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Serum sphingomyelins and ceramides are early predictors of memory impairment

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Abstract

A blood-based biomarker of Alzheimer's disease (AD) progression could be instrumental in targeting asymptomatic individuals for treatment early in the disease process. Given the direct connection between sphingomyelins (SM), ceramides, and apoptosis, these lipids may be indicators of neurodegeneration and AD progression. Baseline serum SM and ceramides from 100 women enrolled in a longitudinal population-based study were examined as predictors of cognitive impairment. Participants were followed up to 6 visits over 9 years. Baseline lipids, in tertiles, were examined in relation to cross-sectional and incident impairment (<1.5 SD below standard norms) on HVLT-immediate and -delayed memory recall and Trails A and B. SM and ceramides varied in relation to the timing of HVLT-delayed impairment: low levels were associated with cross-sectional impairment; high levels predicted incident impairment in asymptomatic individuals. Lipids were not associated with loss-to-follow-up. Results suggest serum SM and ceramides vary according to the timing of the onset of memory impairment and may be good pre-clinical predictors, or biomarkers, of memory impairment: a deficit observed early in AD pathogenesis.

Keywords

Sphingomyelins; Ceramides; Serum lipid markers; Memory; Alzheimer's disease; Biomarker

1. Introduction

While progress has been made identifying diagnostic biomarkers for Alzheimer's disease (AD), including cerebrospinal fluid (CSF) amyloid-beta and phospho-tau, there currently are

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no established biomarkers of AD progression. AD has a long preclinical phase during which the biological changes of the disease affect the brain. Clinical changes are imprecise indicators of disease progression and only observed after substantial pathology has developed. Thus, there is an urgent need to identify AD biomarkers early in the disease process (e.g., that predict transition to early symptoms) before the pathology becomes too great to reverse.

Pathologically, the hallmarks of AD are amyloid plaques and neurofibrillary tangles, associated with widespread neuronal loss. Its fundamental causes and the pathological cascades leading to symptoms, however, remain poorly understood. Lipids and lipid peroxidation products have important roles in central nervous system homeostasis; lipid transport genes and peripheral dyslipidemia are associated with an increased risk of AD [21]. While laboratory and animal studies suggest hypercholesterolemia is a key factor in AD [29,30], epidemiological studies are conflicting [19,22,32]. Other lipids, such as sphingomyelins (SM) or ceramides, may be better indicators of AD progression by reflecting ongoing neurodegeneration.

Sphingomyelins (SM), ubiquitous substances present in cell membranes, are critical to lipid raft formations where cellular processes including signal transduction, membrane trafficking and protein sorting occur [4]. Ceramides, SM metabolites, are second messengers that regulate cellular differentiation, proliferation, and apoptosis by activating signaling cascades and promoting free radical generation [1]. Given the direct connection between these lipids and apoptosis, it is likely they could be indicators of preclinical neurodegeneration. Limited clinical research has examined the role of SM and ceramides in AD pathogenesis. Post-mortem studies and the one CSF study suggest AD cases have higher levels of SM and/or ceramides [7,13, 25,26,28]. Importantly, levels also vary by disease severity [7,13,28], suggesting the possible use of these lipids as biomarkers of AD progression.

In the present study, we extend this clinical research to examine whether serum SM or ceramides are *predictors* of cognitive impairment. Identifying a biomarker of AD progression in serum would be a significant step forward because a blood-based biomarker would meet expert criteria of being “non-invasive, simple to perform, inexpensive” [12], and superior to more invasive and costly CSF-based or brain imaging biomarkers. Using the Women’s Health and Aging Study (WHAS) II, a population-based longitudinal study of older women followed up to nine years, we examined baseline serum SM and ceramides levels as predictors of subsequent cognitive impairment.

2. Methods

2.1. Study population

WHAS II is a prospective study of physical functioning among the two-thirds least disabled 70-79 year-old community-dwelling women in Baltimore, Maryland. The sampling and recruitment of this cohort have been previously described [5,9]. Briefly, WHAS II was designed to be a companion study to WHAS I, a study of the one-third most disabled older women residing in the community. Both cohorts were sampled from the same sampling frame using the Health Care Financing Administration’s Medicare eligibility lists for 12 zip code areas in eastern Baltimore City and County. For WHAS II, age-stratified (70-74, 75-79) random samples were drawn by Westat, Inc in 1994-95. Trained interviewers screened 1,630 women and determined eligibility according to whether women: 1) were 70 to 79 years; 2) had sufficient hearing and English proficiency to be interviewed; 3) could be contacted by telephone; 4) had a MMSE score ≥24; and 5) reported no, or limited, difficulty in only one of the following four domains: mobility and exercise tolerance, upper extremity function, high functioning tasks, and basic self-care. Of 880 women screened eligible, 436 (49.5%) agreed to participate in the baseline examination at the Johns Hopkins Hospital and to prospective follow-up. Those agreeing to participant were more highly educated and had more diseases

than those who refused, but did not differ on other characteristics [5,9]. For the present study, 223 women had adequate serum baseline samples remaining in storage. Due to the limited blood reserved, we randomly selected 100 participants' serum samples for the lipid assays.

