Journal of the American Heart Association

ORIGINAL RESEARCH

Plasma Ceramide Levels Are Elevated in Patients With Early Coronary Atherosclerosis and Endothelial Dysfunction

Nadia Akhiyat, MD, MS; Vlad Vasile, MD, PhD; Ali Ahmad, MD; Jaskanwal Deep Sara, MBChB; Valentina Nardi, MD; Lilach O. Lerman, MD, PhD; Allan Jaffe, MD; Amir Lerman, MD

BACKGROUND: Elevated plasma ceramides are independent predictors of cardiovascular disease and mortality in patients with advanced epicardial coronary artery disease. Our understanding of plasma ceramides in early epicardial coronary artery disease, however, remains limited. We examined the role of plasma ceramides in early coronary atherosclerosis characterized by coronary endothelial dysfunction.

METHODS AND RESULTS: Participants presenting with chest pain and nonobstructive epicardial coronary artery disease underwent coronary endothelial function. Patients (n=90) demonstrated abnormal coronary endothelial function with acetylcholine (≥20% decrease in coronary artery diameter or ≤50% increase in coronary blood flow). A total of 30 controls had normal coronary endothelial function. Concentrations of plasma ceramide 18:0 (P=0.038), 16:0 (P=0.021), and 24:0 (P=0.019) differed between participants with normal and abnormal coronary endothelial function. Ceramide 24:0 (odds ratio [OR], 2.23 [95% CI, 1.07–4.66]; P=0.033) and 16:0 (OR, 1.91×10^{6} [95% CI, $11.93–3.07\times10^{11}$]; P=0.018) were independently associated with coronary endothelial dysfunction. Among participants with endothelium-dependent coronary dysfunction (n=78), ceramides 16:0 (OR, 5.17×10^{5} [95% CI, $2.83–9.44\times10^{10}$]; P=0.033), 24:0 (OR, 2.98 [95% CI, 1.27–7.00]; P=0.012), and 24:1/24:0 (OR, 4.39×10^{-4} [95% CI, 4×10^{-7}–0.48]; P=0.030) were more likely to be elevated.

CONCLUSIONS: The current study demonstrated an association between increased circulating ceramide levels and coronary endothelial dysfunction in the absence of epicardial coronary artery disease. This study supports the role of plasma ceramides as a potential biomarker or a therapeutic target for early coronary atherosclerosis in humans.

Key Words: ceramides ■ coronary artery disease ■ endothelial dysfunction

Early coronary atherosclerosis is characterized by coronary endothelial dysfunction (CED). CED occurs within epicardial arteries and subsequent downstream high-resistance arterial networks that regulate the myocardial blood supply and perfusion.[1] Coronary atherogenesis at its earliest stages segmentally disrupts the endothelial landscape.[2–5] This often diagnostically occult progression of CED evolves to manifest clinically with eventual ventricular dysfunction, myocardial ischemia, increased mortality, and increased risk for major adverse cardiac events (MACE) in the absence of obstructive coronary artery disease.[6–10] Thus, identification of nontraditional risk factors for early coronary atherosclerosis has been an area of increasing clinical and academic interest.

An emerging nontraditional risk factor for coronary atherosclerosis has been identified among known biologically active lipid species. Circulating long-chain and very-long-chain sphingolipids affect functional and structural processes associated with endothelial dysfunction through oxidative, thrombotic, inflammatory, apoptotic, and atherogenic pathways. Theoretically these observations may translate to the progression of coronary artery disease in humans; however, our

Correspondence to: Amir Lerman, MD, 200 First St SW, Rochester, MN 55905. E-mail: lerman.amir@mayo.edu

Supplemental Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.121.022852

For Sources of Funding and Disclosures, see page 14.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- Plasma ceramide levels are elevated in humans with early coronary atherosclerosis and coronary endothelial dysfunction in the absence of epicardial coronary artery disease.

What Are the Clinical Implications?

- Circulating ceramides may be a biomarker and therapeutic target for early coronary atherosclerosis in humans.

Nonstandard Abbreviations and Acronyms

ΔCAD, change in coronary artery diameter;	
ΔCBF	change in coronary blood flow
CED	coronary endothelial dysfunction
CFR	coronary flow reserve
MACE	major adverse cardiac events

current understanding of the direct mechanism underlying the influence of ceramide (Cer) on human coronary atherogenesis relies predominantly on animal and ex vivo studies.[11,12] At present, investigations in humans focus on clinical observations and outcomes. Literature supports a strong association between elevated concentrations in plasma ceramides and advanced coronary atherosclerosis with an emphasis on the strong predictive association between plasma ceramide concentrations and cardiovascular risk.[13–20]

Although studies in humans are currently limited by indirect observations, clinical investigation of individual acyl chain species concentrations sheds light on our understanding of their possible independent roles in the progression of coronary disease. Emerging evidence suggests the role of circulating ceramides in various pathways of atherogenesis with predominant associations between ceramide 18:0, ceramide 16:0, and ceramide 24:1 and their respective roles in the inflammatory, thrombotic, and low-density lipoprotein (LDL)–mediated pathways of atherogenesis. The association of these circulating ceramide acyl species in late coronary atherosclerosis is now robustly described; however, our understanding of the role ceramide acyl species may play in humans with very early coronary disease remains limited.[12,13,17,20–22] The current study was designed to test the hypothesis that elevated levels of circulating plasma ceramides are associated with CED. In this case-control analysis, we examine the relationship between plasma ceramides and a range of coronary function abnormalities associated with early coronary atherosclerosis.

METHODS

Ethical Statement

Supporting data are available from corresponding author upon reasonable request. This study was

Table 1. Demographics and Clinical Characteristics

Variable	Patients* (n=90)	Controls* (n=30)	*P* value
Age, y	56±10	57±9	0.944
Female sex	67 (74)	24 (80)	0.542
BMI, kg/m^2	30±6	28±5	0.410
Systolic blood pressure, mm Hg	122 (112; 138)	120 (112; 130)	0.447
Diastolic blood pressure, mm Hg	77 (70; 82)	74 (70; 80)	0.520
Smoking status			
Never smoked	55 (61)	17 (57)	0.912
Former smoker	31 (34)	10 (33)	0.912
Current smoker	4 (4)	3 (10)	0.265
Hypertension	38 (42)	15 (50)	0.462
Diabetes	11 (12)	4 (13)	0.875
Hyperlipidemia	54 (60)	14 (47)	0.205
eGFR, mL/min per 1.73 m^2	73 (64; 82)	74 (61; 92)	0.967
Aspirin use	58 (64)	19 (63)	0.913
β-blocker use	42 (47)	9 (30)	0.116
Lipid-lowering drug use	48 (53)	12 (40)	0.209
Antihypertensive use	29 (32)	11 (37)	0.658

BMI indicates body mass index; and eGFR, estimated glomerular filtration rate.
*Data are presented as number (percentage), mean±SD, or median (interquartile range).

Table 2. Coronary Angiography and Lipid Data

Variable	Patients*	n	Controls*	n	*P* value
CFR	2.5 (2.2; 2.9)	90	3.2 (2.8; 3.7)	30	<0.000
ΔCBF, %	−1.74 (−32; 24)	90	114 (89; 161)	30	<0.000
ΔCAD, %	−25 (−39; −11)	90	−4 (−11; 1)	30	<0.000
Myocardial infarction–heart ceramide risk score	3 (2; 6)	90	2.5 (1; 4)	30	
Risk category					
Higher risk	1 (1)	90	1 (3)	30	0.415
Increased risk	13 (14)	90	4 (13)	30	0.881
Moderate risk	36 (40)	90	15 (50)	30	0.749
Lower risk	36 (40)	90	15 (50)	30	0.341
Lipid profile					
Total cholesterol, mmol/L	181 (164; 207)	88	177 (160; 208)	29	0.693
LDL cholesterol, mmol/L	101 (83; 123)	88	100 (84.5–107.5)	29	0.494
HDL cholesterol, mmol/L	56 (43; 64)	88	58 (46; 76)	29	0.587
Triglycerides, mmol/L	109 (83; 141)	87	107 (74.5; 131)	29	0.671
Plasma ceramides					
18:0, μmol/L	0.10 (0.08; 0.13)	90	0.08 (0.06; 0.10)	30	0.038
16:0, μmol/L	0.29 (0.25; 0.33)	86	0.27 (0.24; 0.28)	28	0.021
24:1, μmol/L	0.78 (0.66; 0.95)	90	0.77 (0.65; 0.90)	30	0.771
24:0, μmol/L	3.05 (2.73; 3.85)	87	2.82 (2.54; 3.16)	29	0.019
16:0/24:0	0.09 (0.08; 0.11)	90	0.10 (0.08; 0.11)	30	0.854
18:0/24:0	0.03 (0.03; 0.04)	90	0.03 (0.02; 0.04)	30	0.266
24:1/24:0	0.25 (0.21; 0.31)	89	0.28 (0.23–0.32)	29	0.091

ΔCAD indicates change in coronary artery diameter; ΔCBF, change in coronary blood flow; CFR, coronary flow reserve; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

*Data are presented as number (percentage) or median (interquartile range).

approved by the Mayo Clinic Institutional Review Board. Written informed consent was obtained from participants.

