



Published in final edited form as:

*J Alzheimers Dis.* 2017 ; 60(3): 829–841. doi:10.3233/JAD-161292.

## A lipidomics approach to assess the association between plasma sphingolipids and verbal memory performance in coronary artery disease patients undertaking cardiac rehabilitation: a C18:0 signature for cognitive response to exercise

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### Abstract

**Background**—Early subtle deficits in verbal memory, which may indicate early neural risk, are common in patients with coronary artery disease (CAD). While exercise can improve cognition,

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**Conflict of Interest:** The authors have no conflict of interest to report.

cognitive response to exercise is heterogeneous. Sphingolipids have been associated with the development and progression of CAD, and impairments in sphingolipid metabolism may play roles in neurodegeneration, and in the neural adaptation response to exercise. In this study, change in plasma concentrations of sphingolipids were assessed in relation to change in verbal memory performance and in other cognitive domains among CAD subjects undertaking a 6-month cardiac rehabilitation (CR) program.

**Methods**—Patients with CAD (n=120, mean age=64±6 years, 84% male, years of education=16±3 years) underwent CR with neuropsychological assessments and blood collected at baseline, 3-, and 6-months. Z-scores based on age, gender and education were combined for verbal memory, visuospatial memory, processing speed, executive function and global cognition tasks to calculate cognitive domain Z-scores. Plasma sphingolipid concentrations were measured from fasting blood samples using high performance liquid chromatography coupled electrospray ionization tandem mass spectrometry (LC/MS/MS). Mixed models were used to identify sphingolipids significantly associated with performance in verbal memory and other cognitive domains, adjusting for potential confounders.

**Results**—A decrease in ceramide C18:0 concentrations was significantly associated with improvement in verbal memory performance ( $b[SE]=-0.51 [0.25]$ ,  $p=0.04$ ), visuospatial memory ( $b[SE]=-0.44 [0.22]$ ,  $p=0.05$ ), processing speed ( $b[SE]=-0.89 [0.32]$ ,  $p=0.007$ ) and global cognition ( $b[SE]=-1.47 [0.59]$ ,  $p=0.01$ ) over 6 months of CR.

**Conclusions**—Plasma ceramide C18:0 concentrations may be a sensitive marker of cognitive response to exercise in patients with CAD.

### Keywords

ceramides; memory; cognition; coronary artery disease; exercise; sphingolipids

## Introduction

Coronary artery disease (CAD) is the leading cause of mortality and morbidity worldwide affecting as many as 1 in 3 individuals before the age of 70[1]. Patients with CAD are a cognitively at-risk population as evidenced by increased brain atrophy[2], white matter lesions[3-7] increased risk of memory impairment, and incipient neurodegenerative diseases that include mild cognitive impairment (MCI)[8-11], vascular dementia[12-15] and Alzheimer's disease (AD)[10, 12, 16, 17]. While CAD patients show disruptions in multiple cognitive domains[9, 18], subtle changes in verbal memory performance have been associated with mortality[19], physical disability[20], progression to dementia,[21] and interference with secondary prevention[22], suggesting that verbal memory may be a key marker of poor outcomes in patients with CAD.

Exercise is increasingly recognized as an effective intervention to improve cardiac outcomes[23] and has also been shown to delay cognitive decline[24-27]. Regular physical activity in healthy elderly individuals has been associated with reduced risk of MCI, AD, and other dementias[28-30]. Exercise interventions have also been associated with increasing brain volumes[31] and improved memory performance in older adults[26, 27].

However, the cognitive response to exercise can be heterogeneous[32] indicating a need to explore mechanisms that may hinder the cognitive benefits of exercise.

Alterations in circulating lipids are common features associated with the clinical presentations of CAD[33-37], and it has become increasingly recognized that similar perturbations in blood lipid composition are associated with neurodegenerative conditions[38-40] suggesting that dysregulations of lipid metabolic pathways may be fundamental mechanisms underlying cognitive risk in CAD. Sphingolipids have recently emerged as an especially promising class of lipids that have been repeatedly identified as prognostic and associative indicators of cognitive decline[41-47]. Higher peripheral blood sphingolipid concentrations including sphingomyelins, ceramides and lactosylceramides were significantly associated with greater declines in verbal memory, speed of processing, executive function and hippocampal volume loss in subjects with MCI[47], increased risk of AD[44], and faster cognitive progression among AD patients[45]. Recent investigations of post-mortem brain tissue suggest that an increase in ceramides may be a specific indicator of neurodegenerative and pathologic changes relative to other lipid classes[42]. In a targeted pilot study, we have shown preliminary associations between higher plasma ceramide concentrations and less improvement in verbal memory during cardiac rehabilitation (CR) [41]. Conversely, the anti-inflammatory and neuroprotective ceramide metabolite sphingosine-1-phosphate (S1P), has been suggested to promote hippocampal neurogenesis[48, 49] and ameliorate memory deficits in animal studies[50, 51]. Collectively, these findings suggest that circulating sphingolipids may be sensitive markers of early cognitive changes in CAD.