Follow-ups were conducted approximately 1.5, 3, 6, 7.5 and 9 years later. Each examination consisted of a comprehensive medical history, medication inventory, physical and neurological exam, neuropsychological battery, and blood draw. All women provided informed consent and the study was approved by the Johns Hopkins IRB.

2.2. Lipid assays

Non-fasting blood was drawn at baseline and serum was frozen at -80°C until processing. For lipid extraction from serum, three volumes of 100% methanol containing 30mM ammonium acetate were added to each sample and vortexed. Four volumes of chloroform were then added, the mixture was vortexed and centrifuged at 1,000*g* for ten minutes. The bottom (chloroform) layer was removed and analyzed by direct injection into a tandem mass spectrometer. Lipid extractions were performed using borosilicate-coated glass tubes, pipettes, and injectors.

Lipid analyses were performed using methods similar to those reported in previous studies [2]. Samples were injected using a Harvard Apparatus pump at the rate of 15μl/min into a Sciex API3000 electrospray ionization triple stage quadruple tandem mass spectrometer (ESI/MS/MS; Thornhill, Ontario, Canada) operated in the positive mode. The ESI/MS/MS scanned from 300 to 1,000 atomic mass units (amu) per second at a step of 0.1amu. Each lipid species was initially identified by a Q1 mass scan, then by precursor ion scanning or neutral loss scanning of a purified standard. Samples were injected into the ESI/MS/MS for three minutes, where the mass counts accumulate and the sum of the total counts under each peak were used to quantitate each analyate. SM and ceramides were purchased from Avanti Polar Lipids (Alabaster, AL, USA). The coefficient of variations for SM analytes ranged from 5.4-16.4% and for ceramides from 4.6-12.8%.

SM analytes were highly correlated ($p<0.0001$) after adjusting for Bonferroni correction. We therefore summed all SM analytes to create a single (total) SM variable. Ceramides were less correlated, so each analyte was examined separately. Total SM and ceramides were analyzed in tertiles because they were highly skewed to the right.

2.3. Cognitive outcomes

The Trail Making Test was used to evaluate psychomotor speed via Part A (TMT-A), and executive function via Part B (TMT-B). Verbal immediate and delayed memory were assessed using the Hopkins Verbal Learning Test (HVLT-R). Impairment was conservatively defined as the first performance at or below the tenth percentile for each cognitive test using published age-and education-matched norms: TMT-A≥ 81 sec.; TMT-B≥ 225 sec. [15]; HVLT-immediate≤ 16; HVLT-delayed≤ 4 [3]. These cut-points matched well with internal norms, corresponding to 1.4-1.8 standard deviations below mean baseline scores, and between the fifth and twelfth percentiles.

2.4. Covariates

To examine the effects of health and demographic characteristics on lipid levels, we first assessed the association between these variables and tertiles of SM and ceramides using ANOVA for continuous variables and Fischer's Exact Test for dichotomous variables. Covariates included baseline age, race, education, smoking status, and minutes of exercise per week; medical conditions such as systolic and diastolic blood pressure, diabetes, myocardial infarction, stroke, angina, peripheral artery disease, and depression; statins and other medications; serum total and HDL cholesterol, triglycerides, blood glucose, creatinine and

albumin levels. Of these variables, blood glucose was consistently higher and BMI lower in the highest lipid tertile. Multivariate analyses controlled for baseline blood glucose levels, BMI, and age.

2.5. Statistical analysis

T-tests and Fischer's Exact Tests were used to compare the 100 randomly selected women with available baseline samples and the 123 women with available samples who were not selected. Differences between the 100 women with assayed lipids and the rest of the population (n=336), regardless of sample availability, were also examined.

