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Markers of Cholesterol Metabolism in the Brain Show Stronger Associations with Cerebrovascular Disease than Alzheimer's Disease

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Abstract

Cholesterol metabolism is believed to play a role in the development of Alzheimer's disease (AD). Oxysterol metabolites of cholesterol, 24S-hydroxycholesterol (24-OHC, a brain-derived oxysterol) and 27-hydroxycholesterol (27-OHC, a peripherally derived oxysterol) cross the blood brain barrier and have been associated with Alzheimer's disease (AD). We investigated whether oxysterols were associated with markers of cerebrovascular disease prior to the onset of cognitive impairment.

Oxysterols were quantified in 105 participants (average age was 80±4 years) from the Pittsburgh Cardiovascular Health Study Cognition Study (CHS-CS) who remained cognitively normal at blood draw in 2002, had MRI in 1992 and 1998 and annual cognitive assessment for incident AD and mild cognitive impairment (MCI) made by consensus conference between 1998 and 2010.

Higher plasma levels of 24-OHC were associated with age, gender, the presence of high grade white matter hyperintensities (WMH) and brain infarcts on prior MRI. Participants with higher plasma 24-OHC and a greater ratio of 24-OHC/27-OHC were also more likely to develop incident cognitive impairment over 8 years of follow-up.

Higher levels of 24-OHC suggest increased cholesterol metabolism occurring in the brains of participants with cerebrovascular disease prior to the onset of cognitive impairment.

Measurement of oxysterols may provide information about cholesterol metabolism and brain disease over the cognitive impairment process.

Keywords

oxysterols; 24S-hydroxycholesterol; cerebrovascular disease; dementia and Alzheimer's disease

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Introduction

Cholesterol is believed to play a role in the development of Alzheimer's disease (AD). Modifying cholesterol synthesis has been used as an intervention target for dementia without much success[1, 2]. Total cholesterol levels in the blood may have little relevance to cholesterol levels in the brain because cholesterol cannot cross the blood brain barrier (BBB) [3].

Two enzymatically formed metabolites of cholesterol, 24S-hydroxycholesterol (24-OHC, a brain-derived metabolite) and 27-hydroxycholesterol (27-OHC, a peripherally derived metabolite), cross the BBB directly by diffusion and can be measured in the blood[4]. More than 90% of 24-OHC circulating in the blood can be attributed to the central nervous system; meanwhile, less than 10% of blood levels come from peripheral sources including the testes, bone marrow, lungs, eye tissue, adrenals, among others[5]. 24-OHC is the primary metabolite of cholesterol in the brain and is associated with both AD and brain volume[5, 6]. However, associations between plasma 24-OHC levels and AD have been inconsistent in cross-sectional analyses and may depend on disease stage[5]. The levels of plasma 24-OHC are higher in recently diagnosed patients with AD and vascular dementia compared to controls[7], lower in long-term cases of AD[8, 9], and lower with severity of AD[10]. Plasma 24-OHC associated with speed of processing[11] in cross-sectional studies, but not longitudinal change in cognitive performance[12].

Lower plasma levels of 24-OHC are also associated with reduced total gray matter volumes[6] and with smaller hippocampal volumes in cognitively normal individuals[13]. These MRI studies support autopsy findings of a lower synthesis rate of 24-OHC in hippocampus with aging[14] and lower levels of 24-OHC in the tissues of the frontal cortex in AD[15]. However, little is known about the relationship between 24-OHC and structural integrity of the brain during the cognitive impairment process. We investigated whether brain-derived, 24-OHC is associated with incident cognitive impairment and prior evidence of cerebrovascular disease on MRI. Specifically, we quantified the retrospective associations between plasma oxysterol levels and MRI markers of cerebrovascular disease and markers of AD and evaluated the prospective associations between oxysterols and incident cognitive impairment.

