

Original Article

Association Between Sphingolipids and Cardiopulmonary Fitness in Coronary Artery Disease Patients Undertaking Cardiac Rehabilitation

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Abstract

The long-term benefits conferred by cardiac rehabilitation (CR) in those with coronary artery disease (CAD) are strongly linked with an improvement in cardiopulmonary fitness. This study aimed to determine the association between peripheral sphingolipids and cardiopulmonary fitness in CAD subjects undertaking CR. Patients with CAD ($n = 100$, mean age = 64 ± 6 years, 85% male, mean years of education = 17 ± 3 years) underwent 6 months of CR with blood collected at baseline, 3 and 6 months. Cardiopulmonary fitness was assessed by measuring peak oxygen uptake (VO_{2peak}) at all time points. High performance liquid chromatography coupled electrospray ionization tandem mass spectrometry was used to quantify plasma sphingolipid concentrations. Cross-sectional and longitudinal associations between sphingolipids and VO_{2peak} were assessed using linear regressions and mixed models, respectively. Higher concentrations of sphingomyelin C18:1 ($\beta = -0.26$, $p = .01$), ceramides C16:0 ($\beta = -0.24$, $p = .02$), C18:0 ($\beta = -0.29$, $p = .002$), C20:0 ($\beta = -0.24$, $p = .02$) and C24:1 ($\beta = -0.24$, $p = .01$) and monohexylceramide C18:0 ($\beta = -0.23$, $p = .02$) were associated with poorer VO_{2peak} at baseline. An improvement in VO_{2peak} was associated with a decrease in sphingomyelin C18:1 ($b = -10.09$, $p = .006$), ceramides C16:0 ($b = -9.25$, $p = .0003$), C18:0 ($b = -5.44$, $p = .0003$) and C24:1 ($b = -2.46$, $p = .006$) and monohexylceramide C18:0 ($b = -5.37$, $p = .005$). Specific long chain sphingolipids may be useful markers of fitness and response to exercise in CAD.

Keywords: Sphingomyelin, Ceramide, Exercise, Cardiovascular disease

Coronary artery disease (CAD) is the leading cause of mortality and morbidity worldwide (1). CAD can significantly affect quality of life with 60% of those with CAD reporting a reduction in physical activity and 30% reporting disability and unemployment due to illness (2). As one of the most costly diseases, CAD represents a major economic burden; medical costs due to CAD, including hospitalizations, physician visits, and prescription drugs in addition to indirect costs

due to decreased work and household productivity are estimated to be \$182 billion annually in the United States (3).

Secondary prevention strategies such as cardiac rehabilitation (CR) are an important strategy to improve cardiovascular health and reduce the risk of morbidity and mortality in patients with CAD (4). CR consists of a standardized supervised exercise regimen, dietary education, monitoring and psychosocial support to improve fitness, and overall

health and has been shown to confer long-term benefit as exemplified by associations with reduced risk of mortality (4), fewer secondary ischemic cardiac events (5) and hospitalizations (6). The benefits of CR are strongly linked to improvement in cardiopulmonary fitness. For example, each 1% increase in peak oxygen uptake (VO_{2peak}), a reliable measure of cardiopulmonary fitness, is associated with a 2% decrease in the risk of mortality among CAD patients (7). However, there is wide variability in response to CR, suggesting a need to explore the biological correlates of cardiopulmonary fitness in patients with CAD.

CAD is a complex disease with multiple processes contributing to its underlying etiopathophysiology (8,9). In particular, dysregulations of sphingolipid metabolic pathways involved in loss of endothelial integrity and increased permeability (10), inflammatory signaling and oxidative stress (11), may play a central role in the development and progression of CAD. In support of this, subjects with unstable angina (12), acute coronary syndrome (13), and CAD (14), have been reported to have higher blood sphingolipid concentrations compared with healthy controls. Blood sphingolipids have also been extensively associated with vascular risk factors (15), risk of CAD (9,14,15) and poorer prognoses in those with CAD (14).

Recently, higher circulating levels of several long and very long chain sphingolipids were associated with poorer cardiopulmonary fitness in the elderly (16), suggesting that sphingolipids may be important mediators of cardiovascular health. However, the association between sphingolipids and cardiopulmonary fitness in at-risk populations such as those with CAD remains unclear. The present study aimed to assess sphingolipids as predictors of VO_{2peak} and determine if changes in circulating sphingolipids are associated with change in cardiopulmonary fitness in CAD subjects undertaking a 6-month CR program. We tested the hypotheses that higher concentrations of sphingolipids were associated with poorer VO_{2peak} at baseline (diagnostic) and over time (prognostic), and that a decrease in sphingolipids over the course of CR would be associated with an improvement in VO_{2peak} (response).