Participants

Consecutive randomly selected patients presenting for clinically indicated coronary angiographic evaluation of

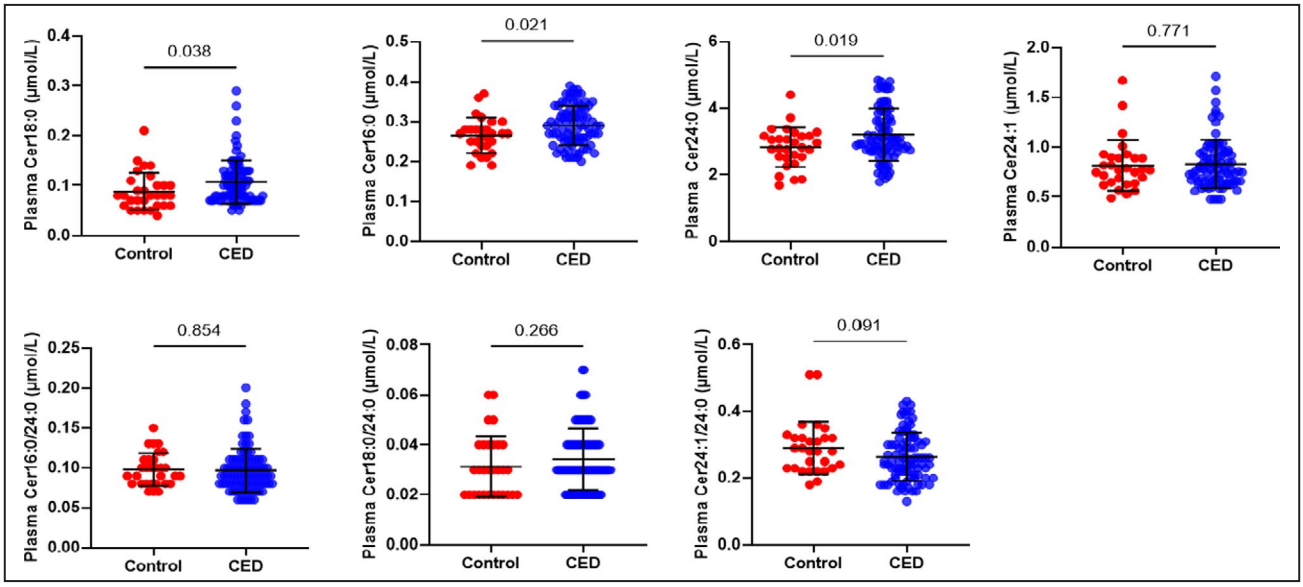


Figure 1. Ceramide levels in patients with and without CED.
CED indicates coronary endothelial dysfunction; and Cer, ceramide.

Table 3. Plasma Ceramide Levels by Sex

Plasma ceramide	Female sex*	n	Male sex*	n	*P* value
18:0, μmol/L	0.09 (0.07; 0.12)	91	0.10 (0.08; 0.13)	29	0.365
16:0, μmol/L	0.27 (0.25; 0.32)	87	0.28 (0.24; 0.31)	27	0.844
24:1, μmol/L	0.77 (0.66;0.94)	90	0.82 (0.74; 0.92)	29	0.374
24:0, μmol/L	2.69 (2.69; 3.38)	90	3.09 (2.79; 3.88)	26	0.414
16:0/24:0	0.09 (0.08; 0.11)	91	0.09 (0.08; 0.11)	29	0.996
18:0/24:0	0.03 (0.02; 0.04)	91	0.03 (0.02; 0.04)	29	0.506
24:1/24:0	0.26 (0.22; 0.31)	90	0.25 (0.21; 0.32)	28	0.795

*Data are presented as median (interquartile range).

chest pain and coronary arteries with <40% obstruction (n=1991) between the years 1992 and 2019 were reviewed. Patients with abnormal CED (n=90) were randomly selected and defined as participants with abnormal coronary endothelial function. These participants had either (1) >20% constriction change in coronary artery diameter following intracoronary acetylcholine injection indicating epicardial endothelial dysfunction or (2) a ≤50% increase change in coronary blood flow (ΔCBF) indicating microvascular endothelial dysfunction.[6,23] Eligible participants underwent indicated angiogram and routine blood sample collection in 2019. Controls (n=30) had normal coronary endothelial function testing and were propensity matched by age and sex.

Endothelial Function Testing

A detailed methodology has been previously published.[24–26] Briefly, patients presenting for clinically indicated angiography underwent invasive coronary function testing. Participants had not received oral nitrates, lipid-lowering drugs, antioxidants, or angiotensin-converting enzyme inhibitors for 2 weeks before intervention. Other medications such as calcium-channel blockers or β-adrenergic blockers were discontinued at least 48 hours before the interventional study. Diagnostic coronary angiography was then performed to assess epicardial coronary artery, microvascular endothelium, and microvascular endothelium-independent function in concordance with the International Microcirculation Working Group expert review.[27] Participants with significant obstructive epicardial coronary artery disease (defined as >40% coronary diameter stenosis) were excluded. During angiography of eligible participants, a Doppler guidewire (FloWire; Philips Healthcare, Andover, MA) was advanced to the mid-portion of the left anterior descending coronary artery.

To assess nonendothelium microvascular function, incremental doses of intracoronary adenosine (18 μg–72 μg) were administrated as consecutive boluses until maximal hyperemia was observed. Endothelium-independent microvascular function was determined by highest observed coronary flow reserve (CFR) in response to increasing doses of intracoronary adenosine.

Endothelium dependent function was assessed by 3 consecutive boluses of acetylcholine in the mid-left anterior descending coronary artery at 3-minute intervals with increasing concentrations of 10^{-6}, 10^{-5}, and 10^{-4} mmol/L. After each dose of acetylcholine, a single operator measured the diameter of the mid-left anterior descending coronary artery 5-mm distal to the Doppler wire tip with a coronary angiogram tool (Medis Cor, Leiden, The Netherlands). We calculated coronary blood flow=Π (mean peak velocity/2)×(coronary artery diameter/2)^2. The ΔCBF, a measure of microvascular function, was calculated as the percentage difference between coronary blood flow at

Table 4. Plasma Ceramide Levels by Sex in Patients With Coronary Endothelial Dysfunction

Plasma ceramide	Female sex*	n	Male sex*	n	*P* value
18:0, μmol/L	0.09 (0.08; 0.13)	67	0.11 (0.07; 0.13)	23	0.787
16:0, μmol/L	0.29 (0.25; 0.33)	64	0.29 (0.25; 0.31)	22	0.664
24:1, μmol/L	0.76 (0.66; 0.96)	67	0.83 (0.74; 0.94)	23	0.381
24:0, μmol/L	3.00 (2.69; 3.71)	66	3.11 (2.85; 3.88)	21	0.570
16:0/24:0	0.09(0.08; 0.11)	67	0.09 (0.08; 0.11)	23	0.814
18:0/24:0	0.03 (0.03; 0.04)	67	0.03 (0.02; 0.04)	23	0.955
24:1/24:0	0.25 (0.21; 0.31)	67	0.24 (0.20; 0.34)	22	0.825

*Data are presented as median (interquartile range).

Table 5. Correlation Between Plasma Ceramides and Age

Plasma ceramide	Correlation with age
18:0, μmol/L	0.08
16:0, μmol/L	0.05
24:1, μmol/L	0.09
24:0, μmol/L	0.04
16:0/24:0	0.05
18:0/24:0	0.13
24:1/24:0	0.11

Correlation coefficients for circulating plasma ceramides and age.

basal flow and maximal hyperemia after acetylcholine injection. We defined microvascular CED as <50% increase in coronary blood flow after intracoronary acetylcholine infusion. We defined endothelium-independent microvascular dysfunction as CFR <2.0.

Clinical and Biochemical Data

Demographic data were obtained at the time of coronary angiography.[5,28] Conventional cardiovascular risk factors were obtained from medical records, including associated comorbidities, smoking status, body mass index, glomerular filtration rate, and pharmacologic therapy. Comorbidities were defined as follows: diabetes was defined as a documented comorbidity and/or use of hypoglycemic agents, hypertension was defined as a documented comorbidity and/or use of antihypertensive agents, hyperlipidemia was defined as elevated lipid laboratory data and/or use of lipid-lowering agents. Blood samples were obtained within 2 weeks before angiography. Routine laboratory analysis (lipid profile, hs-CRP [high-sensitivity C-reactive protein]) and cytokine measurement was performed. Frozen plasma specimens stored in EDTA collected from fasting participants before coronary angiography were analyzed by liquid chromatography–tandem mass spectrometry. The following ceramide acyl chain species were selected based on their association with cardiovascular risk: ceramide 18:0, ceramide 16:0, ceramide 24:0, ceramide 24:1, and ratios of each with ceramide 24:0.[13,17,18] Ceramide concentrations obtained by mass spectrometry were examined for significant confounding outlier data. Among participants, there were significant outliers identified in ceramide 16:0, ceramide 24:01, and ceramide 24:1/24:0 (n=6, n=4, n=1, respectively), which were excluded from further analysis. No significant outliers were observed among other ceramide acyl species (Figure S1).

Statistical Analysis

Continuous variables were listed as mean±SD when data are normally distributed and median with interquartile range when data are skewed. Demographic, clinical, angiographic, and lipid data were compared between participants with and without CED with an unpaired *t* test. Correlations were examined between plasma ceramides and sex, age, hs-CRP, and inflammatory markers (IL [interleukin]-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFNγ [interferon γ], and TNFα [tumor necrosis factor α]). An unpaired *t* test examined plasma ceramide levels between patients who were and were not treated with lipid-lowering therapy. The independent association of each plasma ceramide was then assessed by a multivariable regression analysis with 3 separate variables used to characterize CED: percentage ΔCBF, ΔCAD and CFR. Regressions were adjusted by 3 consecutive models for factors known to affect cardiovascular risk and potential lipid confounders. Model 1 was adjusted for age and sex; model 2 was further adjusted for hypertension, diabetes, hyperlipidemia, and lipid-lowering drug use; and model 3 was further adjusted for LDL and non–high-density lipoprotein (HDL) cholesterol. A receiver operating characteristic curved assessed the predictive accuracy of plasma ceramide concentrations in predicting CED. Area under the curve (AUC) assessed discrimination of CED by plasma ceramide species. A subgroup analysis of participants with endothelial-dependent vascular dysfunction (defined by CFR >2) was conducted in the same manner. Data

Table 6. Correlation Between Plasma Ceramides and hs-CRP

Plasma ceramide	Correlation with hs-CRP
18:0, μmol/L	0.17
16:0, μmol/L	0.02
24:1, μmol/L	0.13
24:0, μmol/L	0.06
16:0/24:0	−0.03
18:0/24:0	0.14
24:1/24:0	0.08

Correlation coefficients for circulating concentrations of ceramides and hs-CRP. hs-CRP indicates high-sensitivity C-reactive protein.