Methods

Subjects

The Pittsburgh Cardiovascular Health Study Cognition Study (CHS-CS) is a sub-study of the Cardiovascular Health Study (CHS). Eligibility for the CHS-CS (n=532) participants included: MRI of the brain and detailed cognitive exam in 1992-93 and a second MRI or detailed cognitive exam in 1998-99. Participants were non-demented in 1998 and followed to 2010 with repeat cognitive tests to determine the incidence of dementia and mild cognitive impairment (MCI). As part of the informed consent in CHS-CS, subjects agreed: 1) to release their findings from previous parts of the CHS and 2) to the storage of blood samples for future laboratory analyses. For the purposes of this study, we selected 105 participants from this CHS-CS who were cognitively normal in 1998-99 and 2002, had blood drawn in 2002, and had repeat cognitive exams between 2002 and 2010 (see Supplementary Figure 1).

Assay of oxysterols and cholesterol

The general blood collection and laboratory methods in the CHS have been previously published [16]. Single tubes of serum for analysis of total plasma lipids and oxysterols were thawed once for each assay. The use of stored samples (at −70 C) may increase the

possibility of autoxidation of 24-OHC and 27-OHC during sample preparation. We addressed this by adding the antioxidant butylated hydroxytoluene (BHT) immediately after thawing the sample. Oxysterols (24S-hydroxycholesterol and 27-hydroxycholesterol) were analyzed by isotope dilution using gas chromatography/mass spectroscopy (GC/MS) using well known and reproducible methods[17]. Briefly, the GC/MS conditions included 2uL injection of sample suspended in heptane. Temperatures held at 180 for 1 minute prior to injection followed by a ramp rate of 10°C per minute to 260°C after injection. The Thermo Scientific® column used in this analysis had a length of 30m with internal diameter of 0.25 mm with a film of 0.25 μm. Each run included two control pool human serum samples and directly derivatized standards (100 ng/mL) for 24-OHC and 27-OHC. Control samples were pooled from multiple individuals from the Pittsburgh Blood Bank to reflect mean levels of various metabolites. Exact retention times for each quantitative ion were identified using directly derivatized standards and internal standards added to each sample. We measured ion peaks at m/z of 413 and 416 for 24-OHC and 456 and 461 for 27-OHC for unlabeled and deuterated compounds, respectively. A commercially available internal standard of 27-OHC (D_5) labeled at 26, 26, 26, 27, 27 was purchased from Medical Isotopes, Inc. (NH, USA). An internal standard of 24RS-OHC (D_4) labeled at 23, 23, 24, 24 was made by RWE in the Heinz lab using the techniques developed by Dzeletovic et al[17].

The reproducibility and reliability of our oxysterol measurements were determined from a random selection of 14 samples with assay repeated one month apart using standard measures of reproducibility and reliability[18]. Variance components analysis determined the variability in oxysterol measures due to the assay, sample preparation and individual sample, reported as intraclass correlation coefficients for 24-OHC and 27-OHC. Intraclass correlation coefficients above 70% are generally interpreted as having excellent reproducibility between repeated measures[18]. We also calculated the coefficient of variation (CV) for duplicate control pool samples included in each run which represent mean oxysterol levels in the general population. The between run CV calculated across all runs was 8.9% for 24-OHC and 13.6% for 27-OHC. The within run CV was 5.6% for 24-OHC and 7.6% for 27-OHC.

Total cholesterol, HDL-C, and triglyceride concentrations were determined at the same time as oxysterols from the same stored plasma samples from 2002 by conventional enzymatic methods from fasting (12-hour) blood samples. Low-density lipoprotein cholesterol level was estimated by the Friedewald equation[19]. All oxysterol and lipid analyses were conducted at the Heinz Nutrition Laboratory in the Department of Epidemiology, University of Pittsburgh.

Diagnosis of cognitive impairment

The diagnosis of dementia and mild cognitive impairment (MCI) was based on cognitive and neuropsychological batteries[20]. The diagnosis of dementia was based on a deficit in performance in two or more cognitive domains that was of sufficient severity to affect the subject's activities of daily living and on history of normal intellectual function before onset of cognitive abnormalities[20]. An adjudication committee classified diagnosis of AD (probable and possible) using NINDS-ARDA criteria. Proxies provided additional information related to time of onset of symptoms. Individuals were considered prevalent AD cases in 2002 if they were diagnosed with AD after 2002 and participant's proxy reported onset of cognitive complaints and symptoms before 2002. In this case, proxy information indicated the neurodegenerative process was more advanced. Prevalent cases were excluded from analyses of incident AD.