Although there is evidence to suggest that the pro-inflammatory[52] and pro-apoptotic[53] properties of most sphingolipid species and the neuroprotective S1P may have opposing effects on the neural adaptation to exercise and neurodegenerative processes[54], sphingolipids have not been comprehensively assessed as prognostic indicators of the cognitive response to exercise in CAD. In the present study we sought to determine if changes in circulating sphingolipids were associated with change in verbal memory performance in CAD subjects undertaking a 6-month CR program. Associations between sphingolipids and other cognitive domains including visuospatial memory, processing speed, executive function and global cognition were also explored.

## Materials and Methods

### Participants

This prospective panel study was approved by the Sunnybrook Health Sciences Centre Research Ethics Board and the University Health Network Research Ethics Board. Written informed consent was obtained from all subjects before enrollment in the study. Eligible subjects who consented to participate in the study were assessed for inclusion/exclusion criteria. All participants had evidence of CAD (previous hospitalization for acute myocardial infarction, coronary angiographic evidence of ≥ 50% blockage in one or more major coronary artery or prior revascularization). All participants also had dyslipidemia and were being treated with statins. Subjects were excluded based on previously diagnosed neurodegenerative illnesses, active cancer, surgery planned within 12 months, schizophrenia,

bipolar affective disorder and substance abuse. Subjects with probable dementia (Mini Mental Status Examination score < 24[55]) were also excluded.

### **Cardiac rehabilitation (CR) program**

The CR program comprised of both aerobic and resistance exercise under the supervision of exercise and medical specialists. Participants attended exercise visits that included an aerobic walk or walk/jog each week for 24 weeks. Participants were also expected to independently exercise 5 out of 7 days of the week. Cardiopulmonary fitness was assessed using a cycle ergometer (Ergoselect 200P, Ergoline, Bitz, Germany) symptom-limited graded exercise test and peak oxygen uptake per minute ( $VO_{2peak}$ ) was obtained at entry into the program and at 3 and 6 months.

### **Demographics and clinical characteristics**

Demographic and clinical characteristics, as well as a detailed medical history including comorbidities independent of CAD, were collected from patient interviews. Cardiac medical history, concomitant medications, cardiac health indicators (body mass index, waist circumference, hyperlipidemia, hypertension, diabetes, waist circumference) and anthropometrics were obtained from patient charts at the Toronto Rehabilitation Institute – University Health Network. Hemoglobin A1c (HbA1c) and lipid profiles, including total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride concentrations were measured using standard clinical assays. Apolipoprotein E (APOE) polymorphism was determined using restriction fragment length polymorphism polymerase chain reaction[56].

### **Cognitive testing**

Cognitive performance was assessed using the 30-minute standardized battery of tests recommended by the National Institute of Neurological Disorders and Stroke-Canadian Stroke Network (NINDS-CSN)[57] for the investigation of vascular cognitive impairment at baseline, 3 and 6 months. A trained researcher administered the battery at a standardized time (0930 hr  $\pm$  30 min) and participants refrained from eating or drinking any caffeine-containing beverages for at least 4 hours before testing. Verbal memory was assessed using the California Verbal Learning Test 2<sup>nd</sup> Ed. (CVLT-II). The CVLT-II yields multiple measures of verbal memory function including verbal learning (recall of a word list over 5 learning trials), short-delay free recall (recall of a word list after an interfering list) and long-delay free recall (recall of a word list after 20 minutes)[57]. Visuospatial memory was assessed using the Brief Visuospatial Memory Test-Revised (BVMTR)[58], which yields a measure of visual learning and delayed recall. Speed of processing was assessed using the Trail-Making Test Part A[59] and the Digit Symbol-Coding task, a measure of complex attention and psychomotor speed from the Wechsler Adult Intelligence Scale 3<sup>rd</sup> Edition[60]. Measures of executive function included the Trail-Making Test Part B[59] and Stroop Color-Word Interference Test. The Montréal Cognitive Assessment (MoCA)[61] was used to assess global cognition. For each cognitive task, a Z-score was determined from age, gender and education matched norms. Z-scores of related tests were summed into composite Z-scores to reflect performance in a cognitive domain and to avoid multiple comparisons[62]. For verbal memory, Z-scores from the three CVLT-II outcomes (verbal

learning, short- and long-delay free recall) were summed. For visuospatial memory, Z-scores for the two BVMT-R outcomes (visual learning and delayed recall) were summed. The sum of the Trail Making Test A and Digit Symbol-Coding task Z-scores were used to represent speed of processing while the Z-scores of the Trail Making Test B and Stroop Test were summed for executive function.

