Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2019 November ; 39(11): 2431–2436. doi:10.1161/ATVBAHA.119.313099.

Elevated Plasma Ceramides are Associated with Higher White Matter Hyperintensity Volume—Brief Report

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Abstract

Objective—Sphingolipids, including sphingosine-1-phosphate (S1P) and ceramides, have been associated with vascular tone, blood pressure regulation, cardiovascular outcomes and mortality. However, the relationship between plasma sphingolipids and cerebrovascular disease has not been examined. We aimed to assess the cross-sectional association between plasma sphingolipids and white matter hyperintensity (WMH) volume, which is a marker of cerebrovascular disease.

Approach and Results—We included 588 participants (302 men and 286 women), aged 60–93, enrolled in the population-based Mayo Clinic Study of Aging who had MRI and plasma sphingolipids at the same study visit. Fasting plasma was obtained and ceramides and S1P were assayed using liquid chromatography electrospray ionization tandem mass spectrometry. Fluid-attenuated inversion recovery (FLAIR) was used to measure WMH volume, defined as percent total intracranial volume. We used linear regression to cross-sectionally examine the relationships

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between plasma sphingolipids and WMH; both were log-transformed. In multivariable analyses adjusting for age, sex, and hypertension, higher levels of ceramide C16:0 (b(95% confidence interval [CI]) = 0.24(0.02, 0.45) and the ceramide ratios C16:0_24:0 (b(95% CI) = 0.30(0.12, 0.48)) and C24:1_24:0 (b(95% CI) = 0.24(0.07, 0.41)) were associated with a higher WMH volume. A higher ceramide score was also associated with higher WMH volume (b(se) = 0.03 (0.01, 0.04)). We did not observe any association between S1P and WMH volume.

Conclusions—Higher plasma ceramide C16:0 and two specific ceramide ratios (C16:0_24:0 and C24:1_24:0) are associated with greater WMH volumes, independent of hypertension, suggesting their utility for measurement of cerebrovascular disease.

Keywords

ceramides; sphingolipids; white matter hyperintensity

Subject Codes

Lipids and Cholesterol; Biomarkers; Clinical Studies; Vascular Biology

Introduction

White matter hyperintensities (WMH), a primary marker of cerebrovascular disease,[1] increase with age and are associated with a two-fold increased risk of dementia and a three-fold increased risk of stroke.[1,2] The etiology and pathophysiology of WMH are not completely understood but are thought to be related to ischemic, hypoxic, and neurodegenerative processes.[3–5] A better understanding of the pathways and mechanisms contributing to WMH is needed to identify potential treatment targets.

Sphingolipids, which contribute to the structure of cell membranes and act as bioactive lipids for multiple cellular pathways, are increasingly being implicated in cardiovascular diseases.[6] Ceramides are bioactive lipids that are pro-inflammatory, pro-apoptotic, and involved in cellular stress. In contrast, sphingosine-1-phosphate (S1P), a metabolite of ceramide, induces proliferation in many cell types, including vascular cells, and attenuates the pro-apoptotic actions of ceramides. However, the exact role of S1P depends on the concentration and receptor; activation of S1P receptor 1 has been associated with vasodilation whereas activation of S1P receptor 3 has been associated with vascular constriction.[7] Both ceramides and S1P have a role in regulating vascular tone, responses to hypoxia, and blood pressure.[8,9]

Recent studies suggest that, among individuals with existing vascular disease, elevated levels of plasma ceramides or S1P are associated with subsequent cardiovascular events, stroke, and death.[10–15] Over the course of these studies, the following ceramides and ceramide ratios were most strongly associated with risk of cardiovascular events: Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:1), Cer(C16:0/24:0) ratio, Cer(C18:0/24:0) ratio, and Cer(C24:1/C24:0) ratio. Based on these ceramides and ceramide ratios, Laaksonen R, et al. [13] developed a ceramide risk score that was found to be better for predicting subsequent cardiovascular events compared to the standard risk factors (e.g., lipids). However, the