ApoE genotyping

The three major allelic forms of the ApoE gene were determined in the Core Molecular Genetics facility at the University of Vermont College of Medicine by the method of Hixson and Vernier as previously described by Kuller et al[21]. Approximately 20% of this sample (n=19) were carriers of the ApoE epsilon 4 allele; of these, only two participants had the 4/4 genotype.

Brain Imaging

Because not all participants had an MRI of the brain in 2002-03, we examined the relationship between oxysterols levels in 2002 and MRI measures in 1997-98. All MRI data acquired in 1998-1999 using a 1.5-T GE Signa system (LX version; Milwaukee, WI) and described in detail by Yue et al.[22]. White matter hyperintensity (WMH) grade was estimated as the total extent of periventricular and subcortical white matter signal abnormality on spin-density- weighted axial images that successively increased from no detectable hyperintensities (grade 0) to almost all white matter involved (grade 9)[22]. In this age group, only 4 participants had no evidence of hyperintensities in the brain; therefore, WMH grades 0 and 1 were combined to produce a reference group of individuals with little to no detectable WMH. Abnormalities interpreted as representing areas of large-vessel cerebral infarction or small-vessel lacunar infarction were coded separately in the database as infarct-like lesions[23]. The degree of ventricular enlargement was also assessed on a 10-point scale from 0 to 9, according to an atlas of predefined visual standards[22].

Statistical Analysis

Reproducibility and reliability of repeated oxysterol measures were estimated by calculating the intra-class correlation coefficients (SAS, PROC VARCOMP) and evaluated outliers and systematic bias using scatter plots and Bland-Altman plots. Both 24-OHC and 27-OHC were found to have approximately normal distributions. Differences in potential covariates between the analytic sample (n=105) and the eligible CHS-CS participants (n=524) were evaluated by *t*- tests and $\chi 2$ tests. The associations between oxysterols, potential covariates and prior MRI measures were estimated using multivariable linear regression. The prospective associations between oxysterols and incident cognitive impairment groups were estimated using multinomial logistic regression. Models were first estimated adjusting only for age and then controlling for age, gender and total cholesterol levels in 2002. Quartiles of oxysterols concentrations were considered for time to cognitive impairment modeling using log-rank tests, Kaplan-Meier curves and Cox proportional hazards models. In time to event models, the proportional hazards assumptions were met for oxysterol predictors of time to cognitive impairment; however the inclusion of age into the model did not meet proportional hazards assumptions. All analyses were conducted using SAS *v*9.2 and level of statistical significance was set at $p<0.05$.

Results

Sample Characteristics

Participants included in this analytic sample were to be younger and more educated than the rest of the participants in the greater CHS-CS (Table 1). The mean age of this analytic samples was 80±4 years in 2002, 55% were women (58 of 105), and 66% (68 of 105) had greater than high school education. All participants (n=105) underwent MRI-1 in 1992-93 and 93 (89%) had a second MRI in 1998-99. No significant differences were observed between this sample and the CHS-CS participants (n=532) with regard to gender, race and cardiovascular disease. Mean lipid levels (mg/dL) measured in 2002 were: 187 for total cholesterol, 49 for HDLc, and 109 for LDLc.

Mean oxysterol levels obtained from 105 samples were 42 ng/mL for 24-OHC and 238 ng/mL for 27-OHC. Measures of 24-OHC and 27-OHC were highly reproducible and reliable, when repeated one month apart, with intra-class correlation coefficients of 0.81 and 0.86, respectively. Evaluation of Bland-Altman plots of repeated measures for each oxysterol indicated no relative bias or outliers between sample preparations. Oxysterols, 24-OHC and 27- OHC were highly correlated with each other (Spearman correlation coefficient (r) = 0.51, $p<0.001$). Oxysterols were also highly correlated with concurrent measures of total cholesterol in 2002 [24-OHC ($r=0.43$, $p<0.001$) and 27-OHC ($r=0.25$, $p=0.01$)] and LDLc [24-OHC ($r=0.37$, $p<0.001$) and 27-OHC ($r=0.24$, $p=0.02$)] but not with HDLc levels [24-OHC ($r=0.07$, $p=0.52$) and 27-OHC ($r=0.6$, $p=0.58$)]. Higher plasma levels of 24-OHC in 2002 were associated with female gender and higher cognitive scores on digit-symbol substitution test. Education, race, MMSE, and white matter grade at MRI-1 (1992-93) were not associated with plasma oxysterols. The use of lipid lowering medications at last assessment in CHS (1998) resulted in significantly lower levels of plasma 24-OHC ($p=0.028$) but not of 27-OHC or MRI outcomes. An inverse correlation between 27-OHC and age was present ($r=0.19$, $p=0.05$); however no correlation between 24- OHC and age was indicated ($r=0.05$ $p=0.39$).