### Sphingolipid measurements

At baseline, 3 and 6 months, fasting blood was drawn at 0900 h  $\pm$  30 min on the same day as the cognitive testing and centrifuged at 1000g for 10 min at 4°C. Plasma was immediately isolated and stored at  $-80^{\circ}\text{C}$  until analysis. Quantification of 45 individual sphingolipid species was accomplished by high performance liquid chromatography coupled electrospray ionization tandem mass spectrometry (LC/MS/MS) using multiple reaction monitoring and processed by the Analyst 1.4.2 software package as previously described[63]. Briefly, a crude lipid extraction from plasma was performed using a modified Bligh and Dyer procedure (Avanti Polar Lipids, Alabaster, AL, USA). Plasma extracts were dried and then re-suspended in methanol before analysis. High performance liquid chromatography (PerkinElmer, MA, USA) with a reverse phase C18 column (Phenomenex, Torrance, CA, USA) was used for temporal resolution of compounds. The eluted samples were then injected into an electrospray ion source coupled to a triple-quadrupole mass spectrometer (API3000, AB Sciex Inc, Thornhill, Ontario, Canada) and analyses were conducted by multiple reaction monitoring. Eight-point calibration curves (0.1-1000 ng/mL) were constructed by plotting area under the curve for different sphingolipids, for each calibration standard normalized to the internal standard. Sphingolipid concentrations (ng/mL) were determined by fitting the identified species to standard curves based on acyl chain length. Instrument control and quantification of spectral data were performed using Analyst 1.4.2 and MultiQuant software (AB Sciex Inc, Thornhill, Ontario, Canada).

### Statistical Analyses

Plasma sphingolipid measurements were skewed, so they were log-transformed prior to analyses as has been done before[41]. Mixed models were used to examine the time-varying relationship between plasma sphingolipids and cognition over the 6-month CR program. Bivariate associations between patient characteristics (fixed and time-varying) and change in verbal memory and other cognitive domains over CR, were assessed using mixed models. Patient characteristics were included as potential confounders in multivariate models if they were significantly associated with the cognitive domains.

Bivariate mixed models were first used to identify associations between  $\text{VO}_{2\text{peak}}$ , verbal memory and other cognitive domain Z-scores over CR to determine change in cognitive performance with increasing fitness. Bivariate mixed models were also used to identify a panel of sphingolipids associated with verbal memory and other cognitive domains over CR. Because some of the sphingolipids may be correlated, multicollinearity was assessed (tolerance statistic $<0.4$ ) before multiple sphingolipid species were included in multivariate mixed models. For lipids that were collinear, only members of a correlated set that maintained the tolerance statistic above 0.4 were included in multivariate analyses. All analyses were finished using the MIXED procedure in SAS University Edition statistical

software (SAS Institute Inc., North Carolina, USA) with the significance level set at a two-tailed  $p = 0.05$ .

## Results

### Patient characteristics

Demographics and clinical characteristics of the 120 CAD participants are reported in Table 1.

### Bivariate associations between clinical characteristics and verbal memory

A higher level of education ( $b=0.09$ ,  $p<0.001$ ) and reduction in waist circumference ( $b=-0.01$ ,  $p=0.02$ ) over CR were associated with better verbal memory performance over time. Absence of the APOE4 allele ( $b=0.26$ ,  $p=0.04$ ) and not taking antiplatelet drugs ( $b=0.64$ ,  $p=0.04$ ) were also associated with better verbal memory performance over CR. There were no other significant associations between change in verbal memory and other sociodemographic characteristics, cardiac risk factors, CAD severity, medical comorbidities, cardiopulmonary fitness parameters or concomitant medications used.

