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RESEARCH ARTICLE

Circulating sphingolipids in relation to cognitive decline and incident dementia: The Cardiovascular Health Study

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Abstract

INTRODUCTION: Whether circulating levels of sphingolipids are prospectively associated with cognitive decline and dementia risk is uncertain.

METHODS: We measured 14 sphingolipid species in plasma samples from 4488 participants (mean age 76.2 years; 40% male; and 25% apolipoprotein E (*APOE*) ε4 allele carriers). Cognitive decline was assessed annually across 6 years using modified Mini–Mental State Examination (3MSE) and Digital Symbol Substitution Test (DSST). Additionally, a subset of 3050 participants were followed for clinically adjudicated dementia.

RESULTS: Higher plasma levels of sphingomyelin-d18:1/16:0 (SM-16) were associated with a faster cognitive decline measured with 3MSE, in contrast, higher levels of sphingomyelin-d18:1/22:0 (SM-22) were associated with slower decline in cognition measured with DSST. In Cox regression, higher levels of SM-16 (hazard ration [HR] = 1.24 [95% confidence interval [CI]: 1.08–1.44]) and ceramide-d18:1/16:0 (Cer-16) (HR = 1.26 [95% CI: 1.10–1.45]) were associated with higher risk of incident dementia.

Rozenn N. Lemaitre and Majken K. Jensen contributed equally to this study.

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DISCUSSION: Several sphingolipid species appear to be involved in cognitive decline and dementia risk.

KEYWORDS
ceramide, cognitive decline, dementia, glycosphingolipid, sphingolipid, sphingomyelin

Highlights

- Plasma levels of sphingolipids were associated with cognitive decline and dementia risk.
- Ceramides and sphingomyelins with palmitic acid were associated with faster annual cognitive decline and increased risk of dementia.
- The direction of association depended on the covalently bound saturated fatty acid chain length in analysis of cognitive decline.

1 | BACKGROUND

Late-onset dementia is a major cause of suffering and disability among older adults and their caregivers.[1] Current treatment options are few and modest in effect, and the irreversible neurodegeneration associated with dementia starts decades before cognitive symptoms are present.[1,2] Thus, an urgent need exists for the discovery of early noninvasive biomarkers tied to its pathophysiology that could help to identify and target high-risk individuals.

Sphingolipids have been implicated in neurodegeneration and neuroinflammation in recent years.[3,4] These membrane constituents are fundamental components of brain structure and function and relay important signals for intra- and intercellular events.[5,6] The ability of sphingolipids to permeate the blood-brain barrier further allows them to be investigated as noninvasive biomarkers of neurological disease.[5,6]

Subspecies of sphingolipids can be defined based on their head groups and include ceramides (with hydrogen), sphingomyelins (with phosphocholine), and glycosphingolipids (with sugars). However, sphingolipid species also differ depending on the fatty acid that is acylated to the sphingoid backbone, and it is becoming increasingly apparent that the length of the fatty acid chain dictates different biological properties.[7] We have previously highlighted the importance of interrogating sphingolipid subspecies by showing that higher levels of circulating ceramides (Cer) and sphingomyelins (SM) with palmitic acid (16:0) were associated with higher risks of cardiovascular disease and mortality, while Cer and SM with very-long saturated fatty acids such as arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0) were associated with lower risk.[8–10] It is unclear in dementia research on sphingolipids whether associations differ dependent on the length of the fatty acid bound to Cer and SM.

Results are conflicting in prospective studies, where blood measures of sphingolipids were obtained before onset of cognitive impairment and diagnosis. In general, higher plasma levels of Cer with 16:0 (Cer-16) have been associated with an increased risk of Alzheimer's disease (AD); however, associations differed between the sexes.[11–14] Many previous studies were directed toward clinical cohorts with AD patients with heterogeneity in clinical states and lacking consistency in case and noncase definitions as a result. Furthermore, many previous studies were conducted with smaller sample sizes ($N < 500$).

To determine the associations of 14 circulating sphingolipid species with cognitive decline, incident dementia, and dementia-specific mortality, we evaluated participants in the Cardiovascular Health Study (CHS), a large prospective cohort of community-living older adults for whom repeated cognitive testing scores and neurologist-adjudicated dementia status was ascertained.

2 | METHODS

2.1 | Study population

CHS is a prospective study of 5888 community-dwelling adults aged 65 years and older who were randomly selected from Medicare eligibility lists at four study sites in the United States: Forsyth County, NC; Sacramento County, CA; Washington County, MD; Allegheny CA. The study design and sampling methods have been previously described.[15] At the initial baseline (1989–1990), 5201 participants were enrolled, and in 1992–1993, an additional 687 predominantly African American participants were recruited.[16] Participants were followed through annual clinic visits between 1989 and 1999 and then by telephone twice per year thereafter. At each clinic visit, thorough physical examinations were conducted, and other data collected, including demographics, anthropometry, blood pressure, psychosocial interviews, medical history, health behaviors, physical function, hematology, and laboratory examinations. In participants who provided genetic consent, DNA was extracted, and apolipoprotein E (*APOE*) genotype was assessed. The Institutional Review Boards at each study site approved the protocol, and all participants provided written informed consent.

Sphingolipids were measured in stored plasma samples from 4026 individuals from the 1994 to 1995 visit and 586 individuals from the 1992 to 1993 visit (total $N = 4612$). The clinic visits from which the sample used for sphingolipid quantification was taken (1992–1993 or 1994–1995) were used as the study baseline in our analyses.

