *et al.* 2021). Cholesterol is also enzymatically converted to 26-hydroxycholesterol (26OHC, also identified as 27-hydroxycholesterol) via 27-hydroxylase (CYP27A1) in neurones and other cell types. The 26OHC is produced mainly by peripheral tissues, although a small amount is also generated by the brain. Brain-derived 26OHC is then transferred to the blood and catabolized towards bile acids in the liver by oxysterol 7α-hydroxylase (CYP7B1). As the most abundant oxysterol in peripheral circulation, 26OHC is found to be associated with the cholesterol content in atherosclerotic lesions and the severity of coronary artery disease (Vaya *et al.* 2001). *In vivo* studies demonstrated that mutations in several HDL genes could redistribute 26OHC levels in HDL and plasma, as well as specific changes in the esterification of 26OHC (Karuna *et al.* 2011). Administration of 26OHC through the tail vein negatively affected cognitive function and cholesterol metabolism in rats (Zhang *et al.* 2015). These observations led to later case–control studies that demonstrated a strong association between high plasma levels of 26OHC and mild cognitive impairment (MCI) (Liu *et al.* 2016). These observations suggest that oxysterols could act as a link between hypercholesterolemia and AD. Considering this background, the aim of this study is to provide an updated systematic review and meta-analysis of available data on oxysterols and the risk related to the development of AD.

Methods

Data acquisition and criteria

This review included a search of six databases: MEDLINE (PubMed), BIOSIS (Web of Science), EMBASE (Elsevier), PsycNET, Scopus and Cochrane for studies measuring oxysterols (24 hydroxycholesterol; 26 hydroxycholesterol/27 hydroxycholesterol and

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7-oxycholesterols) in serum or plasma from MCI/AD patients compared to age- and gender-matched cognitively normal controls published between January 2000 and April 2022. The Boolean terms used were (plasma OR serum OR blood) AND (oxysterols) AND (Alzheimer's disease) NOT review. Authors performed the initial searches and eliminated duplicates, and articles that satisfied the inclusion criteria were selected independently and checked for full text availability. Figure 1 summarises the inclusion and exclusion criteria.

Study selection

Inclusion criteria are:

1. studies on human subject, published between January 2000 and April 2022;
2. case–control study design that reported quantitative measurement of total cholesterol and oxysterols (24OHC, 26OHC and 7-oxycholesterols) in serum or plasma of MCI/AD patients and age- and gender-matched cognitively normal controls.

   Studies that provide data on at least one of the classes of lipids to be analysed were used and there was no limitation based on quantification methodology.

Exclusion criteria are:

1. lack of control groups;
2. other forms of dementia and not AD;
3. intervention studies;
4. review papers, letters, editorials, conference papers or other non-original research articles.

   Studies in any of the listed categories were excluded from the meta-analysis.

Data extraction and assessment

Two independent reviewers performed the search and collected the data. The mean, S.D. and S.E., as well as the adjusted oxysterol levels, were extracted. We explored the year, country of study design, use of quality control, criteria for AD diagnosis, mean age, gender, type of sample, assay method, laboratory kit used, collection process, sample storage, sample preparation and blinding of personnel were assessed for each study. Variables extracted from each of the 14 studies shortlisted for meta-analysis are shown in Table 1 and include mean and S.D. We used the formula $SD=SE*\sqrt{n}$ to calculate S.D. for data reported as S.D., where n is the number of participants.