Table 7. Linear Association Between Plasma Ceramides and High-Sensitivity C-Reactive Protein

Plasma ceramide	Standardized β coefficient	*P* value
18:0, μmol/L	6.20	0.088
16:0, μmol/L	−0.01	0.998
24:1, μmol/L	0.82	0.209
24:0, μmol/L	0.23	0.294
16:0/24:0	−1.51	0.807
18:0/24:0	19.46	0.139
24:1/24:0	1.22	0.825

Linear regression is controlled for age and sex.

Table 8. Correlation Between Plasma Ceramides and Inflammatory Markers

Plasma ceramide	IL-2	IL-4	IL-5	IL-6	IL-8	IL-10	IFNγ	TNFα
18:0 0, μmol/L	−0.08	−0.09	−0.02	0.03	0.23	−0.02	−0.02	−0.07
16:0 0, μmol/L	−0.08	−0.09	0.01	0.02	0.19	0.01	0.01	−0.05
24:1 0, μmol/L	−0.06	−0.07	−0.02	0.00	0.22	−0.01	−0.01	−0.09
16:0/24:0	−0.09	−0.10	−0.01	0.05	−0.06	−0.01	−0.01	−0.06
18:0/24:0	−0.11	−0.12	−0.04	0.03	0.05	−0.04	−0.05	−0.09
24:1/24:0	−0.08	−0.09	−0.02	0.04	0.03	−0.02	−0.01	−0.08

Correlation coefficients for circulating inflammatory markers and plasma ceramides. IFNγ indicates interferon γ; IL, interleukin; and TNFα, tumor necrosis factor α.

were analyzed using Stata software (StataCorp. 2017; Stata Statistical Software: Release 15; StataCorp LLC., College Station, TX).

RESULTS

Ceramides and CED

Between participants with and without CED, there was no significant difference in demographic and clinical characteristics (Table 1). Table 2 summarizes Measures obtained by coronary angiograph and serum lipid data of patients undergoing elective angiography for chest pain Participants with CED had a median CFR of 2.5 (2.2; 2.9), median ΔCBF of –1.74 (–32; 24), and median ΔCAD of –25 (–39; –11). Controls had a median CFR of 3.2 (2.8; 3.7), median ΔCBF of 114 (89; 161), and median ΔCAD of –4 (–11; 1). There was a significant difference in all 3 of these physiologic measures that characterize CED between the patient and control groups. Between participants with normal coronary endothelial function and those with CED, we observed a significant difference in plasma concentrations of ceramides 18:0 (*P*=0.038), 16:0 (*P*=0.021), and 24:0 (*P*=0.019) (Figure 1).

We observed no difference in plasma ceramide levels between women (n=91) and men (n=29; Table 3). When we isolated patients with coronary endothelium-dependent dysfunction (CFR >2), there remained no difference in plasma ceramide concentrations by sex (Table 4). No significant correlation between plasma ceramides and age was observed (Table 5).

Plasma hs-CRP data were available for 100 participants. We did not observe a strong correlation between hs-CRP and plasma ceramides (Table 6). Exploring this further, a linear regression controlled for age and sex showed no significant association between hs-CRP and plasma ceramides (Table 7).

Inflammatory data were available for 58 participants. We did not observe a significant correlation between plasma ceramides and other inflammatory markers (IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFNγ, TNFa; Table 8). There was a significant difference in plasma ceramide 18:0/24:0 levels (*P*=0.037) between patients who were and were not treated with lipid-lowering agents (Table 9).

Table 10 summarizes linear regression models adjusted for cardiovascular risk factors and confounding lipids. When adjusted for cardiovascular comorbidities, LDL cholesterol, and non-HDL cholesterol, lower CFR was associated with elevated circulating ceramides 18:0/24:0 (β, –12.8; *P*=0.016), 16:0/24:0 (β, –5.57; *P*=0.015), and 24:1/24:0 (β, –1.74; *P*=0.022). There was no linear association between change in epicardial coronary artery diameter or coronary blood flow in response to acetylcholine and circulating plasma ceramide levels.

Table 11 summarizes significant multivariable logistic regressions progressively adjusted for age, sex, hypertension, diabetes, hyperlipidemia, lipid-lowering drug use, LDL cholesterol, and non-HDL cholesterol. In each progressive model, ceramides 24:0 (OR, 2.23 [95% CI, 1.07–4.66,]; *P*=0.033) and 16:0 (OR, 1.91×10^{6} [95% CI, $11.93–3.07\times10^{11}$]; *P*=0.018) remained independently associated with CED. When adjusted for age and sex, ceramide 18:0 (OR, 7.26×10^{5} [95% CI, $1.32–3.99\times10^{11}$]; *P*=0.045) was independently associated with CED, but not when further adjusted for cardiovascular risk factors, LDL cholesterol, and non-HDL cholesterol. Ceramides 24:1, 18:0/24:0, 16:0/24:0, and 24:1/24:0 were not associated with CED.

Table 9. Plasma Ceramide Levels Stratified by the Use of Lipid-Lowering Therapy

Plasma ceramide	No lipid therapy* (n=60)	Lipid-lowering therapy* (n=60)	*P* value
18:0, μmol/L	0.08 (0.07; 0.12)	0.10 (0.07; 0.13)	0.096
16:0, μmol/L	0.29 (0.26; 0.35)	0.28 (0.24; 0.31)	0.305
24:1, μmol/L	0.78 (0.69; 0.93)	0.77 (0.63; 0.96)	0.813
24:0, μmol/L	3.05 (2.73; 3.87)	2.93 (2.68; 3.43)	0.950
16:0/24:0	0.09 (0.08; 0.11)	0.09 (0.08; 0.11)	0.337
18:0/24:0	0.03 (0.02; 0.04)	0.03 (0.03; 0.04)	0.037
24:1/24:0	0.25 (0.22; 0.31)	0.26 (0.21; 0.32)	0.620

*Data are presented as median (interquartile range).

Table 10. Association Between Plasma Ceramides and CFR

Adjusted model	Standardized β coefficient	*P* value
1		
Age	−0.01	0.170
Sex	−0.20	0.116
Plasma ceramides 18:0/24:0	−9.78	0.034
2		
Age	−0.01	0.169
Sex	−0.22	0.108
Hypertension	0.09	0.426
Diabetes	−0.06	0.718
Hyperlipidemia	−0.04	0.757
Lipid-lowering drug use	0.02	0.869
Plasma ceramides 18:0/24:0	−9.88	0.040
3		
Age	−0.01	0.114
Sex	−0.19	0.180
Hypertension	0.06	0.599
Diabetes	−0.01	0.964
Hyperlipidemia	−0.02	0.873
Lipid-lowering drug use	−0.01	0.961
LDL cholesterol	−0.01	0.115
Non-HDL cholesterol	0.01	0.097
Plasma ceramides 18:0/24:0	−12.80	0.016
1		
Age	−0.01	0.123
Sex	−0.19	0.148
Plasma ceramides 16:0/24:0	−5.35	0.014
2		
Age	−0.01	0.162
Sex	−0.22	0.115
Hypertension	0.07	0.560
Diabetes	−0.06	0.741
Hyperlipidemia	−0.07	0.637
Lipid-lowering drug use	−0.03	0.803
Plasma ceramides 16:0/24:0		0.016
3		
Age	−0.01	0.091
Sex	−0.19	0.185
Hypertension	0.03	0.783
Diabetes	−0.02	0.941
Hyperlipidemia	−0.05	0.735
Lipid-lowering drug use	−0.05	0.761
LDL cholesterol	−0.00	0.454
Non-HDL cholesterol	−0.00	0.405
Plasma ceramides 16:0/24:0	−5.57	0.015
1		
Age	−0.01	0.154
Sex	−0.20	0.117
Plasma ceramides 24:1/24:0	−1.38	0.044
2		
Age	−0.01	0.175
Sex	−0.23	0.094
Hypertension	0.10	0.409
Diabetes	−0.06	0.727
Hyperlipidemia	−0.06	0.671
Lipid-lowering drug use	−0.02	0.878
Plasma ceramides 24:1/24:0	−1.42	0.044
3		
Age	−0.01	0.113
Sex	−0.20	0.164
Hypertension	0.06	0.613
Diabetes	−0.02	0.908
Hyperlipidemia	−0.02	0.888
Lipid-lowering drug use	−0.08	0.601
LDL cholesterol	−0.01	0.140
Non-HDL cholesterol	0.01	0.146
Plasma ceramides 24:1/24:0	−1.74	0.022

Linear regression models consecutively adjusted for cardiovascular risk factors and lipids. HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.

Ceramides and Endothelial-Dependent Vascular Dysfunction

To assess the relationship between plasma ceramide levels and endothelial function exclusively, we excluded individuals with non-endothelium-dependent CFR defined as CFR ≤2. A subanalysis isolating patients with predominant endothelial-dependent vascular dysfunction (n=78) was then performed with the same age-matched and sex-matched controls (n=30) without CED (Table 12).