In addition, cases of dementia that occurred between 1992 and 1993 and 1998 and 1999 were clinically adjudicated in CHS Cognition study that included all participants free of dementia in 1992–1993 with available Modified Mini–Mental State Examination (3MSE) test scores, *APOE* genotyping, and data from a routine MRI examination performed in 1991–1994 ($N = 3602$).[17]

2.2 | Measurements of sphingolipids

Measurement of 22 sphingolipid species with saturated fatty acid chain was performed using ethylenediaminetetraacetic acid (EDTA)-plasma samples that had been stored at −70°C. Plasma lipids were extracted, and sphingolipids quantified by liquid chromatography-tandem mass spectrometry. A detailed description of the measurement methods and quality control procedures is available.[8] For the current study, we restricted analyses to 14 sphingolipid species with coefficients of variation (CV) < 20% estimated over 52 batches of samples. This included four ceramides (Cer-d18:1/16:0 (Cer-16), Cer-d18:1/20:0 (Cer-20), Cer-d18:1/22:0 (Cer-22), the sum of Cer-d18:1/24:0 and Cerd18:0/24:0 (Cer-24); six sphingomyelins (SM-d18:1/14:0 (SM-14), SM-d18:1/16:0 (SM-16), SM-d18:1/18:0 (SM-18), SM-d18:1/20:0 (SM-20), SM-d18:1/22:0 (SM-22), SM-d18:1/24:0) (SM-24); three hexosylceramides (HexCer-d18:1/16:0 (HexCer-16), HexCer-d18:1/22:0 (HexCer-22), and HexCer-d18:1/24:0 (HexCer-24)); and one lactosylceramide (LacCer-d18:1/16:0 (LacCer-16)). See Table S1 for further information on plasma concentrations and CV values.

2.3 | Assessment of cognitive function

Global cognitive function was evaluated annually in the full cohort using the 3MSE from 1990 to 1991 through 1998–1999[18] and the Digit Symbol Substitution Test (DSST) between 1989 and 1990 and 1998 and 1999.[19] The 3MSE evaluates diverse facets of cognition, including memory, orientation, calculation, verbal fluency, and the ability to follow instructions, resulting in a global score ranging from 0 to 100.[18] In the DSST, participants' ability to quickly and accurately match numbers with symbols in 90 s are used to assess information processing speed.[19] All CHS participants with sphingolipids were included in the cognitive analyses. Depending on the year of sphingolipid measurement, participants' individual datasets comprised either baseline (1992–1993) and six-, or baseline (1994–1995) and four subsequent annual test scores.

RESEARCH IN CONTEXT

1. **Systematic review**: A few epidemiological studies have reported associations of circulating levels of sphingolipids with cognition, all-cause dementia, and Alzheimer´s disease (AD), although with inconsistent study design and results.
2. **Interpretation**: Several different sphingolipid species appear to be involved in cognitive decline and dementia risk. The direction of association seems to depend on the saturated fatty acid chain length.
3. **Future directions**: Further studies are warranted to fully understand the mechanisms of sphingolipids in neurodegenerative diseases and to determine whether these can be used as blood-based biomarkers for risk stratification.

2.4 | Assessment of incident dementia and dementia-specific mortality

A detailed methodology for the evaluation of dementia in the CHS Cognition Study has been described elsewhere.[17,20] Briefly, a committee of neurologists and psychiatrists evaluated all available data to classify dementia status among all study participants. Consistent with DSM-IV criteria,[21] a clinical dementia definition was based on progressive or static cognitive decline that affected activities of daily living with impairments in at least two cognitive domains, not necessarily including memory. The dementia type was classified using multiple standardized criteria and MRI examinations. For the diagnosis of AD, the National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria was used,[22] while vascular dementia [VaD] was diagnosed using criteria from the State of California Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC).[23] The year of onset was determined by a review of the annual cognition assessments in combination with collected measurements on depression, medication use, activities of daily living, hospitalizations, and family member input collected in a standardized dementia questionnaire. Dementia-specific mortality refers to dementia being noted as the adjudicated cause of death.[24]

2.5 | Statistical analysis

Prior to analysis, sphingolipid concentrations were log-transformed to reduce skewness and divided by their standard deviation (SD) to standardize values. We used nonparametric testing to estimate correlations among sphingolipid species. APOE ε4 status was missing for 9% of participants in CHS and was included as a missing category.

We evaluated three sets of outcomes, cognitive decline, incident dementia, and dementia-specific mortality. For each of these, we prespecified two primary sets of multivariable models: Model 1 adjusted for age, sex, race/ethnicity, education, field center, and year of sphingolipid measurement. Model 2 additionally included BMI, physical activity, alcohol intake, smoking status, depression score, prevalent diabetes, prevalent CHD, hypertension, lipid-lowering medication use, *APOE* genotype, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol. Covariates were assessed at the respective baseline visit, except for BMI, physical activity, diabetes status, HDL- and LDL-cholesterol, which were not measured at the 1994–1995 visit. The 1992–1993 visit values for these covariates were used for the participants with sphingolipid measurements at the 1994-1995 visit (see Supplement for description of covariate assessments).

Due to mutual correlation and contrasting biological properties, a third model was included only for Cer and SM with 16-, 20-, 22-, and 24-carbon fatty acid chains, as in our previous work.[8–10,25] In Model 3, Cer and SM with 16-carbon fatty acid chains were adjusted for Cer and SM with 22-carbon fatty acid chains, while Cer and SM with very long fatty acid chains (20-, 22-, and 24-carbon) were adjusted for Cer and SM with 16-carbon fatty acid chain.