### Bivariate associations between clinical characteristics and other cognitive domains

Improvement in visuospatial memory was associated with a higher level of education ( $b=0.05$ ,  $p=0.003$ ) and high dose statin therapy ( $b=0.25$ ,  $p=0.03$ ). Unmarried participants ( $b=0.31$ ,  $p=0.02$ ) also showed improvements in visuospatial memory over CR while participants who did not have a stent worsened in visuospatial memory over CR ( $b=-0.34$ ,  $p=0.004$ ). Participants with longer time since the acute coronary event at time of assessment also exhibited worse visuospatial memory over CR ( $b=-0.003$ ,  $p=0.02$ ). Improvement in processing speed was associated with a higher level of education ( $b=0.06$ ,  $p<0.001$ ), absence of diabetes ( $b=0.5$ ,  $p=0.001$ ), use of antidiabetic agent ( $b=0.44$ ,  $p=0.01$ ) and not being employed ( $b=0.25$ ,  $p=0.03$ ). Participants not taking antioxidants ( $b=0.38$ ,  $p=0.01$ ) nor platelet inhibitors ( $b=1.01$ ,  $p=0.001$ ) also showed improvements in processing speed over CR. Participants who were not Caucasian ( $b=-0.66$ ,  $p<0.001$ ) and those who did not have a stent ( $b=-0.38$ ,  $p=0.001$ ) showed worsening in processing speed over CR. Improvement in executive function was associated with a higher level of education ( $b=0.08$ ,  $p<0.001$ ), absence of both diabetes ( $b=0.30$ ,  $p=0.05$ ) and hypertension ( $b=0.66$ ,  $p=0.005$ ), not being married ( $b=0.40$ ,  $p=0.004$ ), not being employed ( $b=0.36$ ,  $p=0.001$ ) and higher serum HDL concentrations ( $b=0.26$ ,  $p=0.03$ ). Participants who were not taking antiplatelet drugs ( $b=0.86$ ,  $p=0.005$ ) also showed improvement in executive function over CR while non-Caucasian participants ( $b=-0.67$ ,  $p<0.001$ ), those with no angina ( $b=-0.57$ ,  $p=0.005$ ) and those not taking diuretics ( $b=-0.36$ ,  $p=0.02$ ) showed worsening executive function over CR. A higher level of education ( $b=0.20$ ,  $p<0.001$ ) and an increase in weight over CR ( $b=0.02$ ,  $p=0.03$ ) was associated with improvement in global cognition as measured by MoCA score. Participants on high dose statin therapy ( $b=0.67$ ,  $p=0.03$ ) and those not taking  $\beta$ -adrenergic receptor blockers ( $b=0.75$ ,  $p=0.04$ ) also showed improvement in MoCA score over CR while non-Caucasian participants ( $b=-2.39$ ,  $p<0.001$ ) and those with a higher number of stenosed vessels ( $b=-0.35$ ,  $p=0.05$ ) showed a decline in the MoCA score over CR.



### Association between cardiopulmonary fitness and cognitive performance over CR

An increase in  $VO_{2peak}$  over CR was significantly associated with improvements in verbal memory performance ( $b=0.02$ ,  $p=0.05$ ), processing speed ( $b=0.03$ ,  $p=0.001$ ) and executive function ( $b=0.02$ ,  $p=0.05$ ) but not in visual memory ( $b=0.01$ ,  $p=0.37$ ) nor in global cognition as measured by the MoCA score ( $b=0.03$ ,  $p=0.16$ ) in bivariate associations. In multivariate models, increasing fitness was only associated with improved processing speed ( $b(SE) = 0.02 (0.009)$ ,  $p=0.04$ ).

### Identification of a sphingolipid profile associated with cognitive response to CR

As shown in Figure 1, bivariate associations using mixed models identified 9 species that were significantly associated with change in verbal memory performance over CR. These species included sphingomyelins C16:0 ( $b=1.53$ ,  $p=0.05$ ), C20:1 ( $b=1.22$ ,  $p=0.01$ ), and C24:1 ( $b=1.41$ ,  $p=0.02$ ), dihydrosphingomyelins C16:0 ( $b=-0.88$ ,  $p=0.03$ ) and C20:0 ( $b=-1.39$ ,  $p=0.007$ ), ceramide C18:0 ( $b=-0.67$ ,  $p=0.002$ ), lactosylceramides C18:1 ( $b=1.06$ ,  $p=0.002$ ) and C24:1 ( $b=0.70$ ,  $p=0.02$ ) and S1P ( $b=-0.37$ ,  $p=0.02$ ). Figure 1 also shows sphingolipids associated with changes in visuospatial memory, processing speed, executive function and global cognition over CR. Notably, of all the sphingolipids, ceramide C18:0 was most robustly associated with changes in all the cognitive domains over CR.