A 2-tailed *t* test demonstrated a significant difference in the levels of plasma ceramides 16:0 (*P*=0.038), 24:0 (*P*=0.01), and 24:1/24:0 (*P*=0.011) between the patient and control groups (Table 13). A linear regression adjusted for cardiovascular risk factors and confounding lipids examining the independent association of plasma ceramides and CED was performed using the previously described 3 models. Restricted change in coronary artery diameter in response to acetylcholine was associated with high circulating levels of ceramide

Table 11. Association Between Circulating Plasma Ceramides and CED

Adjusted model	Odds ratio	95% CI	*P* value
1			
Age	0.99	0.95–1.04	0.819
Sex	0.82	0.29–2.31	0.704
Plasma ceramide 18:0, μmol/L	7.26×10^{5}	$1.32–3.99\times10^{11}$	0.045
2			
Age	1.00	0.95–1.04	0.897
Sex	0.85	0.28–2.59	0.777
Hypertension	0.61	0.25–2.59	0.285
Diabetes	0.75	0.20–2.88	0.677
Hyperlipidemia	1.25	0.43–3.63	0.679
Lipid-lowering drug use	1.50	0.55–4.04	0.428
Plasma ceramide 18:0, μmol/L	4.73×10^{5}	$0.49–4.55\times10^{11}$	0.063
3			
Age	1.00	0.96 –1.05	0.875
Sex	0.76	0.25–2.35	0.640
Hypertension	0.69	0.27–1.75	0.439
Diabetes	0.68	0.17–2.74	0.592
Hyperlipidemia	1.00	0.30–3.30	0.996
Lipid-lowering drug use	1.82	0.57–5.87	0.311
LDL cholesterol	1.03	0.99–1.08	0.154
Non-HDL cholesterol	0.97	0.93–1.01	0.182
Plasma ceramide 18:0, μmol/L	1.10×10^{7}	$0.87–1.40\times10^{14}$	0.052
1			
Age	0.99	0.95–1.04	0.824
Sex	0.69	0.23–2.10	0.516
Plasma ceramide 24:0, μmol/L	2.12	1.10–4.08	0.024
2			
Age	1.00	0.95–1.05	0.940
Sex	0.80	0.24–2.65	0.716
Hypertension	0.51	0.19–1.34	0.172
Diabetes	0.82	0.21–3.29	0.783
Hyperlipidemia	1.22	0.40–3.75	0.723
Lipid-lowering drug use	1.84	0.64–5.29	0.255
Plasma ceramide 24:0, μmol/L	2.33	1.15–4.72	0.019
3			
Age	1.01	0.96–1.06	0.804
Sex	0.76	0.23–2.53	0.658
Hypertension	0.57	0.21–1.54	0.268
Diabetes	0.77	0.19–3.10	0.709
Hyperlipidemia	1.09	0.33–3.63	0.892
Lipid-lowering drug use	1.86	0.56–6.16	0.309
LDL cholesterol	1.00	0.96–1.05	0.908
Non-HDL cholesterol	1.00	0.96–1.04	0.938
Plasma ceramide 24:0, μmol/L	2.23	1.07–4.66	0.033
1			
Age	1.00	0.96–1.05	0.956
Sex	0.61	0.20–1.84	0.377
Plasma ceramide 16:0, μmol/L	7.64×10^{4}	$4.71–1.24\times10^{9}$	0.023
2			
Age	1.00	0.96–1.05	0.868
Sex	0.67	0.20–2.27	0.521
Hypertension	0.48	0.18–1.27	0.139
Diabetes	0.88	0.22–3.56	0.859
Hyperlipidemia	1.30	0.41–4.07	0.658
Lipid-lowering drug use	1.69	0.57–5.02	0.342
Plasma ceramide 16:0, μmol/L	3.06×10^{5}	7.64–1.22×10 t	0.019
3			
Age	1.01	0.97–1.07	0.565
Sex	0.65	0.19–1.07	0.489
Hypertension	0.54	0.20–1.43	0.214
Diabetes	0.79	0.19–3.28	0.750
Hyperlipidemia	1.28	0.38–4.35	0.689
Lipid-lowering drug use	1.54	0.46–5.13	0.481
LDL cholesterol	1.00	0.96–1.05	0.922
Non-HDL cholesterol	0.99	0.96–1.03	0.736
Plasma ceramide 16:0, μmol/L	1.91×10^{6}	$11.93–3.07\times10^{11}$	0.018

Logistic regression models consecutively adjusted for cardiovascular risk factors and lipids. HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.

18:0/24:0 when adjusted for age, sex, and cardiovascular comorbidities (β, −418; *P*=0.020), but not when further adjusted for LDL cholesterol and non-HDL cholesterol (Table 14). There was no linear association between change in coronary blood flow in response to acetylcholine and circulating plasma ceramide levels.

Table 15 details significant multivariable logistic regression models. We observed a significant association between elevated circulating ceramides 16:0 (OR, 5.17×10^{5} [95% CI, $2.83–9.44\times10^{10}$]; *P*=0.033), 24:0 (OR, 2.98 [95% CI, 1.27–7.00]; *P*=0.012), and 24:1/24:0 (OR, 4.39 $\times10^{-4}$ [95% CI, 4×10^{-7}–0.48]; *P*=0.030) and CED in all models progressively adjusted for age, sex, cardiovascular risk factors, and confounding lipids. Ceramides 18:0, 24:1, 18:0/24:0, and 16:0/24:0 were not associated with CED.

Elevated Ceramides in the Prediction of CED

A receiver operating characteristic curve among all study participants suggested plasma ceramides 18:0

Table 12. Demographics and Clinical Characteristics of Participants With Normal Endothelial Function and Endothelium-Dependent Dysfunction

Variable	Patients* (n=78)	Controls* (n=30)	*P* value
Age, y	54±10	57±9	0.839
Female sex	59 (76)	24 (80)	0.634
BMI, kg/m^2	30±6	28±5	0.274
Systolic blood pressure, mm Hg	125±17	120 (112; 130)	0.540
Diastolic blood pressure, mm Hg	76±9	74 (70; 80)	0.638
Smoking status			
Never smoked	50 (64)	17 (57)	0.800
Former smoker	24 (31)	10 (33)	0.800
Current smoker	4 (5)	3 (10)	0.362
Hypertension	32 (59)	15 (50)	0.404
Diabetes	10 (13)	4 (13)	0.944
Hyperlipidemia	47 (60)	14 (47)	0.206
eGFR, mL/min per 1.73 m^2	75±15	74 (61; 92)	0.681
Aspirin use	50 (64)	19 (63)	0.941
β-blocker use	37 (47)	9 (30)	0.103
Lipid-lowering drug use	41 (53)	12 (40)	0.246
Antihypertensive use	23 (29)	11 (37)	0.476

BMI indicates body mass index; and eGFR, estimated glomerular filtration rate.

*Data are presented as number (percentage), mean±SD, or median (interquartile range).

(AUC, 0.64 [95% CI, 0.52–0.76]; *P*=0.019) and 16:0 (AUC, 0.64 [95% CI, 0.53–0.75]; *P*=0.027) predicted CED with reasonable accuracy (Figure 2). Endothelial-dependent coronary dysfunction was predicted with reasonable accuracy by plasma ceramides 18:0 (AUC, 0.63 [95% CI, 0.51–0.75]; *P*=0.034), 24:0 (AUC, 0.64 [95% CI, 0.53–0.76]; *P*=0.021), and 24:1/24:0 (AUC, 0.64 [95% CI, 0.53–0.76]; *P*=0.022; Figure 3). A receiver operator curve was not significant when combining subtypes of ceramide acyl species among all study participants (AUC, 0.53 [95% CI, 0.27–0.59]; *P*=0.360) and with endothelial-dependent coronary dysfunction (AUC, 0.52 [95% CI, 0.44–0.59]; *P*=0.675).

DISCUSSION

Summary of Findings

First, elevated levels of plasma ceramides 18:0, 24:0, and 16:0 are independently associated with early coronary atherosclerosis in the absence of epicardial coronary artery disease. Second, higher plasma levels of circulating ceramides 18:0, 18:0/24:0, 16:0/24:0, and 24:1/24:0 are associated with abnormal microvascular function. Third, in participants with predominant endothelium-dependent dysfunction, plasma ceramides 16:0, 24:0, and 24:1/24:0 are associated with CED. Thus, we present the first study associating elevated plasma ceramides with early coronary atherosclerosis. The current study supports a potential role of serum ceramides as a marker and a potential therapeutic target in early coronary atherosclerosis in humans.

Ceramides and Early Atherosclerosis

Despite our growing understanding of potential mechanisms and specific associations of individual ceramide acyl species and cardiovascular disease, literature associating individual ceramides with aspects of atherosclerosis remains largely inconsistent.[14,29] Scientists postulate that specific acyl species may be more associated with varying atherogenic pathways in humans–that is, ceramide is 18:0 associated with the inflammatory pathway, ceramide 16:0 is associated with the thrombotic pathway, and ceramide 24:1 is associated with the LDL pathway of atherogenesis.[11,17,29,30] In support of these associations, we present data that further suggest an association between elevated circulating ceramide 18:0 and ceramide 16:0 in humans with observed endothelial dysfunction related to early atherosclerosis. Furthermore, a linear association shows a positive association between hs-CRP and circulating ceramide 18:0, which may be in part attributed to its role in the inflammatory pathway of atherosclerosis.

Studies examining the association between ceramide 24:0 and cardiovascular disease are widely inconsistent, and our overall understanding of their role in atherogenesis in humans remains limited; however, there may be a role in early atherosclerosis as alluded to by our findings. Traditionally, the abundant very-long-chain sphingolipid ceramide 24:0 has been measured to normalize other circulating ceramide species relative to its abundance. Interestingly, our findings suggest an inverse correlation between ceramide 24:0 and early coronary atherosclerosis. When we examine patients with endothelium-dependent dysfunction (CFR >2), the normalized ratio ceramide 24:1/24:0 is further associated with early atherosclerosis, which may suggest the role of ceramides in the LDL pathway in these participants. We interpret these findings with the understanding that atherogenesis is a progressive and dynamic process over time and that these associations may be unique to participants with early coronary atherosclerosis.

