Plasma Ceramides

A Novel Predictor of Major Adverse Cardiovascular Events After Coronary Angiography

Jeffrey W. Meeusen, Leslie J. Donato, Sandra C. Bryant, Linnea M. Baudhuin, Peter B. Berger, Allan S. Jaffe

Objective—Ceramides are sphingolipids involved with cellular signaling. Synthesis of ceramides occurs in all tissues. Ceramides accumulate within tissues and the blood plasma during metabolic dysfunction, dyslipidemia, and inflammation. Elevations of ceramides are predictive of cardiovascular mortality. We sought to verify the utility of plasma concentrations of 4 ceramides: N-palmitoyl-sphingosine [Cer(16:0)], N-stearoyl-sphingosine [Cer(18:0)], N-nervonoyl-sphingosine [Cer(24:1)], and N-lignoceroyl-sphingosine [Cer(24:0)] in predicting major adverse cardiovascular events in a diverse patient population referred for coronary angiography.

Approach and Results—Plasma ceramides were measured in 495 participants before nonurgent coronary angiography. Coronary artery disease, defined as >50% stenosis in ≥1 coronary artery, was identified 265 (54%) cases. Ceramides were not significantly associated with coronary artery disease. Patients were followed for a combined primary end point of myocardial infarction, percutaneous intervention, coronary artery bypass, stroke, or death within 4 years. Ceramides were significantly predictive of outcomes after adjusting for age, sex, body mass index, hypertension, smoking, LDL (low-density lipoprotein) cholesterol, HDL (high-density lipoprotein) cholesterol, triglycerides, serum glucose, and family history of coronary artery disease. The fully adjusted per SD hazard ratios (95% confidence interval) were 1.50 (1.16–1.93) for Cer(16:0), 1.42 (1.11–1.83) for Cer(18:0), 1.43 (1.08–1.89) for Cer(24:1), and 1.58 (1.22–2.04) for the ceramide risk score.

Conclusions—Elevated plasma concentrations of ceramides are independently associated with major adverse cardiovascular events in patients with and without coronary artery disease.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2018;38:1933-1939. DOI: 10.1161/ATVBAHA.118.311199.)**

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Ceramides are complex sphingolipids that play a central role in cell membrane integrity, cellular stress responses, inflammatory signaling, and apoptosis.[1] All tissues can synthesize ceramides de novo from saturated fats and sphingosine.[2] Thus, ceramide moieties are found in all tissues. However, arterial plaque is enriched with certain ceramides by as much as 50-fold.[3] This is, in part, because of inflammatory cytokines, such as interferon-γ, TNF-α (tumor necrosis factor-α), and interleukin-1ß, all of which stimulate ceramide synthesis.[4] These cytokines also participate in the interleukin-β inflammasome what has recently been documented to be instrumental to the development of atherosclerotic events.[5]

In the circulation, ceramides are transported by lipoproteins. Plasma concentrations of ceramides are elevated in patients with hypertension, type 2 diabetes mellitus, and insulin resistance.[6–10] In arteries, ceramides promote lipoprotein infiltration into the vessel wall.[11] Finally, ceramides are implicated in platelet activation and endothelial dysfunction via uncoupling of NO signaling pathways.[6,12–14]

Untargeted metabolomic analysis has identified specific plasma ceramides as significantly linked to cardiovascular disease.[15,16] Risk conferred by these ceramides was independent of age, body mass index, smoking status, statin use, triglycerides (TGs), and LDL (low-density lipoprotein) cholesterol (LDL-C).

Subsequent studies have supported the link between ceramides and cardiovascular death or acute coronary syndrome hospitalizations within 1 to 5 years. A ceramide risk score was developed in these studies, which combined the values of N-palmitoyl-sphingosine [Cer(16:0)], N-stearoyl-sphingosine [Cer(18:0)], and N-nervonoyl-sphingosine [Cer(24:1)], and N-lignoceroyl-sphingosine [Cer(24:0)] into a single score.[15–19] However, an evaluation of patients in the United States has not yet been published. The objective of this study was to investigate the predictive utility of ceramides

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From the Department of Laboratory Medicine and Pathology (J.W.M., L.J.D., L.M.B., A.S.J.), Department of Biomedical Statistics (S.C.B.), and Department of Cardiology (P.B.B., A.S.J.), Mayo Clinic, Rochester, MN.