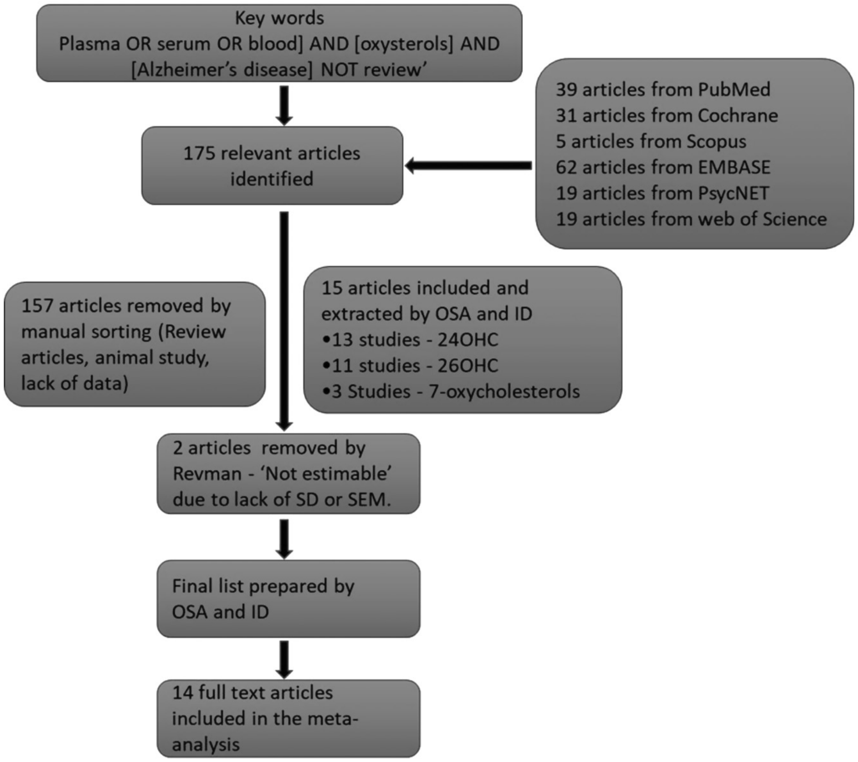

Figure 1
Flow diagram of the study selection process for studies included in the meta-analysis. All included studies were controlled trials.

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Table 1 Quantitative data and characteristics of the selected studies. Summary details of the 14 published studies included in the meta-analyses.

	Control	MCI	AD	Detection method	Reference
Costa *et al.* 2018				GC-MS	Costa *et al.* 2018
Sample numbers	33		30		
Age ± s.d.	73.88 ± 5.35		73.93 ± 6.46		
Hughes *et al.* 2012				GC-MS	Hughes *et al.* 2012
Sample numbers	26	36	37		
Age ± s.d.	80.2 ± 3.9		80.2 ± 3.8		
Hughes *et al.* 2014				GC-MS	Hughes *et al.* 2014
Sample numbers	137	37			
Liu *et al.* 2016				LC-MS	Liu *et al.* 2016
Sample numbers	140	70			
Age, range	62–69	61–72			
Liu *et al.* 2021				LC-MS	Liu *et al.* 2021
Sample numbers	209	209			
Age, range	60–67	59–66			
Iuliano *et al.* 2010				GC-MS	Iuliano *et al.* 2010
Sample numbers	24	29	37		
Age ± s.d.	83 ± 6.4	70.86 ± 6.6	76.03 ± 7.9		
Lütjohann *et al.* 2000				GC-MS	Lütjohann *et al.* 2000
Sample numbers	30		30		
Age (range)	49–91		52–87		
Mateos *et al.* 2011				GC-MS	Mateos *et al.* 2011
Sample numbers	28	10	21		
Age ± s.d.	57.81 ± 1.27	61.2 ± 2.33	67.3 ± 1.70		
Popp *et al.* 2012				GC-MS	Popp *et al.* 2012
Sample numbers	43		53		
Age ± s.d.	67.33 ± 9.04		71.23 ± 8.29		
Popp *et al.* 2013				GC-MS	Popp *et al.* 2013
Sample numbers	87		106		
Age ± s.d.	67.7 ± 9.13		71.1 ± 7.87		
Qureischie *et al.* 2008				GC-MS	Qureischie *et al.* 2008
Sample numbers	78		108		
Age ± s.d.	72.4± 7.9		72.5± 8.8		
Roy *et al.* 2019				Sandwich ELISA	Roy *et al.* 2019
Sample numbers	40		40		
Age ± s.d.	71.95 ± 6.27		70.20 ± 5.17		
Zuliani *et al.* 2011				LC-MS	Zuliani *et al.* 2011
Sample numbers	40	25	60		
Age ± s.d.	73 ± 8.7	74 ± 8.2	78 ± 5.5		
Zarrouk *et al.* 2020				GC-MS	Zarrouk *et al.* 2020
Sample numbers	42		40		
Age, range	52–74		64–79		

Data analysis

Statistical analyses were performed using the Review manager (RevMan) version 5.4.1 (provided by www.cochrane.org). The meta-analysis compared the mean differences and standard deviations within the study groups using inverse variance for the continuous data. Chi-square test was used to investigate heterogeneity within the studies, and sensitivity analysis was used to identify the source of heterogeneity. A random-effects model was used for analysis with heterogeneity ($P \leq 0.1$, $I^2 \geq 50\%$). We used the standardised mean difference (SMD), and 95% CI was calculated to analyse statistical data effectiveness for the continuous data. The difference was statistically significant when $P < 0.05$.

