ORIGINAL RESEARCH

Association of Circulating Ceramides With Cardiac Structure and Function in the Community: The Framingham Heart Study

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Background—A higher circulating plasma ceramide ratio (C16:0/C24:0) is associated with an increased risk of heart failure, even after accounting for standard risk factors including lipid markers. However, the pathobiological mechanisms that underlie this association are incompletely understood. We tested the hypothesis that plasma ceramide ratio (C16:0/C24:0) is associated with adverse cardiac remodeling in the community.

Methods and Results—We evaluated 2652 Framingham Offspring Study participants (mean age, 66±9 years; 55% women) who attended their eighth examination cycle and underwent routine echocardiography and liquid chromatography–tandem mass spectrometry–based assays for circulating ceramide concentrations. We used multivariable linear regression models to relate C16:0/C24:0 (independent variable) to the following echocardiographic measures (dependent variables; separate models for each): left ventricular mass, left ventricular ejection fraction, left atrial emptying fraction, left atrial end-systolic volume, E/e′ (a measure of left ventricular diastolic function), and left ventricular global circumferential and longitudinal strain by speckle-tracking echocardiography. In multivariable-adjusted analyses, higher C16:0/C24:0 per standard deviation increment was associated with lower left ventricular ejection fraction (0.991-fold change in left ventricular ejection fraction; P=0.0004), worse global circumferential strain (β=0.34, P=0.004), higher left atrial end-systolic volume (β=2.48, p<0.0001), and lower left atrial emptying fraction (0.99-fold change; P<0.0001). The C16:0/C24:0 ratio was not associated with either E/e′ or global longitudinal strain, and the association with higher left ventricular mass was rendered statistically nonsignificant upon correction for multiple comparisons.

Conclusions—Our cross-sectional observations in a large community-based sample are consistent with a potential detrimental impact of higher ceramide ratio (C16:0/24:0) on cardiac remodeling traits, which may partly explain the associations of these molecular species with clinical heart failure. **(*J Am Heart Assoc*. 2019;8:e013050. DOI: 10.1161/JAHA.119.013050.)**

Key Words: ceramides • lipids and lipoproteins • cardiac remodeling • left ventricle • left atrium • cardiac function

Heart failure (HF) remains a leading cause of morbidity and mortality, affecting 6.5 million Americans, and its prevalence is expected to rise with the aging of the US population.[1] Given the substantial and rising burden of HF, ongoing efforts are needed to uncover novel mechanistic pathways that may underlie disease pathogenesis and progression. One area of recent interest is the role of plasma ceramide biomarkers in the pathogenesis of cardiovascular disease (CVD), including HF. Ceramides are bioactive lipids with a sphingoid base and a fatty acyl chain that are present in cell membranes and plasma, and have major influences on cellular signaling, differentiation, senescence, and programmed cell death.[2] Ceramide synthases facilitate variable acetylation of the sphingoid base, producing a spectrum of ceramide molecular species.[2] Expression and biomolecular effects of ceramides can vary on the basis of detectable relative proportions of distinct circulating ceramide

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An accompanying Figure S1 is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.013050

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Received April 23, 2019; accepted August 21, 2019.

Clinical Perspective

What Is New?

- We used a high-throughput liquid chromatography–mass spectrometry assay to quantify ratios of ceramide molecular species in the plasma and subsequently assessed their relations to echocardiographic measures of cardiac remodeling.
- Data from the Framingham Offspring Study demonstrate that higher plasma ceramide ratio (C16:0/24:0) is associated with potentially detrimental changes in echocardiographic measures of cardiac structure and function.

What Are the Clinical Implications?

- Our study observations support the notion that the association between higher ceramide ratio (C16:0/24:0) and the subsequently increased risk of clinical heart failure may be partly explained by relations with unfavorable subclinical cardiac structural and functional alterations.

species and the CVD risk profile.[3] Recent studies have focused, therefore, on analyzing the ratio of select ceramides, such as the ratio of long-chain to very-long-chain ceramide species.[4,5] In the FHS (Framingham Heart Study), higher circulating levels of ceramide 16:0 relative to ceramide 24:0 were associated with an increased HF risk even after adjusting for standard CVD risk factors, including blood lipid levels.[5] These findings have been supported recently by a report from the CHS (Cardiovascular Health Study), which noted that lower circulating concentrations of fatty acids with 24 carbons and no unsaturated bonds, was associated with a higher risk of HF.[6] However, the mechanisms underlying these relations are incompletely understood. In this context, it is widely accepted that antecedent alterations in cardiac structure and function (cardiac remodeling) antedate the onset of overt HF.[7,8]Accordingly, we investigated the association between the plasma ceramide ratio (C16:0/C24:0) and echocardiographic measures of cardiac structure and function in a large community-based sample where both sets of measures were contemporaneously assessed.