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Correspondence to Jeffrey W. Meeusen, PhD, Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First St SW, Rochester, MN 55905. E-mail meeusen.jeffrey@mayo.edu

Nonstandard Abbreviations and Acronyms

apoB	apolipoprotein B
BMI	body mass index
CAD	coronary artery disease
Cer(16:0)	N-palmitoyl-sphingosine
Cer(18:0)	N-stearoyl-sphingosine
Cer(24:0)	N-lignoceroyl-sphingosine
Cer(24:1)	N-nervonoyl-sphingosine
CI	confidence interval
CRP	C-reactive protein
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
MI	myocardial infarction
PREDIMED	Prevention With Mediterranean Diet
TG	triglyceride
TNF-α	tumor necrosis factor-α

and the ceramide risk score in a diverse cohort of US patients referred for coronary angiography.

Materials and Methods

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

Study Populations

The Mayo Clinic Institutional Review Board approved this study, and all subjects provided written informed consent. We designed the current study on the basis of a previous study in which we had enrolled a total of 504 consecutive patients between 18 and 75 years of age, who were undergoing clinically indicated coronary angiography at Mayo Clinic between June 1998 and December 1998.[20] Patients with diabetes mellitus (defined by use of oral or injectable diabetes mellitus medication or fasting glucose >110 mg/dL at enrollment), smoking history >50 pack-years, organ transplantation, prior coronary revascularization, bleeding disorders, HIV, renal failure, prior radiation therapy, pregnancy, congenital heart disease, or <18 years of age were not eligible.

Four-Year Outcomes

Clinical outcomes were evaluated by chart review, mail-in survey, and phone questionnaire at 4 years postenrollment. The primary outcome was combined incidence of myocardial infarction (MI), coronary artery bypass graft, percutaneous intervention, stroke, and death. The remaining 26 patients either refused to participate in the follow-up (16; 62%) or could not be contacted (10; 38%). The first time point covered a median and interquartile range follow-up for the combined outcome of 4.0 (3.8–4.1) years and documented 67 events among 59 patients. There were 18 deaths (4 cardiac), 14 MIs, 26 coronary revascularizations, and 9 strokes.

Eighteen-Year Outcomes

Data for a second outcome of death by any cause at 18 years postenrollment was generated by reviewing national death records in June 2016. At the second time point, median (interquartile range) follow-up for death was 12.8 (5.6–16.5) years, and there were 166 events.

Biochemical Analysis

Mayo Clinic Cardiovascular Laboratory Medicine—a clinical testing laboratory and participant in the Centers for Disease Control and Prevention Lipid Standardization Program—performed all testing. Total cholesterol, HDL (high-density lipoprotein) cholesterol (HDL-C), TGs, and apoB (apolipoprotein B) were measured using a Cobas c501 per manufacturer's instructions (Roche Diagnostics, Indianapolis, IN).

Plasma ceramides were measured by high-pressure liquid chromatography coupled tandem mass spectrometry. EDTA plasma samples were diluted in ethyl acetate and isopropanol (20:80 v:v) with 0.1% formic acid before addition of deuterium-labeled internal standards (Avanti Polar Lipids, Alabaster, AL). Ceramides were separated using an X-bridge C18 3.5, 3.1×50-mm column (Waters, Milford, MA) and detected using an API 5000 MS/MS (AB Sciex, Framingham, MA).