Method

Participants

This study was a subgroup analysis of CAD patients undergoing CR from a previously published prospective longitudinal cohort study (17). Briefly, participants with evidence of CAD (previous hospitalization for acute myocardial infarction, coronary angiographic evidence of $\geq 50\%$ blockage in one or more major coronary arteries or prior revascularization) were included. 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, substance abuse and probable dementia (Mini-Mental Status Examination (18) score < 24). Participants with diabetes were also excluded because insulin resistance is an independent predictor of VO_{2peak} in patients with CAD (19). In addition, the use of antidepressants was an exclusion criterion due to possible interactions with sphingolipids (20). This study was approved by the Sunnybrook Health Sciences Centre Research Ethics Board and the University Health Network Research Ethics Board and written informed consent was obtained from all study participants before any study procedures. Demographic and clinical characteristics, as well as a detailed medical history including comorbidities independent of CAD were collected from patient interviews. Concomitant medications, cardiac health indicators including left ventricular ejection

fraction grade and cumulative stenosis of coronary arteries, and anthropometrics were obtained from patient charts at the Toronto Rehabilitation Institute, University Health Network at baseline, 3 months, and 6 months. Participants also underwent a blood draw at all three time points.

Cardiac Rehabilitation

CR consisted of both aerobic and resistance training supervised by medical and exercise specialists. Participants were required to complete five aerobic training and 2–3 resistance training sessions weekly for 6 months. Both aerobic and resistance training was performed once per week during supervised exercise visits and the rest was conducted in the home/community. Walking/jogging over-ground was the primary mode of aerobic training prescribed. For those with musculoskeletal issues that precluded walking, other modalities such as stationary recumbent/upright cycling were prescribed. Aerobic training intensity was based on the cardiopulmonary exercise test (CPET) results conducted at baseline and 3 months. The goal was to progress participants to 20–60 min of exercise, 5 times/wk. A walking/jogging pace that elicited an oxygen uptake that occurred at the ventilatory anaerobic threshold during the CPET was used to prescribe exercise intensity. In the absence of a discernable ventilatory anaerobic threshold, a combination of the following was used; 60%–80% of heart rate reserve or peak oxygen uptake and/or rating of perceived exertion of 11–16 on the Borg 6–20 Scale (21). Prescriptions were initially progressed by increasing duration and then increasing intensity. Thereafter, training intensity was adjusted based on the mid-point CPET.

Cardiopulmonary Fitness

A symptom-limited CPET was performed on a cycle ergometer (Ergoline, Ergoselect 1000, Blitz, Germany) or upright cycle (Ergoselect 200P, Ergoline, Blitz, Germany) at baseline, 3 and 6 months. The CPET was conducted on the same modality on subsequent assessments. Breath-by-breath gas samples were collected via calibrated metabolic cart (SensorMedics Vmax Encore, San Diego, CA) to determine VO_{2peak} , mL/kg/min. Twelve-lead ECG, RPE, and blood pressure was monitored throughout. VO_{2peak} was calculated as the highest 20 second average recorded during the CPET (22). The expected VO_{2peak} mL/kg/min was calculated from established age and sex-matched norms (23).

Sphingolipid Measurements

At baseline, 3 months, and 6 months, blood for sphingolipid measurements was drawn at 0900 hours \pm 30 minutes and centrifuged at 1,000g for 10 minutes at 4°C. Plasma was immediately isolated and stored at -80°C until analysis. Quantification of 45 different sphingolipid species was accomplished by high performance liquid chromatography coupled electrospray ionization tandem mass spectrometry (LC/MS/MS) using multiple reaction monitoring as previously described (24). Briefly, a crude lipid extraction from plasma was performed using a modified Bligh and Dyer procedure (Avanti Polar Lipids, Alabaster, AL). Plasma extracts were dried and then resuspended in methanol before analysis. High performance liquid chromatography (PerkinElmer, MA) with a reverse phase C18 column (Phenomenex, Torrance, CA) 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 (0.1–1,000 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.).

Inflammatory Marker and Reactive Oxygen Species Measurements

Tumor necrosis factor (TNF) was measured in serum obtained at baseline and 6 months using multiplex magnetic bead immunoassay (EMD Millipore, Germany). Lipid peroxidation markers were also measured in serum at baseline and 6 months as previously described (25). Briefly, lipid hydroperoxides (LPH) was measured based on absorbance relative to hydroperoxide at 500 nm using a colorimetric assay (Cayman Chemical, Michigan). The intra-assay coefficient of variation (CV) of this assay ranged from 2% to 15% and inter-assay CV was 9%. A standard competitive sandwich enzyme-linked immunosorbent assay (ELISA) was used to assay 8-isoprostane (8-ISO). The intra-assay CV was 1%–19% and the inter-assay CV was 9.1%. Serum concentrations of 4-hydroxy-2-nonenal (4-HNE) were measured using an ELISA utilizing a Michael addition to lysine, histidine, or cysteine (Cell Biolabs, Inc. San Diego, CA). The intra-assay CVs were between 1% and 16% and inter-assay CV was 7.5%.

Statistical Analyses

Associations between fixed and time-varying patient characteristics and VO_{2peak} were assessed using Pearson correlations and ANOVAs at baseline and bivariate mixed models, respectively. Patient characteristics were included as potential confounders in multivariate models if they were significantly associated with VO_{2peak} at baseline and/or change in VO_{2peak} . All measurements of sphingolipids, inflammatory markers and lipid peroxidation markers were log-transformed to approximate normal distribution before analysis.