Of the 100 women included, two were missing HVLT-delayed scores at all visits, one was missing HVLT-immediate scores, two were missing TMT-A scores, and one was missing TMT-B scores, leaving between 98-99 women for all analyses. The baseline, cross-sectional, association between SM and ceramides, in tertiles, and cognitive impairment on each test was examined using logistic regression. After excluding prevalent cases of impairment, discrete time Cox proportional hazards regression with generalized linear modeling and a complementary log-log link was used to assess the effect of baseline lipid levels on the risk of developing impairment on each cognitive test. Modeling the dichotomous outcome using a complementary log-log link yielded comparable results to those produced by the usual continuous time proportional hazard model [6]; exponentiated coefficients can be interpreted as hazard ratios. Subjects were included in longitudinal analyses if they received a baseline evaluation and at least one additional follow-up. For each outcome, subjects contributed information up to the examination at which they first developed impairment on that test, died, or were lost to follow-up and therefore censored. Both univariate and multivariate models, controlling for age, blood glucose and BMI, were examined. The *a-priori* p-value for cross-sectional and longitudinal models was $p<0.05$. Analyses were conducted using Stata Version 9.2 (StataCorp, College Station, TX).

3. Results

The 100 participants with lipid assays were younger (74.0 vs 74.7; $p = 0.036$) and had lower baseline systolic blood pressure (148.6 vs 153.5, $p = 0.047$) compared to the rest of the sample (n=336) (Table 1). No other health or demographic characteristics differed between the two groups, including baseline cognitive test scores. Among the 223 women with available baseline serum samples, there were no differences between the 100 women randomly selected for the study and the 123 women not selected.

At baseline, 8 participants were cognitively impaired on HVLT-delayed, 10 on HVLT-immediate, 10 on TMT-A, and 11 on TMT-B. Using logistic regression to examine the cross-sectional association between lipids and cognition, each tertile increase in total SM (odds ratio [OR], 0.31, $p = 0.047$) was associated with reduced odds of impairment on HVLT-delayed. Controlling for baseline age, blood glucose, and BMI slightly attenuated this relationship (OR 0.32, $p = 0.069$). Higher levels of the ceramide analytes were also associated with reduced odds of HVLT-delayed impairment, but results did not reach significance. There was no association between SM or ceramides and cross-sectional impairment on any other test.

After excluding women with baseline impairment on each cognitive test, there were 23 incident cases of impairment on HVLT-delayed (23/90, 25.6%); 27 on HVLT-immediate (27/89, 30.3%); 24 on TMT-A (24/88, 27.3%); and 34 on TMT-B (34/88, 38.6%). Using multivariate discrete time survival analysis, higher levels of total SM and ceramides C16:0, C18:0, C22:0, C24:1, C24:0, stearoyl, and sulfatide were associated with a significantly increased risk of incident impairment on HVLT-delayed (Table 2). In fact, none of the participants in the lowest tertile of ceramide C22:0, and only one participant in the lowest tertiles of C16:0 and C24:0

developed HVLT-delayed impairment over 9-years of follow-up. Notably, while total SM increased stepwise in risk per tertile, there was a threshold effect for most ceramides such that both the second and third tertiles exhibited increased risk of HVLT-delayed impairment. Figures 1 and 2 presents Kaplan-Meier graphs for tertiles of total SM, ceramide C22:0 and incident HVLT-delayed impairment.

Examining other outcomes, compared to the lowest tertile, the second tertile of ceramides C16:0 (hazard ratio [HR], 13.73, p = 0.001), C20:0 (HR 4.04, p = 0.025), C22:0 (HR 8.12, p = 0.007), and stearoyl (HR 3.14, p = 0.032) were associated with a greater risk of impairment on HVLT-immediate recall, while the effects of the highest tertiles were attenuated (Table 3). Similarly, there was a U-shaped curve for these lipids and risk of TMT-A impairment such that the second tertiles of C16:0 (HR 3.80, p = 0.021), C20:0 (HR 3.63, p = 0.048), and stearoyl (HR 3.09, p = 0.047), but not the highest tertile, was associated with increased risk (Table 4). There was no association between SM or ceramides and risk of TMT-B impairment (Table 5).

We examined baseline total and HDL cholesterol and triglycerides, but did not find associations between these sterols and incident cognitive impairment. We also examined the SM/total cholesterol and SM/ceramide ratios. Higher levels of SM/total cholesterol were associated with an increased risk of HVLT-delayed impairment; the highest tertile exhibited a 3-fold increased risk (Table 2), but the association was borderline significant (p = 0.074). There was no association between the SM/ceramide ratio and incident impairment. Further, baseline SM and ceramide levels were not associated with loss-to-follow-up.

4. Discussion

In this population-based study of older women, *low* levels of serum total SM and ceramides were associated with cross-sectional memory impairment on delayed recall while *high* levels predicted incident impairment up to nine years later. Three specific ceramides (C16:0, C20:0, and stearoyl) also predicted impairment on immediate recall and psychomotor speed. These findings suggest that serum SM and ceramides vary according to proximity to onset of memory impairment and therefore may be good pre-clinical predictors, or biomarkers, of memory impairment: a deficit observed early in AD pathogenesis.