Existing literature remains inconsistent regarding the specific consequence of elevated individual ceramides. Clinically, ceramide 16:0 has repeatedly been shown to be associated with recurrent MACE and acute coronary syndrome, whereas ceramide 18:0 appears to be associated with MACE alone.[16,21,31,32]

Table 13. Coronary Angiography and Lipid Data of Participants With Normal Endothelial Function and Endothelium-Dependent Dysfunction

Variable	Patients*	n	Controls*	n	*P* value
CFR	2.7 (2.3–2.9)	78	3.2 (2.8; 3.7)	30	<0.000
ΔCBF, %	−8.7 (−34; 24)	78	114 (89; 161)	30	<0.000
ΔCAD, %	−27 (−40; −11)	78	−4 (−11; 1)	30	<0.000
Myocardial infarction–heart ceramide risk score	3.5 (2; 5)	78	2.5 (1; 4)	30	
Risk category					
Higher risk	1 (1)	78	1 (3)	30	0.483
Increased risk	8 (10)	78	3 (10)	30	0.919
Moderate risk	34 (44)	78	11 (37)	30	0.518
Lower risk	35 (45)	78	15 (50)	30	0.636
Lipid profile					
Total cholesterol, mmol/L	185 (165; 206)	76	177 (160; 208)	29	0.681
LDL cholesterol, mmol/L	105 (84; 121)	76	100 (84.5; 107.5)	29	0.437
Non-HDL cholesterol, mmol/L	129 (103; 151)	76	58 (46; 76)	29	0.501
Triglycerides, mmol/L	112 (82; 140)	75	107 (74.5; 131)	29	0.454
Plasma ceramides					
18:0, μmol/L	0.09 (0.07; 0.13)	78	0.08 (0.06; 0.10)	30	0.060
16:0, μmol/L	0.29 (0.25; 0.33)	74	0.27 (0.24; 0.28)	28	0.038
24:1, μmol/L	0.77 (0.66; 0.94)	78	0.77 (0.65; 0.78)	30	0.945
24:0, μmol/L	3.07 (2.78; 3.85)	75	2.82 (2.54; 3.16)	29	0.007
16:0/24:0	0.09 (0.08; 0.10)	78	0.10 (0.08; 0.11)	30	0.563
18:0/24:0	0.03 (0.02; 0.04)	78	0.03 (0.02; 0.04)	30	0.466
24:1/24:0	0.24 (0.20; 0.29)	77	0.28 (0.23; 0.32)	29	0.011

ΔCAD indicates change in coronary artery diameter; ΔCBF, change in coronary blood flow; CFR, coronary flow reserve; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

*Data are presented as number (percentage) or median (interquartile range).

The most inconsistent data exist for the association of circulating ceramide 24:0 and cardiovascular disease. Most large studies investigating the association between cardiovascular disease and ceramide 24:0 remain consistent and observe no association.[13,22,31] Few other studies suggest an inverse association between ceramide 24:0 and coronary atherosclerosis. An examination of ceramide 24:0 in patients from the Framingham Heart Study and Study of Health in Pomerania in participants with coronary artery disease observed the relative risk of coronary artery disease for each 3-unit increase in ceramide 24:0 was 0.79 when adjusted for confounding lipids and cardiovascular risk factors.[19] Additional studies have supported that elevated levels of ceramide 24:0 may be associated with a lower risk of cardiovascular event in patients with preexisting coronary artery disease.[22,30,33] Our study interestingly finds a positive association between ceramide 24:0 in participants with early coronary atherosclerosis. Discrepancies in specific associations may be in part secondary to population selection (ie, healthy participants with stable coronary disease, participants with heart failure, population studies). Our particular cohort with early coronary disease and endothelial dysfunction remains unique and distinct from the populations, which likely underlie our specific findings.

Elevated Plasma Ceramides and Non-Endothelium-Dependent Dysfunction

The mechanistic role of ceramides in endothelial and microvascular dysfunction is likely multifactorial. Ceramides are biologically active lipids key to cell membrane integrity and are either synthesized de novo or transported by LDL into the endothelium where they facilitate various metabolic pathways.[34–37] Notably, ceramides have been observed to mediate endothelium-dependent flow-induced dilation by facilitating the production of endothelium-derived hydrogen peroxide rather than the preferred nitrous oxide.[38] Although this process may to an extent preserve flow-induced dilation in an early atherosclerotic artery, hydrogen peroxide leads to adverse consequences to endothelial function over time.[39–41] In addition to the chemical consequence of these bioactive lipids, ceramides add structural consequence to arteries by

Table 14. Association Between Plasma Ceramides and Change in Coronary Artery Diameter in Study Participants With Endothelium-Dependent Vascular Dysfunction (CFR >2)

Adjusted model	Standardized β coefficient	*P* value
1		
Age	0.44	0.032
Sex	10.20	0.033
Plasma ceramides 18:0/24:0	−428.86	0.016
2		
Age	0.53	0.011
Sex	9.25	0.056
Hypertension	−4.68	0.262
Diabetes	9.44	0.149
Hyperlipidemia	−5.12	0.378
Lipid-lowering drug use	−6.59	0.242
Plasma ceramides 18:0/24:0	−418.38	0.020
3		
Age	0.45	0.031
Sex	9.11	0.072
Hypertension	−3.69	0.389
Diabetes	10.28	0.149
Hyperlipidemia	−6.61	0.275
Lipid-lowering drug use	−2.53	0.676
LDL cholesterol	0.59	0.017
Non-HDL cholesterol	−0.51	0.020
Plasma ceramides 18:0/24:0	−322.55	0.084

HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.

progressively infiltrating plaque, further impairing microvascular function.

In early coronary artery disease, lower CFR is associated with higher levels of circulating plasma ceramides. Impaired non-endothelium-dependent dysfunction (typically when CFR <2.5) has been used as a marker to predict the development of cardiovascular events such as heart failure, chest pain, ischemia, recurrent hospitalizations, long-term adverse cardiovascular outcomes, and acute coronary syndrome.[5,42,43] In our cohort with no coronary artery stenosis, impaired dilation represents non-endothelium-dependent dysfunction (typically when CFR <2.5). We found lower CFR to be associated with higher plasma levels of ceramides 18:0/24:0, 16:0/24:0, and 24:1/24:0. This association may be in part secondary to changes in endothelial reactive oxygen species related to vascular ceramide infiltration. Ceramide-infiltrated endothelium favors hydrogen peroxide rather than physiologically preferred NO as a mediator of flow-induced dilation. Compounded structural change of endothelium by early lipid infiltration

Table 15. Association Between Elevated Circulating Plasma Ceramides and Endothelium-Dependent Vascular Dysfunction (CFR >2)

Adjusted model	Odds ratio	95% CI	*P* value
1			
Age	1.00	0.92–1.04	0.919
Sex	0.62	0.20–1.92	0.407
Plasma ceramide 16:0, μmol/L	3.05×10^{4}	$1.79–5.20\times10^{8}$	0.038
2			
Age	1.00	0.95–1.05	0.996
Sex	0.70	0.20–2.43	0.573
Hypertension	0.46	0.17–1.23	0.123
Diabetes	0.93	0.22–3.84	0.917
Hyperlipidemia	1.50	0.45–4.96	0.511
Lipid-lowering drug use	1.36	0.43–4.31	0.595
Plasma ceramide 16:0, μmol/L	7.97×10^{4}	$2.04–2.88\times10^{9}$	0.036
3			
Age	1.01	0.96–1.06	0.692
Sex	0.67	0.19–2.36	0.535
Hypertension	0.53	0.20–1.44	0.214
Diabetes	0.81	0.19–3.49	0.781
Hyperlipidemia	1.48	0.42–5.23	0.545
Lipid-lowering drug use	1.33	0.38–−4.60	0.653
LDL cholesterol	1.01	0.96–1.06	0.689
Non-HDL cholesterol	0.99	0.95–1.03	0.546
Plasma ceramide 16:0, μmol/L	5.17×10^{5}	$2.83–9.44\times10^{10}$	0.033
1			
Age	0.99	0.94–1.03	0.564
Sex	0.77	0.25–2.44	0.664
Plasma ceramide 24:0, μmol/L	2.59	1.26–5.32	0.010
2			
Age	0.99	0.94–1.04	0.664
Sex	0.98	0.28–3.42	0.973
Hypertension	0.45	0.16–1.22	0.117
Diabetes	1.06	0.24–4.55	0.942
Hyperlipidemia	1.25	0.38–4.16	0.715
Lipid-lowering drug use	1.71	0.54–5.38	0.362
Plasma ceramide 24:0, μmol/L	3.02	1.35–6.77	0.007
3			
Age	1.00	0.95–1.05	0.874
Sex	0.96	0.27–3.41	0.949
Hypertension	0.50	0.18–1.39	0.184
Diabetes	0.97	0.22–4.24	0.969
Hyperlipidemia	1.20	0.34–4.25	0.779
Lipid-lowering drug use	1.68	0.48–5.93	0.418

(*Continued*)