Table 1. Baseline Demographic Data for Patients With Clinically Ordered Coronary Angiography

	No CAD	CAD
Demographics		
n	230	265
Age, y; mean±SD	58±11	62±10
Women, n (%)	129 (56.1%)	61 (23.0%)
BMI, kg/m^2; median (IQR)	28 (25–32)	29 (26–32)
Hypertension, n (%)	88 (38.3%)	140 (52.8%)
Ever smoked, n (%)	113 (49.1%)	162 (61.1%)
Statin at enrollment, n (%)	35 (15.2%)	106 (40.0%)
Family history of CAD	50 (21.7%)	76 (28.7%)
Traditional biomarkers; median (IQR)		
Total cholesterol, mg/dL	201 (177–231)	207 (180–236)
LDL-C, mg/dL	113 (95–139)	128 (106–152)
HDL-C, mg/dL	50 (41–62)	42 (37–51)
TGs, mg/dL	148.5 (109–202)	155 (114–208)
ApoB, mg/dL	93 (79–106)	100 (85–114)
Apolipoprotein A1, mg/dL	132 (117–153)	125 (113–141)
hsCRP, ng/mL	2.9 (1.1–6.2)	2.9 (1.3–7.6)
Glucose, mg/dL; mean±SD	88±14	91±15
Ceramides, μmol/L; median (IQR)		
Ceramide (16:0)	0.30 (0.26–0.34)	0.30 (0.26–0.36)
Ceramide (18:0)	0.11 (0.09–0.14)	0.12 (0.10–0.15)
Ceramide (24:0)	3.17 (2.61–3.70)	3.27 (2.75–3.89)
Ceramide (24:1)	1.14 (0.96–1.35)	1.18 (1.02–1.40)
Ceramide (16:0)/(24:0)	0.09 (0.08–0.11)	0.09 (0.08–0.11)
Ceramide (18:0)/(24:0)	0.04 (0.03–0.05)	0.04 (0.03–0.05)
Ceramide (24:1)/(24:0)	0.37 (0.30–0.45)	0.37 (0.31–0.45)
Ceramide score, 0–12	5 (2–7)	5 (3–8)
Ceramide score, n (%)		
0–2	59 (25.7%)	61 (23.0%)
3–6	93 (40.4%)	107 (40.4%)
7–9	51 (22.2%)	59 (22.3%)
10–12	27 (11.7%)	38 (14.3%)

CAD was defined as stenosis >50% in ≥1 artery. ApoB indicates apolipoprotein B; BMI, body mass index; CAD, coronary artery disease; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; and TG, triglyceride.

Within laboratory, analytic imprecision ranged between 5.1% and 11.8%.[21] Samples were stored in aliquots of 0.5 mL at −80°C from date of collection in 1998 until testing in 2015. The ceramide testing method used was developed in 2014, and internal studies have demonstrated ceramides are stable at −80°C for at least 3 years.

The ceramide risk score was developed as an interpretive aide.[19] The score is calculated as a sum with 1 point added for each Cer(16:0), Cer(18:0), or Cer(24:1) value above the median and an additional point for each value in the fourth quartile. Additionally, the score adds 1 point for each ratio Cer(16:0)/Cer(24:0), Cer(18:0)/Cer(24:0), and Cer(24:1)/Cer(24:0) value in the third quartile and 2 points are added for values in the fourth quartile. Quartiles were established from a combined pool of angiography patients (n=477) and healthy donor subjects (n=168). Detailed explanation on the derivation of the ceramide risk score is presented below.

Statistical Analysis

General linear models were used to assess the univariate association of ceramides with age, sex, body mass index (BMI), smoking status, hypertension, MI at presentation, total LDL and HDL-C, and TGs. Kaplan-Meier survival with a log-rank test was used to assess the univariate impact of ceramide score groups (0–2, 3–6, 7–9, and 10–12) on 4-year major adverse events and 18-year death. Univariate and multivariable Cox proportional hazards models were used to assess the association of 4-year major adverse events and 18-year overall survival with ceramides. Hazard ratios were calculated per SD change with 95% confidence intervals (CIs). Pearson correlation was also used to assess possible associations between ceramides and continuous covariates from the models. All hypothesis tests were 2 tailed with a 0.05 type I error rate. Statistical analyses were performed using SAS 9.4 software (SAS, Inc, Cary, NC).