### Multivariate models predicting cognitive performance over CR

We focused on sphingolipid species significantly associated with change in memory and other cognitive domains over CR, which were not collinear in multivariate analyses. Over the 6-month CR program, in a model including sphingomyelin C16:0, sphingomyelin C20:1, sphingomyelin C24:1, dihydrosphingomyelin C20:0, lactosylceramide C18:1, lactosylceramide C24:1, total years of education, waist circumference, presence of APOE4 allele and  $VO_{2peak}$ , each log-unit decrease in ceramide C18:0 was associated with a 0.51 standard deviation improvement in verbal memory performance ( $b(SE)=-0.51 [0.25]$ ,  $p=0.04$ ) and each log-unit decrease in S1P was associated with a 0.53 standard deviation improvement in verbal memory performance ( $b(SE)=-0.53 [0.17]$ ,  $p=0.002$ ) (Table 2). As shown in Table 3, a decrease in ceramide C18:0 over the 6-month CR was associated with improvements in visuospatial memory ( $b(SE)=-0.44[0.22]$ ,  $p=0.05$ ), processing speed ( $b(SE)=-0.89[0.32]$ ,  $p=0.007$ ) and MoCA score ( $b(SE)=-1.47[0.59]$ ,  $p=0.01$ ). There was also a trend towards a decrease in ceramide C18:0 and improvement in executive function over CR ( $b(SE)=-0.59[0.31]$ ,  $p=0.06$ ). Ceramide C18:0 ( $b=-0.006$ ,  $p=0.005$ ) levels were inversely associated with increases in  $VO_{2peak}$  over CR in bivariate analyses (Supplementary Table 1).

### Discussion

This study assessed the relationships between plasma sphingolipids and cognition over a 6-month CR program among patients with CAD. Decreasing plasma ceramide C18:0 concentration was consistently associated with improved performance in verbal memory, visuospatial memory, processing speed and global cognition as well as an increase in  $VO_{2peak}$  over CR, suggesting that plasma ceramide C18:0 may be a surrogate measure of the cognitive response to exercise.

Overall, participants experienced a subtle yet significant improvement in processing speed performance with increasing fitness over the course of the 6-month CR program. Previous findings suggest that certain cognitive domains, such as processing speed and attention[64, 65], may be more amenable to exercise effects; however, effects of exercise on cognition have not been consistent[66]. While adaptations to exercise are incompletely understood, they are associated with markers of neurogenesis and angiogenesis in patients with CAD[67]. These processes may underlie increases in gray and white matter volumes[31, 68] as well as cerebral blood flow[69, 70], which may partly mediate exercise-induced cognitive improvement[71, 72].

We hypothesized that sphingolipids may be interfering with exercise-induced improvements in cognitive performance due to their role in inducing inflammatory signals and damaging neural progenitor cells directly by activating apoptotic cascades[73] in patients with CAD. In the present study, bivariate analyses identified associations between several sphingolipid species and cognitive performance in CAD patients undergoing an exercise intervention (Figure 1). While mechanisms underlying the physiological effects of sphingolipids remain unclear, several species identified in this study have been consistently associated with metabolic disease[74-76] and neurodegenerative disorders[40, 44, 47]. Glycosphingolipids, including monohexylceramides and lactosylceramides have been previously associated with atherosclerosis and arterial stiffness in animal studies[74]. In particular, lactosylceramides have been associated with increased risk of AD[44]. Long-chain (C16:0 and C18:0) and very long-chain (C24:1) ceramides are higher in obese individuals and patients with diabetes[75, 77, 78] and accumulation of these species in the skeletal muscle is inversely associated with insulin sensitivity[75] which plays an important role in the development of AD[79]. High HbA1c levels consistent with a diagnosis of prediabetes and diabetes in the present study suggest ceramide-mediated insulin insensitivity as an important mechanism underlying cognitive decline in CAD. Long-chain and very long-chain ceramides have also been linked to systemic metabolic health (C16:0, C18:0)[76], depression (C16:0, C18:0, C20:0, C24:1) [80], cognitive impairment in Parkinson's disease (C16:0, C18:0, C20:0, C22:0, C24:1)[40], hippocampal volume loss in MCI (C22:0)[47] and increased risk of AD (C16:0)[44]. Preliminary findings from a pilot study also showed an association between higher ceramide C22:0 and C24:0 and less improvement in verbal memory in CAD patients undertaking CR[41]. These published data combined with the findings from this study suggest that long-chain ceramides, very long-chain ceramides and glycosphingolipids of certain chain lengths may be especially relevant to cognitive changes in CAD.

Our results suggest that ceramide C18:0 is most significantly and robustly associated with the cognitive response to exercise in CAD patients. These results are consistent with other studies suggesting that ceramide C18:0 may be of etiopathological importance in AD[81]. Recently, ceramide C18:0 was shown to be positively associated with amyloid- $\beta$  and tau in cognitively normal individuals with a confirmed parental history of AD[46]. Those associations were stronger in individuals aged 54 years or older, consistent with the age range in this study of another at-risk population.

