

ORIGINAL RESEARCH

Plasma Ceramide C24:0/C16:0 Relates to Outcomes in Patients With Heart Failure With Preserved Ejection Fraction



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ABSTRACT

BACKGROUND Ceramides are complex sphingolipids with pleiotropic effects. The ratio of specific ceramides (plasma C24:0/C16:0) is inversely related to *incident* heart failure (HF) and all-cause death in large, community-based cohorts *without pre-existing HF*. Whether plasma C24:0/C16:0 relates to outcomes in patients with HF with preserved ejection fraction (HFpEF) is unclear. We hypothesized plasma C24:0/C16:0 would be inversely related to, and independently predict, outcomes in HFpEF patients.

OBJECTIVES To test our hypothesis, we used plasma samples, baseline, and outcomes data from the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist) trial. Findings were extended to a community-based cohort of HFpEF patients from the SHIP (Study of Health in Pomerania).

METHODS Plasma C24:0/C16:0 was measured using well-validated liquid chromatography/tandem mass spectrometry. For TOPCAT, our primary endpoint was the composite of time to cardiovascular disease (CVD) death, hospitalization for HF, or aborted cardiac death episode. Secondary endpoints were time to CVD death and to HF hospitalization. For SHIP, CVD death was the primary endpoint and total mortality a secondary endpoint.

RESULTS In 419 TOPCAT subjects (mean follow-up 3.3 years), lower plasma C24:0/C16:0 was associated with a higher risk of the primary endpoint; and HF hospitalization. In SHIP (N = 292; median follow-up 15.7 years), lower plasma C24:0/C16:0 was associated with a higher risk of CVD death and all-cause mortality in the SHIP cohort.

CONCLUSIONS Low plasma C24:0/C16:0 is independently associated with CVD death, all-cause mortality, and HF hospitalization in patients with HFpEF and may provide insight into novel treatment targets. (JACC Adv. 2025;4:102183) © 2025 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the [Author Center](#).

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**ABBREVIATIONS
AND ACRONYMS****BMI** = body mass index**CVD** = cardiovascular disease**HF** = heart failure**HFpEF** = heart failure with
preserved ejection fraction**MI** = myocardial infarction

Hear failure (HF) affects approximately 64 million persons worldwide.¹ Nearly half have HF with preserved ejection fraction (HFpEF),^{1,2} making it an “emerging epidemic.”² Patients with HFpEF have poor survival rates and high hospitalization rates. However, HFpEF is a heterogeneous condition comprised of multiple phenotypes, making risk prediction for this syndrome particularly challenging.³

Risk prediction in patients with HFpEF is also difficult given the different echocardiographic indices,⁴ sex-related differences in outcomes,⁵ comorbidities, and the wide spectrum of genetic expression signatures.⁶ Cardiopulmonary exercise testing is useful for risk prediction in HFpEF;⁷ however, it is limited to patients who are able to exercise and is not commonly used in HFpEF patients. Other tests, for example, plasma natriuretic peptide levels, are often lower in patients with HFpEF than patients with HF with reduced ejection fraction. Moreover, many patients with HFpEF have comorbidities, such as obesity and renal disease, that affect plasma natriuretic peptide concentrations and their interpretation.⁸ Troponins are even less extensively studied as prognostic markers in patients with HFpEF.⁹

Recent data suggest that plasma ceramides are an important new biomarker for HF risk prediction,¹⁰ cardiovascular outcomes, and mortality.^{10,11} Ceramides are complex lipids containing a sphingosine base and a fatty acyl chain of varying lengths. They are in cell membranes and affect cell signaling, apoptosis, differentiation, senescence/proliferation, and hormonal and stress responses.¹² Our group developed a high-throughput and reproducible assay for the most abundant very-long-chain and long-chain ceramides, C24:0 and C16:0, respectively.¹³ Using this robust assay, we observed that *lower* plasma C24:0/C16:0 ceramide ratio was associated with higher *incident* HF, CVD death, and all-cause mortality rates, even after adjusting for known risk factors in large, community-based cohorts, including the SHIP (Study of Health in Pomerania) (N = 3,134).¹⁰

Thus, we hypothesized that plasma C24:0/C16:0 would be inversely and independently related to the primary combined outcome (HF hospitalization, CVD, and sudden death) in patients with HFpEF from the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist) study, a clinical trial of spironolactone vs placebo. We further sought to determine if plasma C24:0/C16:0 is inversely related to any of the individual components of the combined outcome. Exploratory

cardiovascular endpoints were myocardial infarction (MI) and stroke. We also sought to extend our findings regarding the predictive value of the plasma C24:0/C16:0 ratio in a community-based cohort of patients with HFpEF from the SHIP.