Sphingolipids were assessed as diagnostic, prognostic and response markers of VO_{2peak} as defined by the National Institute on Aging–Alzheimer’s Association Research Framework and the Biomarkers, EndpointS, and other Tools Resource of the Food and Drug Association–National Institutes of Health Biomarker Working Group (26). Crude associations between sphingolipid species and VO_{2peak} at baseline were explored using Pearson correlations. Bivariate mixed models were used to identify baseline sphingolipid species that significantly predicted change in VO_{2peak} . Bivariate mixed models were also used to identify significant associations between change in sphingolipid species and change in VO_{2peak} . Multivariate linear regression models were used to test the cross-sectional associations between individual sphingolipid species and VO_{2peak} while adjusting for potential confounders (diagnostic). Multivariate mixed models were then used to test whether individual sphingolipid species predicted change in VO_{2peak} (prognostic) and to assess associations between change in sphingolipid species and change in VO_{2peak} (response) while adjusting for potential confounders. Multicollinearity was assessed (tolerance statistic < 0.4) before multiple variables were included in the model. All analyses were performed using SPSS statistical software (version 24.0, IBM, NY) and the SAS University Edition statistical software (SAS Institute Inc.). Corrections for multiple comparisons were conducted using Benjamini–Hochberg procedures.

Post Hoc analyses

Post hoc analyses were conducted to assess whether possible confounders such as medications (statins, angiotensin converting enzyme inhibitors [ACE-I], angiotensin II receptor blockers [ARB] and calcium channel blockers), CAD severity (cumulative stenosis and LVEF grade) or mechanistic correlates, such as inflammatory (TNF) and lipid peroxidation markers (LPH, 8-ISO, and 4-HNE) influenced the diagnostic, prognostic and response associations between sphingolipids and VO_{2peak} . Bivariate cross-sectional and time-varying analyses were conducted to determine associations between potential confounders and mechanistic correlates and sphingolipids associated with VO_{2peak} . A sphingolipid × confounder or correlate interaction term was then added to multivariate models as a covariate for variables that were significantly associated with sphingolipids. Since all participants were being treated with statins, we compared differences between CAD patients using high (atorvastatin, pravastatin, or fluvastatin 40–80 mg/d and rosuvastatin or simvastatin 20–40 mg/d) versus low doses of statins (atorvastatin, pravastatin, or fluvastatin 10–20 mg/d and rosuvastatin or simvastatin 5–10 mg/d).

Results

Participant Characteristics

A total of 100 participants (mean age = 64 ± 6 , 85% male) completed this study. Participant characteristics and associations with VO_{2peak} at baseline and change in VO_{2peak} over the 6 months of CR are shown in [Supplementary Table 1](#). At baseline, males had higher VO_{2peak} compared with females and a higher level of education was associated with higher VO_{2peak} . Older age at baseline was associated with less improvement in VO_{2peak} . VO_{2peak} improved more in males compared with females and in those with a higher level of education at baseline. Higher cumulative stenosis at baseline was associated with less improvement in VO_{2peak} . Participants taking aspirin had greater gains in VO_{2peak} while those with a history of past or current smoking and those using calcium channel blockers and diuretics showed less improvement in VO_{2peak} . An increase in total cholesterol was associated with less improvement in VO_{2peak} . Higher LPH at baseline was associated with an improvement in VO_{2peak} .

Physiological and Cardiopulmonary Characteristics

Participant physiological and exercise characteristics are summarized in [Table 1](#). The mean VO_{2peak} at baseline was 21.7 ± 5.5 ml/kg/min, which is approximately 89% of age and sex-predicted normative values. Over the course of CR, VO_{2peak} increased significantly to 27.4 ± 6.9 ml/kg/min ($b = 1.14$, $p < .0001$). At baseline, higher BMI, body fat percentage, and waist circumference were significantly associated with lower VO_{2peak} . However, an improvement in VO_{2peak} was associated with a decrease in BMI, body fat percentage, waist circumference, resting heart rate, and systolic blood pressure.

Associations Between Sphingolipids and VO_{2peak} at Baseline (Diagnostic)

Pearson correlations identified eight sphingolipid species that were negatively correlated with VO_{2peak} cross-sectionally. These species include sphingomyelins C18:0 ($r = -.26$, $p = .01$), C16:1 ($r = -.22$, $p = .03$) and C18:1 ($r = -.34$, $p = .001$), ceramides C16:0

($r = -.21, p = .04$), C18:0 ($r = -.32, p = .001$), C20:0 ($r = -.24, p = .02$) and C24:1 ($r = -.25, p = .01$), and monohexylceramide C18:0 ($r = -.25, p = .01$). A complete list of correlations can be found in [Supplementary Table 2](#). Higher concentrations of sphingomyelin C18:1, ceramides C16:0, C18:0, C20:0, and C24:1, and monohexylceramide C18:0 remained significantly associated with poorer VO_{2peak} after adjustment for sex, years of education and BMI. These results were retained after correcting for multiple comparisons ([Table 2](#) and [Figure 1](#)).