Methods

Study Design and Participant Selection

The objectives and study design of the FOS (Framingham Offspring Study) have been published previously.[9] Briefly, offspring of the original FHS cohort and their spouses were enrolled in the FOS in 1971, and participants have been evaluated approximately every 4 years. Of the 3021 attendees at the eighth examination cycle (2005–2008), we excluded participants who did not have plasma samples for ceramide quantification (n=177) and those with missing covariates (n=192), yielding an analytic sample size of 2652 study participants who underwent routine transthoracic echocardiography and phlebotomy to obtain plasma samples for targeted measurement of circulating ceramide species. The institutional review boards at the Boston University Medical Center and the Washington University School of Medicine, St. Louis, Missouri, approved the study protocols, and all participants provided written informed consent. FOS data and materials in the current study will be made publicly available through the Biologic Specimen and Data Repositories Information Coordinating Center (BioLINCC) of the National Heart Lung and Blood Institute, Bethesda.

Measurement of Plasma Ceramides

The targeted plasma ceramide assay and quantification of C24:0 and C16:0 in the FHS Offspring cohort have been previously reported and demonstrated an excellent reproducibility profile.[5] Briefly, plasma ceramides were obtained using a sensitive, accurate, and high-throughput liquid chromatography–mass spectrometry assay to quantify the long-chain (C16:0) and the very-long-chain (C24:0) ceramides in fasting plasma samples. The coefficients of variation for C16:0 and C24:0 assays were 7.8% and 6.9%, respectively, whereas the interassay and intra-assay accuracy values were within $\pm$3.2% and $\pm$4.9% deviation for C16:0 and C24:0 ceramides, respectively. Ten percent of samples were tested in duplicate for quality assurance purposes.

Covariates

FHS Offspring study participants underwent a detailed medical history, physical examination, and laboratory assessment for CVD risk factors using methods described previously.[10,11] Briefly, systolic and diastolic blood pressure were assessed by an FHS physician, and weight and height by technicians using standardized protocols. Participants were considered to be current smokers if they reported smoking $\geq$1 cigarettes on a daily basis during the year preceding their FHS examination. Assays were performed on fasting biosamples using standard enzymatic methods for blood glucose, serum creatinine, serum total cholesterol, and high-density lipoprotein cholesterol. Estimated glomerular filtration rate was calculated using the chronic kidney disease epidemiology collaboration equation.[12]

Echocardiographic Measures

Echocardiographic assessment in the FHS Offspring Study at the eighth examination cycle has been described previously.[11,13,14] For our investigation, we evaluated the following

echocardiographic measures: left ventricular (LV) mass (LVM), LV ejection fraction (LVEF), left atrial (LA) emptying fraction (LAEF), LA end-systolic volume (LAV_{es}), ratio of mitral inflow velocity to early diastolic mitral annular velocity (E/e′), LV global longitudinal strain (GLS), and global circumferential strain (GCS). Using Digisonics DigiView System Software (version 3.7.9.3; Digisonics Inc, Houston, TX) and digitized images, LV volumes were measured by Simpson's method. LA maximum and minimum volumes were obtained by averaging the respective volumes in apical 2- and 4-chamber views that were measured using the area-length method.[15] Early peak systolic mitral annulus velocity was measured at the lateral mitral annulus using Doppler tissue imaging. Transmitral Doppler flow velocities were recorded using a standardized protocol. Repeated analysis of LV diastolic function measures yielded interobserver correlation with coefficients of >0.97. We used an offline speckle-tracking software package (2D Cardiac Performance Analysis v1.1; TomTec Imaging Systems, Unterschleißheim, Germany) to analyze LV myocardial deformation, including GLS and GCS, according to a standardized protocol with excellent reproducibility.[16] GCS and GLS are markers of myocardial deformation, and more positive values indicate worse systolic function. Lower values of LAEF indicate worse LA function.

Statistical Analyses

Descriptive characteristics of study participants were summarized as mean±SD or as median (25th, 75th percentile) for continuous variables, while categorical variables were summarized using proportions. Skewed echocardiographic measures were transformed by using natural logarithms to normalize their distributions.

We used multivariable linear regression to relate the plasma ceramide ratio C16:0/24:0 (independent variable) to the following echocardiographic measures (dependent variables, separate model for each): LVM, LVEF, GLS, GCS, E/e′, LAV_{es}, and LAEF. Multivariable regression models were adjusted for the following covariates: age, sex, resting heart rate, height, weight, diabetes mellitus, systolic blood pressure, antihypertensive medication use, current smoking, and estimated glomerular filtration rate the ratio of total to high-density lipoprotein cholesterol. We plotted cubic splines with 4 knots at the 5th, 25th, 75th, and 95th percentiles with a reference equal to the median C16:0/C24:0 value (0.07) to visualize the associations between C16:0/24:0 and echocardiography measures. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). A Bonferroni corrected 2-sided $P<0.007$ (0.05/7) was used to indicate statistical significance to account for multiple testing. Authors (V.X. and M.D.) had access to all the data and take responsibility for the integrity of the analyses.