Oxysterols and MRI Markers of Prevalent Cerebrovascular Disease

Plasma oxysterols were associated with evidence of cerebrovascular disease on MRI-2 in 1997-1998. Higher levels of 24-OHC in 2002 were significantly associated with higher WMH grade (WMH<2=39 vs. WMH≥2=45, $p=0.01$) and the presence of MRI-defined infarcts (no infarcts=41 vs. infarcts=47, $p=0.05$) at MRI-2. Associations between 24-OHC and WMH grade were independent of age, gender, and total cholesterol levels in 2002(Table 2). Additional adjustment for statin use and diagnosis of cognitive impairment over the study period (1998- 2010) had little effect on these associations (both $p=0.04$). The inclusion of ventricle size and ApoE-4 carrier status produced only mild attenuation of these relationships ($p=0.06$). The 17 participants with MRI-defined brain infarcts at MRI-2 also had significantly elevated 24-OHC and 27-OHC in 2002 (Table 2). Plasma 24-OHC and 27-OHC were not associated with: evidence of cerebrovascular disease on MRI in 1992-1993, age-related size of the ventricles on MRI (1992 or 1998) or with ApoE-4 carrier status (Table 2).

Oxysterols and the Onset of Incident Cognitive Impairment

During mean follow-up of 7.4 years, 37 participants developed incident AD and 36 developed MCI between 2002 and 2010 (table 3). An additional 6 participants (5.7% of the analytic sample) were considered to have prevalent dementia in 2002 and were excluded from this analysis. Only 26 participants remained cognitively normal over the entire observation period (1998-2010) and had a mean age of 86 years in 2010. Incident AD was associated with older age, gender and greater ventricular grade in 1998-99. Incident MCI (n=36, between 2002- 2010) was associated with age and having an increased WMH grade in 1992-93, but not WMH grade in 1998-99. Neither 24-OHC nor 27-OHC was significantly associated with incident MCI or AD when considered as separate groups compared to normal controls. Participants with AD and MCI were combined into a single group, incident cognitive impairment (CI), to provide a larger number of incident cases and improve power to detect small differences in cholesterol metabolism. Compared to participants who remained cognitively normal (CN) over follow-up, participants who developed incident CI had significantly higher levels 24-OHC (CN=0.39, MCI=0.43, AD=0.43, $p=0.05$), slightly lower levels of 27-OHC (CN=268, MCI=234, AD=227, $p=0.18$), resulting in a significantly higher ratio of 24-OHC/27-OHC (CN=0.17, MCI=0.19, AD=0.19, $p=0.02$)(Table 3).

Results from time to event analyses (Supplementary Figure 2) supported results obtained from logistic regression models. Quartiles of 24-OHC were marginally associated with time to cognitive impairment over follow-up period (log-rank test, p=0.065). The lowest quartile of plasma 24-OHC was significantly less likely to develop dementia over the follow-up period in unadjusted proportional hazards models [HR(95%CI) = 0.52(0.27-0.98)]. This difference in survival time held after adjustment for age; however, inclusion of age caused to model to fail proportional hazards assumptions. Quartiles of 27-OHC were not associated with differential time to cognitive impairment (log-rank test, p=0.876). The ratio of 24-OHC/27-OHC was marginally associated with time to cognitive impairment in log-rank tests (log-rank test, p=0.055); however, individual quartiles of 24-OHC/27-OHC were not significantly different in unadjusted proportional hazards models.