RESEARCH DESIGN AND METHODS

TOPCAT COHORT. The TOPCAT cohort has been well-described.¹⁴ Briefly, subjects in TOPCAT had a diagnosis of HFpEF (ie, left ventricular ejection fraction $\geq 45\%$, and either hospitalization within the previous 12 months, with HF management a major component of the care provided, or an elevated natriuretic peptide level within 60 days before randomization). All subjects were $\geq$ age 50 years and with ≥ 3 years life expectancy. Exclusions included age < 50 years, serum potassium level > 5.0 mmol/L, uncontrolled hypertension, severe systemic illness, and severe renal dysfunction.¹⁴ Participants enrolled between 2006 and 2012 at sites in the United States, Canada, Brazil, Argentina, Russia, and Georgia. Subjects were randomly assigned to receive spironolactone or placebo. Study drug was given for 8 months.¹⁴

Plasma samples for baseline lipidomic analysis from the Biologic Specimen and Data Repository Information Coordinating Center database were available in 421 subjects. Of the subjects with an evaluable plasma ceramide concentration, only 74 subjects also had a brain natriuretic peptide or NT-pro-brain natriuretic peptide level available, so these were not included in the analyses. All subjects signed informed consent for TOPCAT.¹⁴ This study of the deidentified samples and data was also approved by the Washington University School of Medicine Human Research Protection Office.

SHIP COHORT. Data and plasma samples were used from the first SHIP cohort, a community-based study of health in a northern European population.¹⁰ The University Medicine Greifswald's Ethics Committee and Washington University Institutional Review Board approved this study.¹⁰ Subjects (N = 264) with HF symptoms were studied at the baseline time point from the SHIP-START 1 cohort (2002-2006) and followed for a median of 15.7 years (14.8-16.9 years). Patients were excluded if they did not: have evaluable plasma samples; meet the Rotterdam criteria for HFpEF (prevalent HF symptoms, ejection fraction $> 50\%$ by echocardiogram, and no chronic obstructive lung disease); or had incomplete follow-up data or missing covariables.

LIPIDOMIC ANALYSES. All samples were analyzed using the same exact Federal Drug Administration-

compliant method, by the same laboratory (at Washington University), and the same personnel for SHIP and TOPCAT samples.^{13,15} Briefly, we used a robust, reproducible, and fully validated, liquid chromatography/tandem mass spectrometry assay including deuterated internal standards. This method's reproducibility (correlation coefficient) for C24:0 at 1.5 years is 0.9214.¹³ This highly targeted, Federal Drug Administration-compliant assay¹³ study was used to quantify C24:0 and C16:0 ceramides from frozen plasma samples. Five samples were run in duplicate for quality control. The correlation coefficients (r) of the standard curves for C16:0 and C24:0 were >0.99, and the accuracies of back-calculated concentrations of all of calibrators were 89 to 109% and 91.2 to 108% for C16:0 and C24:0, respectively. The precisions for C16:0 and C24:0 in quality control samples were ≤5.2% CV and ≤5.0% CV, respectively. The accuracies were 92.1% to 116% and 91.4% to 113% for C16:0 and C24:0 in quality control samples, respectively. One sample failed measurement, and one sample had a ceramide measure of 42—more than 3 SDs above the mean (11.8)—and was therefore considered an outlier and was removed from further analysis. The final sample size of 419 was used for association analyses.

OUTCOMES. The primary endpoint for the analysis of the TOPCAT study was the TOPCAT primary endpoint: that is, the composite endpoint of time to hospitalization for HF, time to CVD death, or time to aborted cardiac sudden death episode—whichever came first.⁹ Prespecified secondary outcomes were the composite endpoint components: time to hospitalization for HF, time to CVD death. (Since only one subject experienced an event for aborted sudden cardiac death, further analysis was not performed for this event). Time to MI and stroke were evaluated as exploratory endpoints.

For the community-based cohort, SHIP, time to HF hospitalization was not an evaluable endpoint. However, CVD death (a secondary endpoint for TOPCAT) was available as was all-cause mortality, so they were the primary endpoints. Time to stroke and MI, and non-CVD death were exploratory endpoints.

STATISTICAL ANALYSIS. Baseline and follow-up characteristics for TOPCAT were compared as follows: categorical data were reported as frequencies. Continuous data were reported as mean ± SD. Unadjusted survival estimates were evaluated through Kaplan-Meier analysis ceramide ratios below the median vs above the median. The log-rank test was used to identify differences in clinical outcomes between the groups. A Cox proportional hazards model

TABLE 1 Baseline Characteristics of TOPCAT and SHIP Subjects With Plasma Ceramides Available for Analysis

	TOPCAT Subjects With Plasma Ceramides (n = 419)	SHIP Subjects With Plasma Ceramides (n = 264)
Age (y)	69.34 (9.6)	63.47 (13.3)
Sex		
Male	222 (53.0%)	111 (42.0%)
Race		
White	385 (91.9%)	264 (100%)
Black	28 (6.7%)	
Other/Mixed	6 (1.4%)	
Country		
US	180 (43.0%)	
Canada	58 (13.8%)	
Russia	181 (43.2%)	
Germany		264 (100.0%)
Smoking		
Yes	39 (9.3%)	34 (12.9%)
Body mass index (kg/m ²)	32.7 (6.7)	30.0 (4.7)
Medical history		
Myocardial infarction	132 (31.5%)	50 (19.2%)
Stroke	33 (7.9%)	12 (4.6%)
CABG	79 (18.8%)	13 (4.9%)
PCI	74 (17.7%)	71 (27.0%)
Angina	213 (50.8%)	96 (36.4%)
COPD	47 (11.2%)	27 (10.2%)
Dyslipidemia	298 (71.1%)	167 (63.3%)
Atrial fibrillation	177 (42.2%)	26 (9.8%)
Hypertension	396 (94.5%)	223 (84.5%)
Ceramide C16:0 plasma (μg/mL)	0.19 (0.06)	0.22 (0.05)
Ceramide C24:0 plasma (μg/mL)	2.20 (0.83)	2.43 (0.68)
Ceramide ratio C24:0/C16:0	11.82 (3.02)	11.40 (2.39)