2.6 | Analysis of cognitive decline

The analysis of cognitive decline included 4488 participants after exclusion for missing covariates ($N = 124$, see Figure S1 for flow chart). We used mixed-effects linear regression models to evaluate the association between one SD in log sphingolipid concentration and cognitive function over time, estimated by the annual rate of change in mean 3MSE and DSST scores over follow-up. All repeated measures of cognition available during follow-up through 1998–1999 were used. Models included terms for each sphingolipid specie, years from sphingolipid measurement to each examination during follow-up (time under observation), the interaction between the sphingolipid specie concentration and time under observation (the primary measure of association), covariates as fixed effects, and participants as random effects with a compound symmetry covariance structure.

2.7 | Analysis of incident dementia and dementia-specific mortality

After excluding 52 individuals with missing covariate information and 259 participants with prevalent dementia at sphingolipid measurement, our analytical dataset included 3050 participants (Figure S1). To examine the associations of each sphingolipid specie with incident all-cause dementia and dementia-specific mortality, we used Cox proportional hazards regression with entry at the time of sphingolipid measurement and time-at-risk until first diagnosis, death, dropout, or last date of follow-up through the 1998–1999 study visit, the last visit of the CHS Cognition Study. Associations are reported as hazard ratios (HR) for dementia per one SD higher log sphingolipid specie concentration (μg/mL) (continuous) and according to tertiles (categorical). The linear trend across tertiles was tested using the median of each tertile as a continuous variable. Schoenfeld residual tests were used to evaluate the proportional hazards assumption for each sphingolipid of interest, and models stratified by quartile of survival time were used to check for nonlinearity.

We also investigated the associations with incident dementia subtypes and tested for equal associations across types, accounting for multiple events per person, with Wald test as described before.[26]

In sensitivity analyses, we further adjusted for history of stroke and estrogen medication use. We examined the potential interaction of each sphingolipid species with age, sex, race, prevalent stroke, and *APOE* genotype at the time of the sphingolipid measurement to investigate whether these factors modified the association of sphingolipids and incident dementia risk.

To correct the analyses for multiple comparisons, we applied a false discovery rate (FDR) adjustment and considered $P_{FDR} < 0.05$ as statistically significant. All statistical analyses were conducted using R version 4.1.3[27] and SAS software version 9.4.[28]

3 | RESULTS

CHS participants ranged from 70 to 86 years old (mean 76 years, SD 4.7) at the time of sphingolipid measurement. The majority of the participants were female and white (Table 1, see Table S1 for baseline characteristics stratified by year of sphingolipid measurement, sex, and *APOE* genotype). The measured sphingolipid species were weak to moderately correlated with each other, with stronger correlations observed among those with very long fatty acid chains (20-, 22-, and 24-carbon) (Table S2).

3.1 | Sphingolipid species differ in their associations with cognitive decline

Higher plasma levels of SM-16 were associated with a sharper decline in 3MSE scores (time × sphingolipid interaction $\beta = -0.08$, $P_{FDR} = 0.004$), but not DSST scores (Figures 1 and 2, Tables S3 and S4). In contrast, higher plasma levels of SM-22 associated with slower decline measured with DSST scores ($\beta = 0.08$, $P_{FDR} = 0.04$).

When we examined these associations across tertiles of sphingolipid concentrations, we observed a dose-response relationship between SM16 and 3MSE scores in that the highest tertile of SM-16 was associated with faster decline in 3MSE scores compared to the first ($\beta = -0.17$, P_{FDR}-linear trend across tertiles = 0.03) (Figure 1). In contrast, the third versus first SM-24 tertile was significantly associated with slower decline in 3MSE scores over time ($\beta = 0.16$, P_{FDR}-linear trend across tertiles = 0.04) (Figure 1).

Finally, we explored associations with circulating glycosphingolipids. Higher plasma levels of LacCer-16 and HexCer-16 were associated with a sharper decline in 3MSE (Figure 1), in contrast, higher levels of

TABLE 1 Baseline characteristics of the study participants in the Cardiovascular Health Study.