Discussion

To our knowledge, this is the first study to show that plasma oxysterols are higher in cognitively normal participants with evidence of small vessel cerebrovascular disease on MRI and higher levels of plasma 24-OHC is associated with the development of incident cognitive impairment. It should be noted that these differences in plasma oxysterols occurred in cognitively normal individuals at least four years prior to the onset of cognitive impairment. These associations were independent of age, gender and concurrent circulating cholesterol levels. Adjusting for future cognitive impairment did not attenuate the relationship between oxysterols and cerebrovascular disease. Specifically, 24-OHC was significantly higher among cognitively normal participants with evidence of cerebrovascular disease as defined by then presence of brain infarcts and high white matter grade at MRI-2 (1998-99) at 4 years prior to blood draw and cognitive impairment. In turn, higher levels of 24-OHC in 2002 were associated with the development of incident cognitive impairment over 8 years of follow-up. Interestingly, both plasma oxysterols were higher in participants with evidence of cerebrovascular disease on MRI; however, only 24-OHC was higher in individuals who went onto develop incident cognitive impairment. Participants who went on to develop incident CI had higher levels of 24-OHC in the plasma relative to 27-OHC. The resulting higher ratio of 24-OHC/27-OHC suggests a higher cholesterol metabolism to oxysterols occurs in the brain versus the periphery of those who go on to develop cognitive impairment.

These findings are compelling in the context of our understanding of the structural and metabolic changes occurring early in the neurodegenerative disease process and may have implications to understanding the underlying metabolic changes occurring in the aging brain. Our findings point to ongoing cerebrovascular disease as a potential mechanism leading to elevated 24-OHC occurring early in the neurodegenerative disease process. The increase in 24- OHC levels could be due to the greater BBB permeability seen in cerebrovascular disease. However, such changes are very likely to be small as indicated by prior studies of 24-OHC levels following stroke[9, 24]. Studies of 24-OHC and demyelinating neurodegerative diseases, suggest disruption of the white matter is highly correlated with increased 24-OHC, particularly in the CSF[5]. The white matter is particularly vulnerable to cerebrovascular disease and the aging process[22]. The white matter holds vast reserves of cholesterol in the brain [25]. Cholesterol provides essential insulation to the axonal myelin of the white matter[25]. The integrity of the myelinated axon is essential to the speed of communication between neurons[25]. Degradation of the myelin is believed to be a normal component of aging and is accelerated in cerebrovascular disease and AD[22]. Myelin breakdown releases excess cholesterol into the extracellular space[25].

Excess cholesterol in the cell membrane has been shown to lead to the production of Aβ and its deposition as plaques *in vitro*[26]. The brain must eliminate excess cholesterol to prevent

Aβ formation. Excess cholesterol is metabolized to 24-OHC by 24-hydroxylase in the neuronal body and crosses the BBB directly by diffusion. Plasma levels of 24-OHC are regarded as a surrogate marker for the number of metabolically active neurons located in the grey matter of the brain[4]. However, in AD patients, an abnormal induction of 24-hydroxylase enzyme in glial cells has also been observed [27], suggesting a compensatory response to the net loss of 24-hydroxylase enzyme resulting from neuronal loss. Taken together, an increased demand to eliminate excess cholesterol from the brain likely results in: the increased production of 24-OHC, a higher diffusion of 24-OHC across the BBB, and higher levels of plasma 24-OHC. Therefore, plasma 24-OHC may be a sensitive marker of increased cholesterol metabolism occurring during acute demyelination[28] early in the neurodegenerative process prior to neuronal loss.