association between these ceramides and WMH is not known. Therefore, the aim of the current study was to examine the relationship between plasma ceramides and S1P with WMH volume in a population-based study of aging and cognition. Although we hypothesized that the same ceramides and ceramides ratios found to be associated with cardiovascular events would also be most strongly associated with WMH, we examined all individual ceramides and S1P because the association between these lipids and WMH had not previously been examined. Thus, assessing all lipids would allow us to confirm whether the same ceramides and ceramide ratios were most associated with WMH or whether additional ceramides or S1P were also important. Because hypertension is one of the major risk factors for WMH, we also determined whether the associations were independent of hypertension. In addition, both WMH volume and ceramide levels have been shown to be higher in women after menopause compared to men.[28,29] Therefore, in additional analyses we determined whether the relationship between ceramides and S1P interacted with sex in relation to WMH volume.

Materials and Methods

Data Sharing

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Participants

All participants were enrolled in the Mayo Clinic Study of Aging (MCSA), a population-based study of Olmsted County, Minnesota residents.[16] The Olmsted County population was enumerated using the Rochester Epidemiology Project medical records-linkage system.[17] Complete details of the MCSA study design have been previously described.[16,18] MCSA clinical visits included an interview by a study coordinator, a neurological examination, neuropsychological testing, and a blood draw. All participants are invited to participate in a brain MRI; metal in the body (e.g., pacemaker) and claustrophobia are the only exclusionary criteria. About 70% of participants consent. Plasma sphingolipids were assayed on a randomly selected group of participants for another project. The current analysis included 588 participants, aged 60–92, who had MRI and plasma sphingolipids concurrently measured at the same study visit between August, 2008 and March, 2015. Compared to the overall MCSA cohort, individuals with a MRI were significantly younger and healthier (defined as less comorbidities, including hypertension) compared to those without a MRI. The study was approved by Mayo Clinic and Olmsted Medical Center Institutional Review Boards. Written informed consent was obtained from all participants.

Sphingolipid Analyses

During the in-clinic exam, participants' blood (serum and EDTA plasma) was collected in the fasting state. The blood was centrifuged, aliquoted, and stored at −80°C. LC/ESI/MS/MS analysis of plasma sphingolipids was performed using an AB Sciex quadrupole mass spectrometer 6500 (Sciex, Framingham, MA) equipped with an ESI probe and interfaced with the Agilent 1290 infinity LC system (Agilent, Palo Alto, CA). The UPLC system consisted of an Agilent 1290 binary pump, thermostat, TCC, and sampler. The injection

volume was 10 μL for extracted sample. Sphingolipids were separated with a Poroshell 120 EC- C8 column, 2.1×50 mm, 2.7 μm (Agilent, Palo Alto, CA). Mobile phase A was water:methanol:formic acid:ammonium formate (45/55/0.5%/5 mM by v/v). Mobile phase B was acetonitrile:methanol:formic acid:ammonium formate (50/50/0.5%/5 mM by v/v). The valve, sample loop, and needle were washed with acetonitrile:methanol (50/50 by v/v) for 20 sec. Mass spectrometric analyses were performed online using electrospray ionization tandem mass spectrometry in the positive mode.

Samples were prepared using Biomek FX (Beckman Coulter, Brea, CA). A small amount of plasma sample was added to a 2-mL 96-well plate. Internal standard mixture was added to the samples. Sphingolipids were extracted using 1 phase extraction with methanol-dichloromethane. Lipid levels were quantified by the ratio of analyte and internal standard and calibration curves obtained by serial dilution of a mixture of lipid standards. Pure synthetic standards of sphingolipids were purchased from Avanti Lipids. Isotope labeling synthetic standards were synthesized internally at Eli Lilly and Company. All sphingolipids were expressed in ng/ml.

Calculation of the Ceramide Ratios and Score

We utilized the ceramide ratios and calculated the ceramide score based on Laaksonen R, et al.[13] Briefly, for each of three ceramides (16:0, 18:0, 24:1) and three ceramide ratios (C16:0/24:0, C18:0/24:0, C24:1/C24:0), individuals received 1 point for being in the third quartile of the whole study population and 2 points for being in the fourth quartile. Thus, the ceramide score ranged from 0–12.