Characteristics	Cognition sub-study cohort	Full cohort
n	3050	4488
Age, years; mean (SD)	76.2 (4.7)	76.5 (5.2)
Male, *n* (%)	1244 (40.8)	1842 (41.0)
African American, *n* (%)	422 (13.8)	688 (15.3)
Education, years; mean (SD)	12.8 (2.9)	12.5 (3.1)
BMI, kg/m^2; mean (SD)	26.6 (4.4)	26.7 (4.7)
Physical activity, kcal/week; median [IQR]	960 [340, 2014]	864 [274, 1913]
Alcohol intake, drinks/week; mean (SD)	2.2 (5.2)	2.1 (5.4)
Current smoker, *n* (%)	260 (8.5)	404 (9.0)
Depression score[a]; mean (SD)	5.4 (4.8)	5.7 (5.0)
Prevalent diabetes, *n* (%)	409 (13.4)	659 (14.7)
History of coronary heart disease, *n* (%)	671 (22.0)	1074 (23.9)
Prevalent hypertension, *n* (%)	1702 (55.8)	2609 (58.1)
Prevalent stroke, *n* (%)	153 (5.0)	300 (6.7)
Lipid-lowering medication use, *n* (%)	203 (6.7)	291 (6.5)
Estrogen therapy use, *n* (%)	320 (10.5)	438 (9.8)
APOE ε4 allele carrier, *n* (%)	674 (24.0)[b]	1035 (25.3)[c]
HDL cholesterol, mg/dL; mean (SD)	53.6 (14.5)	53.5 (14.4)
LDL cholesterol, mg/dL; mean (SD)	127 (33.4)	128 (33.9)
Sphingolipids, μg/mL; (median [IQR])		
Cer-16	0.26 [0.22, 0.30]	0.26 [0.22, 0.30]
Cer-20	0.08 [0.06, 0.10]	0.08 [0.06, 0.10]
Cer-22	0.60 [0.49, 0.73]	0.60 [0.49, 0.73]
Cer-24	4.4 [3.8, 5.1]	4.4 [3.7, 5.1]
SM-14	35.3 [28.9, 42.7]	35.0 [28.7, 42.5]
SM-16	123 [112, 135]	124 [112, 136]
SM-18	36.3 [31.1, 42.9]	36.7 [31.3, 43.3]
SM-20	17.6 [15.4, 20.0]	17.5 [15.3, 19.9]
SM-22	26.2 [22.8, 30.0]	26.2 [22.6, 30.1]
SM-24	14.0 [12.0, 16.4]	14.0 [11.9, 16.4]
HexCer-16	0.17 [0.13, 0.22]	0.17 [0.14, 0.23]
HexCer-22	0.18 [0.14, 0.21]	0.18 [0.15, 0.21]
HexCer-24	0.17 [0.14, 0.20]	0.17 [0.14, 0.20]
LacCer-16	0.70 [0.58, 0.86]	0.70 [0.58, 0.86]
DSST score[d]; mean (SD)	40.4 (13.1)	38.0 (14.5)
3MSE score[d]; mean (SD)	92.2 (6.9)	89.7 (11.0)
MCI (<88 3MSE score), *n* (%)	557 (18.3)	1202 (26.8)
Incident dementia during follow-up, *n* (%)	360 (11.8)	360 (8.0)
Alzheimer's disease, *n* (%)	191 (6.3)	191 (4.3)
Vascular dementia, *n* (%)	44 (1.4)	44 (0.98)
Mixed dementia, *n* (%)	106 (3.5)	106 (2.4)
Unknown, *n* (%)	19 (0.62)	19 (0.42)
Dementia-specific deaths during follow-up, *n* (%)	492 (16.3)	492 (11.0)

Note: 14, 16, 18, 20, 22, and 24 stand for the number of carbons of the saturated fatty acid acylated to the sphingolipid backbone.

Abbreviations: 3MSE, modified Mini–Mental State Examination; *APOE E4*, apolipoprotein E4; BMI, body mass index; Cer, ceramide; DSST, Digit Symbol Substitution Test; HDL, high-density lipoprotein; HexCer, hexosylceramide; IQR, interquartile range; LacCer, lactosylceramide; LDL, low-density lipoprotein; MCI, mild cognitive impairment; SM, sphingomyelins.

[a]Depression Score measured with the 10-item version of the Center for Epidemiological Studies Depression Scale with maximum of 30 possible points.

[b]$N = 2806$.

[c]$N = 4089$.

[d]Score ranges from 1 to 100.

HexCer-16 were associated with slower cognitive decline measured with DSST scores (Figure 2).

3.2 | Cer-16 and SM-16 are associated with increased risk for incident dementia

Among the 3050 participants free of dementia at baseline, a total of 360 (12%) developed dementia during maximum follow-up of 7 years (median follow-up = 4.5 [4.1–4.8] years) and 492 (16%) dementia-specific deaths were ascertained during maximum follow-up of 30 years (median follow-up = 12 [6.5–17.1] years).

Higher plasma levels of Cer-16 and SM-16 were associated with a higher risk of incident all-cause dementia after full covariate adjustment and adjustments for Cer-22 and SM-22, respectively. Per SD increase in circulating levels, Cer-16 was associated with 26% increase in risk of incident dementia [HR = 1.26 (95% CI: 1.10–1.45, P_{FDR} = 0.02)] and SM-16 with 24% [HR = 1.24 (95% CI: 1.08–1.44, P_{FDR} = 0.03)] (Figure 3, Table S5). Comparing those in the third versus first tertiles of sphingolipid concentration, we observed HR = 1.63 (95% CI: 1.20–2.23, P_{FDR}-linear trend across tertiles = 0.04) for Cer-16 and HR = 1.79 (95% CI: 1.28–2.49, P_{FDR}-linear trend across