Results

Plasma Ceramide Associations With Lipids and Conventional Biomarkers

Demographic and biomarker data are presented in Table 1. Plasma ceramide concentrations were not associated with sex, smoking status (current or ever), hypertension, and MI at presentation (all R^2 <4%). Ceramides were not clinically associated with angiographic coronary artery disease (CAD), defined as stenosis of ≥50% in at least 1 coronary artery (all R^2 <1%). Age was minimally associated with only the 3 ceramide ratios (R^2<1%), whereas BMI was minimally associated with only Cer(18:0) and its ratio (R^2<3%), but these were not considered clinically relevant. All 4 ceramides were inversely correlated with HDL-C (0.2%<R^2<3%) and directly correlated with total cholesterol (7%<R^2<17%), LDL-C (4%<R^2<10%), and TGs (7%<R^2<18%; Table III in the online-only Data Supplement), but these associations were also not clinically relevant.

Four-Year Outcomes

Cer(16:0), Cer(18:0), and Cer(24:1) were significantly predictive for a combined outcome of MI, stroke, revascularization, and death from any cause (Table 2) at 4 years of follow-up. Cer(24:0) was not predictive. However, normalizing Cer(16:0), Cer(18:0), and Cer(24:1) as a ratio with Cer(24:0) increased

Table 2. Adjusted Hazard Ratios (per SD) for Plasma Ceramide Concentrations

	Univariate		Model 1*		Model 2†	
	Hazard Rate (95% CI)	*P* Value	Hazard Rate (95% CI)	*P* Value	Hazard Rate (95% CI)	*P* Value
Combined outcome within 4 y (59 events in 469 patients)‡						
Ceramide (16:0)	1.34 (1.07–1.68)	0.01	1.41 (1.10–1.80)	0.007	1.50 (1.16–1.93)	0.002
Ceramide (18:0)	1.44 (1.16–1.79)	0.001	1.43 (1.13–1.83)	0.004	1.42 (1.11–1.83)	0.01
Ceramide (24:0)	0.96 (0.87–1.05)	0.33	0.94 (0.84–1.04)	0.24	0.95 (0.85–1.06)	0.32
Ceramide (24:1)	1.32 (1.06–1.64)	<0.001	1.37 (1.05–1.79)	0.02	1.43 (1.08–1.89)	0.01
Ceramide (16:0)/(24:0)	1.62 (1.32–1.98)	<0.001	1.70 (1.36–2.12)	<0.001	1.75 (1.40–2.19)	<0.001
Ceramide (18:0)/(24:0)	1.61 (1.31–1.97)	<0.001	1.54 (1.25–1.90)	<0.001	1.54 (1.24–1.91)	<0.001
Ceramide (24:1)/(24:0)	1.46 (1.22–1.76)	<0.001	1.52 (1.22–1.89)	<0.001	1.58 (1.25–1.99)	<0.001
Ceramide score	1.61 (1.26–2.07)	<0.001	1.56 (1.21–2.02)	<0.001	1.58 (1.22–2.04)	<0.001
Overall death at 18 y (166 deaths in 495 patients)§						
Ceramide (16:0)	1.24 (1.08–1.42)	0.002	1.30 (1.12–1.52)	<0.001	1.31 (1.12–1.53)	<0.001
Ceramide (18:0)	1.11 (0.96–1.28)	0.15	1.15 (0.97–1.35)	0.10	1.14 (0.97–1.35)	0.11
Ceramide (24:0)	0.98 (0.93–1.03)	0.39	0.96 (0.90–1.02)	0.21	0.96 (0.90–1.02)	0.20
Ceramide (24:1)	1.24 (1.07–1.43)	0.003	1.25 (1.06–1.48)	<0.001	1.25 (1.06–1.48)	0.009
Ceramide (16:0)/(24:0)	1.42 (1.22–1.66)	<0.001	1.48 (1.26–1.74)	<0.001	1.49 (1.27–1.76)	<0.001
Ceramide (18:0)/(24:0)	1.23 (1.06–1.43)	0.007	1.26 (1.08–1.47)	0.004	1.26 (1.08–1.48)	0.004
Ceramide (24:1)/(24:0)	1.34 (1.15–1.56)	<0.001	1.30 (1.11–1.52)	0.001	1.30 (1.11–1.53)	0.001
Ceramide score	1.36 (1.16–1.59)	<0.001	1.35 (1.15–1.59)	<0.001	1.36 (1.15–1.60)	<0.001