Values are n (%) or mean (SD).
CABG = coronary artery bypass graft surgery; COPD = chronic obstructive lung disease; PCI = percutaneous coronary intervention; SHIP = Study of Health in Pomerania; TOPCAT = Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist.

was performed for each outcome with ceramide ratio and for ceramide ratio adjusted for age, sex, and country. The Cox proportional hazards model is very sensitive for the model assumptions: 1) the hazards are proportional for all subjects, and 2) the residuals of each covariate remain linear over time. All outcomes met the proportional hazards assumptions. Some outcomes in TOPCAT (ie, the primary outcome, time to HF hospitalization) did not meet the linearity assumption. For those outcomes, we log transformed the ceramide ratio and this resolved the linearity assumption for time to hospitalization for these outcomes. Because the log transformation did not resolve the linearity assumption for the secondary outcome of time to CVD death, ceramide ratio was categorized into a dichotomous value (“lower” below the median, “upper” above the median) and run as a

TABLE 2 Univariate and Multivariable Analyses Between Plasma C24:0/C16:0 and Primary and Secondary Endpoints in TOPCAT

Outcome	HR (95% CI)	P Value
Primary outcome ^a		
Unadjusted	0.10 (0.05, 0.18)	<0.0001
Adjusted	0.17 (0.09, 0.34)	<0.0001
Hospitalization for HF		
Unadjusted	0.78 (0.71, 0.87)	<0.0001
Adjusted	0.85 (0.77, 0.94)	0.0012
CVD death ^b		
Unadjusted	0.67 (0.36, 1.24)	0.20
Adjusted	0.82 (0.43, 1.55)	0.54

Primary outcome = hospitalization for HF, CVD mortality, or aborted cardiac arrest. Multivariable adjusted analyses are adjusted for age, sex, and country. **Bolded** values reached statistical significance ($P < 0.05$). ^aCeramide ratio was log transformed to meet assumptions of Cox PH model. ^bCeramide ratio dichotomized to meet assumptions of Cox PH model.

CVD = cardiovascular disease; HF = heart failure; other abbreviation as in Table 1.

categorical covariate for this outcome for TOPCAT. This resolved all assumptions of the models. For the analysis of main effects, P values <0.05 were considered statistically significant; Analyses were

performed with SAS version 9.4 (SAS Institute, Inc) and R version 3.0.1.

In SHIP, we performed univariate analyses and multivariable regression Cox modeling to address confounding in that observational cohort. We further performed a sensitivity analysis adjusting only for age, sex, and body mass index (BMI) to avoid model overfitting. Lastly, in SHIP, we performed analyses for competing risk of non-CVD death (vs CVD death) and used a Fine-Gray model as a sensitivity analysis for the association of C24:0/C16:0 ratio with the risk of CVD death. Analyses were performed with Stata version 18 (StataCorp LLC).

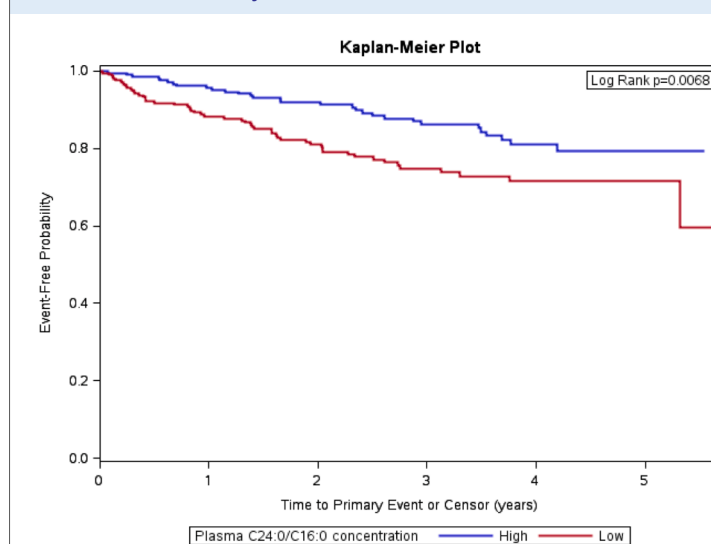
RESULTS

THE TOPCAT PLASMA COHORT IS REPRESENTATIVE OF THE ENTIRE TOPCAT COHORT. The mean age in the original TOPCAT cohort was reported as 68.7 years, with 52% female, and 89% White.¹⁴ The mean BMI was 31 kg/m².¹⁴ As seen in Table 1, the TOPCAT subjects' mean age in this analysis was 69.34 years; nearly half were female and the majority were White. The mean BMI was 32.7 kg/m². Most subjects came from the Americas or Russia, and the most common medical comorbidities for the patients in the current study's subset included dyslipidemia, hypertension, and coronary artery disease, again similar to the entire TOPCAT cohort.¹⁴

THE SHIP COHORT BASELINE CHARACTERISTICS.