Table 15. Continued

Adjusted model	Odds ratio	95% CI	*P* value
LDL cholesterol	1.01	0.96–1.06	0.737
Non-HDL cholesterol	0.99	0.95–1.03	0.664
Plasma ceramide 24:0, μmol/L	2.98	1.27–7.00	0.012
1			
Age	0.99	0.95–1.04	0.804
Sex	0.93	0.32–2.70	0.888
Plasma ceramides 24:1/24:0	4.63×10^{-4}	8.80×10^{-7}–0.24	0.016
2			
Age	0.99	0.95–1.04	0.759
Sex	1.06	0.34–3.31	0.921
Hypertension	0.54	0.21–1.41	0.212
Diabetes	0.76	0.19–3.08	0.702
Hyperlipidemia	1.93	0.60–6.24	0.272
Lipid-lowering drug use	1.27	0.41–3.90	0.676
Plasma ceramides 24:1/24:0	3.65×10^{-4}	5.64×10^{-7}–0.24	0.017
3			
Age	1.00	0.95–1.05	0.967
Sex	1.05	0.32–3.43	0.931
Hypertension	0.60	0.23–1.59	0.306
Diabetes	0.73	0.17–3.06	0.666
Hyperlipidemia	1.64	0.47–5.66	0.438
Lipid-lowering drug use	1.28	0.37–4.44	0.698
LDL cholesterol	1.00	0.95–1.05	0.975
Non-HDL cholesterol	1.00	0.96–1.04	0.949
Plasma ceramides 24:1/24:0	4.39×10^{-4}	4×10^{-7}–0.48	0.030

HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.

further limits the dilatory capacity of coronary vessels and overall results in limited CFR.[44]

Few other studies highlight the association of circulating ceramides and impaired vascular hemodynamics in early coronary disease. A prospective community-based cohort study demonstrated decreased survival free of stroke and myocardial infarction participants with an average burden of coronary artery disease and elevated plasma ceramides 18:0/24:0 (hazard ratio [HR], 3.00 [95% CI, 1.17–7.68]) and 24:1/24:0 (HR, 2.93 [95% CI, 1.52–5.67]).[21] Interestingly, ceramide 24:1/24:0 was identified in this study as a marker for high cardiovascular risk in patients with a low atherosclerotic cardiovascular disease risk score. This highlights the suspicion that ceramides are not only constituents of obstructive plaque but are also drivers of endothelial disease, which carries a risk for adverse cardiac outcomes independent of coronary plaque burden.[17,20,21,45] In the The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis-Intravascular Ultrasound study examining 581 patients with coronary artery disease, elevated plasma ceramide 16:0/24:0 was associated with vulnerable plaque characteristics determined by intravascular ultrasound and MACE.[46] Endothelial dysfunction is known to be associated with characteristics of vulnerable plaque. Although mounting clinical science increases our suspicion for ceramides as possible independent drivers of endothelial dysfunction and eventual coronary atherosclerosis, further direct investigation in humans is needed to support these observations. Our novel findings associate elevated circulating ceramides in a unique cohort of patients at the earliest detectable stages of coronary artery disease and emphasize their potential role in the contribution of impaired vascular hemodynamics.

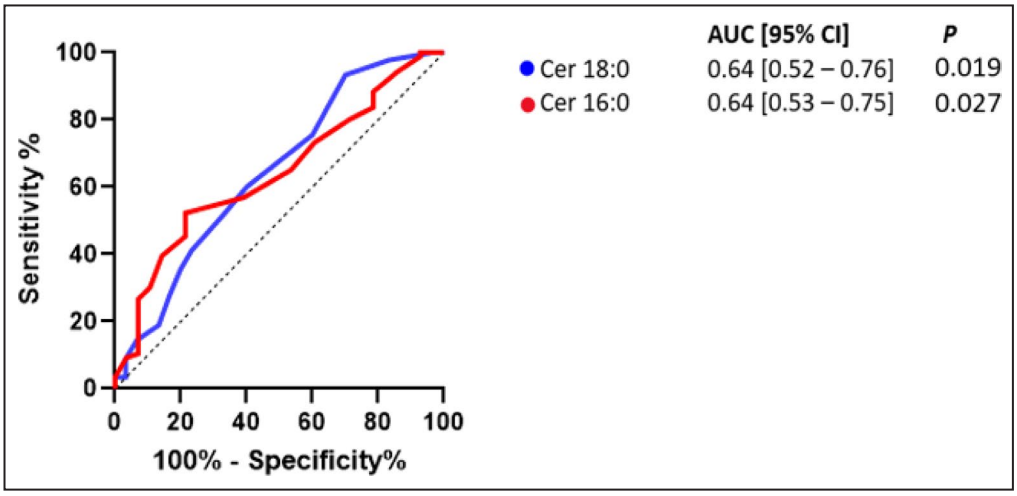


Figure 2. Predictive assessment of plasma ceramides levels in coronary endothelial dysfunction.
AUC indicates area under the curve; and Cer, ceramide.

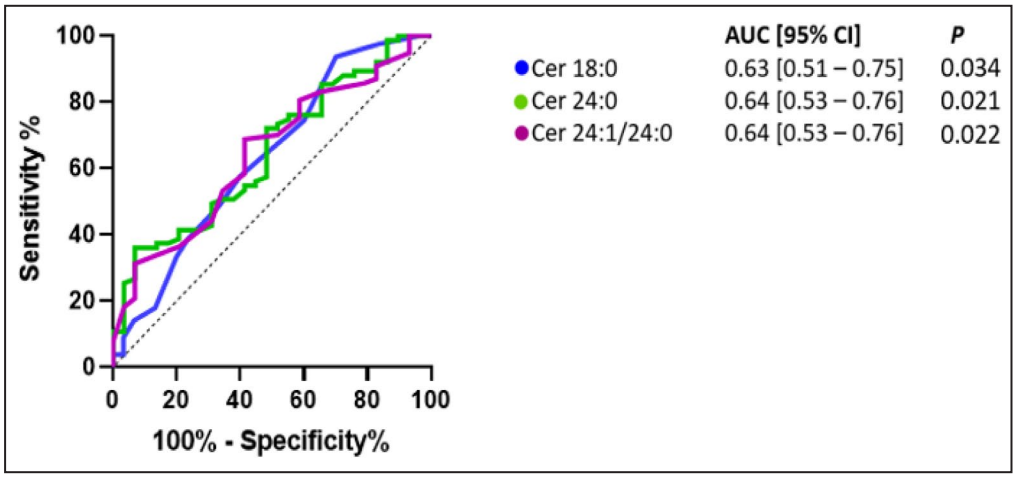


Figure 3. Predictive assessment of plasma ceramide levels in coronary endothelial dysfunction in patients with endothelium-dependent dysfunction.
AUC indicates area under the curve; and Cer, ceramide.

Coronary Artery Diameter

We observe impaired coronary artery dilation related to elevated plasma ceramide levels. Change in coronary artery diameter in response to intracoronary administration of acetylcholine can be used to evaluate the endothelium-dependent flow–diameter relationship of coronary arteries. A normal endothelial response to acetylcholine is vasodilation, whereas an abnormal response is either no change in coronary artery diameter or vasoconstriction.[47] This normal coronary vasodilation occurs under balanced circumstances. Intravascular acetylcholine acts on endothelial muscarinic receptors that facilitate smooth muscle vasoconstriction and endothelial-mediated NO release; the summative effect in normal physiology is vessel dilation. In normal endothelium, this smooth muscle vasoconstriction is counteracted by NO-mediated vasodilation, resulting in overall coronary dilation following acetylcholine exposure. If a coronary artery vasoconstricts following acetylcholine exposure, we presume that acetylcholine acts on smooth muscle muscarinic receptors to vasoconstrict in an environment unopposed by NO activity. The consequent imbalance signifies endothelial dysfunction.[12] Among patients with predominant endothelium-dependent dysfunction, we observed an association between elevated levels of ceramides 18:0/24:0 and coronary artery dilation in response to intracoronary acetylcholine. These findings suggest endothelial cell rather than smooth muscle dysfunction. Recent evidence supports the potential role of ceramides 18:0/24:0 in advanced coronary atherosclerosis. Meeusen et al observed an association between elevated plasma ceramides 18:0/24:0 and MACE when adjusted for cardiovascular risk factors in participants undergoing nonurgent coronary angiography. Of these participants, 54% were observed to have obstructive coronary artery disease.[13] Our findings extend previous observations by highlighting an association between CED and specifically elevated plasma ceramide species implicated in adverse outcomes of advanced coronary disease.

Limitations

Several limiting factors of this case-control analysis should be discussed. First, our retrospective analysis examined the clinical association between elevated plasma ceramides and early coronary atherosclerosis. We did not examine the biologic and pathophysiologic mechanisms that may underly these associations. Regarding angiographic evaluation, occult atherosclerosis not observed by the procedural operator may confound vascular hemodynamic data and may not be specific to microcirculation abstraction. The implications of the associations highlighted by this study require future validation. Lastly, in the current analysis, multiplicity control methodologies were not employed.

CONCLUSIONS

In conclusion, we present evidence that supports the association between elevated plasma ceramides and early coronary atherosclerosis defined by endothelial dysfunction and absence of obstructive coronary artery disease. We speculate the mechanism of injury to be related to smooth muscle damage as suggested by our presented association of plasma

ceramides and CFR. Emerging data further suggest systemic vascular implications of elevated plasma ceramide levels, including stroke.[48–50] Circulating plasma ceramides may be a novel therapeutic target and could be used to stratify risk. Emerging studies suggest therapies that reduce circulating plasma ceramides; however, further studies are required to determine the long-term outcomes of ceramide-lowering therapy on outcomes.[51,52] Further studies are needed to investigate the effects of lowering plasma ceramide levels in patients with CED on cardiovascular outcomes.

ARTICLE INFORMATION

Received June 11, 2021; accepted February 1, 2022.

Affiliations

Division of Internal Medicine (N.A.); Division of Cardiovascular Disease (V.V., A.A., J.D.S., V.N., A.J., A.L.) and Division of Nephrology and Hypertension (L.O.L.), Department of Medicine, Mayo Clinic, Rochester, MN.