Our results, examining blood lipids as predictors of memory impairment, build on findings from post-mortem and CSF studies comparing ceramides and SM in AD patients and controls. AD patients had higher levels of ceramides in the middle frontal cortex [7] and white matter [13] and these levels peaked in early mild dementia (CDR = 0.5) [13]. Elevated SM levels were found in the inferior parietal lobe of AD patients, and had a strong positive correlation with the number of amyloid-beta plaques [25]. In contrast, one study reported lower SM levels in the middle frontal gyrus of AD patients [7]. The conflicting results could be due to the stage of the disease process studied. One CSF study reported higher ceramide levels in moderate versus mild or severe AD [28], while another examining brain tissue reported that the gene expression patterns of enzymes participating in the sphingolipid metabolism pathway varied by AD severity [18]. Similarly, we found serum SM and ceramides varied by time to onset of memory impairment, such that levels were higher pre-symptomatically but lower at the time of impairment.

While the present results are promising, the exact mechanism(s) by which these peripheral blood lipids are associated with memory impairment is not well understood. A recent study examining plasma signaling proteins as predictors of AD diagnosis suggested changes in blood may be representative of structural and metabolic changes in the brain, and specific to AD [27]. We have a similar hypothesis for SM and ceramides because the homeostasis of these lipids are critical to both the brain and periphery. SM are important structural compounds that

give cells their asymmetric shape and marked curvature [20]; loss of this membrane symmetry is an early event in apoptosis. SM are metabolized into ceramides, second messengers that regulate cellular differentiation, proliferation, and apoptosis [1]. At low levels, ceramides promote cell survival and division and play a fundamental role in injury-induced cytokine production. At high levels, they inhibit cell division, promote stress signaling cascades and induce apoptosis [11]. Ceramides are also intermediates linking inflammatory cytokines to insulin resistance [31] and subclinical atherosclerosis [14,24]; all of which are associated with an increased risk of AD.

Cholesterol and SM preferentially interact in cell membranes. This interaction has a direct effect on the structure and permeability of the membranes [23] and is important for lipid raft formation. These domains contribute to a variety of cellular processes and second messenger systems, including signal transduction, membrane trafficking and protein sorting [4]. In this context, it is important to highlight that the serum SM/total cholesterol ratio was predictive of memory impairment in our study. This ratio may be a better pre-clinical predictor of AD pathogenesis than total cholesterol alone.

Recent studies have suggested a direct association between ceramides and AD. Amyloid-beta (1-42) induces ceramide production, leading to neuronal apoptosis [16]. Alternatively, ceramides modulate BACE activity [17]. One post-mortem study reported high SM levels were specific to AD; there were no differences between SM levels in normal control brains and those with amyotrophic lateral sclerosis, striato-nigral degeneration, primary progressive aphasia, or progressive supranuclear palsy[25]. This specificity is in line with our main findings suggesting that ceramides and SM are important predictors of memory impairment, the first domain affected in AD. However, given their role in apoptosis, it is still possible that serum SM and ceramides may be indicators of neurodegeneration and, therefore, not specific to AD. For example, elevated CSF SM and ceramides were predictive of HIV dementia progression [2], but blood lipid levels were not examined. Nonetheless, lack of specificity does not preclude these serum lipids from being biomarkers of AD progression, and suggests future avenues of research for other neurodegenerative disorders.

SM and ceramide levels have been reported to increase with age [7], the major risk factor for AD. As we currently do not have specific clinical diagnoses of MCI and AD in our population, it is possible that SM and ceramides could reflect accelerated age-associated impairment. However, previous studies have found that cognitive decline in normal aging, in contrast to neurodegenerative disease, is not associated with a significant loss of neurons [10] but that age-related deficits are more reflective of subtle changes in synaptic connectivity [8]. Thus, the apoptotic affects of these lipids suggest they are more indicative of neurodegenerative processes and not cognitive aging. Additionally, lipids did not vary by age in the present analysis.

Several limitations warrant consideration. First, this sample was composed of women and may not be generalizable to men. It is small from an epidemiological perspective, but large when compared to post-mortem and CSF studies conducted to date. Second, serum lipids were only assayed at baseline, leaving open the possibility that c*hange* in these biomarkers may be a better indicator of progression. Third, lipids were non-fasting and the effect of fasting status on SM and ceramides is not clear. Correcting for fasting status by controlling for blood glucose had little effect on point estimates. Fourth, information on MCI or dementia diagnosis is not currently available. Examining specific impairments in memory and attention has been important to investigating these lipids as preclinical biomarkers, and offering a promising venue for future research to assess their relationship to incident dementia. Last, APOE genotype was not available. The ε4 allele is associated with peripheral cholesterol and may, therefore, affect observed associations.