Associations Between Baseline Sphingolipids and Change in VO_{2peak} (Prognostic)

Bivariate mixed models identified 14 sphingolipid species that were significantly associated with change in VO_{2peak} . Higher sphingomyelins C18:0 ($b = -17.87, p < .001$), C16:1 ($b = -16.81, p < .001$), C18:1 ($b = -17.69, p < .001$), C20:1 ($b = -8.78, p = .02$) and C22:1 ($b = -10.62, p = .04$), ceramides C16:0 ($b = -8.11, p = .007$), C18:0 ($b = -6.31, p < .001$), C20:0 ($b = -5.93, p = .005$), C22:1 ($b = -5.63,$

Table 1. Physiological and Cardiopulmonary Characteristics of Study Participants ($n = 100$) and Associations With VO_{2peak} at Baseline and Change in VO_{2peak} More Than 6 Months of Cardiac Rehabilitation

Variable	Baseline	3 Mo	6 Mo	Association With VO_{2peak} at Baseline		Association With Change in VO_{2peak}	
	Mean \pm SD or n (%)			r	p -Value	b	p -Value
Anthropometric characteristics							
Body mass index, kg/m ²	28.5 \pm 4.5	28.2 \pm 4.0	28.0 \pm 4.9	-.27	.007*	-0.39	<.0001*
Percentage body fat	30.4 \pm 8.3	29.4 \pm 8.0	28.0 \pm 8.2	-.29	.004*	-0.32	<.0001*
Waist circumference, cm	97.5 \pm 10.4	97.0 \pm 12.8	95.5 \pm 11.0	-.24	.02*	-0.14	<.0001*
Resting physiology							
Heart rate, bpm	68 \pm 12	66 \pm 11	66 \pm 10	-.15	.15	-0.08	.02*
Systolic blood pressure, mmHg	125 \pm 17	122 \pm 14	121 \pm 16	-.06	.58	-0.07	.01*
Diastolic blood pressure, mmHg	77 \pm 9	75 \pm 8	76 \pm 8	-.09	.36	-0.02	.72
Fitness parameters							
Maximum heart rate, bpm	123 \pm 20	129 \pm 20	133 \pm 24	.47	<.0001*	0.15	<.0001*
Maximum systolic blood pressure, mmHg	170 \pm 24	172 \pm 24	176 \pm 22	.17	.09	0.05	.005*
Maximum diastolic blood pressure, mmHg	78 \pm 11	77 \pm 10	78 \pm 10	-.21	.04*	-0.06	.11
VO_{2peak} , mL/kg/min	21.7 \pm 5.5	25.3 \pm 6.4	27.4 \pm 6.9				
Fraction of norm VO_{2peak}	0.89 \pm 0.24	1.04 \pm 0.25	1.10 \pm 0.25				

* $p < .05$.

Table 2. Cross-sectional and Longitudinal Associations Between Individual Sphingolipids (SPH) and VO_{2peak} in Coronary Artery Disease Patients Undergoing 6 Months of Cardiac Rehabilitation

SPH	Lipid	SPH \times VO_{2peak} at Baseline (Diagnostic)		Baseline SPH \times Change in VO_{2peak} (Prognostic)		Change in SPH \times Change in VO_{2peak} (Response)	
		β	p -Value	b	p -Value	b	p -Value
Sphingomyelins	C18:0	-0.19	.05	-7.27	.09	-7.28	.07
	C16:1	-0.05	.70	-5.21	.26	-2.86	.54
	C18:1	-0.26	.01*#	-11.94	.001*#	-10.09	.006*#
	C20:1			-5.30	.18	-3.86	.28
	C22:1			8.29	.17		
Ceramides	C16:0	-0.24	.02*#	-9.54	.001*#	-9.25	.0003*#
	C18:0	-0.29	.002*#	-5.11	.001*#	-5.44	.0003*#
	C20:0	-0.24	.02*#	-5.79	.004*#		
	C22:1			-3.33	.09		
	C24:1	-0.24	.01*#	-2.39	.008*#	-2.46	.006*#
Monohexylceramides	C18:0	-0.23	.02*#	-6.05	.002*#	-5.37	.005*#
	C20:0			-3.86	.11		
Lactosylceramides	C22:0			-5.75	.02*#		
	C26:1					2.05	.09
Sphingosine-1-phosphate	S1P			-1.22	.32		

Note: Multivariate linear regressions and mixed models used to assess cross-sectional and longitudinal associations between individual sphingolipids and VO_{2peak} respectively. Only values for sphingolipid species that were significantly associated with VO_{2peak} in diagnostic, prognostic, and response bivariate analyses are shown.

* $p < .05$; # $p < .05$ at Benjamini-Hochberg procedure.