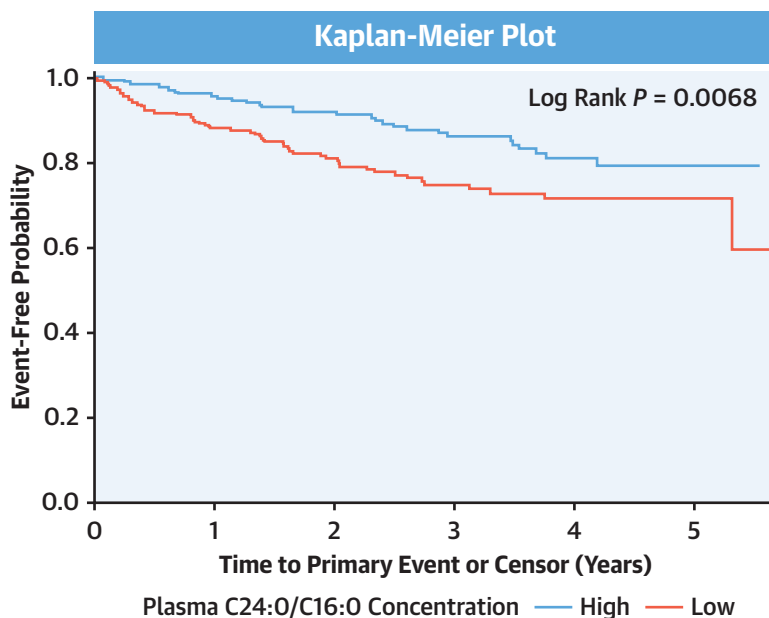
Table 2 lists SHIP cohort baseline characteristics. There were 264 subjects with HFpEF. The average age in SHIP was like that of the TOPCAT group. Seventy-three percent were women, higher than in TOPCAT (53% women), but like TOPCAT, the vast majority in SHIP (>99%) were White. Mirroring TOPCAT, the most common comorbidities included dyslipidemia, hypertension, and coronary artery disease.

PRIMARY OUTCOME ANALYSIS: TOPCAT. Plasma C24:0 concentration is nearly 12-fold higher than the plasma C16:0 concentration (Table 1). There were more primary events ($n = 50$) in the group with C24:0/C16:0 plasma concentrations *below* the median than in those who had concentrations above the median ($n = 30$). The Kaplan-Meier curve in Figure 1 graphically depicts this *higher risk* for those with a *lower* C24:0/C16:0 (Central Illustration). Cox proportional hazard models confirmed that the group with the higher C24:0/C16:0 concentrations had a 90% decreased hazard of the primary outcome in unadjusted analyses and an 83% decreased hazard in

FIGURE 1 Time to Primary Combined Outcome of TOPCAT

The red line in the Kaplan-Meier curves shows the significantly increased risk of primary outcomes (HF hospitalization, CVD death, or sudden death) in patients with HFpEF ($n = 209$) for those who have below-the-median concentrations of plasma C24:0/C16:0 compared with those with plasma C24:0/C16:0 concentrations above the median ($n = 210$; blue line). CVD = cardiovascular disease; HF = heart failure; HFpEF = heart failure with preserved ejection fraction; TOPCAT = Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist.

CENTRAL ILLUSTRATION Relationship Between Plasma C24:0/C16:0 Ceramide Ratio and Event-Free Survival in Patients With Heart Failure With Preserved Ejection Fraction



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Primary endpoint: An "event" was CVD death, HF hospitalization, or aborted sudden cardiac death. *Population studied:* Patients with HFpEF who were in the TOPCAT trial and who had plasma samples available. *Results:* High plasma C24:0/C16:0 ceramide ratio independently related to event-free survival even after adjustment for age, sex, and country. Abbreviations as in [Figure 1](#).

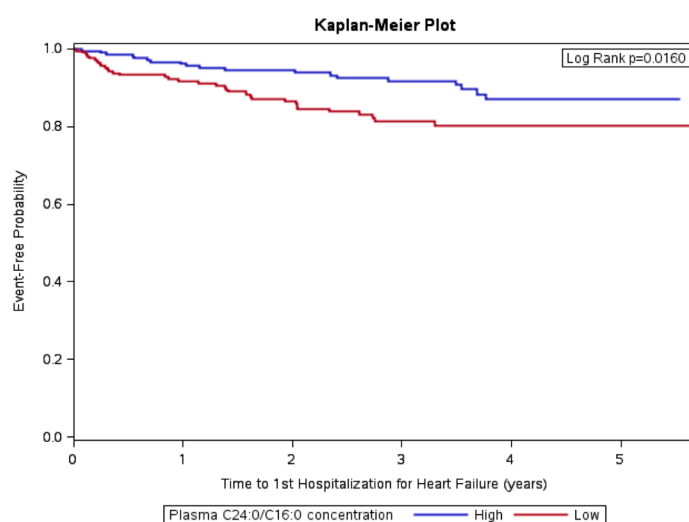
multivariable adjusted (for age, sex, and country) analyses ([Table 2](#)).