These findings support the hypothesized trajectory of the 24-OHC across the neurodegenerative disease process[5]. Specifically, this study examines the relationship between oxysterols, 24-OHC and 27-OHC, and the longitudinal development of cognitive impairment. Our findings suggest a temporal elevation in plasma 24-OHC occurs prior to the development of incident cognitive impairment. These results would appear to contrast with cross-sectional studies in the literature that show that plasma oxysterol levels are lower in patients with neurodegenerative diseases including patients with AD compared to controls[6, 8, 9, 29]. However, our results support findings from studies of 24-OHC and neurodegenerative diseases[5] as well prior cross-sectional studies of oxysterols and AD, MCI and dementia [5, 7, 29] that suggest plasma 24-OHC is elevated early in the neurodegenerative disease process and maybe lower in patients with a longer duration of AD[6, 9]. Our study provides temporal associations between 24-OHC and incident cognitive impairment. Case-control studies of 24- OHC and AD should be interpreted with caution because they lack temporality, may be confounded by duration of disease and underlying neuropathology. Neurodegeneration, with loss of neurons, would be expected to lead to net reduction in 24-hydroxylase in the brain, a subsequent reduction in the formation of 24-OHC and lower levels of 24-OHC in the circulation. Studies reporting lower levels of 24-OHC in the plasma of patients with dementia have been unable to demonstrate that the lower levels of 24-OHC cannot be attributed to brain atrophy.

The high correlations between oxysterols and lipids (LDLc and total cholesterol) in the blood observed here were previously reported by Burkard *et al.* [30]. They quantified free and esterified 24-OHC and 27-OHC in lipoprotein sub-fractions in healthy volunteers and found that oxysterols were highly correlated with total cholesterol, HDLc and LDLc with spearman correlation coefficients >0.21 for 24-OHC and >0.55 for 27-OHC, respectively. The levels of oxysterols and plasma lipids are affected by statin use; however, controlling for statins did not attenuate the associations between oxysterols and cerebrovascular disease and incident cognitive impairment.

It is possible that high correlations between oxysterols and lipids may be attributed to artificial generation of oxysterols in the stored blood. Autoxidation of cholesterol is a concern for lipid analysis, particularly during storage and processing of samples. However, 24-OHC is not considered a significant autoxidation product of cholesterol in the blood[31]. It is enzymatically produced in a subset of neurons that express 24-hydroxylase. Expression of 24-hydroxylase is responsible for the conversion of excess cholesterol to 24-OHC and is largely confined to the brain and CNS. Specifically, 24-hydroxylase is found in neurons of the cortex, including Purkinje cells of the cerebellum and some neurons of hippocampus and thalamus[32] and maybe expressed the glial cells of AD patients[27]. There is no evidence to date that of its expression in the peripheral nervous system[33]. Furthermore, we prevent potential autoxidation during sample preparation by adding BHT, a potent antioxidant to the serum before processing. If autoxidation occurred in samples during storage, we would

expect that the mean levels of 24- OHC and 27-OHC would have been higher than those previously published in the literature. In fact, the levels of 24-OHC and 27-OHC observed in these samples were comparable to those reported in ng/mL by van den Kommer[12], Teunissen[11], Koschak[13]and Solomon[6]. We note, the levels of 24-OHC measured in this study are somewhat lower than those published in the literature. This is likely to use of an unstandardized technique.

A potential limitation of this study is that the associations between oxysterols and MRI markers of subclinical cerebrovascular disease were not measured at the same time, with MRI occurring four years prior to the measurement of oxysterols. While this design does not facilitate cross-sectional associations, it potentially provides additional temporal information regarding the relationship between subclinical cerebrovascular disease and oxysterol formation. These retrospective associations indicate that MRI defined subclinical cerebrovascular disease is a predictor of oxysterol formation four years later. Because cerebrovascular disease is likely to remain stable or increase with time, it would be expected that subsequent cross sectional associations would be stronger than our retrospective findings. This should be confirmed with future studies.

Strengths of this study include the use of extensive measures of neurological disorders and cognition and the long follow-up time. The CHS-CS participants were followed with repeat cognitive testing from 1998 to 2010. However, the small sample size of this sample limits interpretation of the data. This study did not measure change in oxysterols over time. The hypothesized change in oxysterols over the dementia process is essential to assessing its validity as a marker and has yet to be investigated. We are currently completing the evaluation of the longitudinal change in oxysterols and its relationship to amyloid deposition in brain measured *in vivo* by PET scan using the Pittsburgh compound B (PiB-PET)[34].