BMI indicates body mass index; CAD, coronary artery disease; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; and TG, triglyceride.

*Model 1 is adjusted for age, sex, BMI, hypertension, ever smoked, HDL-C, LDL-C, and TGs.

†Model 2 is adjusted for all variables in model 1 plus serum glucose and family history of CAD.

‡Combined 4-y outcomes included death of any cause, MI, revascularization, or stroke.

§Overall death was abstracted from 18 y of follow-up.

the association with outcome (Table 2). In all cases, ceramides remained significantly predictive after adjusting for age, sex, BMI, hypertension, smoking, HDL-C, LDL-C, TGs, glucose, and family history of CAD.

A 12-point risk score combining the values from Cer(16:0), Cer(18:0), Cer(24:1), and the ratios with Cer(24:0) was developed previously (Table II in the online-only Data Supplement).[19] The ceramide risk score was ≤6 for most patients (n=320/495). The rate of events at 4 years was significantly higher (20% versus 11%) among the 65 individuals with a score ≥10 (P=0.04). The ceramide risk score was significantly predictive of 4-year outcomes with a fully adjusted per SD hazard ratio of 1.58 (95% CI, 1.22–2.04).

Event rates increased as a function of ceramide score among patients with or without CAD at baseline (Figure 1). Kaplan-Meir survival analysis revealed the risk of major adverse events at any time was significantly greater (P<0.001) during 4 years for individuals with increased ceramide risk score (Figure 2). The area under the receiver operating characteristic curve (C statistic) for the combined outcome increased from 0.68 (95% CI, 0.61–0.74) to 0.72 (95% CI, 0.65–0.78) when the ceramide risk score was added to the fully adjusted model. The ceramide risk score reclassified 34 of 59 patients with events as higher risk and 285 of 436 patients without events as lower risk for an overall net reclassification index of 131.0% (95% CI, 114.7–147.2). Additional C statistic and net reclassification index data for specific ceramides and individual end points are available in Table V in the online-only Data Supplement.

Eighteen-Year Outcomes

Death of any cause was reassessed at 18 years postenrollment. Cer(16:0) and Cer(24:1) were significantly predictive for all-cause death at 18 years (P<0.005; Table 2). The ratios Cer(16:0)/Cer(24:0), Cer(18:0)/Cer(24:0), and Cer(24:1)/Cer(24:0) were significantly predictive (P<0.002) and remained significant after adjusting for age, sex, BMI, hypertension, smoking, HDL-C, LDL-C, TGs, serum glucose, and family history of CAD (P<0.004).

A ceramide risk score ≥10 was associated with a >2-fold increase in risk of all-cause death at 18 years compared with patients with a score ≤2 (P<0.001; Table 3). The 2-fold increased risk remained even after adjusting for age, sex, BMI, smoking, hypertension, HDL-C, LDL-C, TGs, glucose, and family history of CAD (P<0.001). Incidence of death was significantly higher among patients with higher risk score regardless of baseline CAD (Figure 1). Kaplan-Meir survival analysis revealed the risk of death at any time from any cause was significantly greater (P<0.001) during 18 years for individuals with increased ceramide risk score (Figure 2). Multivariable hazard ratios for covariates and