Despite these limitations, there are several strengths. WHAS II is a longitudinal, population-based study that allowed us to examine specificity of associations between blood SM and ceramides and incident impairments across domains of cognition. Women had up to six examinations, and nine years of follow-up, allowing for serial cognitive assessments. Second, while previous studies have focused on diagnostic biomarkers of AD and/or memory impairment, this study identified possible predictive biomarkers that may be more sensitive to preclinical memory decline. This is important because the earlier we can identify at-risk, asymptomatic individuals, the earlier treatments can be initiated. Lastly, despite the small sample size, effect sizes were quite large, consistent, and specific to SM and ceramides.

In conclusion, serum levels of SM and ceramides may be predictive of subsequent memory impairment and thus indicators of ongoing AD pathology and progression in asymptomatic individuals. The finding that low SM were associated with memory impairment in cross-sectional analyses, but high levels predicted impairment longitudinally suggests that these lipids could be biomarkers of AD progression. Indeed, post-mortem and CSF studies indicate levels vary by AD severity. While this study is a first step in examining these sphingolipids as AD biomarkers, serial blood samples are needed to determine how these lipids change over time in response to, or predictive of, the disease pathology and progression.

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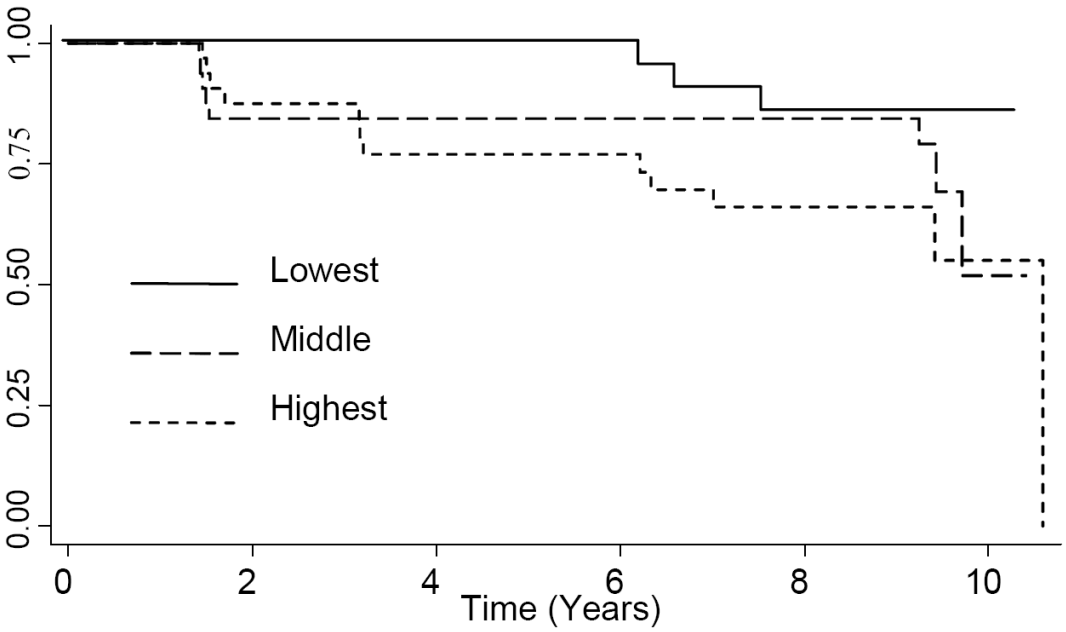

Fig 1.
Kaplan-Meier survival graph showing baseline total SM, in tertiles, and incident HVLT-delayed memory impairment.