Sources of Funding

This work was supported by funding from the Mayo Foundation.

Disclosures

None.

Supplemental Material

Figure S1

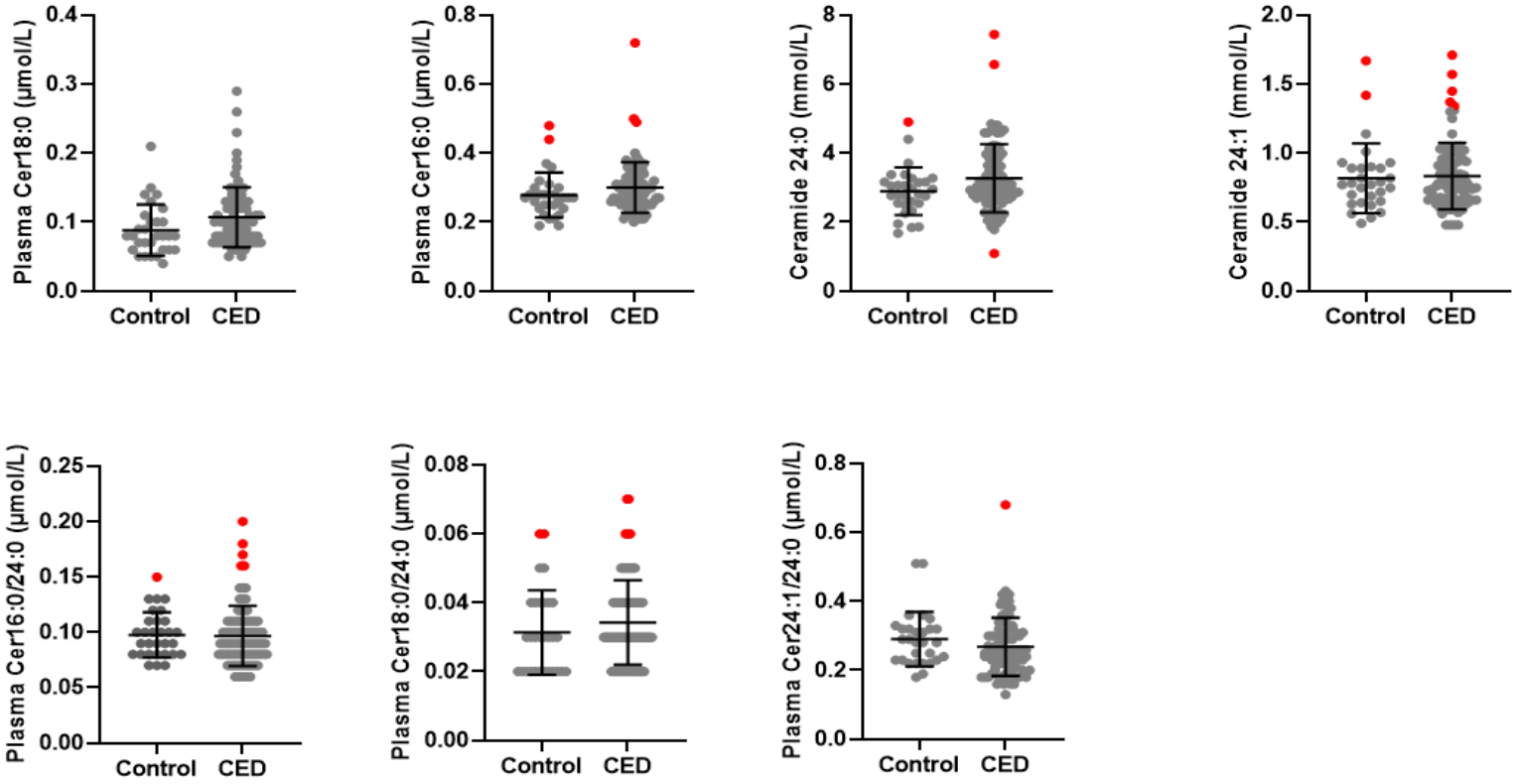
REFERENCES

1. Camici PG, Crea F. Coronary microvascular dysfunction. *N Engl J Med*. 2007;356:830–840. doi: 10.1056/NEJMra061889
2. Godo S, Corban MT, Toya T, Gulati R, Lerman LO, Lerman A. Association of coronary microvascular endothelial dysfunction with vulnerable plaque characteristics in early coronary atherosclerosis. *EuroIntervention*. 2020;16:387–394. doi: 10.4244/eij-d-19-00265
3. Choi BJ, Prasad A, Gulati R, Best PJ, Lennon RJ, Barsness GW, Lerman LO, Lerman A. Coronary endothelial dysfunction in patients with early coronary artery disease is associated with the increase in intravascular lipid core plaque. *Eur Heart J*. 2013;34:2047–2054. doi: 10.1093/eurheartj/eht132
4. Gössl M, Yoon MH, Choi BJ, Rihal C, Tilford JM, Reriani M, Gulati R, Sandhu G, Eeckhout E, Lennon R, et al. Accelerated coronary plaque progression and endothelial dysfunction: serial volumetric evaluation by IVUS. *JACC Cardiovasc Imaging*. 2014;7:103–104. doi: 10.1016/j.jcmg.2013.05.020
5. Sara JD, Widmer RJ, Matsuzawa Y, Lennon RJ, Lerman LO, Lerman A. Prevalence of coronary microvascular dysfunction among patients with chest pain and nonobstructive coronary artery disease. *JACC Cardiovasc Interv*. 2015;8:1445–1453. doi: 10.1016/j.jcin.2015.06.017
6. Gutiérrez E, Flammer AJ, Lerman LO, Elízaga J, Lerman A, Fernández-Avilés F. Endothelial dysfunction over the course of coronary artery disease. *Eur Heart J*. 2013;34:3175–3181. doi: 10.1093/eurheartj/eht351
7. Herrmann J, Lerman A. The endothelium: dysfunction and beyond. *J Nucl Cardiol*. 2001;8:197–206. doi: 10.1067/mnc.2001.114148
8. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*. 2000;101:948–954. doi: 10.1161/01.CIR.101.9.948
9. Marks DS, Gudapati S, Prisant LM, Weir B, diDonato-Gonzalez C, Waller JL, Houghton JL. Mortality in patients with microvascular disease. *J Clin Hypertens (Greenwich)*. 2004;6:304–309. doi: 10.1111/j.1524-6175.2004.03254.x
10. Gdowski MA, Murthy VL, Doering M, Monroy-Gonzalez AG, Slart R, Brown DL. Association of isolated coronary microvascular dysfunction with mortality and major adverse cardiac events: a systematic review and meta-analysis of aggregate data. *J Am Heart Assoc*. 2020;9:e014954. doi: 10.1161/jaha.119.014954
11. Bismuth J, Lin P, Yao Q, Chen C. Ceramide: a common pathway for atherosclerosis? *Atherosclerosis*. 2008;196:497–504. doi: 10.1016/j.atherosclerosis.2007.09.018
12. Cogolludo A, Villamor E, Perez-Vizcaino F, Moreno L. Ceramide and regulation of vascular tone. *Int J Mol Sci*. 2019;20:411. doi: 10.3390/ijms20020411
13. Meeusen JW, Donato LJ, Bryant SC, Baudhuin LM, Berger PB, Jaffe AS. Plasma ceramides. *Arterioscler Thromb Vasc Biol*. 2018;38:1933–1939. doi: 10.1161/atvbaha.118.311199
14. Tippetts TS, Holland WL, Summers SA. The ceramide ratio: a predictor of cardiometabolic risk. *J Lipid Res*. 2018;59:1549–1550. doi: 10.1194/jlr.C088377
15. Mantovani A, Bonapace S, Lunardi G, Canali G, Dugo C, Vinco G, Calabria S, Barbieri E, Laaksonen R, Bonnet F, et al. Associations between specific plasma ceramides and severity of coronary-artery stenosis assessed by coronary angiography. *Diabetes Metab*. 2020;46:150–157. doi: 10.1016/j.diabet.2019.07.006
16. de Carvalho LP, Tan SH, Ow G-S, Tang Z, Ching J, Kovalik J-P, Poh SC, Chin C-T, Richards AM, Martinez EC, et al. Plasma ceramides as prognostic biomarkers and their arterial and myocardial tissue correlates in acute myocardial infarction. *JACC Basic Transl Sci*. 2018;3:163–175. doi: 10.1016/j.jacbts.2017.12.005
17. Hilvo M, Vasile VC, Donato LJ, Hurme R, Laaksonen R. Ceramides and ceramide scores: clinical applications for cardiometabolic risk stratification. *Front Endocrinol (Lausanne)*. 2020;11. doi: 10.3389/fendo.2020.570628
18. Hilvo M, Wallentin L, Ghukasyan Lakic T, Held C, Kauhanen D, Jylhä A, Lindbäck J, Siegbahn A, Granger CB, Koenig W, et al. Prediction of residual risk by ceramide-phospholipid score in patients with stable coronary heart disease on optimal medical therapy. *J Am Heart Assoc*. 2020;9:e015258. doi: 10.1161/jaha.119.015258
19. Peterson LR, Xanthakis V, Duncan MS, Gross S, Friedrich N, Völzke H, Felix SB, Jiang H, Sidhu R, Nauck M, et al. Ceramide remodeling and risk of cardiovascular events and mortality. *J Am Heart Assoc*. 2018;7. doi: 10.1161/jaha.117.007931
20. Meeusen JW, Donato LJ, Kopecky SL, Vasile VC, Jaffe AS, Laaksonen R. Ceramides improve atherosclerotic cardiovascular disease risk assessment beyond standard risk factors. *Clin Chim Acta*. 2020;511:138–142. doi: 10.1016/j.cca.2020.10.005
21. Vasile VC, Meeusen JW, Medina Inojosa JR, Donato LJ, Scott CG, Hyun MS, Vinciguerra M, Rodeheffer RR, Lopez-Jimenez F, Jaffe AS. Ceramide scores predict cardiovascular risk in the community. *Arterioscler Thromb Vasc Biol*. 2021;41:1558–1569. doi: 10.1161/atvbaha.120.315530
22. Laaksonen R, Ekroos K, Sysi-Aho M, Hilvo M, Vihervaara T, Kauhanen D, Suoniemi M, Hurme R, März W, Scharnagl H, et al. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. *Eur Heart J*. 2016;37:1967–1976. doi: 10.1093/eurheartj/ehw148
23. Taqueti VR, Di Carli MF. Coronary microvascular disease pathogenic mechanisms and therapeutic options: JACC state-of-the-art review. *J Am Coll Cardiol*. 2018;72:2625–2641. doi: 10.1016/j.jacc.2018.09.042
24. Widmer RJ, Flammer AJ, Herrmann J, Rodriguez-Porcel M, Wan J, Cohen P, Lerman LO, Lerman A. Circulating humanin levels are associated with preserved coronary endothelial function. *Am J Physiol Heart Circ Physiol*. 2013;304:H393–H397. doi: 10.1152/ajpheart.00765.2012
25. Cannon RO III. Does coronary endothelial dysfunction cause myocardial ischemia in the absence of obstructive coronary artery disease? *Circulation*. 1997;96:3251–3254.
26. Ahmad A, Corban MT, Toya T, Sara JD, Lerman B, Park JY, Lerman LO, Lerman A. Coronary microvascular endothelial dysfunction in patients with angina and nonobstructive coronary artery disease is associated with elevated serum homocysteine levels. *J Am Heart Assoc*. 2020;9:e017746. doi: 10.1161/jaha.120.017746
27. Widmer RJ, Samuels B, Samady H, Price MJ, Jeremias A, Anderson RD, Jaffer FA, Escaned J, Davies J, Prasad M, et al. The functional assessment of patients with non-obstructive coronary artery disease: expert review from an international microcirculation working group. *EuroIntervention*. 2019;14:1694–1702. doi: 10.4244/eij-d-18-00982