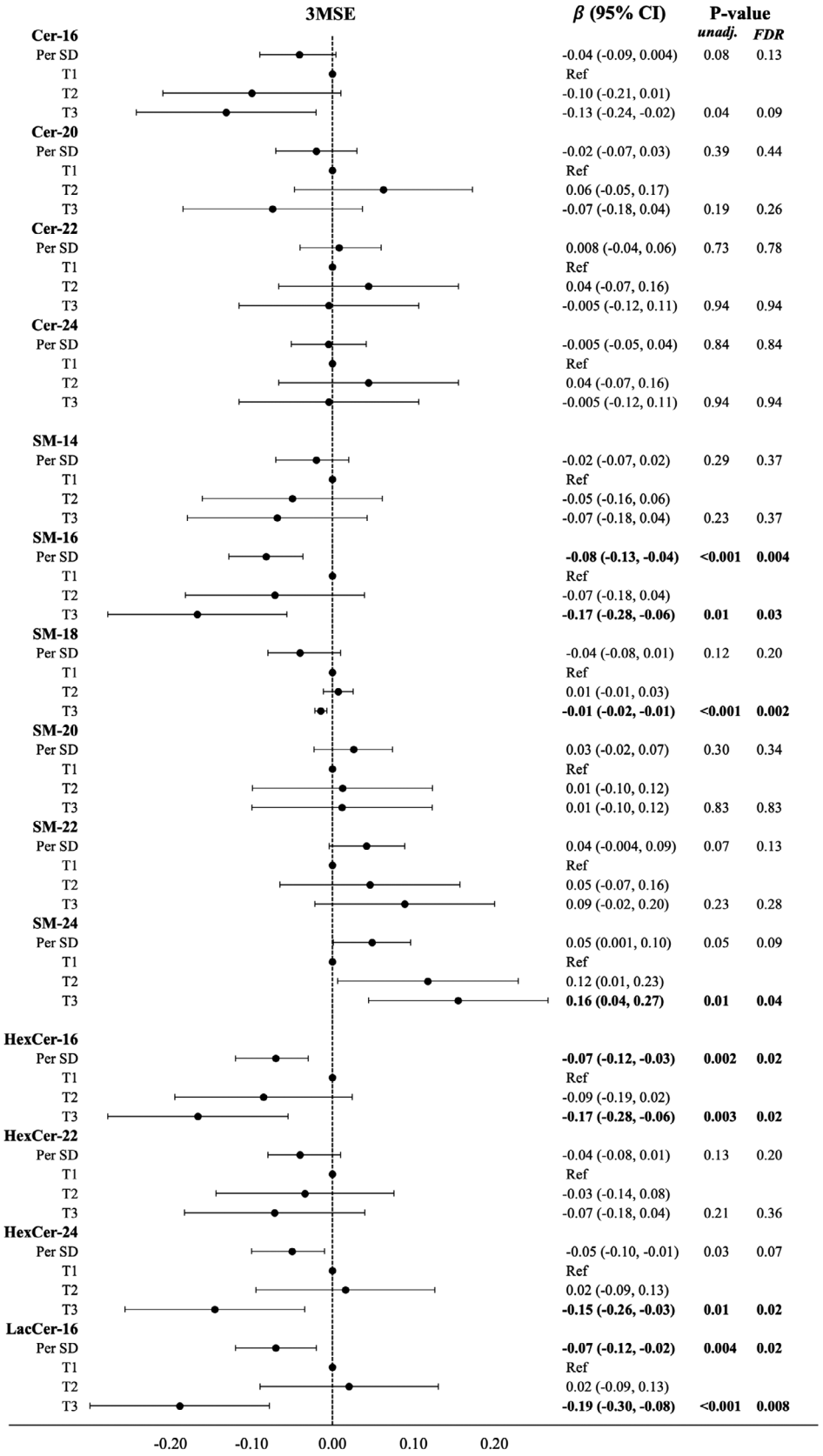

FIGURE 1 The associations between sphingolipids and annual rate of cognitive decline measured with 3MSE scores per SD higher plasma log-sphingolipid concentrations and across tertile, $N = 4488$. Adjusted for age, sex, race/ethnicity, education, field center, year of blood sample measurement, BMI, physical activity, alcohol intake, smoking status, depression score, prevalent diabetes, prevalent coronary heart disease,

tertiles = 0.02) for SM-16 (Figure 3). Including adjustments for prevalent stroke and estrogen therapy did not change the results and no interactions were observed for any sphingolipid specie.

In dementia subtype-specific analyses, we observed 41% higher risk of incident VaD/mixed dementia after full adjustment in Model 3 per SD higher Cer-16 [HR = 1.41 (95% CI: 1.17–1.70, $P_{FDR} = 0.007$)] (Table S6). No associations were observed for risk of AD (Table S6). However, when testing for equal association across AD and VaD/mixed dementia cases, the difference in risk across dementia types for Cer-16 was nominally significant ($p = 0.08$) (Table S6).

We observed no statistically significant associations with dementia-specific mortality (Table S7).

4 | DISCUSSION

Our study represents one of the largest comprehensive investigations of the relationship between several sphingolipid species with the rate of cognitive decline and incident dementia. In this prospective study of older participants, higher plasma levels of Cer-16 and SM-16 were robustly associated with higher incident all-cause dementia risk, and SM-16 with faster cognitive decline after full adjustment. In contrast, higher levels of SM-22 were associated with a slower cognitive decline over time, although this did not translate to a statistically significant reduction in dementia risk. LacCer-16 is associated with faster cognitive decline measured with 3MSE scores, while HexCer-16 showed contrasting associations in the two neuropsychological tests.

Prospective evidence related to sphingolipids, cognition, and dementia has been inconsistent. In prospective studies in 100 women (mean age of 74 years), higher levels of Cer-16, Cer-22, and Cer-24 associated with increased risk of cognitive impairment over maximum 9 years[11] and Cer-16 further associated with an increased risk of AD.[12] In contrast, sex-stratified analysis of individuals from the Baltimore Longitudinal Study of Aging (BLSA) (N = 992, mean age of 63 years) reported that higher levels of Cer-16 associated with increased risk of AD in men and higher levels of SM-16 associated with decreased risk of AD in women.[13] A metabolomics study in a subset of the same cohort (N = 207, mean age of 79 years, 4.3 years follow-up), reported that higher levels of SM-16 associated with an increased risk of conversion from MCI to AD.[29] In the Framingham Offspring study (N = 1892, mean age of 70 years), higher plasma ratios of very long-chain Cer (Cer-22 and Cer-24) to long-chain Cer (Cer-16) were associated with a lower risk of incident all-cause dementia.[14] Indicating that either high levels of Cer-16 are harmful, or that very long-chain Cer are protective. Our findings are supportive of this interpretation. However, in contrast to examining the association of sphingolipid ratios, which assumes equivalence of higher levels of Cer-22/Cer-24 to lower levels of Cer-16, our study shows that the sphingolipid associations are independent.