A significant association between decreasing ceramide C18:0 and increasing fitness over CR suggests that ceramide metabolism may be responsive to exercise. Previously, better



cardiopulmonary fitness was associated with lower plasma ceramide concentrations in healthy elderly subjects[82] and muscle ceramide concentrations were decreased following chronic aerobic exercise[83, 84]. The present study shows similar effect of exercise in CAD, a disease characterized by aberrant ceramide metabolism. Plasma ceramides were also shown to be reduced following 12 weeks of aerobic exercise training in obese individuals and those with diabetes[85]. A decrease in ceramide concentrations imply increased insulin sensitivity[78], which may partly contribute to the cognitive benefits of exercise.

Exercise may influence ceramide production through various mechanisms. Exercise training has been shown to reduce plasma inflammatory markers including tumor necrosis factor[78], a key inducer of ceramide synthesis[86]. Reduced inflammatory signaling with exercise has been associated with decreased *de novo* synthesis of ceramides[87]. Lipid utilization during exercise has also been hypothesized to reduce ceramide production by reducing the availability of substrates needed for ceramide synthesis[83, 84]. In addition, exercise may induce ceramide degradation and clearance by increasing the expression of genes responsible for ceramide clearance, including acidic and alkaline ceramidase 1 and 3, glucosylceramide synthase, and sphingosine kinase 1[78].

Exercise also induces the release of S1P[88]. S1P concentrations increase in blood as a result of release from erythrocytes, muscular tissue and vascular endothelium following exercise[89], inducing VEGF receptor activation[90], angiogenesis, neural progenitor cell proliferation and morphogenesis and long term potentiation[73]. However, change in S1P was not associated with cardiopulmonary fitness and improvement in verbal memory was associated with a decrease in S1P over CR. It is possible that plasma S1P concentrations are inversely related to cerebrospinal fluid concentrations as previously suggested for other sphingolipids[47]. Decreasing plasma concentrations over the course of CR may be indicative of higher S1P concentrations in the brain, which has been previously associated with hippocampal neurogenesis and memory improvement[48-51]. Future studies should examine plasma- cerebrospinal fluid correlations to further understand the utility of plasma S1P concentrations as markers of cognitive response to exercise.

This study was strengthened by a methodologically robust analysis of sphingolipids to determine species associated with cognitive response to exercise. The use of a rigorous statistical pipeline including use of composite Z-scores representing important cognitive domains and use of mixed models that allowed for modeling of the complex temporal relationships between cognitive outcomes, sphingolipids, effects of exercise and important clinical confounders also strengthened the findings. The use of an objective measurement of fitness was also a strength of this study.

This study was limited by the lack of a control group; as such, independent influence of CAD and exercise on cognitive outcomes and sphingolipids could not be determined. However, CAD patients who have aberrant lipid metabolism and who are as of yet free of overt cognitive impairment but show early subtle changes in cognitive function[22, 91] are an ideal population to study these associations as they may be representative of a preclinical stage. Future randomized prospective trials are needed to clarify the direction of cause and effects of these results. Practice effects may have contributed to the overall improvement in

verbal memory and other cognitive outcomes; however, a 3-month interval between testing would be expected to minimize such effects on tests of verbal memory[92]. The study sample included participants who were referred to and agreed to enter CR, which may have introduced selection bias. In addition, the study sample was predominantly Caucasian, male and highly educated, which may not be representative of all CAD patients. However, the study population was representative of CR participants[93-96] and CR is a standard of care, which may contribute to the generalizability of the results to CAD patients undertaking CR.

Future work should look at associations between S1P and cognition in this population to further elucidate mechanisms underlying exercise-induced neural adaptation and to better develop blood sphingolipids as clinically useful markers of early cognitive changes.

## Conclusions

In CAD patients undertaking CR, lower plasma ceramide C18:0 was associated with improvements in verbal memory, visuospatial memory, processing speed and global cognition. Bivariate analysis showed that ceramide C18:0 decreased with increasing fitness over CR. These findings suggest that ceramide C18:0 (and possibly other related sphingolipids) may be modulated by lifestyle modifications such as exercise, and underscores the importance of exercise in preserving cognitive function in an at-risk population such as individuals with CAD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This study was supported by a research grant from the Canadian Institutes of Health Research (Lancot/MOP-114913). MS was supported by a doctoral grant from the Alzheimer's Society of Canada. MM was supported by grants from the National Institute on Aging (U01 AG37526, R01 AG49704). NJH is supported by the National Institutes of Health (MH105280, MH075673, DA040390, MH096630 and MH110246).