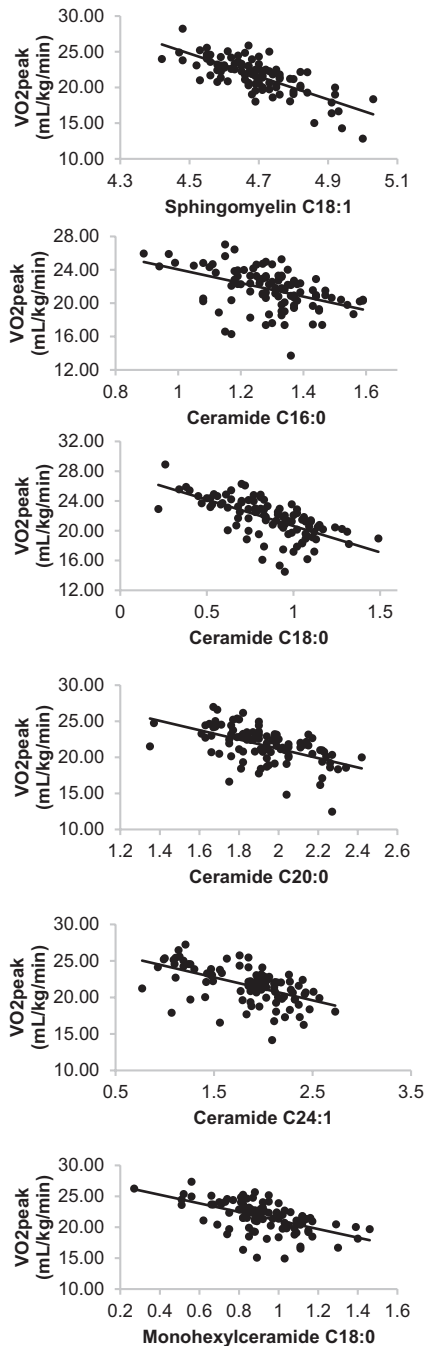


Figure 1. Associations between plasma sphingolipids and cardiopulmonary fitness at baseline. VO_{2peak} is based on predicted values obtained in linear regression models with sphingolipid concentrations predicting VO_{2peak} controlling for additional covariates ($p < .05$).

$p = .006$) and C24:1 ($b = -3.02, p = .002$), monohexylceramides C18:0 ($b = -7.96, p < .001$), and C20:0 ($b = -6.04, p = .02$), lactosylceramide C22:0 ($b = -6.27, p = .02$) and sphingosine-1-phosphate ($b = -2.61, p = .03$) at baseline were associated with less improvement in VO_{2peak} . A complete list of correlations can be found in [Supplementary Table 2](#). Higher concentrations of sphingomyelin C18:1, ceramides C16:0, C18:0, C20:0, and C24:1, monohexylceramide C18:0 and lactosylceramide C22:0 remained significantly associated with less improvement in VO_{2peak} after adjustment for age, sex,

BMI, smoking history, total cholesterol, resting heart rate, systolic blood pressure, and use of calcium channel blockers and diuretics. These results were retained after correcting for multiple comparisons ([Table 2](#)).

Associations Between Change in Sphingolipids and Change in VO_{2peak} (Response)

Bivariate mixed models identified eight sphingolipid species that were significantly associated with change in VO_{2peak} over 3 months. An improvement in VO_{2peak} was associated with a decrease in sphingomyelins C16:0 ($b = -16.43, p = .01$), C16:1 ($b = -14.85, p < .001$), and C18:1 ($b = -18.21, p < .001$), ceramides C16:0 ($b = -8.73, p = .006$), C18:0 ($b = -6.39, p < .001$), and C24:1 ($b = -2.40, p = .03$), and monohexylceramide C18:0 ($b = -7.69, p = .001$) and an increase in lactosylceramide C26:1 ($b = 3.46, p = .03$). Associations between improvement in VO_{2peak} and decrease in sphingomyelin C18:1, ceramides C16:0, C18:0, and C24:1, and monohexylceramide C18:0 remained significant after adjustment for age, sex, BMI, smoking history, total cholesterol, resting heart rate, systolic blood pressure, and use of calcium channel blockers and diuretics. These results were retained after correcting for multiple comparisons.

Bivariate mixed models identified nine sphingolipid species that were significantly associated with change in VO_{2peak} over 6 months. An improvement in VO_{2peak} was associated with a decrease in sphingomyelins C18:0 ($b = -17.07, p < .001$), C16:1 ($b = -16.35, p < .001$), C18:1 ($b = -18.00, p < .001$) and C20:1 ($b = -8.99, p = .02$), ceramides C16:0 ($b = -8.54, p = .003$), C18:0 ($b = -6.25, p < .001$), and C24:1 ($b = -2.54, p = .01$), and monohexylceramide C18:0 ($b = -7.34, p < .001$) and an increase in lactosylceramide C26:1 ($b = 3.44, p = .01$). A complete list of time-varying associations can be found in [Supplementary Table 2](#). Associations between improvement in VO_{2peak} and decrease in sphingomyelin C18:1, ceramides C16:0, C18:0, and C24:1, and monohexylceramide C18:0 remained significant after adjustment for age, sex, BMI, smoking history, total cholesterol, resting heart rate, systolic blood pressure, and use of calcium channel blockers and diuretics. These results were retained after correcting for multiple comparisons ([Table 2](#) and [Figure 2](#)).