We categorised the studies into three groups for separate meta-analyses: (i) total 24OHC composition of plasma/serum in studies comparing AD or MCI to cognitively normal individuals; (ii) 26OHC composition of plasma/serum in studies comparing AD or MCI to cognitively normal individuals; (iii) 7-oxycholestrol composition of plasma/serum in studies comparing AD or MCI to cognitively normal individuals. We performed a total of 1935 meta-analyses (957 control vs 469 MCI

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and 509 AD). Oxysterol summary including all units and sources is shown in Table 1. The overall effect results of meta-analysis were accepted as statistically significantly different if they have $P \leq 0.05$.

Results

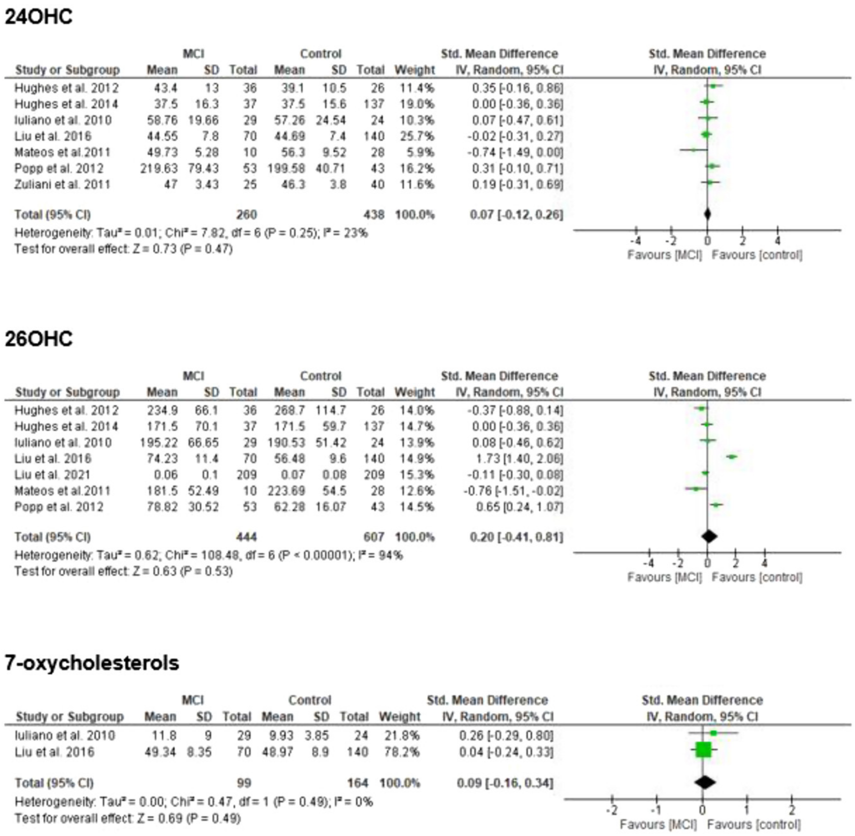
Literature search results

The flow diagram of the literature searches and study selection is shown in Fig. 1. Overall, 175 studies between January 2000 and April 2022 were identified by two independent researchers. Finally, 14 observational studies met the inclusion criteria and were included in the meta-analysis. Sample size ranged from 10 to 140 subjects and analysed with a total of 957 controls, 469 MCI and 509 AD cases. Populations in the included studies were from Pittsburgh (USA), Germany, Spain, Tunisia, China, Brazil, India and Sweden. Data of the included studies are summarised in Table 1. The full list of plasma/serum levels of oxysterols reported in each paper is in the Forest plot summary tables. Only studies reporting MCI and dementia of the Alzheimer's type were used for meta-analysis. Highly specific mass spectrometric approaches (either LCMS or GCMS) were used for the analysis of oxysterols in all the studies except for Roy *et al.* (2019) who used sandwich ELISA.

Studies on oxysterols and MCI

To understand the relationship between circulating 24OHC, 26OHC and 7-oxycholesterols in disease progression, first, we investigated data from subjects with MCI (Fig. 2). The data used in the meta-analysis were calculated and converted from the original data. Among seven studies included in this analysis, no change was reported for 24OHC levels in four studies (Iuliano *et al.* 2010, Hughes *et al.* 2012, Liu *et al.* 2016). While Hughes *et al.* (2012) and Popp *et al.* (2012) reported increased levels of circulating 24OHC in MCI, Mateos *et al.* (2011) reported decreased levels. For 26OHC, Hughes *et al.* (2012) and Popp *et al.* (2012) reported decreased circulating levels in MCI but Liu *et al.* (2016) and Zuliani *et al.* (2011) reported 26OHC is higher in MCI plasma.