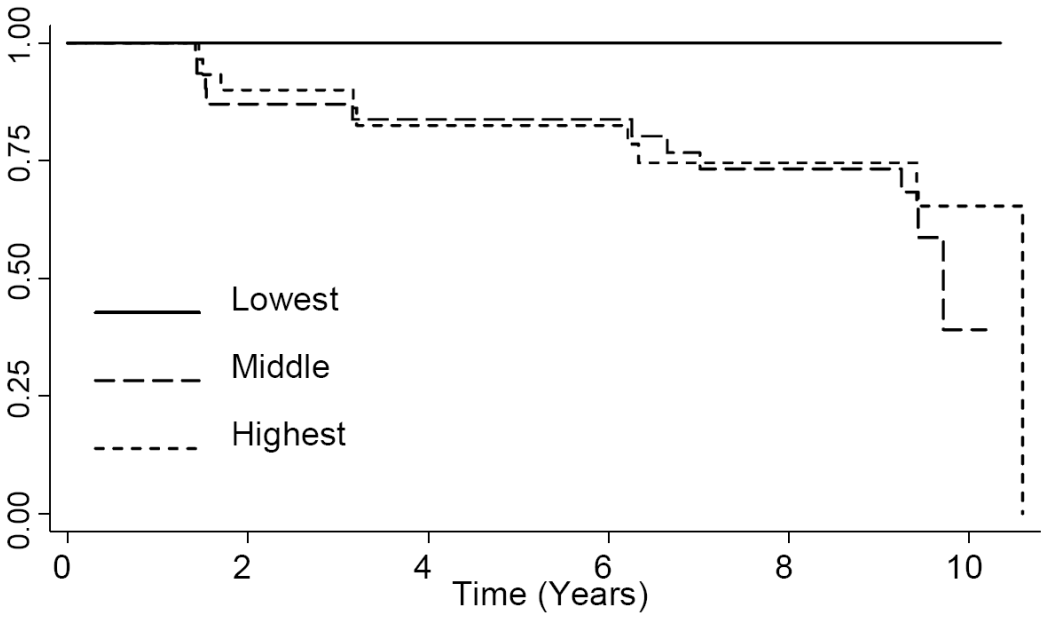

Fig 2.
Kaplan-Meier survival graph showing baseline ceramide C22:0, in tertiles, and incident HVLT-delayed memory impairment.

Table 1

Baseline Characteristics of Women with Available Serum Samples

Variables	Blood Available and Lipids Assayed (n=100)		Blood Not Assayed (n=336)	
	N	N(%)/mean(SD)	N	N(%)/mean(SD)
Demographic				
Age	100	74.0 (2.5)	336	74.7 (2.9)[a]
African American	100	23 (23.0%)	336	60 (17.9%)
Education (yrs)	100	12.7 (3.2)	336	12.7 (3.4)
Married	100	41 (41.0%)	336	125 (37.2%)
Health-Related				
Systolic Blood Pressure	100	148.6 (21.7)	334	153.5 (21.7)[a]
Diastolic Blood Pressure	100	78.6 (16.9)	334	76.3 (15.8)
Myocardial Infarction	100	10 (10.0%)	333	17 (5.1%)
Angina	100	11 (11.0%)	333	33 (9.9%)
Stroke	100	6 (6.0%)	333	17 (5.1%)
Diabetes	100	4 (4.0%)	333	28 (8.4%)
Cancer	100	15 (15.0%)	333	43 (12.9%)
Body Mass Index	100	26.9 (5.4)	334	26.7 (5.2)
Ever smoked	100	40 (40.0%)	333	156 (46.9%)
Minutes exercise/week	100	68.3 (55.7)	333	69.4 (62.6)
Statin Use	100	11 (11.0%)	336	39 (11.6%)
Total Cholesterol (mg/dl)	99	237.0 (40.3)	327	232.5 (38.2)
Triglycerides (mg/dl)	99	151.2 (84.0)	327	153.4 (90.4)
Blood Glucose (mg/dl)	99	108.3 (38.3)	327	109.6 (47.6)
Creatinine (mg/dl)	99	1.0 (0.2)	327	1.0 (0.2)
Cognitive Tests				
MMSE	97	28.1 (1.7)	329	28.1 (1.9)
HVLT-delayed	98	8.3 (2.6)	314	8.1 (2.7)
HVLT-immediate	99	23.1 (5.2)	318	22.5 (5.0)
Trails A	98	49.1 (31.3)	311	48.1 (24.4)
Trails B	99	131.4 (72.6)	309	132.1 (78.5)
GDS (30-item)	100	4.2 (4.6)	333	4.0 (3.5)

[a] $p<0.05$

Table 2

Baseline Serum Lipids, in Tertiles, Predicting Impairment on HVLT-Delayed Recall[a]