Post Hoc Analyses

Medication Effects

Bivariate analyses at baseline showed that sphingomyelin C18:1 was significantly higher in patients taking calcium channel blockers ($\beta = 0.22, p = 0.03$). In repeated measures bivariate analyses, calcium channel blocker use was associated with a decrease in ceramides C16:0 ($b = -0.06, p = .04$), C18:0 ($b = -0.10, p = .05$), and C20:0 ($b = -0.08, p = .04$). In addition, ceramide C16:0 was higher at baseline ($\beta = 0.25, p = .03$) and increased over time ($b = 0.06, p = .003$) in patients using high dose statins. In diagnostic post hoc analyses, neither the sphingomyelin C18:1 \times calcium channel blocker use ($F = 0.55, p = .46$) nor the ceramide C16:0 \times statin dose interaction ($F = 2.91, p = .09$) significantly predicted VO_{2peak} at baseline. The sphingomyelin C18:1 \times calcium channel blocker use ($b = -6.60 [13.70], p = .63$) and the ceramide C16:0 \times statin dose interaction ($b = -0.08 [0.72], p = .91$) also were not prognostic predictors of change in VO_{2peak} . Neither of the ceramide C16:0 ($b = -1.80 [10.22], p = .86$), C18:0 ($b = -8.52 [5.21], p = 0.10$) or C20:0 \times calcium channel blocker use ($b = 0.86 [5.81], p = .88$) and the ceramide C16:0 \times statin dose interaction ($b = -4.48 [5.06], p = .37$) interaction terms were associated with improvement in VO_{2peak} in response post hoc models.

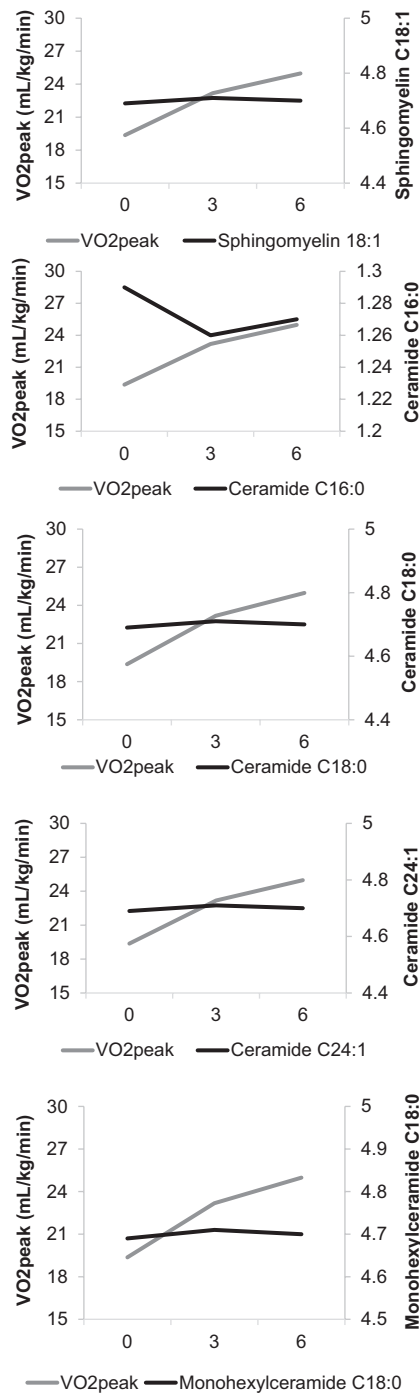


Figure 2. Associations between change in plasma sphingolipids and change in cardiopulmonary fitness over 6 months of cardiac rehabilitation. VO_{2peak} is based on predicted values obtained in mixed models with sphingolipid concentrations predicting VO_{2peak} controlling for additional covariates ($p < .05$).

CAD Severity

At baseline, sphingomyelin C18:1 was significantly higher in patients with higher cumulative stenosis ($\beta = 0.35$, $p = .01$). Higher cumulative stenosis at baseline was also significantly associated with an increase in sphingomyelin C18:1 ($b = 0.001$, $p < .001$) and ceramides C16:0 ($b < 0.001$, $p = .009$), C18:0 ($b = 0.001$, $p < .001$), C20:0 ($b < 0.001$, $p = .004$) and C24:1 ($b = 0.001$, $p = .02$) over time. LVEF

grade 4 was associated with a decrease in sphingomyelin C18:1 ($b = -0.10$, $p = .009$) and ceramides C16:0 ($b = -0.15$, $p = .002$) and C18:0 ($b = -0.26$, $p = .002$) compared with Grade 1. In post hoc analyses, a sphingomyelin C18:1 \times cumulative stenosis interaction was not a diagnostic ($F = 0.56$, $p = .46$) or prognostic ($b = 0.05$ [0.06], $p = .37$) predictor of VO_{2peak} . In response post hoc analyses, the interaction between cumulative stenosis and ceramides C18:0 ($b = 0.05$ [0.02], $p = .01$) and C20:0 ($b = 0.06$ [0.03], $p = .03$) but not sphingomyelin C18:1 ($b = 0.05$ [0.05], $p = .33$) and ceramides C16:0 ($b = 0.04$ [0.03], $p = .18$), and C24:1 ($b = 0.01$ [0.01], $p = .45$) were associated with change in VO_{2peak} . Sphingolipid \times LVEF grade interactions were not associated with change in VO_{2peak} .