Two similar metabolomics studies in Alzheimer's Disease Neuroimaging Initiative (ADNI) study investigated sphingolipids and the risk of AD but did not observe any clear associations[29,30] and a large prospective metabolomics study of 274,160 individuals in the UK Biobank (mean age of 58 years) found that higher levels SM (specific species not reported) were associated with decreased risk of all-cause dementia, AD, and vascular dementia.[31]

Most studies report on the associations of sphingolipids with risk of AD. We did not confirm this in subanalysis of type of dementia that included two categories: AD and VaD/mixed dementia. Cer-16 was observed to be associated with an increased risk of VaD/mixed dementia and not AD, but the test of equal associations across type was not statistically significant. This shows that the diagnoses of dementia (in particular, AD and VaD) highly overlap and that it can be difficult to distinguish, which also could explain some of the discrepancies in previous studies.

Our results complement the existing literature by showing robust results on the individual concentration of each sphingolipid subspecies from a large cohort. The results are novel regarding the differences in associations with cognitive decline and incident dementia dependent on the structure of the sphingolipid.

Experimental evidence suggests that low levels of Cer-16, high levels of Cer-22, and Cer-24 appear to promote cell survival and proliferation, while high levels of Cer-16 prevent cell proliferation and induce stress and apoptosis, potentially leading to neurodegeneration.[7,32,33] Genome-wide association studies have identified multiple genetic variants that are associated with AD liability[34–36] many of which are in gene regions related to lipid metabolism including *APOE*, *TREM2*, and *ABCA7*.[37–39] By integrating genomics and metabolomics Dehghan et al. 2022 identified associations between LacCer and AD-related genetic variations in the *ABCA7* gene and showed that higher LacCer plasma concentration was related to poorer cognitive performance, and genetically modified levels of LacCer were associated with AD risk.[39] Additionally, experiments with *ABCA7* knockout mice showed altered concentrations of Cer, SM, HexCer in brain tissue compared to wild-type mice.[39]

Besides investigations of AD, sphingolipids have been associated with other types of neurodegenerative diseases such as Huntington's and Parkinson's disease[40,41] and are involved in neuroinflammation.[42] Cer have been identified as dysregulated in cerebrovascular diseases, including stroke and cerebrovascular small vessel diseases.[43–46]

prevalent hypertension, lipid-lowering medication use, *APOE* genotype, HDL-cholesterol, LDL-cholesterol (Model 2). Mutual adjustments were done for ceramides and sphingomyelins with 16-, 20-, 22-, and 24-carbon fatty acid chain (Model 3). *p*-value for tertile 3 refers to the P-linear trend across tertiles. 3MSE, Modified Mini–Mental State Examination; Cer, ceramide; CI, confidence interval; FDR, false discovery rate; HexCer, hexosylceramide; HDL, high-density lipoprotein; LacCer, lactosylceramide; LDL, low density lipoprotein; SD, standard deviation; SM, sphingomyelins; T1, tertile 1; T2, tertile 2; T3, tertile 3; unadj., unadjusted. 14, 16, 18, 20, 22, and 24 refer to the number of carbons of the saturated fatty acid acylated to the sphingolipid backbone.