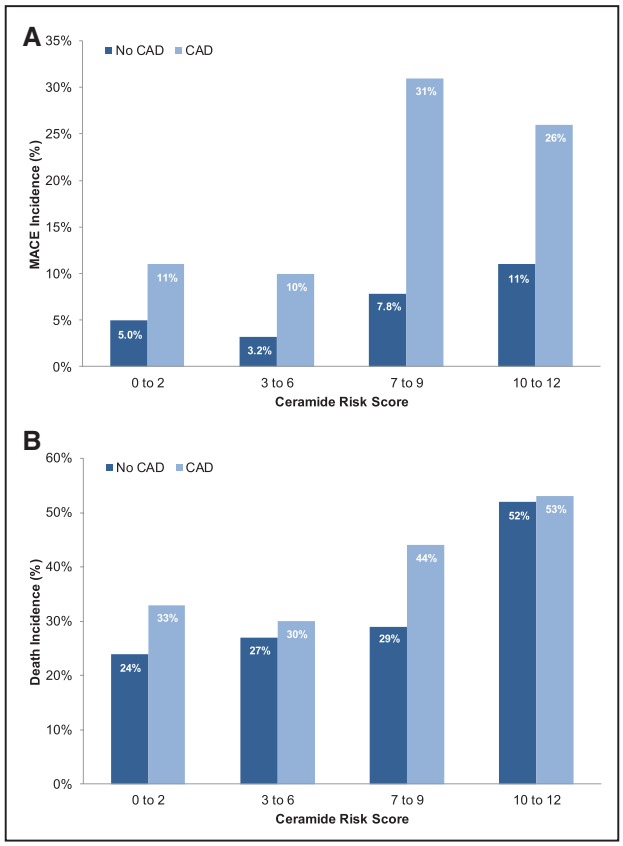

Figure 1. Incident cardiovascular events as a function of ceramide risk score. Events were more prevalent among patients with higher risk score regardless of coronary artery disease (CAD) for (**A**) myocardial infarction, revascularization, stroke, and mortality within 4 y or (**B**) death by any cause within 18 y. MACE indicates major adverse cardiovascular events.

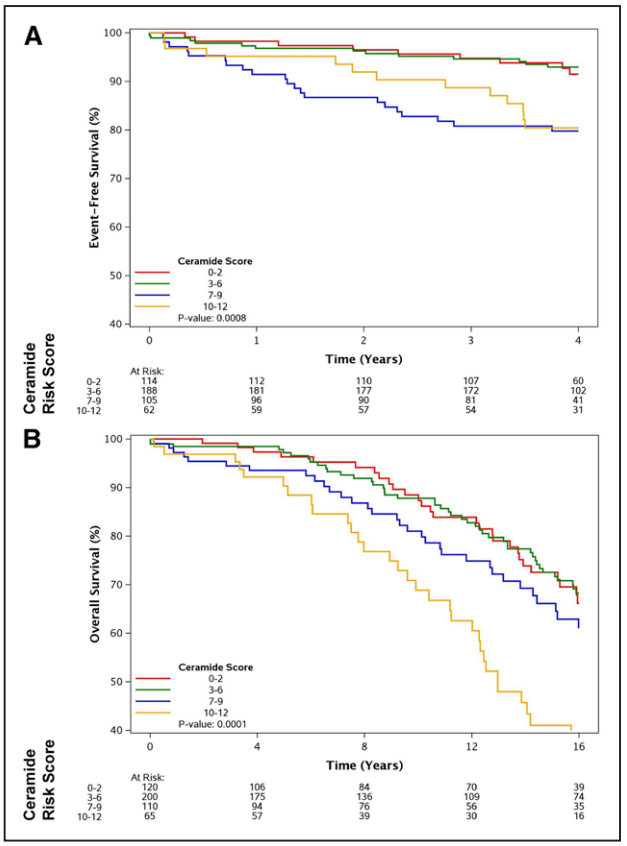

Figure 2. Event-free survival among patients with clinically ordered coronary angiography. Risk of cardiovascular events increased significantly with increased ceramide risk score among all patients. **A**, Combined outcome of myocardial infarction, revascularization, stroke, or death within 4 y (P<0.001). **B**, Death by any cause within 18 y (P<0.001).