Measurement of oxysterols may provide information about cholesterol metabolism and brain disease preceding cognitive impairment. As a marker of excess cholesterol metabolism in the brain, plasma 24-OHC may also have applications to understanding cholesterol metabolism and beta amyloid neuropathology occurring early in the cognitive impairment process. It is possible that measures of oxysterols can provide a tool to investigate both drug and non- pharmacological interventions that may affect cholesterol metabolism in brain and risk of AD. These novel associations need to be replicated in other larger elderly populations. Future research investigating oxysterols as potential markers of AD should consider the structural and metabolic changes occurring in the brain, in particular during the development of cerebrovascular disease and amyloid deposition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of CHS-CS participants and analytic sample in 2002

	CHS-CS cohort (n=524)		Analytic sample (n=105)		p-value
Age, mean, std	82.1	3.9	80.2	3.8	<0.001
Women, *n, %*	265	62.1	58	55.2	0.199
Education >12 years, *n, %*	228	53.4	68	64.7	0.035
White, *n, %*	335	78.5	80	76.2	0.764
ApoE-4 allele*, *n, %*	87	23.0	19	20.0	0.499
Coronary Heart Disease, *n, %*	79	18.5	16	15.5	0.435
Cholesterol in 1991 (mg/dL), *mean, std*	213.0	38.8	214.9	38.5	0.683
Cholesterol in 2002 (mg/dL), *mean, std*					
Total	--	--	186.7	40.0	
HDLc	--	--	49.2	13.0	
LDLc	--	--	109.3	32.0	
Triglycerides	--	--	140.9	66.6	
Oxysterols in 2002 (ng/mL), *mean, std*					
24-OHC	--	--	42.2	11.8	
ratio 24/27	--	--	0.2	0.1	
27-OHC	--	--	238.0	81.1	

*ApoE genotyped on n=382 in CHS-CS and n=97 in analytic sample.

Table 2

Plasma oxysterol concentrations and MRI measures of cerebrovascular disease and AD

	Oxysterol measured in 2002 (ng/mL)									
		24S-hydroxycholesterol			27-hydroxycholesterol			Ratio 24-OHC/27-OHC		
	n	LSmeans	SE	p-value	LSmeans	SE	p-value	LSmeans	SE	p-value
MRI-defined markers of cerebrovascular disease (1998)										
White Matter Grade, n=93										
<2	41	39.3	1.6	0.014	226.5	11.2	0.053	0.18	0.01	0.907
≥2	52	44.7	1.4		255.9	10.0		0.18	0.01	
Infarcts, n=93										
No infarcts	76	41.2	1.2	0.028	233.4	8.1	0.010	0.18	0.01	0.567
Infarcts	17	47.3	2.4		283.9	17.1		0.18	0.01	
Markers of AD (1998)										
Size of ventricles on MRI, n=93										
<5	82	41.8	1.2	0.225	241.3	8.2	0.577	0.18	0.01	0.397
≥5	11	46.1	3.3		255.1	23.1		0.19	0.01	
ApoE genotyping, n=97										
e4 −	86	42.1	1.3	0.870	239.3	8.73	0.345	0.19	0.01	0.623
e4 +	19	41.6	2.6		220.9	17.65		0.19	0.01	

*Least squared means (LSmeans), standard errors (SE) and p?values from general linear models for each oxysterol adjusted for age, gender and total cholesterol levels in 2002.

Table 3

Plasma oxysterol concentrations by cognitive status

		Normal (1998-2010) (n=26)		Mild Cognitive Impairment (2002-2010) (n=36)		Alzheimer's disease (2002-2010) (n=37)			Incident CI (AD + MCI)	
		mean	std	mean	std	mean	std	age-adjusted trend	age-adjusted p-value	Model 2 p-value
24-OHC	ng/mL	39.1	10.5	43.4	13.0	43.3	11.5	0.153	0.054	0.155
27-OHC	ng/mL	268.7	114.7	234.9	66.1	227.4	63.8	0.821	0.185	0.478
24-OHC/27-OHC		0.17	0.05	0.19	0.06	0.19	0.05	0.021	0.019	0.159

Unadjusted means and standard deviations (std) of oxysterol distributions presented for each category of cognitive status.

Model 2 - p-value adjusted for age, gender and total cholesterol

* Prevalent cases of dementia in 2002 (n=6) excluded from analysis of incident cognitive impairment.