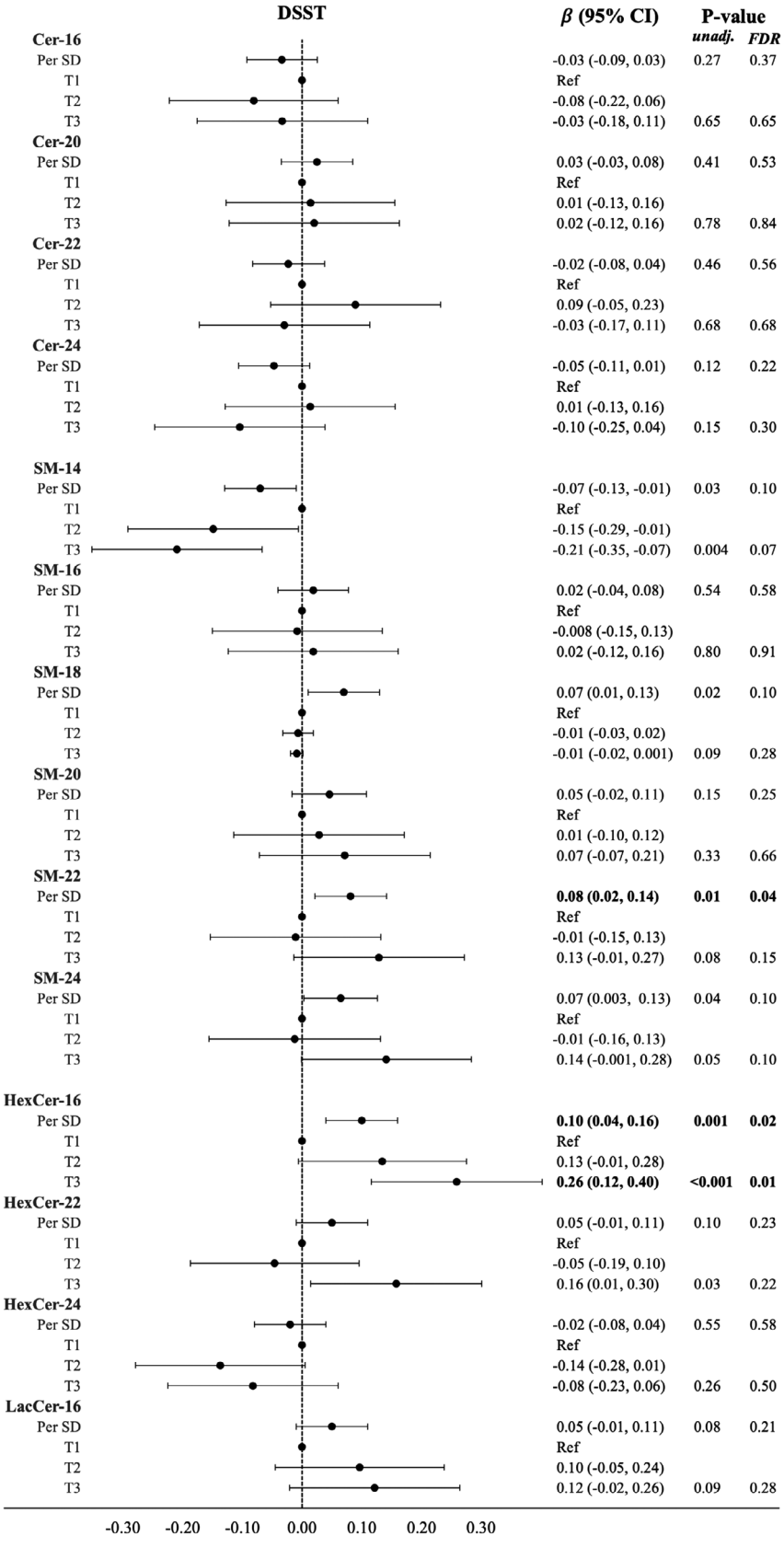

FIGURE 2 The associations between sphingolipids and annual rate of cognitive decline measured with DSST per SD higher plasma log-sphingolipid concentrations and across tertile, $N = 4488$. Adjusted for age, sex, race/ethnicity, education, field center, year of blood sample measurement, BMI, physical activity, alcohol intake, smoking status, depression score, prevalent diabetes, prevalent coronary heart disease, prevalent hypertension, lipid-lowering medication use, *APOE* genotype, HDL-cholesterol, LDL-cholesterol (Model 2). Mutual adjustments were done for ceramides and sphingomyelins with 16-, 20-, 22-, and 24-carbon fatty acid chain (Model 3). *p*-value for tertile 3 refers to the P-linear trend across tertiles. *APOE*, apolipoprotein E; BMI, body mass index; Cer, ceramide; CI, confidence interval; DSST, Digit Symbol Substitution Test; FDR, false discovery rate; HexCer, hexosylceramide; HDL, high-density lipoprotein; LacCer, lactosylceramide; LDL, low-density lipoprotein; SD, standard deviation; SM, sphingomyelins; T1, tertile 1; T2, tertile 2; T3, tertile 3; unadj., unadjusted. 14, 16, 18, 20, 22, and 24 refer to the number of carbons of the saturated fatty acid acylated to the sphingolipid backbone.