Overall, 24OHC showed no difference between control and MCI; the standardised mean difference was 0.07 (95% CI: −0.12 to 0.26, $P=0.47$, Fig. 2). No statistical heterogeneity was found ($\chi^2=7.82$, $P=0.25$, $I^2=23\%$). Likewise, 26OHC



24OHC

	MCI			Control				Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Hughes et al. 2012	43.4	13	36	39.1	10.5	26	11.4%	0.35 [-0.16, 0.86]
Hughes et al. 2014	37.5	16.3	37	37.5	15.6	137	19.0%	0.00 [-0.36, 0.36]
Iuliano et al. 2010	58.76	19.66	29	57.26	24.54	24	10.3%	0.07 [-0.47, 0.61]
Liu et al. 2016	44.55	7.8	70	44.69	7.4	140	25.7%	-0.02 [-0.31, 0.27]
Mateos et al.2011	49.73	5.28	10	56.3	9.52	28	5.9%	-0.74 [-1.49, 0.00]
Popp et al. 2012	219.63	79.43	53	199.58	40.71	43	16.2%	0.31 [-0.10, 0.71]
Zuliani et al. 2011	47	3.43	25	46.3	3.8	40	11.6%	0.19 [-0.31, 0.69]
Total (95% CI)			**260**			**438**	**100.0%**	**0.07 [-0.12, 0.26]**

Heterogeneity: Tau² = 0.01; Chi² = 7.82, df = 6 (P = 0.25); I² = 23%
Test for overall effect: Z = 0.73 (P = 0.47)

26OHC

	MCI			Control				Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Hughes et al. 2012	234.9	66.1	36	268.7	114.7	26	14.0%	-0.37 [-0.88, 0.14]
Hughes et al. 2014	171.5	70.1	37	171.5	59.7	137	14.7%	0.00 [-0.36, 0.36]
Iuliano et al. 2010	195.22	66.65	29	190.53	51.42	24	13.9%	0.08 [-0.46, 0.62]
Liu et al. 2016	74.23	11.4	70	56.48	9.6	140	14.9%	1.73 [1.40, 2.06]
Liu et al. 2021	0.06	0.1	209	0.07	0.08	209	15.3%	-0.11 [-0.30, 0.08]
Mateos et al.2011	181.5	52.49	10	223.69	54.5	28	12.6%	-0.76 [-1.51, -0.02]
Popp et al. 2012	78.82	30.52	53	62.28	16.07	43	14.5%	0.65 [0.24, 1.07]
Total (95% CI)			**444**			**607**	**100.0%**	**0.20 [-0.41, 0.81]**

Heterogeneity: Tau² = 0.62; Chi² = 108.48, df = 6 (P < 0.00001); I² = 94%
Test for overall effect: Z = 0.63 (P = 0.53)

7-oxycholesterols

	MCI			Control				Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Iuliano et al. 2010	11.8	9	29	9.93	3.85	24	21.8%	0.26 [-0.29, 0.80]
Liu et al. 2016	49.34	8.35	70	48.97	8.9	140	78.2%	0.04 [-0.24, 0.33]
Total (95% CI)			**99**			**164**	**100.0%**	**0.09 [-0.16, 0.34]**

Heterogeneity: Tau² = 0.00; Chi² = 0.47, df = 1 (P = 0.49); I² = 0%
Test for overall effect: Z = 0.69 (P = 0.49)

Figure 2
Forest plots of concentration of oxysterols in plasma/serum in MCI vs control meta-analyses. Results from the meta-analysis of 14 studies using random-effect model did not show significant difference for oxysterols (24OHC, 26OHC and 7-oxycholesterols) tested in subjects with MCI compared to healthy controls.

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showed no difference between control and MCI; the standardised mean difference was 0.20 (95% CI: –0.41 to 0.81, $P=0.53$, Fig. 2). However, statistical heterogeneity was found ($\chi^2=108.48$, $P < 0.00001$, $I^2=94\%$). Subgroup analysis (not shown) did not show statistical significance for 24OHC and 26OHC either. 7-oxycholesterols showed no difference between control and MCI; the standardised mean difference was 0.09 (95% CI: –0.16 to 0.34, $P=0.49$, Fig. 2). No statistical heterogeneity was found ($\chi^2=0.47$, $P=0.49$, $I^2=0\%$).