TIME TO FIRST HOSPITALIZATION FOR HF: TOPCAT. Time to first hospitalization for HF was evaluated as a secondary endpoint. The number of HF hospitalizations was *higher* ($n = 34$) in the group that had a C24:0/C16:0 plasma concentration *below* the median compared with the group that had plasma C24:0/C16:0 concentrations above the median ($n = 19$). This significant difference is depicted in [Figure 2](#). Cox proportional hazard models confirm that those with higher plasma C24:0/C16:0 had a significant, 22% decreased risk of hospitalization for HF in unadjusted analyses and a 15% decreased risk in multivariable-adjusted analyses ([Table 2](#)).

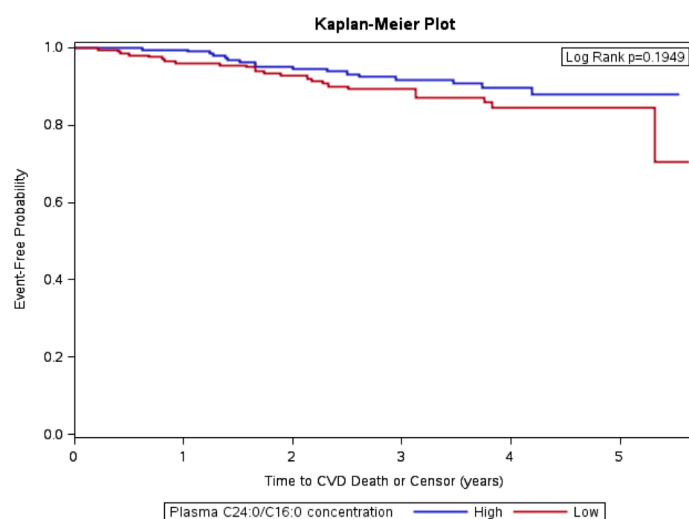
TIME TO CVD DEATH: TOPCAT. Time to CVD death was also a secondary endpoint. There were fewer

CVD deaths ($n = 41$) than HF hospitalization events ($n = 53$). There were more CVD deaths in the group with the lower plasma C24:0/C16:0 concentrations ($n = 24$) and fewer CVD deaths in the group with high plasma C24:0/C16:0 concentrations ($n = 17$). However, this difference was not statistically significant ([Figure 3](#)) ($P = 0.19$). This was confirmed in Cox proportional hazards models in unadjusted and adjusted analyses ([Table 2](#)).

OTHER ENDPOINTS: TOPCAT. Stroke-free survival time trended toward being worse in the patients who had lower plasma C24:0/C16:0 concentrations ($P = 0.075$) ([Supplemental Figure 1](#)). In multivariable analyses ([Supplemental Table 1A](#)), this relationship remained insignificant. Paradoxically, in univariate analyses subjects who had a lower C24:0/C16:0 plasma concentration related to better MI-free

FIGURE 2 Time to HF Hospitalization: TOPCAT

The red line in the Kaplan-Meier curves shows the significantly increased risk of HF hospitalization in patients with HFpEF ($n = 209$), who have below-the-median concentrations of plasma C24:0/C16:0 compared with those with plasma C24:0/C16:0 concentrations above the median ($n = 210$; blue line). Abbreviations as in Figure 1.

FIGURE 3 Time to CVD Death: TOPCAT

Kaplan-Meier curves show the nonsignificantly increased CVD death rates in TOPCAT in patients with HFpEF, who have below-the-median concentrations of plasma C24:0/C16:0 ($n = 209$; red line) compared with those with plasma C24:0/C16:0 concentrations above the median ($n = 210$; blue line). Abbreviations as in Figure 1.

survival ($P = 0.033$) (Supplemental Figure 2); however, this relationship lost significance in multivariable analyses (Supplemental Table 1A).

PRIMARY OUTCOME (CVD DEATH) ANALYSES: SHIP.

Baseline plasma concentrations for individual ceramides and the ratio of C24:0/C16:0 were like those in TOPCAT (Table 1). There were 30 CVD deaths in the SHIP cohort. (There were 10 deaths with an undetermined cause; hence, the denominator for calculations regarding CVD death was 254 instead of 264). There were more CVD deaths (Figure 4) ($P = 0.0034$) in the group with plasma C24:0/C16:0 below the median ($n = 22$) than in the group with plasma C24:0/C16:0 concentrations above the median ($n = 8$). This association with C24:0/C16:0 is further highlighted by unadjusted analyses, showing a 52% decrease in risk of CVD death with each SD increase in C24:0/C16:0 (Table 3). In multivariable analyses (Table 3), plasma C24:0/C16:0 was still associated with freedom from CVD mortality with each SD increase corresponding to a 44% lower risk of CVD mortality ($P = 0.025$). This association was still present in a less adjusted model (only age, sex, BMI) during sensitivity analysis: 34% lower risk of CVD mortality ($P = 0.057$) with each SD increase in plasma C24:0/C16:0. We also adjusted for competing risks of non-CVD death. In these analyses, the HR was consistent with the cause-specific Cox models for CVD death (HR: 0.642; 95% CI: 0.388–1.003; $P = 0.052$).