Table 3. Outcome Incidence and Plasma Concentrations of Biomarkers Among Patients Classified by Ceramide Risk Score

Ceramide Risk Score	0–2	3–6	7–9	10–12	*P* Value
Subjects, n (%)	120 (24.2%)	200 (40.4%)	110 (22.2%)	65 (13.1%)	…
Death, MI, revascularization, stroke at 4 y, n (%)	10 (8.3%)	14 (7.0%)	22 (20.0%)	13 (20.0%)	…
Overall death at 18 y, n (%)	34 (28.3%)	57 (28.5%)	41 (37.3%)	34 (52.3%)	…
Outcome hazard ratios					
Death, MI, revascularization, stroke					
Univariate	ref	0.87 (0.39–1.97)	2.64 (1.25–5.58)	2.54 (1.11–5.80)	0.002
Model 1*	ref	0.87 (0.38–1.97)	2.55 (1.17–5.53)	2.31 (0.98–5.42)	0.005
Model 2†	ref	0.87 (0.38–1.99)	2.62 (1.20–5.70)	2.36 (0.99–5.60)	0.004
Overall death					
Univariate	ref	0.92 (0.60–1.42)	1.32 (0.83–2.07)	2.33 (1.45–3.75)	<0.001
Model 1*	ref	0.93 (0.61–1.44)	1.32 (0.83–2.10)	2.23 (1.36–3.66)	0.001
Model 2†	ref	0.94 (0.61–1.45)	1.35 (0.84–2.14)	2.24 (1.36–3.71)	<0.001
Biomarkers, median (IQR)					
BMI, kg/m^2	28 (26–31)	28 (25–32)	29 (26–33)	28 (26–33)	0.42
Glucose, mg/dL	88 (82–95)	87 (80–96)	90 (81–97)	88 (84–98)	0.71
LDL-C, mg/dL	115 (97–136)	123 (99–147)	120 (100–146)	132 (108–154)	0.052
HDL-C, mg/dL	48 (39–58)	46 (38–56)	44 (37–53)	46 (38–49)	0.18
TGs, mg/dL	125 (93–189)	156 (114–201)	153 (120–237)	167 (124–234)	<0.001

BMI indicates body mass index; CAD, coronary artery disease; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; and TG, triglyceride.

*Model 1 is adjusted for age, sex, BMI, hypertension, ever smoked, HDL-C, LDL-C, and TGs.

†Model 2 is adjusted for all variables in model 1 plus serum glucose and family history of CAD.

other discrete outcomes are presented in Table IV in the online-only Data Supplement.

Discussion

Our data in a cohort of patients undergoing angiography are consistent with reports establishing the relationship of plasma ceramides and cardiovascular disease risk[15–19] and extend the literature to a more diverse population. Specifically, we found a strong signal between ceramides and the ceramide risk score even among patients in whom fixed coronary atherosclerosis was not present.

The risk conferred by ceramides was independent of traditional risk factors, including age, sex, BMI, smoking status, and blood cholesterol. Additionally, the predictive value remained significant after adjusting for serum glucose and family history of CAD. Uniquely, our data suggest that when ceramide levels are elevated among patients without significant coronary artery stenosis, the risk of death is equal to that of patients with known CAD and abnormal ceramides ratios, and the risk of adverse cardiovascular events in patients without significant stenosis in the highest ceramide levels is equivalent to that of patients with known CAD at the lowest ceramide levels. However, the fact that they were referred for coronary angiography likely suggests they were at increased risk for CAD.