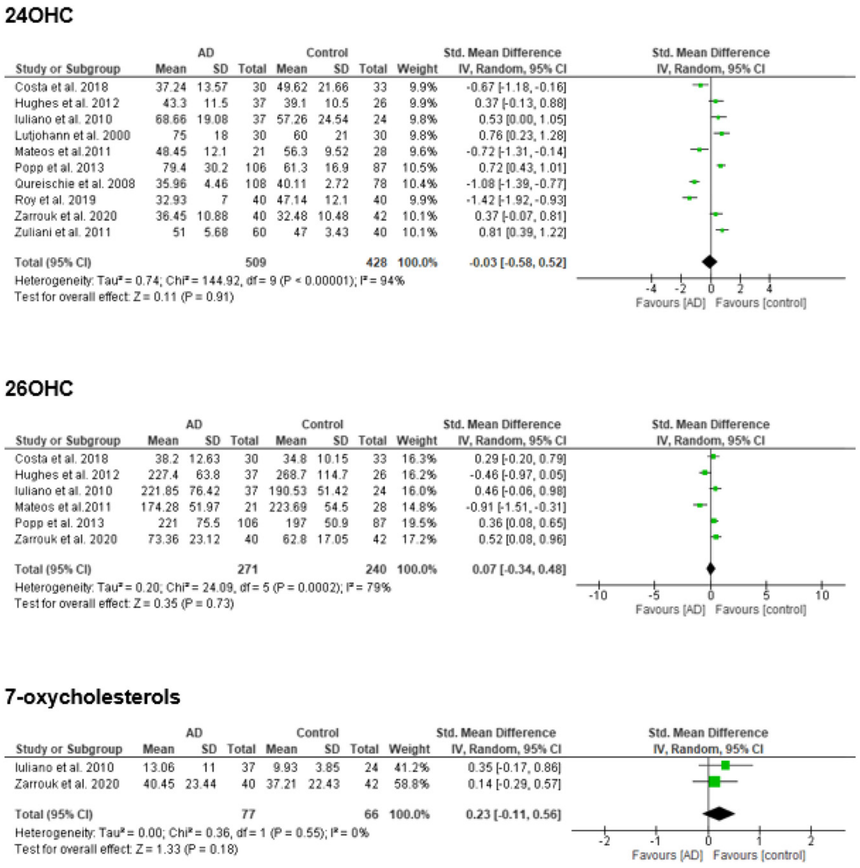
The overall effect of the oxysterols analysed between control and MCI was not significant.

Studies on oxysterols and AD

To explore the relationship between circulating oxysterols levels and risk of AD, next, we investigated data from subjects with AD (Fig. 2). Data of 24OHC, 26OHC and 7-oxycholesterol levels were also inconsistent in literature. Out of ten studies analysed for 24OHC, six studies reported high circulating levels (Lütjohann *et al.* 2000, Iuliano *et al.* 2010, Zuliani *et al.* 2011, Hughes *et al.* 2012, Popp *et al.* 2013, Zarrouk *et al.* 2020) and four studies reported low circulating levels (Qureischie *et al.* 2008, Mateos *et al.* 2011, Costa *et al.* 2018, Roy *et al.* 2019) in AD compared to control. For 26OHC, three studies reported high circulating levels (Iuliano *et al.* 2010*b*, Popp *et al.* 2013, Zarrouk *et al.* 2020), two studies reported low levels (Mateos *et al.* 2011, Hughes *et al.* 2012) and one study reported no change (Costa *et al.* 2018) compared to control. 7-oxycholesterol levels were reported as high in both studies but not statistically significant (Iuliano *et al.* 2010*b*, Zarrouk *et al.* 2020).

Overall, 24OHC showed no difference between control and AD; the standardised mean difference was –0.03 (95% CI: –0.58 to 0.52, $P=0.91$, Fig. 3). However, statistical heterogeneity was found ($\chi^2=144.92$, $P < 0.00001$, $I^2=94\%$). 26OHC showed no difference between control and AD; the standardised mean difference was 0.07 (95% CI: –0.34 to 0.48, $P=0.73$, Fig. 3). However, statistical heterogeneity was found ($\chi^2=24.09$, $P=0.0002$, $I^2=79\%$) for 26OHC. 7-oxycholesterols showed no difference between control and AD; the standardised mean difference was 0.23 (95% CI: –0.11 to 0.56, $P=0.18$, Fig. 3). No statistical heterogeneity was found ($\chi^2=0.36$, $P=0.55$, $I^2=0\%$).