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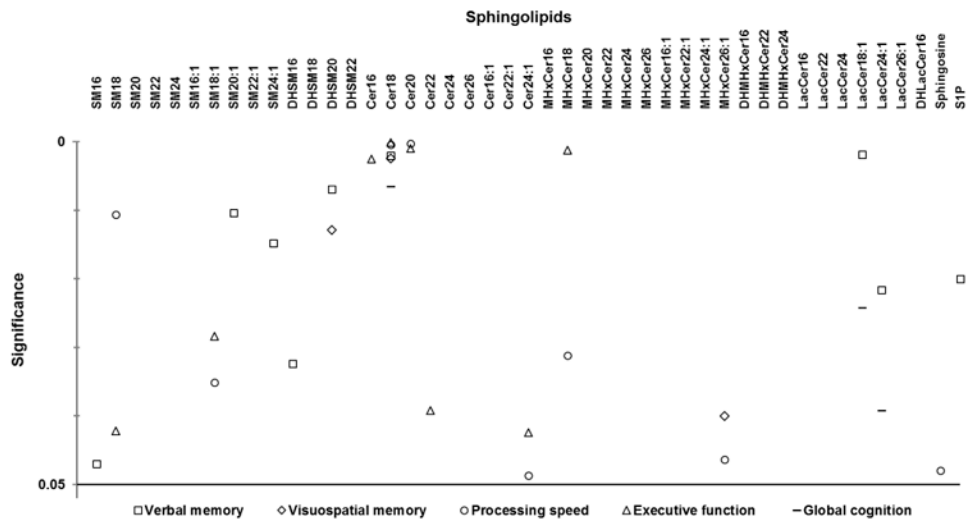


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**Figure 1.** Sphingolipids associated with change in cognitive domains over 6 months of cardiac rehabilitation in patients with coronary artery disease. Symbols denote p-values < 0.05 from bivariate associations using mixed models between cognitive domain Z-scores and log sphingolipid concentrations over cardiac rehabilitation; all identified species were included in multivariate mixed models. For Cer18, overlapping symbols in the top cluster include a circle representing processing speed and a triangle representing executive function, and the two overlapping symbols in the bottom cluster include a square representing verbal memory and a diamond representing visuospatial memory. For Cer20, overlapping symbols include a circle representing processing speed and a triangle representing executive function. Abbreviations: SM, sphingomyelin; DHSM, dihydrosphingomyelin; Cer, ceramide; MHxCer, monohexylceramide; DHMHxCer, dihydromonohexylceramide; LacCer, lactosylceramide; DHLacCer, dihyrdolactosylceramide; S1P, sphingosine-1-phosphate.

**Table 1**  
**Characteristics of study participants (n=120) over 6 months of cardiac rehabilitation (CR)**

Characteristic	CAD (n=120) Mean±SD or n (%)
<b>Sociodemographics</b>	
Age, years	64±6
Sex, male	101 (84)
Ethnicity, Caucasian	99 (83)
Marital status, married	93 (78)
Years of education, years	16±3
Employed	66 (55)
Smoking history, smoker or quit smoking	74 (62)
APOE4 allele carrier	33 (28)
<b>Lipid Profile and HbA1c</b>	
Low density lipoprotein (LDL; mmol/L)	
Baseline	1.60±0.61
3 months	1.72±0.66
6 months	1.66±0.63
High density lipoprotein (HDL; mmol/L)	
Baseline	1.29±0.34
3 months	1.33±0.34
6 months	1.36±0.39
Total cholesterol (mmol/L)	
Baseline	3.49±0.83
3 months	3.16±1.14
6 months	3.56±0.81
Triglycerides (mmol/L)	
Baseline	1.31±0.74
3 months	1.66±1.12
6 months	1.31±1.47
Hemoglobin A1c (HbA1c)	
Baseline	0.059±0.007
3 months	0.063±0.053
6 months	0.067±0.081
<b>Body Composition</b>	
Body mass index (kg/m <sup>2</sup> )	
Baseline	29.2±5.1
3 months	28.9±4.8
6 months	28.3±5.1
Body fat percentage	
Baseline	31.7±10.5
3 months	30.3±8.5
6 months	28.5±8.3