TIME TO ALL-CAUSE DEATH: SHIP. There were 91 deaths in SHIP ($N = 264$) over the follow-up period in SHIP. There were more deaths in the group with plasma C24:0/C16:0 concentrations below the median ($n = 58$) than in those with concentrations above the median ($n = 33$) (Figure 5) ($P = 0.0004$). Moreover, in the multivariable analyses, higher plasma C24:0/C16:0 was associated improved survival, with each SD increase corresponding to a 26% lower risk of death ($P = 0.008$). This association was still present in a less adjusted model (only age, sex, BMI) during sensitivity analysis, which showed a 10% lower risk of CVD mortality ($P = 0.026$) with each SD increase in plasma C24:0/C16:0.

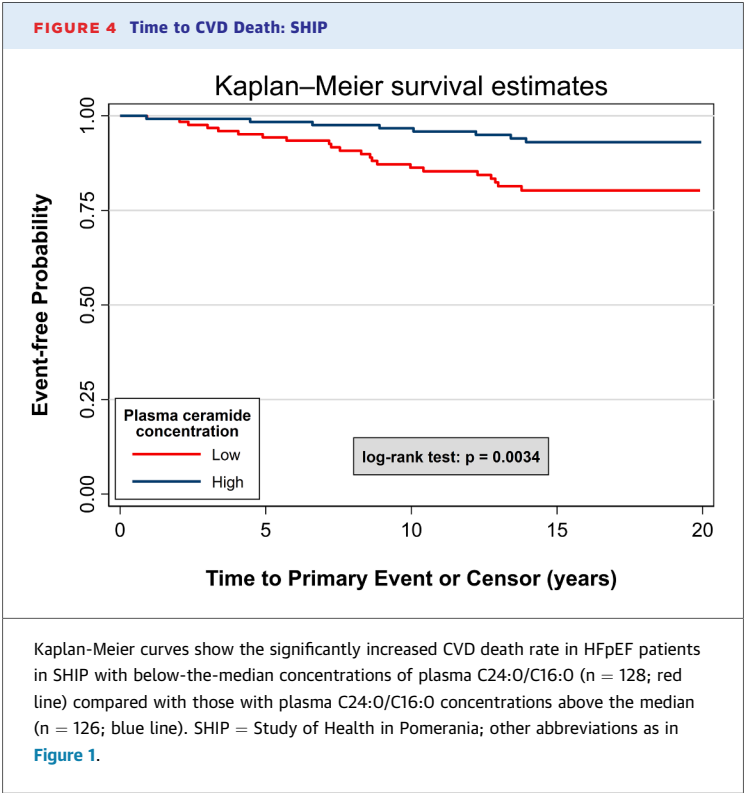
OTHER ENDPOINTS: SHIP. Stroke occurred in only 21 subjects in the SHIP cohort (denominator: $n = 218$) over the follow-up period. Still, for every SD increase plasma C24:0/C16:0 concentration, there was a 48% lower risk of stroke ($P = 0.020$) after multivariable adjustment for age, sex, BMI, systolic blood pressure, diabetes, total cholesterol/high-density

lipoprotein ratio, lipid-lowering medication, and triglyceride concentration. This was still present in a sensitivity analysis using less adjustments (only age, sex, BMI) to avoid model overfitting: 45% lower risk per each SD increase in C24:0/C16:0 ($P = 0.026$). Incident MI occurred in 31 subjects in SHIP (denominator: $n = 206$). In contrast to the results from TOPCAT, higher plasma ceramide concentration was associated with a 28% lower risk of MI, but this difference was not statistically significant ($P = 0.099$) in multivariable analysis. In sensitivity analysis with less adjustments (only age, sex, BMI) to avoid model overfitting, this was still nonsignificant (10.1% lower risk, $P = 0.55$).

DISCUSSION

Our analyses demonstrated for the first time that plasma concentrations of C24:0/C16:0 independently related to secondary cardiovascular events in patients with HFpEF. A low C24:0/C16:0 concentration independently related to a higher risk of the primary outcome of the TOPCAT study. This was primarily driven by the significant association between low plasma C24:0/C16:0 concentrations and higher HF hospitalization risk. Though the association with CVD death was not significant in multivariable analyses in TOPCAT, in the SHIP cohort, a lower plasma C24:0/C16:0 was significantly associated with CVD death. In TOPCAT, there was a trend toward a higher stroke risk with a plasma C24:0/C16:0 below the median, but in SHIP, the association with stroke was significant, even in multivariable analyses.