Quantification of White Matter Hyperintensities

Structural magnetic resonance imaging (MRI) was acquired using standardized Magnetization Prepared – Rapid Gradient Echo (MPRAGE) sequences on 3T GE scanners (GE Medical Systems, Milwaukee, WI). WMH on standard 2-dimensional Fluid-attenuated inversion recovery (FLAIR) imaging were segmented and edited by a trained imaging analyst using a semi-automated method.[19] WMH volume is presented as the percentage of total intracranial volume (TIV).

Covariates

Age and sex were determined at the in-person clinical examination. Medical conditions (eg, hypertension, diabetes) were determined for each participant by medical record abstraction using the REP medical records-linkage system, which is more accurate than self-report.[17] Hypertension was defined as 1) systolic blood pressure ≥ 140 mmHg or diastolic ≥ 90 mmHg on two occasions, or 2) treatment for hypertension. We did not adjust for diabetes, dyslipidemia, smoking and obesity because we previously determined that these factors were not associated with WMH volume in the MCSA.[28]

Statistical Analyses

Both the plasma sphingolipids and WMH volume were log-transformed. Linear regression models were run using the plasma sphingolipids to predict WMH volume as a continuous variable. Assumptions of linear regression were checked by examining plots of the residuals

versus fitted values and also QQ plots of the residuals; the assumptions were met. Multivariable models adjusted for age, sex, and hypertension. In additional analyses, we examined whether an interaction with sex existed by adding an interaction term in the linear regression model consisting of the plasma sphingolipid and sex. Statistical analysis was completed using SAS, version 9.4 (SAS Institute Inc., Cary, NC). A *P* value of <0.05 was considered to represent statistical significance.

Results

The clinical characteristics of the 588 participants are shown in Table 1. The median (interquartile range [IQR]) age was 68.0 (64.2, 71.9) years and 302 (51.4%) were men. The median (IQR) WMH volume, as a percent of the TIV, was 0.005 (0.003, 0.010) and all participants had some degree of WMH. There were 335 (57.0%) participants with a medical-record confirmed diagnosis of hypertension.

Table 2 provides the univariable and multivariable models for associations between the plasma sphingolipids (in ng/ml) or ratios with WMH volume as a percent of TIV. In multivariable analyses adjusting for age, sex, and hypertension, higher ceramide C16:0 (b(95% confidence interval [CI]) = 0.24(0.02, 0.45) and higher ceramide ratios C16:0_24:0 (b(95% CI) = 0.30(0.12, 0.48)) and C24:1_24:0 (b(95% CI) = 0.24(0.07, 0.41)) were associated with a greater WMH volume. Each one point higher in the ceramide score was also associated with higher WMH volume (b(se) = 0.03 (0.01, 0.04)). We did not observe any association between S1P and WMH volume. Hypertension was the variable associated with the greatest attenuation of significance between the univariable and multivariable models.

We further determined whether there was an interaction between the plasma sphingolipids and sex for WMH volume. However, there were no significant interaction terms.

Discussion

Although plasma ceramides and S1P have been associated with cardiovascular disease outcomes, the relationship between these plasma sphingolipids and WMH volume has not been examined. In this population-based sample, higher ceramide ratios of C16:0/C24:0 and C24:1/C24:0 and a higher ceramide score were cross-sectionally associated with greater WMH volume. These associations were slightly attenuated after adjustment for covariates but remained statistically significant. There was no association between plasma S1P and WMH volume. These results suggest that elevated plasma ceramide levels are associated with greater WMH volumes, independent of hypertension and may be a useful plasma marker for predicting cerebrovascular disease.

Ceramides and S1P have key roles in vascular-related pathways. S1P activates endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) production, which is important for blood pressure regulation.[20] This lipid is also an important regulator of vascular growth,[21,22] angiogenesis,[23] permeability,[24] and vascular tone.[20,25] Studies of cellular and animal models have shown that ceramides increase endothelial permeability and either induce vasodilation or vasoconstriction depending on the vascular beds, cell types, vascular diameter, and animal

model.[8,26] Plasma ceramide levels have been shown to be elevated among patients with hypertension, and increases in ceramides were found to correlate with increased severity of hypertension.[27]

Although previous studies have reported associations of ceramides, ceramide ratios, and the ceramide risk score and multiple cardiovascular outcomes,[10–15] studies have not examined the relationship between plasma ceramides and WMH volume. Given the association between these sphingolipids, hypertension, and vascular pathology, we hypothesized that elevated ceramides and S1P would be associated with greater WMH volume. However, we did not know whether this association would be fully mediated by hypertension. Although we found that hypertension partly attenuated the association between the ceramides and WMH volume, there was still a significant association. Thus, elevated levels of plasma ceramides are independently associated with WMH volume.