			Lipids in Tertiles					
		Total N	Categorical		2 vs. 1		3 vs. 1	
Lipid	**Events**		**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Total SM	23	89	**1.88 (1.05-3.38)**	**0.034**	2.29 (0.58-9.03)	0.238	3.83 (1.04-14.08)	0.043
Ceramides								
C16:0[b]	21	83	**2.12 (1.17-3.84)**	**0.013**	**15.36 (1.86-126.68)**	**0.011**	**12.39 (1.57-97.97)**	**0.017**
C18:0	18	69	1.89 (0.95-3.77)	0.071	**9.03 (1.06-76.67)**	**0.044**	7.35 (0.88-61.18)	0.065
C20:0	20	70	1.61 (0.90-2.86)	0.108	3.64 (0.91-14.54)	0.068	3.14 (0.81-12.16)	0.098
C22:0	20	86	**2.12 (1.10-4.08)**	**0.025**		**no events in lowest tertile**		
C24:1	23	88	1.67 (0.96-2.88)	0.069	**6.32 (1.35-29.66)**	**0.019**	**4.94 (1.05-23.37)**	**0.044**
C24:0[b]	23	90	**2.23 (1.23-4.05)**	**0.008**	**12.75 (1.54-105.41)**	**0.018**	**13.25 (1.69-103.96)**	**0.014**
Galactosyl C12:0	21	73	1.79 (0.99-3.24)	0.053	2.79 (0.67-11.59)	0.158	3.71 (0.98-14.07)	0.054
Lactosyl C12:0	19	67	**2.26 (1.15-4.44)**	**0.018**	**9.58 (1.14-80.43)**	**0.037**	**10.48 (1.28-85.72)**	**0.028**
Stearoyl	23	87	**1.86 (1.05-3.27)**	**0.032**	**13.71 (1.75-107.44)**	**0.013**	**9.83 (1.24-78.11)**	**0.031**
Sulfatide	20	76	**2.06 (1.08-3.96)**	**0.029**	1.75 (0.43-7.13)	0.433	**4.04 (1.07-15.28)**	**0.040**
SM/Cholesterol ratio	22	88	1.66 (0.94-2.92)	0.081	3.03 (0.81-11.33)	0.100	3.35 (0.89-12.59)	0.074
Total cholesterol	23	89	0.86 (0.64-1.15)	0.317	0.74 (0.44-1.25)	0.263	0.76 (0.43-1.34)	0.345

HR = Hazard Ratio; CI = Confidence Interval

[a] Models control for baseline age, blood glucose level, and BMI

[b] 1 case in lowest tertile

Table 3

Baseline Serum Lipids, in Tertiles, Predicting Impairment on HVLT-Immediate Recall[a]

			Lipids in Tertiles					
		Total N	Categorical		2 vs. 1		3 vs. 1	
Lipid	**Events**		**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Total SM	27	88	1.22 (0.76-1.95)	0.414	1.07 (0.40-2.85)	0.885	1.45 (0.57-3.69)	0.434
Ceramides								
C16:0	23	81	1.56 (0.95-2.56)	0.077	**13.73 (3.01-62.56)**	**0.001**	**5.38 (1.12-25.82)**	**0.035**
C18:0	21	67	1.29 (0.75-2.20)	0.354	3.03 (0.91-10.10)	0.071	1.81 (0.54-6.05)	0.335
C20:0	21	66	1.55 (0.92-2.62)	0.098	**4.04 (1.19-13.72)**	**0.025**	2.95 (0.86-10.11)	0.085
C22:0	24	84	1.70 (0.99-2.92)	0.056	**8.12 (1.76-37.56)**	**0.007**	**5.49 (1.13-26.60)**	**0.034**
C24:1	27	89	1.15 (0.73-1.81)	0.554	2.28 (0.87-5.98)	0.095	1.44 (0.52-3.98)	0.487
C24:0	27	87	1.48 (0.93-2.34)	0.096	3.48 (1.14-10.61)	0.028	2.79 (0.95-8.21)	0.062
Galactosyl C12:0	22	70	1.23 (0.74-2.06)	0.426	1.89 (0.63-5.68)	0.256	1.62 (0.54-4.88)	0.394
Lactosyl C12:0	21	64	1.39 (0.81-2.38)	0.229	2.87 (0.95-8.66)	0.061	2.11 (0.63-7.09)	0.226
Stearoyl	35	85	1.35 (0.83-2.17)	0.223	**3.14 (1.11-8.92)**	**0.032**	2.12 (0.69-6.51)	0.191
Sulfatide	23	74	1.36 (0.81-2.27)	0.243	0.82 (0.29-2.28)	0.703	1.78 (0.68-4.62)	0.238
SM/Cholesterol ratio	27	88	1.29 (0.80-2.07)	0.302	1.17 (0.44-3.07)	0.752	1.64 (0.64-4.22)	0.303
Total cholesterol	27	88	0.94 (0.58-1.52)	0.790	0.43 (0.16-1.14)	0.089	0.91 (0.38-2.17)	0.832

HR = Hazard Ratio; CI = Confidence Interval

[a]Models control for baseline age, blood glucose level, and BMI

Table 4

Baseline Serum Lipids, in Tertiles, Predicting Impairment on TMT-A[a]