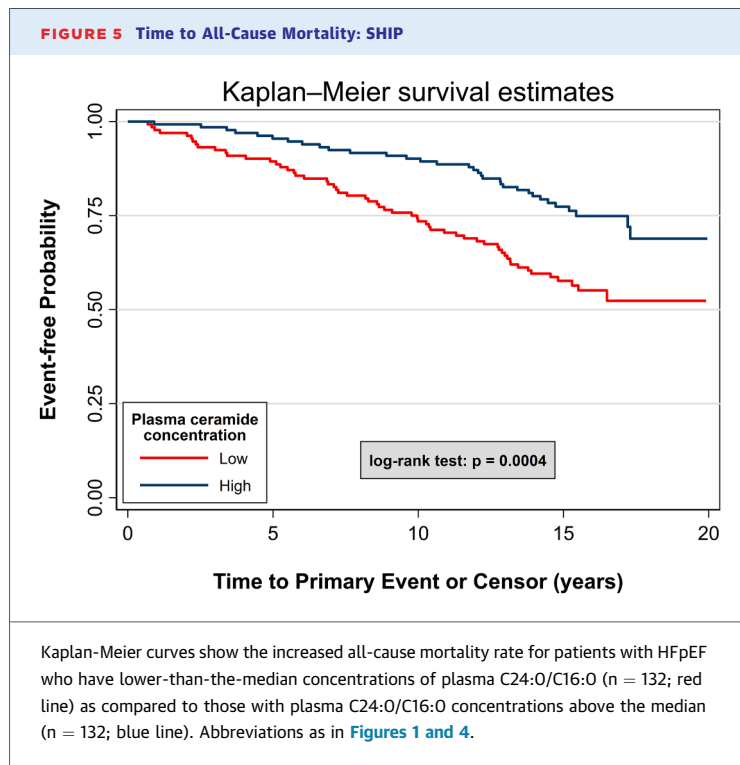
Our finding that low plasma C24:0/C16:0 concentrations independently associated with the primary outcome in TOPCAT is especially notable given the heterogeneity of HFpEF and limitations of current predictors.¹⁶ There are heterologous etiologies for HFpEF, and it is associated with comorbidities, such as obesity, hypertension, and atrial fibrillation, that can contribute to symptoms, phenotypes, pathophysiology, and outcomes.^{16,17} Nearly one-third of patients with HFpEF have normal natriuretic peptides levels.^{18,19} Thus, many have suggested that new outcome predictors must be identified for HFpEF.²⁰ It is therefore notable that the significant association we found between low plasma C24:0/C16:0 concentrations and the composite endpoint was identified in a cohort that has a mean BMI of 32.7 kg/m², 95% with hypertension, and 42% with a history of atrial fibrillation. It is also important that adjustment for age, sex, and country did not change the significance of this association, especially given the regional



discrepancies in study drug compliance, patient characteristics, and outcomes in TOPCAT.²¹

Our finding that plasma C24:0/C16:0 independently associated with the primary composite outcome is partially consistent with another study of plasma sphingolipidomics in patients with HFpEF. That study²² evaluated a slightly larger cohort ($N = 433$) from the TOPCAT study and used a less

TABLE 3 Univariate and Multivariable Analyses Between Plasma C24:0/C16:0 and Primary and Secondary Endpoints in SHIP		
Outcome	HR (95% CI) per 1SD Change	P Value
Primary outcome		
Unadjusted	0.48 (0.31, 0.76)	0.002
Adjusted	0.56 (0.33, 0.93)	0.025
All-cause mortality		
Unadjusted	0.64 (0.49, 0.83)	0.001
Adjusted	0.74 (0.59, 0.93)	0.008
Primary outcome = CVD mortality. Secondary endpoint = all-cause mortality. Multivariable adjusted analyses are adjusted for age, sex, body mass index, systolic blood pressure, diabetes, smoking, hypertensive medication, total cholesterol/high-density lipoprotein ratio, lipid-lowering medication, and triglycerides. Bolded values reached statistical significance ($P < \text{at least } 0.05$). Abbreviations as in Tables 1 and 2.		



targeted approach, evaluating 76 sphingolipid species in comparison to our highly targeted evaluation of the prespecified ratio of 2 ceramides.¹⁰ The study²² that evaluated 76 sphingolipid species in patients with HFpEF in TOPCAT found that plasma C16:0 (and C18:0) concentrations were associated with death or HF hospitalization.²² C24:0/C16:0 in that study was not evaluated nor was the composite TOPCAT endpoint, differing from our study's prespecified endpoint; therefore, results from the 2 studies cannot be directly compared but are complementary. Our prespecified ceramide ratio was based on our previous work that showed that low plasma C24:0/C16:0 correlated with prevalent CVD, *incident* HF, and all-cause mortality.¹⁰ Another study also implicated plasma C16:0 in increased *incident* HF risk.²³

The ratio of plasma C24:0/C16:0 concentration in the TOPCAT samples also significantly related to a prespecified secondary outcome: HF hospitalization. HF hospitalization is an important as it is associated with an increased risk of readmission, mortality²⁴ and has a substantial economic cost: \$11 billion in 2014.²⁵ Thus, the predictive utility of C24:0/C16:0 for patients with HFpEF could potentially help clinicians direct increased efforts and advanced interventions toward their most at-risk patients.