In univariate analyses, we did observe a possible association between higher plasma S1P and lower WMH volume, which might be expected given the vascular protective effects of S1P. However, after adjustment for hypertension and other covariates, the results were attenuated and essentially null. It is possible that hypertension more strongly attenuated the association between S1P and WMH compared to ceramides and WMH. Of note, the impact of S1P on the cardiovascular system depends on the S1P concentration and receptor; activation of S1P receptor 1 has been associated with vasodilation whereas activation of S1P receptor 3 has been associated with vascular constriction.[7] It is difficult to determine how blood S1P levels impact each receptor in the current study to obtain the observed result.

Recent studies have suggested that ratios of Cer(d18:1/16:0), Cer(d18:1/18:0) and Cer(d18:1/24:1) to Cer(d18:1/24:0), and the corresponding ceramide risk score, are better predictors of vascular outcomes compared to individual chain lengths.[13,14] These studies have found that Cer(d18:1/16:0), Cer(d18:1/18:0) and Cer(d18:1/24:1) were associated with increased risk of the outcomes whereas Cer(d18:1/24:0) was associated with reduced risk. Thus, the ratios resulted in stronger associations. In the current study, the only ceramide chain length associated with greater WMH volume was Cer d18:1/16:0. However, the ceramide ratios C16:0_C24:0 and C24:1_C24:0, and the ceramide risk score, were more strongly associated with WMH volume. These results further support the use of the ceramide ratios and risk score for cardio- and cerebrovascular outcomes compared to individual ceramide chain lengths.

WMH volume has been shown to be greater in women compared to men.[28] In addition, we have previously shown longitudinal sex-specific trajectories of increasing plasma ceramides with greater increases in ceramide levels for women compared to men.[29] Therefore, we determined whether the relationship between ceramide and S1P interacted with sex in relation to WMH volume. We did not find an interaction. This may indicate that the association between plasma ceramides and WMH volume is similar for both women and men. However, since women have higher ceramide levels, they are more affected with WMH. Future research examining sex differences in the relationship between sphingolipids and cerebrovascular pathology is warranted.

In conclusion, the present results suggest that the sphingolipid pathway, specifically plasma ceramide ratios and the ceramide risk score, is associated with WMH volume and may be useful for detection of cerebrovascular disease. Because MCSA participants who do not consent to a MRI are older and are more frequently diagnosed with hypertension, the observed association is likely a conservative estimate and replication is needed. A limitation of the study is the cross-sectional study design, which can only infer an association. Future studies are needed to evaluate if plasma ceramides have prognostic utility for cerebrovascular damage and to assess the longitudinal association between serial plasma ceramide levels and WMH volume changes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

This work was supported by the National Institutes of Health/National Institute on Aging grants U01 AG006786, R01 AG011378, and R01 AG049704, R01 NS097495, the GHR Foundation, and was made possible by the Rochester Epidemiology Project (R01 AG034676).

Disclosures

Dr. Mielke receives unrestricted research grants from Biogen and Lundbeck. Dr. Bai is an employee of Eli Lilly. Dr. Knopman serves on a Data Safety Monitoring Board for Lundbeck Pharmaceuticals and for the DIAN study, and is an investigator in clinical trials sponsored by Biogen, Lilly Pharmaceuticals, and the Alzheimer's Disease Cooperative Study. Dr. Jack has provided consulting services for Eli Lilly and serves on a DSMB for Roche but he receives no personal compensation from any commercial entity. Dr. Petersen is a consultant for Roche, Inc., Merck, Inc., Biogen, Inc., Genentech, Inc., and GE Healthcare. All other authors have nothing to disclose.