			Lipids in Tertiles					
		Total N	Categorical		2 vs. 1		3 vs. 1	
Lipid	**Events**		**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Total SM	24	87	1.32 (0.79-2.20)	0.295	0.71 (0.22-2.28)	0.566	1.57 (0.59-4.14)	0.362
Ceramides								
C16:0	23	79	1.19 (0.73-1.92)	0.489	**3.80 (1.23-11.75)**	**0.021**	1.64 (0.52-5.10)	0.396
C18:0	19	66	0.93 (0.50-1.74)	0.821	1.26 (0.36-4.44)	0.720	0.86 (0.23-3.16)	0.821
C20:0	19	66	1.22 (0.71-2.11)	0.473	**3.63 (1.01-13.02)**	**0.048**	1.63 (0.45-5.86)	0.456
C22:0	23	82	1.16 (0.68-1.96)	0.593	2.00 (0.64-6.24)	0.232	1.46 (0.46-4.68)	0.521
C24:1	24	86	1.17 (0.72-1.89)	0.524	2.32 (0.79-6.85)	0.127	1.51 (0.52-4.36)	0.445
C24:0	24	88	1.40 (0.86-2.28)	0.174	1.60 (0.48-5.38)	0.447	2.02 (0.73-5.54)	0.174
Galactosyl C12:0	20	70	0.92 (0.53-1.59)	0.756	0.73 (0.22-2.46)	0.609	0.83 (0.28-2.44)	0.736
Lactosyl C12:0	20	65	1.60 (0.87-2.93)	0.132	2.37 (0.69-8.13)	0.171	2.66 (0.72-9.77)	0.142
Stearoyl	24	84	1.31 (0.81-2.11)	0.279	**3.09 (1.02-9.41)**	**0.047**	1.90 (0.63-5.74)	0.253
Sulfatide	20	73	1.28 (0.70-2.35)	0.428	1.21 (0.38-3.88)	0.745	1.63 (0.49-5.47)	0.425
SM/Cholesterol ratio	24	86	1.36 (0.81-2.28)	0.249	1.40 (0.46-4.21)	0.552	1.85 (0.65-5.28)	0.251
Total cholesterol	24	87	0.82 (0.48-1.39)	0.460	0.98 (0.36-2.67)	0.967	0.66 (0.22-1.97)	0.461

HR = Hazard Ratio; CI = Confidence Interval

[a]Models control for baseline age, blood glucose level, and BMI

Table 5

Baseline Serum Lipids, in Tertiles, Predicting Impairment on TMT-B[a]

			Lipids in Tertiles					
		Total N	Categorical		2 vs. 1		3 vs. 1	
Lipid	Events		HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Total SM	34	87	1.33 (0.86-2.06)	0.196	1.25 (0.50-3.13)	0.630	1.76 (0.73-4.21)	0.205
Ceramides								
C16:0	33	80	1.15 (0.76-1.73)	0.514	2.18 (0.90-5.28)	0.085	1.35 (0.56-3.28)	0.507
C18:0	26	65	0.84 (0.50-1.40)	0.499	1.06 (0.38-2.94)	0.908	0.68 (0.23-2.00)	0.488
C20:0	27	67	1.14 (0.71-1.83)	0.576	1.86 (0.67-5.21)	0.236	1.39 (0.51-3.80)	0.525
C22:0	32	82	1.17 (0.74-1.83)	0.506	1.95 (0.77-4.92)	0.156	1.43 (0.54-3.83)	0.475
C24:1	34	86	1.15 (0.76-1.74)	0.502	2.29 (0.94-5.54)	0.067	1.44 (0.58-3.59)	0.437
C24:0	34	86	1.25 (0.83-1.90)	0.288	1.54 (0.60-3.95)	0.372	1.61 (0.69-3.79)	0.274
Galactosyl C12:0	26	69	1.02 (0.63-1.65)	0.936	1.07 (0.37-3.12)	0.904	1.04 (0.40-2.74)	0.931
Lactosyl C12:0	29	67	1.28 (0.78-2.08)	0.330	1.38 (0.55-3.51)	0.494	1.62 (0.60-4.35)	0.341
Stearoyl	33	84	1.13 (0.75-1.72)	0.559	1.89 (0.78-4.59)	0.159	1.33 (0.54-3.27)	0.536
Sulfatide	29	73	1.51 (0.91-2.52)	0.110	0.75 (0.27-2.04)	0.568	2.20 (0.87-5.53)	0.094
SM/Cholesterol ratio	34	86	1.34 (0.86-2.07)	0.192	1.43 (0.58-3.49)	0.434	1.80 (0.74-4.37)	0.193
Total cholesterol	34	87	0.88 (0.58-1.34)	0.564	1.49 (0.66-3.40)	0.340	0.75 (0.30-1.89)	0.540

HR = Hazard Ratio; CI = Confidence Interval

[a]Models control for baseline age, blood glucose level, and BMI