The study was powered for a combined outcome at 4 years, but no unique end points (death, MI, revascularization, and stroke) were statistically significant. However, ceramides, their ratios, and the risk score were predictive of death by any cause at 18 years. The hazard ratios for individual ceramides and ratios were similar for both follow-up scenarios, suggesting that an adequately powered shorter term study may provide significant associations for discrete end points. Indeed, this is the case in albeit higher risk cohorts published previously.[19]

Interpreting Elevated Ceramides

Several factors should be considered when evaluating novel methods.[22] Does the new risk marker provide new information independent of established risk factors? Are results clear regarding each patient's unique risk? Is there a clear action to be taken given the result?

Plasma ceramides are able to stratify risk among patients even after adjustment for multiple risk factors. Thus, it provides unique independent prognostic information. Our data, and other reports,[15–19] substantiate that 3 plasma ceramides and their ratios to a fourth ceramide are all independently linked to increased risk. Thus, 6 unique results, all individually predictive of cardiovascular disease via different mechanistic pathways, are available for interpretation. More work in more diffuse populations is needed to define whether there are unique subsets of patients in whom one ceramide or the other is optimal. However, individually parsing these 6 results may engender more confusion than clarity. These considerations prompted development of a ceramide risk score.[19]

The ceramide risk score proposed by Laaksonen et al and evaluated in the present study incorporates the values from all 4 ceramide results into a clearly defined risk category. A

single point is added for each result above the median and 2 points for values at or above the 75th percentile. This technique accounts for the potential risk attributable to all measured ceramides in a simple 12-point scale. In our cohort, the incidence of events and time of event-free survival were both significantly linked with the ceramide risk score. Other biomarkers increased as the ceramide risk score did, and there was considerable overlap in concentrations of LDL-C, HDL-C, and TGs between each discrete ceramide risk category (Table 3). However, ceramides continued to manifest significant prognostic information.

The ceramide risk score was initially developed and applied in 2 secondary prevention studies.[19] The ceramide risk score also was predictive of cardiovascular death in a large primary prevention study[18] and in a dietary intervention trial.[16] All studies have indicated that patients with a ceramide risk score of ≥10 points had a 4- to 6-fold increase in risk of cardiovascular mortality compared with subjects with a ceramide risk score of ≤2. The present data show similar results, despite excluding high-risk patients with previous coronary revascularization or diabetes mellitus.

Caveats Associated With Novel Testing

We have corrected our data for a large number of variables and biomarkers, and the significant risk associated with elevated ceramide values remains. More analyses are planned, including additional covariates and interrogation of different populations. Multiple reports have suggested an association between ceramides and CRP (C-reactive protein).[14,19,23] This link between ceramides and inflammatory cardiovascular risk is of particular interest in light of the success of canakinumab.[24] Additional studies evaluating plasma CRP and ceramide levels over time, as well as their relationship to acute or chronic inflammation, would be helpful.

Ceramides Are Modifiable

A biomarker is only useful in the clinic when it is able to guide effective interventions. Randomized clinical trials based on measured plasma ceramides have not been reported. However, the PREDIMED study (Prevention With Mediterranean Diet) demonstrated that dietary changes among patients with elevated ceramides could significantly mitigate adverse cardiovascular events.[16] Several other studies have reported that ceramide concentrations are modifiable by gastric bypass,[8] aerobic exercise,[7] and both simvastatin[15] and rosuvastatin.[25] Although dietary intervention is the only study that demonstrated that outcomes tracked with ceramide changes (which is a critical step), these data are promising in that many therapies already known to be effective at reducing risk of heart disease are also able to modify plasma ceramide concentrations. Additionally, several small molecule inhibitors of key enzymes in ceramide synthesis are currently being investigated.[26]

Conclusions

In conclusion, plasma ceramides are a promising new clinical diagnostic tool for the identification of patients at risk of adverse cardiovascular events.

Sources of Funding

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Highlights

- Ceramides are lipids that play an active role in atherosclerotic disease.
- Increased plasma concentrations of ceramides are associated with increased risk of major adverse cardiovascular events and death by any cause.
- Elevations of plasma ceramides may provide beneficial information about a patient's risk of adverse cardiovascular events, despite normal conventional risk factors and the absence of coronary artery stenosis.