The overall effect of the oxysterols analysed between control and AD was not significant.



24OHC

Study or Subgroup	AD Mean	AD SD	AD Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
Costa et al. 2018	37.24	13.57	30	49.62	21.66	33	9.9%	-0.67 [-1.18, -0.16]
Hughes et al. 2012	43.3	11.5	37	39.1	10.5	26	9.9%	0.37 [-0.13, 0.88]
Iuliano et al. 2010	68.66	19.08	37	57.26	24.54	24	9.8%	0.53 [0.00, 1.05]
Lutjohann et al. 2000	75	18	30	60	21	30	9.8%	0.76 [0.23, 1.28]
Mateos et al.2011	48.45	12.1	21	56.3	9.52	28	9.6%	-0.72 [-1.31, -0.14]
Popp et al. 2013	79.4	30.2	106	61.3	16.9	87	10.5%	0.72 [0.43, 1.01]
Qureischie et al. 2008	35.96	4.46	108	40.11	2.72	78	10.4%	-1.08 [-1.39, -0.77]
Roy et al. 2019	32.93	7	40	47.14	12.1	40	9.9%	-1.42 [-1.92, -0.93]
Zarrouk et al. 2020	36.45	10.88	40	32.48	10.48	42	10.1%	0.37 [-0.07, 0.81]
Zuliani et al. 2011	51	5.68	60	47	3.43	40	10.1%	0.81 [0.39, 1.22]
Total (95% CI)			509			428	100.0%	-0.03 [-0.58, 0.52]

Heterogeneity: Tau^2 = 0.74; Chi^2 = 144.92, df = 9 (P < 0.00001); I^2 = 94%
Test for overall effect: Z = 0.11 (P = 0.91)

26OHC

Study or Subgroup	AD Mean	AD SD	AD Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
Costa et al. 2018	38.2	12.63	30	34.8	10.15	33	16.3%	0.29 [-0.20, 0.79]
Hughes et al. 2012	227.4	63.8	37	268.7	114.7	26	16.2%	-0.46 [-0.97, 0.05]
Iuliano et al. 2010	221.85	76.42	37	190.53	51.42	24	16.0%	0.46 [-0.06, 0.98]
Mateos et al.2011	174.28	51.97	21	223.69	54.5	28	14.8%	-0.91 [-1.51, -0.31]
Popp et al. 2013	221	75.5	106	197	50.9	87	19.5%	0.36 [0.08, 0.65]
Zarrouk et al. 2020	73.36	23.12	40	62.8	17.05	42	17.2%	0.52 [0.08, 0.96]
Total (95% CI)			271			240	100.0%	0.07 [-0.34, 0.48]

Heterogeneity: Tau^2 = 0.20; Chi^2 = 24.09, df = 5 (P = 0.0002); I^2 = 79%
Test for overall effect: Z = 0.35 (P = 0.73)

7-oxycholesterols

Study or Subgroup	AD Mean	AD SD	AD Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
Iuliano et al. 2010	13.06	11	37	9.93	3.85	24	41.2%	0.35 [-0.17, 0.86]
Zarrouk et al. 2020	40.45	23.44	40	37.21	22.43	42	58.8%	0.14 [-0.29, 0.57]
Total (95% CI)			77			66	100.0%	0.23 [-0.11, 0.56]

Heterogeneity: Tau^2 = 0.00; Chi^2 = 0.36, df = 1 (P = 0.55); I^2 = 0%
Test for overall effect: Z = 1.33 (P = 0.18)

Figure 3
Forest plots of concentration of oxysterols in plasma/serum in AD vs control meta-analyses. Random-effect model did not show significant difference in oxysterols measured in AD patients compared to healthy controls.

Discussion

Oxysterols, especially neuronal-derived 24OHC and peripheral 26OHC, have been of interest as biomarkers for neurodegeneration including AD. A large portion of the total 24OHC in the periphery is known to be produced in the brain (Björkhem *et al.* 1998). During brain cholesterol metabolism, 99% of brain-derived 24OHC is estimated to enter into the plasma and excreted via liver (Lütjohann *et al.* 1996), reflecting brain cholesterol metabolism. Plasma levels of 24OHC seem to be stable in healthy subjects regardless of the sex differences, but there are mixed results on circulating 24OHC levels in AD (Dzeletovic *et al.* 1995). Defective BBB permeability in AD has been suggested to increase the permeability of many brain biomarkers into the circulation including oxysterols (Dias *et al.* 2014*b*). To ascertain 24OHC as a possible biomarker for neurodegeneration, many previous studies investigated 24OHC levels in the brain as well as in the blood and the results have been reviewed before (Leoni & Caccia 2011, 2013*a*,*b*). Therefore, the aim of this study was to incorporate recent findings and to analyse available data to investigate any changes to circulating oxysterol levels in AD. This meta-analysis shows that circulating levels of 24OHC, 26OHC and 7-oxycholesterols are not changed between healthy subjects and patients with MCI or AD.