Inflammatory and Oxidative Stress Markers

TNF and lipid peroxidation markers were not associated with sphingolipids of interest in bivariate analyses at baseline. In bivariate repeated measures analyses, an increase in LPH was associated with a decrease in lactosylceramide C22:0 over time ($b = -0.06$, $p = .02$). However, a lactosylceramide C22:0 \times LPH interaction term ($b = -1.78$ [6.74], $p = 0.79$) was not associated with VO_{2peak} change in response post hoc analyses.

Discussion

Cardiopulmonary fitness is an independent predictor of mortality in CAD (7). This study assessed plasma sphingolipids as diagnostic, prognostic and response biomarkers of cardiopulmonary fitness in patients with CAD. Higher plasma concentrations of sphingomyelin C18:1, ceramides C16:0, C18:0, C20:0, and C24:1 and monohexylceramide C18:0 were associated with poorer VO_{2peak} at baseline. Higher baseline plasma concentrations of these sphingolipids and lactosylceramide C22:0 were also predictive of less improvement in VO_{2peak} . In addition, an improvement in VO_{2peak} was associated with a decrease in these sphingolipids, suggesting that sphingolipid metabolism may be responsive to exercise.

Reduced cardiac output and impaired skeletal muscle mitochondrial capacity and efficiency are considered to be the primary contributors to age-related decline in VO_{2peak} (27). Evidence suggests that sphingolipids accumulate in the skeletal muscle and induce mitochondrial damage and impair mitochondrial oxidative capacity (28). In addition, both animal (29) and human (30) studies show a correlation between sphingolipids and impaired myocardial contractility. As such, sphingolipids may be important mediators of cardiovascular health.

Cross-sectional findings from this study are consistent with the only other study assessing the relationship between sphingolipids and VO_{2peak} , in which higher levels of circulating ceramides C18:0, C20:0, and C24:1 were associated with lower VO_{2peak} in an elderly population (16). We further expand on those findings by showing that higher concentrations of these sphingolipids also predict less improvement in VO_{2peak} . Notably, our results also showed associations between higher levels of sphingomyelin C18:1, ceramide C16:0, and monohexylceramide C18:0 and poorer VO_{2peak} at baseline and higher levels of these species and lactosylceramide C22:0 and less improvement in VO_{2peak} in patients with CAD. While all sphingolipids have been shown to increase with age in a large sample of elderly subjects (24,31), these additional associations may be generally attributable to the specific contributions of CAD etiology. Compared with a sample of healthy elderly (24,31), study participants had higher sphingomyelin but slightly lower ceramide blood

concentrations consistent with other studies in elderly patients with CAD (12). Sphingomyelins are intimately associated with the formation of atherosclerotic plaques substantiated by higher blood concentrations of sphingomyelins among subjects with unstable angina (12), acute coronary syndrome (13), and CAD (12,14) compared with healthy controls. In addition, long and very long chain ceramides, such as ceramides C16:0, C18:0, C20:0, and C24:1 are also enriched in aggregated LDL in atherosclerotic lesions (9) and ceramides identified in this study have also been previously associated with cardiovascular risk factors, myocardial infarction, percutaneous coronary intervention (12) and cardiomyocyte damage in those with a coronary artery bypass graft (32). In particular, ceramide C16:0 has been previously associated with the progression of CAD (33), and glycosphingolipids, including monohexylceramides and lactosylceramides, have been previously associated with atherosclerosis and arterial stiffness in animal studies (34).

Findings from this study suggest that sphingomyelin C18:1 and ceramides C16:0 and C18:0 were most responsive to improvements in cardiopulmonary fitness in patients with CAD. These sphingolipids have been consistently associated with metabolic disease (35), psychiatric disorders (36), and neurodegenerative diseases (37). Our own studies have previously shown associations between these sphingolipids and depressive symptoms (38) and cognitive response to exercise in patient with CAD (17). As such, sphingolipids may be important markers of early pathophysiological changes in at-risk populations such as those with CAD.

An inverse relationship found between ceramides and VO_{2peak} in a previous study (16) implied that improvement in cardiopulmonary fitness following exercise may modify sphingolipid levels. We confirm that hypothesis in the present study; an improvement in VO_{2peak} was associated with a decrease in circulating sphingolipids. While there are limited data on the time-varying relationship between sphingolipids and cardiopulmonary fitness, these findings are supported by previous reports of a decrease in plasma sphingomyelins, ceramides and glucosylceramides following a bout of acute exercise in patients with obesity and diabetes (39). In previous studies, 12 weeks of exercise training in obese individuals and those with diabetes (40) and 16 weeks of exercise in overweight to obese adults (41) resulted in a significant reduction in sphingolipid species including ceramides C18:0, C20:0, and C24:1. Importantly, we recently showed that a decrease in ceramide C18:0 with an improvement in VO_{2peak} was associated with improved cognition (17), highlighting the importance of circulating sphingolipids as markers of response to exercise in patient with CAD. Those results were independent of change in BMI.