28. Targonski PV, Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr, Lerman A. Coronary endothelial dysfunction is associated with an increased risk of cerebrovascular events. *Circulation*. 2003;107:2805–2809. doi: 10.1161/01.cir.0000072765.93106.ee
29. Mah M, Febbraio M, Turpin-Nolan S. Circulating ceramides- are origins important for sphingolipid biomarkers and treatments? *Front Endocrinol (Lausanne)*. 2021;12. doi: 10.3389/fendo.2021.684448
30. Song JH, Kim GT, Park KH, Park WJ, Park TS. Bioactive sphingolipids as major regulators of coronary artery disease. *Biomol Ther (Seoul)*. 2021;29:373–383. doi: 10.4062/biomolther.2020.218
31. Havulinna AS, Sysi-Aho M, Hilvo M, Kauhanen D, Hurme R, Ekroos K, Salomaa V, Laaksonen R. Circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 cohort. *Arterioscler Thromb Vasc Biol*. 2016;36:2424–2430. doi: 10.1161/atvbaha.116.307497
32. Yu J, Pan W, Shi R, Yang T, Li Y, Yu G, Bai Y, Schuchman EH, He X, Zhang G. Ceramide is upregulated and associated with mortality in patients with chronic heart failure. *Can J Cardiol*. 2015;31:357–363. doi: 10.1016/j.cjca.2014.12.007
33. Sigruener A, Kleber ME, Heimerl S, Liebisch G, Schmitz G, Maerz W. Glycerophospholipid and sphingolipid species and mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *PLoS One*. 2014;9:e85724. doi: 10.1371/journal.pone.0085724
34. Bikman BT, Summers SA. Ceramides as modulators of cellular and whole-body metabolism. *J Clin Invest*. 2011;121:4222–4230. doi: 10.1172/jci57144
35. Zhou K, Blom T. Trafficking and functions of bioactive sphingolipids: lessons from cells and model membranes. *Lipid Insights*. 2015;8:11–20. doi: 10.4137/lpi.s31615
36. Bartke N, Hannun YA. Bioactive sphingolipids: metabolism and function. *J Lipid Res*. 2009;50:S91–S96. doi: 10.1194/jlr.R800080-JLR200
37. Jadczyk T, Baranski K, Syzdol M, Nabialek E, Wanha W, Kurzelowski R, Ratajczak MZ, Kucia M, Dolegowska B, Niewczas M, et al. Bioactive Sphingolipids, complement cascade, and free hemoglobin levels in stable coronary artery disease and acute myocardial infarction. *Mediators Inflamm*. 2018;2018:1–11. doi: 10.1155/2018/2691934
38. Freed JK, Beyer AM, LoGiudice JA, Hockenberry JC, Gutterman DD. Ceramide changes the mediator of flow-induced vasodilation from nitric oxide to hydrogen peroxide in the human microcirculation. *Circ Res*. 2014;115:525–532. doi: 10.1161/circresaha.115.303881
39. Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. *Am J Physiol Regul Integr Comp Physiol*. 2004;287:R1014–R1030. doi: 10.1152/ajpregu.00124.2004
40. Cai H. NAD(P)H oxidase-dependent self-propagation of hydrogen peroxide and vascular disease. *Circ Res*. 2005;96:818–822. doi: 10.1161/01.RES.0000163631.07205.fb
41. Brandes RP, Kreuzer J. Vascular NADPH oxidases: molecular mechanisms of activation. *Cardiovasc Res*. 2005;65:16–27. doi: 10.1016/j.cardiores.2004.08.007
42. Nakanishi K, Fukuda S, Shimada K, Miyazaki C, Otsuka K, Maeda K, Miyahana R, Kawarabayashi T, Watanabe H, Yoshikawa J, et al. Impaired coronary flow reserve as a marker of microvascular dysfunction to predict long-term cardiovascular outcomes, acute coronary syndrome and the development of heart failure. *Circ J*. 2012;76:1958–1964. doi: 10.1253/circj.cj-12-0245
43. Tsukamoto O, Kitakaze M. Reserve of coronary flow deserves predictor of cardiovascular events. *Circ J Japan*. 2012;76:1834–1835. doi: 10.1253/circj.CJ-12-0724
44. Weil BR, Canty JM Jr. Ceramide signaling in the coronary microcirculation: a double-edged sword? *Circ Res*. 2014;115:475–477. doi: 10.1161/CIRCRESAHA.114.304589
45. Poss AM, Maschek JA, Cox JE, Hauner BJ, Hopkins PN, Hunt SC, Holland WL, Summers SA, Playdon MC. Machine learning reveals serum sphingolipids as cholesterol-independent biomarkers of coronary artery disease. *J Clin Invest*. 2020;130:1363–1376. doi: 10.1172/jci131838
46. Cheng JM, Suoniemi M, Kardys I, Vihervaara T, de Boer SPM, Akkerhuis KM, Sysi-Aho M, Ekroos K, Garcia-Garcia HM, Oemrawsingh RM, et al. Plasma concentrations of molecular lipid species in relation to coronary plaque characteristics and cardiovascular outcome: results of the ATHEROREMO-IVUS study. *Atherosclerosis*. 2015;243:560–566. doi: 10.1016/j.atherosclerosis.2015.10.022
47. Horio Y, Yasue H, Rokutanda M, Nakamura N, Ogawa H, Takaoka K, Matsuyama K, Kimura T. Effects of intracoronary injection of acetylcholine on coronary arterial diameter. *Am J Cardiol*. 1986;57:984–989. doi: 10.1016/0002-9149(86)90743-5
48. Ighodaro ET, Graff-Radford J, Syrjanen JA, Bui HH, Petersen RC, Knopman DS, Jack CR Jr, Zuk SM, Vemuri P, Mielke MM. Associations between plasma ceramides and cerebral microbleeds or lacunes. *Arterioscler Thromb Vasc Biol*. 2020;40:2785–2793. doi: 10.1161/atvbaha.120.314796
49. Gui YK, Li Q, Liu L, Zeng P, Ren RF, Guo ZF, Wang GH, Song JG, Zhang P. Plasma levels of ceramides relate to ischemic stroke risk and clinical severity. *Brain Res Bull*. 2020;158:122–127. doi: 10.1016/j.brainresbull.2020.03.009
50. Fiedorowicz A, Kozak-Sykała A, Bobak Ł, Kałas W, Strządała L. Ceramides and sphingosine-1-phosphate as potential markers in diagnosis of ischaemic stroke. *Neurol Neurochir Pol*. 2019;53:484–491. doi: 10.5603/PJNNS.a2019.0063
51. Ye Q, Svatikova A, Meeusen JW, Kludtke EL, Kopecky SL. Effect of proprotein convertase subtilisin/kexin type 9 inhibitors on plasma ceramide levels. *Am J Cardiol*. 2020;128:163–167. doi: 10.1016/j.amjcard.2020.04.052
52. Tarasov K, Ekroos K, Suoniemi M, Kauhanen D, Sylvänne T, Hurme R, Gouni-Berthold I, Berthold HK, Kleber ME, Laaksonen R, et al. Molecular lipids identify cardiovascular risk and are efficiently lowered by simvastatin and PCSK9 deficiency. *J Clin Endocrinol Metab*. 2014;99:E45–E52. doi: 10.1210/jc.2013-2559

SUPPLEMENTAL MATERIAL

Figure S1. Plasma ceramide levels between case and reference groups including outliers



Plasma ceramide levels between case and reference groups without statistical significance were examined. Red dots illustrate data beyond two standard deviations from the mean.