Some studies observed increased 24OHC at the early stage of the disease due to increased brain cholesterol turnover, but majority of the studies did not observe changes to circulating 24OHC levels in AD (Iuliano *et al.* 2010, Hughes *et al.* 2014, Liu *et al.* 2016). Lütjohann *et al.* (2000) reported significantly higher concentration of 24OHC in AD and non-Alzheimer demented patients compared to healthy controls and depressed patients. Agreeing with this, Popp *et al.* reported increased 24OHC in circulation (Popp *et al.* 2013). In contrast, several studies reported decreased levels of 24OHC in AD and MCI (Bretillon *et al.* 2000, Kölsch *et al.* 2004, Roy *et al.* 2019) and a correlation between severity of AD and decrease of 24OHC (Ruthirakuhan *et al.* 2019). Plasma 24OHC was also found to be significantly reduced in some other human neurodegenerative diseases such as multiple sclerosis (MS) (Teunissen *et al.* 2003) and Huntington's disease (Solomon *et al.* 2009, Leoni *et al.* 2013). Since AD progression is coupled with loss of 24-hydroxylase-expressing neurons, brain 24OHC levels are found to be significantly lower than in healthy subjects (Testa *et al.* 2016, Dias *et al.* 2022). The reduction of neuronal cell-rich grey matter during neurodegeneration could be mirrored by a parallel reduction of peripheral 24OHC (Solomon *et al.* 2009). However, Solomon *et al.* described that 24OHC was significantly related to brain volumes in the control group but not with MCI or AD groups despite the significant decrease in plasma 24OHC levels. Previous work found a differential expression of the CYP46 enzyme in glial cells in AD (Brown *et al.* 2004). Abnormal induction of the cholesterol-catabolic enzyme CYP46 in glial cells may act as a compensatory mechanism in neurodegeneration masking the direct correlation between brain volume and the evolution of the disease. It is also possible that the differences observed in circulating 24OHC may be due to individual variance in hepatic clearance rates of 24OHC. It was estimated that 24OHC is eliminated from human circulation with half-lives of 10–14 h (Björkhem *et al.* 1998). However, there are no data available for differential clearance of 24OHC in AD patients. Another confounder to varied levels of 24OHC would be polymorphism in 24-hydroxylase gene. Previous reports suggest that polymorphisms in CYP46A1 are associated with an increased load of amyloid beta (Aβ) or earlier onset of AD (Kölsch *et al.* 2002, Papassotiropoulos *et al.* 2003).

Hughes *et al.* (2014) have shown that plasma oxysterol concentrations were higher among normal individuals who progressed onto developing MCI or AD by an 8-year follow-up study. This study demonstrated that blood lipid levels were not associated with Aβ status in the non-demented elderly group. However, higher levels of oxysterols and, in particular, significantly greater ratios of 24OHC to 26OHC and cholesterol were related to Aβ deposition in individuals with MCI. This might reflect alterations in cholesterol metabolism occurring early-on in the cognitive impairment process and may be indicative for individuals experiencing cognitive deterioration. However, it was uncertain whether all the subjects with MCI would progress onto AD or if 24OHC levels were directly associated with AD pathology. Roy *et al.* (2019) reported that serum 24OHC does not correlate with APOE4 genotype in AD subjects. Recently, Gamba *et al.* (2021) discussed the ambiguity of 24OHC levels related to AD pathogenesis and the controversial role it plays in the brain.