An improvement in cardiopulmonary fitness may influence sphingolipid metabolism in various ways. An increase in VO_{2peak} following CR has been previously associated with a reduction in oxidative stress and inflammation (42), which are potent inducers of sphingolipid synthesis (43). Exercise can also reduce sphingolipid levels due to utilization of lipids as substrates during sphingolipid synthesis (41). In addition, ceramide sphingolipids have been previously positively associated with BMI and waist-hip-ratio (31). A decrease in BMI, body fat percentage and waist circumference with an improvement in VO_{2peak} in the present study implies indirect attenuating effects on sphingolipid concentrations through changes in cardiopulmonary fitness parameters.

Post hoc findings suggest that inflammatory and oxidative stress markers did not influence the relationships between sphingolipids and cardiopulmonary fitness. However, as found in this study, other factors such as CAD severity may be important contributors in this

population. Future work should elucidate the mechanisms underlying the relationship between sphingolipids and cardiopulmonary fitness to develop sphingolipids as clinically useful diagnostic, prognostic, and response markers.

Findings from this study suggest that higher long chain sphingolipids may be important biomarkers of poorer aerobic capacity in patients with CAD. Given the mechanistic role of sphingolipids in age-related decline in VO_{2peak} , findings from this study also identify potential drug targets. This work is timely and has clinical implications since several newly developed synthetic inhibitors of enzymes involved in sphingolipid metabolism are in preclinical or clinical development (44). For example, modification of the immunosuppressive drug FTY720 (fingolimod) into different oxy and amine derivatives has yielded certain compounds that may be promising in modulating ceramide synthases 4 and 6 involved in the production of ceramides C16:0, C18:0, and C20:0 and one compound that may be more specific to ceramide C18:0 synthesis (44).

Commonly used cardiovascular medications such as calcium channel blockers and anti-arrhythmics have been classified as functional inhibitors of acidic sphingomyelinase (FIASMA), an enzyme that converts sphingomyelins into ceramides (20). While calcium channel blockers did not influence the results in this study, the availability of a large variety of cardiovascular medications that can modulate sphingolipid metabolism may be advantageous in improving and maintaining cardiopulmonary fitness in this at-risk population. Furthermore, while the effects of antidepressants on sphingolipid concentrations and VO_{2peak} could not be assessed in the present study since antidepressant use was an exclusion criterion, antidepressants are known to be potent FIASMA (20). Depression is a common comorbidity associated with CAD (45). Effects of aspirin could also not be assessed since almost all patients were being treated with low dose aspirin. In a previous study in healthy volunteers, low dose aspirin had no effect on plasma or platelet sphingolipid level but increased ceramide levels in erythrocytes (46). In addition, sphingomyelins C20:0, C22:0, C16:1, C18:1, C20:1, C22:1, and C24:1 and ceramides C18:0, C20:0, and C24:1 were significantly higher ($p < 0.01$) among statin users compared with nonusers at baseline after adjustment for age, sex, and comorbidities in the BLSA study ($n = 992$) (24,31), consistent with findings from this study. Future work should explore interactions between commonly used medications in CAD and sphingolipid metabolism in this population.

This study was strengthened by the measurement of a comprehensive sphingolipid profile. Study findings also add to a growing body of evidence with novel longitudinal associations between sphingolipids and cardiopulmonary fitness in an at-risk population. Limitations of this study include a small sample size in comparison to epidemiological studies. In addition, study subjects were predominantly highly educated Caucasian males from a single center and may have been highly motivated to enter CR. While this precluded assessment of sex differences and may limit the generalizability of the results to all CAD patients, the relative homogeneity of the sample may be beneficial as the cohort is representative of patients undergoing CR, which is a standard of care. Future studies in larger and more diverse cohorts are needed to confirm these findings and enhance the generalizability of these results. Since perturbations in sphingolipid metabolism may differ in patients with acute coronary syndrome compared with stable CAD such as in this study (47), future studies should also explore sphingolipid changes in that patient population and their effects on important outcomes such as VO_{2peak} . In addition, evidence suggests that skeletal muscle adaptations occur following resistance training, which may be attenuated by sphingolipids (48).

Although resistance training is a component of the CR program at UHN Toronto Rehab, the relationship between resistance training and sphingolipids could not be assessed due to missing data.

Conclusion

In patients with CAD, higher baseline plasma concentrations of long chain sphingolipids were associated with poorer VO_{2peak} and less improvement in VO_{2peak} . Over time, an improvement in VO_{2peak} was associated with a decrease in these sphingolipids. These findings suggest that sphingolipids that have been consistently associated with CAD and other medical disorders can be modulated by exercise, underscoring the importance of lifestyle modifications in at-risk populations such as those with CAD.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None reported.

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