Low plasma C24:0/C16:0 also independently related to increased risk of CVD death and all-cause

death in SHIP but not TOPCAT. A longer-term follow-up for SHIP, different cohort size, and/or other factors, likely contributed to the significant finding in SHIP but not TOPCAT. However, in both studies, the findings showed the same *inverse* correlation between plasma C24:0/C16:0 and CVD death, and in SHIP, this inverse relationship extended to all-cause mortality. This predictive utility for CVD and all-cause death, also is consistent with findings from our previous study¹⁰ that showed a low C24:0/C16:0 independently associated with higher CVD death and all-cause mortality rates in 2 large community-based cohorts (not HFpEF patients).

Stroke and MI were exploratory endpoints. An inverse association was seen with plasma C24:0/C16:0 and stroke risk in the larger SHIP cohort with longer follow-up, but this relationship did not meet significance in the TOPCAT group. This dovetails with a previous study's finding of an inverse relationship between low plasma C24:0/C16:0 and increased dementia risk and more white matter hyperintensity burden on magnetic resonance imaging (often caused by microvascular disease).²⁶ The lack of a significant independent relationship between plasma C24:0/C16:0 and MI risk in multivariable analyses in both groups is somewhat surprising given that *incident* coronary heart disease was less likely in the Framingham and SHIP cohorts.¹⁰ However, the current study evaluated secondary events in a cohort with previously established HFpEF rather than community-based cohorts. Second, "coronary heart disease," in Framingham and SHIP, encompassed more than MIs.¹⁰ Other patient factors, including country of origin, may also have led to a difference between the coronary heart disease results in TOPCAT and in Framingham/SHIP.¹⁰

In addition to the significance of identifying a new prognostic biomarker, the implications of improving our understanding of the pathophysiologic relationships between specific ceramides and HFpEF worsening are profound. Other studies in HF with reduced ejection fraction have shown that serum C16:0 (and 5 other ceramides) is increased in patients with HF and that after myocardial unloading, C16:0 decreases and C24:0 is increased.²⁷ A recent review detailed several pathophysiologic mechanisms by which C16:0 and C24:0 and others, may be involved in, or cause of cardiac dysfunction including: apoptosis, mitochondrial dysfunction, cardiac metabolism and inflammation, calcium handling, and ion channel function changes.²⁸ Interestingly, C24:0 appears to counteract mitochondrial pore formation, cytochrome C release, and apoptosis associated with C16:0.²⁸ In short, there are several pathways of interest that are ripe for

further investigation into the links between C24:0/C16:0 and HF outcomes.

STUDY LIMITATIONS. Our findings should be considered in the context of several potential limitations. First, our cohorts were predominantly White. Our findings cannot be extrapolated to other racial groups, and additional studies of plasma C24:0/16:0 ceramide concentrations should be examined independently in adequately powered, racially diverse cohorts. It should also be noted that the mechanism of the association between plasma C24:0/16:0 ceramide concentrations and outcomes in TOPCAT is not thoroughly understood and caution is warranted in attributing lower plasma C24:0/16:0 ceramides as directly causing the worse outcomes. Lastly, while our primary analysis was highly statistically significant, our secondary analyses were not adjusted for multiple comparisons.

CONCLUSIONS

We identified a novel biomarker that is prognostic for secondary events for individuals with HFpEF. This association with the composite endpoint, and for HF hospitalization in specific, was robust and remained statistically significant even after multivariable adjustment. Findings from a second community-based cohort extended the finding of the significant associations between plasma C24:0/C16:0 ratio to CVD death and all-cause mortality for patients with HFpEF. Further characterization of the mechanisms by which plasma C24:0/16:0 ceramides are associated with improved outcomes in subjects with HFpEF may identify novel therapeutic targets and/or interventions for those with this disease.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: This study's results suggest that plasma ceramide C24:0/C16:0 concentrations are a robust marker of the combined endpoint of HF hospitalization and CVD death in patients with HFpEF. Plasma C24:0/C16:0 independently associated with the combined endpoint and HF hospitalization alone. In a community-based cohort, low plasma C24:0/C16:0 also independently associated with CVD death and all-cause mortality in patients with HFpEF. Thus, plasma C24:0/C16:0 may prove to be a useful tool for the management of patients with HFpEF and may point to novel targetable pathophysiologic pathways, including possible modulation of ceramide synthases.

TRANSLATIONAL OUTLOOK: Based on these results, future studies should prospectively evaluate the utility of plasma C24:0/C16:0 concentrations for the prediction of outcomes and hospitalization in patients with HFpEF. Additional prospective studies are also needed to determine how plasma C24:0/C16:0 concentrations may be best combined with other current predictive measures to personalize in improve prognostication in patients with HFpEF.

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APPENDIX For supplemental tables and figures, please see the online version of this paper.