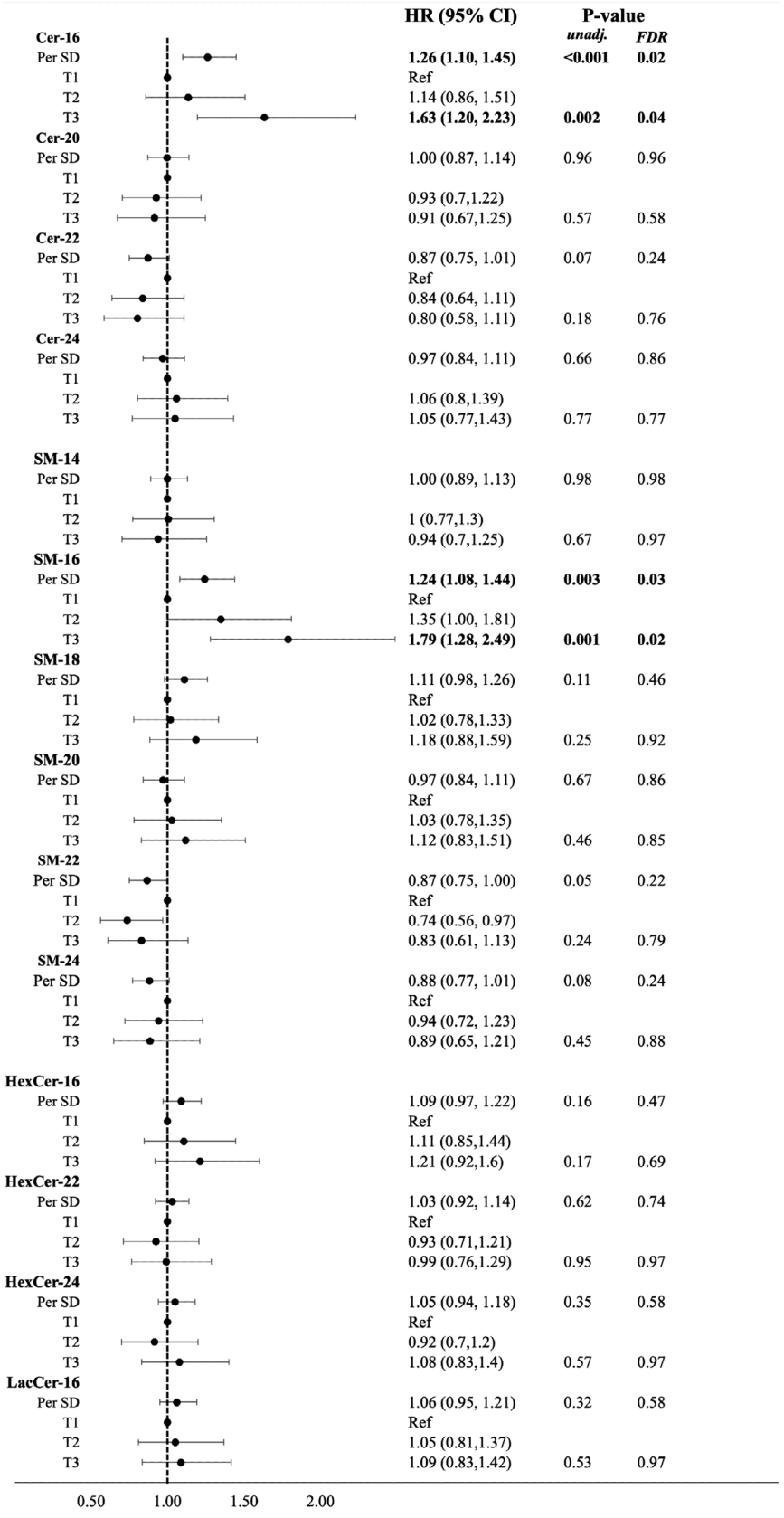

FIGURE 3 Adjusted hazard ratios of incident dementia per SD higher plasma log-sphingolipid concentrations and across tertiles, N = 3,050. Adjusted for age, sex, race/ethnicity, education, field center, year of blood sample measurement, BMI, physical activity, alcohol intake, smoking status, depression score, prevalent diabetes, prevalent coronary heart disease, prevalent hypertension, lipid-lowering medication use, *APOE* genotype, HDL-cholesterol, LDL-cholesterol (Model 2). Mutual adjustments were done for ceramides and sphingomyelins with 16-, 20-, 22-, and 24-carbon fatty acid chain (Model 3). *p*-value for tertile 3 refers to the P-linear trend across tertiles. *APOE*, apolipoprotein E; BMI, body mass index; Cer, ceramide; CI, confidence interval; FDR, false discovery rate; HDL, high-density lipoprotein; HexCer, hexosylceramide; HR, hazard ratio; LacCer, lactosylceramide; LDL, low-density lipoprotein; SD, standard deviation; SM, sphingomyelins; T1, tertile 1; T2, tertile 2; T3, tertile 3; unadj., unadjusted. 14, 16, 18, 20, 22, and 24 refer to the number of carbons of the saturated fatty acid acylated to the sphingolipid backbone.

Hydrolysis of SM by sphingomyelinases activated by stress, inflammation, or ischemia, creates Cer, which is involved in the pathway connecting inflammatory cytokines with insulin resistance and subclinical atherosclerosis,[43,44] which are risk factors for dementia. Moreover, sphingolipids are highly enriched in myelin in the brain and may play a role in neuronal function.[5] Thus, altered sphingolipid metabolism may reflect neurodegeneration, neuroinflammation or both and might be used in conjunction with other emerging biomarkers like glial fibrillary acidic protein (GFAP) and neurofilament light chain (NfL).[47] Blood-based biomarkers can provide a window into the mechanisms of emerging disorders of the brain and provides a minimally invasive and accessible means to access molecular changes. Therefore, circulating sphingolipids as biomarkers for cognitive decline and onset of dementia might advance our understanding of the underlying mechanism and thereby identify susceptible individuals.

Our study has several strengths, including the large sample size of community-living participants recruited from diverse geographic regions and demographic backgrounds in the United States; the prospective study design; the cohort was well-characterized with extensive phenotyping; the comprehensive assessment of multiple outcomes comprising neurologist-adjudicated dementia diagnoses and changes in cognitive function measured using two repeatedly administered standardized neuropsychological tests over at least 4 years; the long follow-up on dementia-specific mortality; as well as control for several potential confounders such as education, comorbidities, and lifestyle factors.

Limitations of our study include the older age of the participants (mean baseline age is 75 years). While this age group represents those at the highest risk of clinically diagnosed dementia, incident cases would have likely been in a prodromal phase of the disease, starting even decades before the clinical symptoms resulting in diagnosis may have arisen. As a result, we cannot tell whether altered plasma levels of sphingolipids are involved in disease onset or merely biomarkers of preclinical disease progression. In addition, adjudication of dementia-specific deaths might have been incomplete, as deaths were indirectly assessed. Furthermore, the study participants were followed during the 1990s, and the socioeconomic, cultural, and healthcare context of the 1990s may differ from contemporary settings. Also, CHS did not include routine positron emission tomography or postmortem examinations, so we are not able to relate the levels of sphingolipids with the buildup of amyloid or other proteinopathies in the brain. Last, the study participants were primarily white, thus our findings may not generalize to other ethnicities or younger populations. Further studies are warranted to determine whether the timing of the measurements (midlife vs. late life) is important. Levels of sphingolipids change with age[48,49]; thus, multiple measurements over a long time will be needed to fully understand the dynamics of sphingolipid metabolism with relation to disease.

In summary, several different sphingolipid species appear to be involved in cognitive decline and dementia risk, although the direction of association depends on the saturated fatty acid chain length. Further studies are warranted to fully understand the mechanisms of sphingolipids in neurodegenerative diseases and to determine whether these can be used as blood-based biomarkers for risk stratification.

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CONFLICT OF INTEREST STATEMENT

Manja Koch is currently employed by Biogen and was previously employed by Harvard School of Public Health during the period when the research was conducted. Héléne T. Cronjé is currently employed by Lane Clark & Peacock LLP. Manja Koch and Héléne T. Cronjé have no financial or non-financial conflicts of interest to declare regarding the research or manuscript presented herein. None of the other authors have competing interests to disclose. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All CHS participants provided voluntary written informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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