Enzymatically derived 26OHC is mainly produced in peripheral tissues and transported to the brain via BBB. This transport may be limited in healthy people due to intact BBB but could be increased during compromised BBB functions in AD. Therefore, multiple research studies have investigated the potential role of increased 26OHC in brain cell activity. Generally, 26OHC levels in the brain are about ten-fold less than that of 24OHC (Hughes *et al.* 2013). Even though the low-abundant 26OHC might not severely influence the brain cholesterol metabolism, there is copious evidence to suggest its neuronal toxicity (Gamba *et al.* 2014, Dias *et al.* 2015). At low concentrations, 26OHC induced neuronal cell oxidative stress and increased Aβ production (Dias *et al.* 2014*a*, Gamba *et al.* 2014). Animal studies also indicated that 26OH levels alter synaptic potentiation and could lead to dysfunction of fine-tuned processing of information in hippocampal circuits resulting in cognitive impairment (Loera-Valencia *et al.* 2021). These studies confirmed neuronal toxicity exerted by 26OHC at high concentrations. Leoni *et al.* investigated the possibility of using 24OHC and 26OHC to inform differences between different conditions that affect neuronal damage (MS, AD, viral meningitis, chronic demyelinating polyneuropathies and subarachnoid haemorrhage) (Leoni *et al.* 2004). The study concluded no significant change to plasma levels of 24OHC and 26OHC, but neuronal damage and demyelination could increase oxysterol levels in cerebrospinal fluid (CSF) (Leoni *et al.* 2004). These studies clearly show that there is oxysterol involvement in neuronal cell damage in the brain but less likely that oxysterol levels would mirror this effect in the circulation. A meta-analysis to investigate 24OHC and 26OHC as surrogate biomarkers in CSF demonstrated both 24OHC and 26OHC are increased in MCI and AD (Wang *et al.* 2016). Altered cholesterol metabolism and increased cholesterol levels in CSF during MCI (Papassotiropoulos *et al.* 2002, Mateos *et al.* 2011) may account for increased oxysterols found in CSF and compromised BBB functions may explain the accumulation of oxysterols at later stage. This data suggested along with Aβ, total tau and phospho-tau, CSF 24OHC and 26OHC could be appropriate biomarkers for AD screening.

In contrast to the brain cholesterol metabolism, peripheral cholesterol metabolism is greatly influenced by diet, physical activity and medications. Therefore, 26OHC levels may be variable between and within individuals during sampling. As a result, circulating 26OHC levels affected by these confounders may complicate the use of it as a disease biomarker. Hence, interpretation of 26OHC levels in biomarker studies should be carefully considered. A recent randomized controlled trial suggests that reducing serum 26OHC levels by managing lifestyle and vascular factors over 2 years is beneficial for improving cognitive function related to memory (Sandebring-Matton *et al.* 2021). The study suggested that the intervention is beneficial to reduce 27OHC, particularly in individuals with the highest 27OHC levels and younger age. This suggests that managing oxysterol levels from young is beneficial to improve cognition, but it is possible that increased oxidative stress in ageing and neurodegeneration may overcome the benefits of managing vascular and lifestyle factors. Therefore, we also compared autooxidised circulating 7-oxycholesterol levels between healthy, MCI and AD. However, these were not significantly different in our meta-analysis. In a systematic analysis of oxysterols in the brains of patients with different stages of AD development, they identified increased levels of oxysterols including 7-oxycholesterols, 4β-hydroxycholesterol, 5α,6α-epoxycholesterol and 5β,6β-epoxycholesterol (Testa *et al.* 2016). Toxicity of 7-oxycholesterols on neuronal cells is well documented in both *in vivo* and *in vitro* studies (Okabe *et al.* 2013, Debbabi *et al.* 2017, Yammine *et al.* 2020). In addition, 7-ketocholesterol levels have been reported to increase in AD CSF (Iriondo *et al.* 2020). However, it is not clear if circulating 7-oxycholesterols would transfer through BBB to add oxidative stress burden in the brain.

Conclusions

In summary, this meta-analysis does not show significant changes in circulating oxysterols between healthy individuals and people with MCI and AD. One important factor comparing biomarker results across decades is the changes to the classification between MCI and AD. While early studies were mainly based on clinical examinations, later studies included other biomarkers such as Aβ or Tau to categorise disease groups. This will have significant impact on reported data on oxysterols. Another confounding factor for oxysterol data could be the level of medication between healthy controls and AD patients. While most of the studies that enrol healthy controls do not undergo medications, AD patients could be on many different types of medications to control cognitive functions such as neuropsychiatric drugs, vitamins, anti-hypertensive agents or statins. These drugs may have confounding effects on oxysterol levels. In addition, diet and physical performance play important role in peripheral cholesterol levels that will affect cholesterol metabolites. The context of this data and evidence from previous work suggest that the use of oxysterols as biomarkers in AD is a complex process and it may need careful analysis and modelling to find trends to predict AD risk.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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