Nonstandard Abbreviations and Acronyms

eNOS	endothelial nitric oxide synthase
MCSA	Mayo Clinic Study of Aging
MRI	magnetic resonance imaging
NO	nitric oxide
S1P	sphingosine-1-phosphate
SD	standard deviation
WMH	white matter hyperintensity

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Highlights

- Plasma ceramides have been associated with risk of major adverse cardiovascular events and death, but have not been examined in relation to cerebrovascular pathology.
- Higher levels of specific plasma ceramide ratios are associated with greater white matter hyperintensity volumes.
- Elevated levels of specific plasma ceramide ratios may be indicative of brain vascular burden.

Table 1.

Baseline Characteristics, Plasma Lipid Levels and WMH Volume (N=588)

Characteristics	Median (IQR)/N(%)
General Information	
Age	68.0 (64.2, 71.9)
Male	302 (51.4%)
Hypertension	335 (57.0%)
WMH volume (% TIV)	0.005 (0.003, 0.010)
Plasma lipid levels, ng/ml	
Sphingosine-1-phosphate	223.1 (185.8, 265.1)
Ceramides, ng/ml	
C14:0	9.0 (6.8, 12.5)
C16:0	88.8 (74.3, 105.3)
C18:0	78.1 (59.4, 98.3)
C18:1	2.3 (1.4, 3.6)
C20:0	226.1 (190.7, 274.9)
C22:0	701.9 (574.1, 859.8)
C23:0	418.7 (344.0, 505.9)
C24:0	1214.8 (993.8, 1461.8)
C24:1	314.8 (257.1, 376.0)
Ceramide ratios	
C16:0_24:0	0.07 (0.06, 0.09)
C18:0_24:0	0.06 (0.05, 0.08)
C24:1_24:0	0.26 (0.21, 0.32)
Ceramide score	4.0 (2.0, 7.0)

IQR, Interquartile range; WMH, white matter hyperintensity; TIV, Total intracranial volume. WMH is expressed as the percent of the total intracranial volume.

Table 2.

Associations Between Plasma Sphingolipids and WMH Volume

	Univariate		Multivariate	
Log Lipid (ng/ml)	**b(95% CI)**	**P value**	**b(95% CI)**	**P value**
Sphingosine-1-phosphate	−0.22 (−0.49, 0.04)	0.099	−0.02 (−0.25, 0.20)	0.857
Ceramides				
C14:0	0.07 (−0.07, 0.20)	0.341	−0.01 (−0.13, 0.10)	0.816
C16:0	0.34 (0.09, 0.60)	0.007	0.24 (0.02, 0.45)	0.035
C18:0	0.10 (−0.07, 0.27)	0.242	0.04 (−0.10, 0.19)	0.565
C18:1	0.04 (−0.05, 0.14)	0.386	0.005 (−0.08, 0.09)	0.907
C20:0	0.18 (−0.04, 0.39)	0.104	0.11 (−0.07, 0.30)	0.231
C22:0	−0.04 (−0.26, 0.19)	0.742	0.05 (−0.14, 0.25)	0.578
C23:0	−0.09 (−0.31, 0.12)	0.393	−0.15 (−0.33, 0.03)	0.110
C24:0	−0.35 (−0.57, -0.13)	0.002	−0.14 (−0.33, 0.04)	0.130
C24:1	0.27 (0.04, 0.50)	0.023	0.16 (−0.04, 0.35)	0.119
Ceramide ratios				
C16:0_24:0	0.56 (0.36, 0.77)	<0.0001	0.30 (0.12, 0.48)	0.001
C18:0_24:0	0.26 (0.10, 0.41)	0.001	0.11 (−0.02, 0.24)	0.108
C24:1_24:0	0.48 (0.29, 0.68)	<0.0001	0.24 (0.07, 0.41)	0.006
Ceramide score	0.05 (0.03, 0.07)	<0.0001	0.03 (0.01, 0.04)	0.003

WMH, white matter hyperintensity; CI, confidence interval. Multivariate models adjust for age, sex, and hypertension. WMH is defined as the percent of total intracranial volume.