Characteristic	CAD (n=120) Mean±SD or n (%)
<b>Body mass (kg)</b>	
Baseline	86.4±16.6
3 months	85.0±16.3
6 months	83.8±17.6
<b>Waist circumference (cm)</b>	
Baseline	99.2±12.2
3 months	99.0±14.6
6 months	96.9±12.8
<b>CAD Severity</b>	
Cumulative stenosis, percent	150.2±67.2
Number of vessels stenosed	2±1
Time since acute coronary event, weeks	22.3±43.4
<b>Cardiac History</b>	
Myocardial infarction (MI)	58 (48)
Coronary artery bypass graft surgery (CABG)	39 (33)
Stent	77 (64)
Angina	9 (8)
Hypertension	112 (93)
<b>Comorbidities</b>	
Diabetes	20 (17)
Depression	18 (15)
Hypercholesterolemia	120 (100)
<b>Cardiopulmonary Fitness Parameters</b>	
<b>Maximum heart rate (bpm)</b>	
Baseline	121.9±20.3
3 months	128.6±20.1
6 months	133.0±23.9
<b>Maximum systolic blood pressure (mm Hg)</b>	
Baseline	170.1±23.9
3 months	171.7±23.7
6 months	175.8±21.9
<b>Maximum diastolic blood pressure (mm Hg)</b>	
Baseline	78.4±11.0
3 months	76.7±9.9
6 months	77.9±9.4
<b>Peak oxygen consumption (VO<sub>2</sub> peak; mL/kg/min)</b>	
Baseline	20.9±5.6
3 months	24.5±6.6
6 months	26.8±7.2
<b>Medications</b>	
β-adrenergic receptor blockers	96 (80)
Diuretics	19 (16)

Characteristic	CAD (n=120) Mean±SD or n (%)
Anti-hypertensive	
Angiotensin-converting enzyme inhibitors	63 (53)
Angiotensin II receptor blockers	23 (19)
Calcium channel blocker	16 (13)
Antidiabetic	16 (13)
Antioxidants	17 (14)
Platelet inhibitors	116 (97)
Statins	
High dose <sup>I</sup>	74 (63)
Low dose	43 (37)

<sup>I</sup>High statin dose was defined as atorvastatin, pravastatin, or fluvastatin 40–80 mg/day and rosuvastatin or simvastatin 20–40 mg/day. Low dose statin was defined as 10–20 mg/day and 5–10 mg/day of those medications, respectively.



**Table 2**  
**Multivariate mixed model showing the association between change in sphingolipids of interest and change in verbal memory domain Z-score over 6 months of cardiac rehabilitation (CR) in patients with coronary artery disease (CAD)**

Variable	<i>b</i> (SE)	p-value (p 0.05)*
Years of education	0.08 (0.02)	<0.0001*
Waist circumference	0.0005 (0.005)	0.91
APOE4 allele	0.19 (0.14)	0.06
VO <sub>2peak</sub>	0.003 (0.009)	0.76
Sphingomyelin C16:0	0.76 (1.14)	0.51
Sphingomyelin C20:1	0.95 (0.65)	0.07
Sphingomyelin C24:1	-0.46 (0.83)	0.58
Dihydro sphingomyelin C20:0	-1.13 (0.63)	0.07
Ceramide C18:0	-0.51 (0.25)	0.04*
Lactosylceramide C18:1	0.006 (0.48)	0.99
Lactosylceramide C24:1	0.85 (0.45)	0.06
Sphingosine-1-phosphate	-0.53 (0.17)	0.002*

**Table 3**  
**Multivariate mixed models showing associations between change in ceramide C18:0 and change in 4 cognitive domain Z-scores over 6 months of cardiac rehabilitation (CR) in patients with coronary artery disease (CAD)**

Cognitive domain (outcome variable)	Ceramide C18:0	
	<i>b</i> (SE)	p-value (p 0.05)*
Visuospatial memory <sup>2</sup>	-0.44 (0.22)	0.05*
Processing speed <sup>3</sup>	-0.89 (0.32)	0.007*
Executive function <sup>4</sup>	-0.59 (0.31)	0.06
Global cognition (MoCA score) <sup>5</sup>	-1.47 (0.59)	0.01*

<sup>2</sup> model also included years of education, stent procedure, statin dose, dihydro sphingomyelin C20:0 and mono hexyl ceramide C26:1

<sup>3</sup> model also included years of education, ethnicity, stent procedure, diabetes, VO<sub>2</sub>peak, sphingomyelin C18:1, ceramide C22:0, ceramide C24:1, mono hexyl ceramide C18:0, mono hexyl ceramide C26:1 and sphingosine

<sup>4</sup> model also included years of education, ethnicity, diabetes, VO<sub>2</sub>peak, serum HDL concentration, sphingomyelin C18:1, ceramide C22:0, ceramide C24:1 and mono hexyl ceramide C18:0

<sup>5</sup> model also included years of education, ethnicity, weight, β-adrenergic receptor blocker use, statin dose, lactosyl ceramide C18:1 and lactosyl ceramide C24:1