Results

Baseline Characteristics

The baseline clinical, biochemical, and echocardiographic characteristics of our study sample are shown in Table 1. Study participants in our sample had a mean age of 66±9 years, and 48.1% were men. The mean plasma concentration of C16:0 was roughly one tenth as abundant as that of plasma C24:0.

Table 1. Characteristics of Study Sample

Characteristics	N=2652
Age, y	66±9
Men, %	44.8
Height, cm	166.9±10
Weight, kg	78.8±18
Systolic blood pressure, mm Hg	128±17
Diastolic blood pressure, mm Hg	74±10
Use of antihypertensive medication, %	47.5
Heart rate, bpm	59.6±10
Diabetes mellitus, %	3.1
Current smoking, %	9.1
LDL-C, mg/dL	105.8±31
HDL-C, mg/dL	57.7±18
Triglycerides, mg/dL	117.5±69
Total cholesterol, mg/dL	186.6±37
Lipid lowering medication use, %	42.1
eGFR, mL/min/1.73 m^2	77.8±16
Prior heart failure, %	2.38
Plasma ceramide concentrations	
Plasma C16:0 ceramide, μg/mL	0.2±0.04
Plasma C24:0 ceramide, μg/mL	2.3±0.7
Plasma C16/24 ceramide, μg/mL	0.08±0.02
Echocardiographic measures	
LV mass index, g	162.2 (134.5, 162.6)
LV ejection fraction, %	67.3 (62.9, 71.7)
Left atrial emptying fraction, %	48 (46, 50)
Left atrial end-systolic volume, mL	56.8±19.3
LV global longitudinal strain, %	−20.6±3
LV global circumferential strain, %	−31.9±6
E/e′	6.6 (5.5, 8.1)

Values are mean±standard deviation, median (Q1, Q3), or percentage. E/e′ indicates mitral inflow velocity to early diastolic mitral annular velocity; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LV, left ventricular.

Circulating Ceramide Ratio Cardiac Structure and Function

Results from multivariable linear regression models relating ceramide ratio to echocardiographic traits are shown in Table 2. In multivariable-adjusted models, higher ceramide ratio C16:0/C24:0 was associated with lower LVEF, worse (more positive) LV GCS, lower LAEF, higher LAV_{es}, and a higher LVM, although the association with LVM was no longer statistically significant after adjustment for multiple comparisons. C16:0/24:0 ratio was not associated with either E/e′ or GLS. Figure S1 shows the least squared mean plots per tertile of C16:0/24:0 ratio.

In the Figure, restricted cubic splines illustrating the association between C16:0/24:0 ratio and echocardiography measures (LAEF, GCS, LVM, and LVEF) are shown. Higher C16:0/24:0 ratio above the set reference level showed an approximately linear relation with LAEF, LVM, and LVEF, whereas the association with GCS was monotonic.

Discussion

Principal Findings

In a large community-based sample, we observed that a higher circulating plasma C16:0/C24:0 ceramide ratio was cross-sectionally associated with alterations in cardiac structure and function, including higher LAV_{es}, lower LV systolic function (determined by LVEF and LV GCS), lower LA function (assessed by LAEF), and a propensity toward an association with LVM upon correction for multiple comparisons. However, a higher C16:0/C24:0 ceramide ratio was not significantly associated with either E/e′ or GLS. Our findings raise the possibility that the association of higher circulating ceramide ratio (C16:0/C24:0) with an increased HF risk may in part be mediated by the association between specific ceramide species and measures of LV as well as LA remodeling.

Table 2. Ceramide Ratio (C16:0/24:0) and Cardiac Structure and Function

Echocardiography Indices	Beta Estimate (Standard Error)	e^{β}	*P* Value
LV ejection fraction*	−0.009 (0.002)	0.991	0.0004†
LV global circumferential strain	0.34 (0.12)	NA	0.004†
LV global longitudinal strain	0.07 (0.06)	NA	0.26
E/e′*	0.006 (0.005)	1.006	0.26
Left atrial emptying fraction*	−0.008 (0.002)	0.992	<0.0001†
Left atrial end-systolic volume	2.48 (0.4)	NA	<0.0001†
LV mass*	0.009 (0.004)	1.009	0.02

Multivariable linear regression showing the relation between C16:0/C24:0 and echocardiographic measures (dependent variable). Beta estimates represent change in echocardiography variable per 1 SD increment in ceramide ratio. Models were adjusted for age, sex, heart rate, height, weight, diabetes mellitus, systolic blood pressure, antihypertensive medication use, current smoking, estimated glomerular filtration rate, and the ratio of total to high-density lipoprotein cholesterol. E/e′ indicates mitral inflow velocity to early diastolic mitral annular velocity; LV, left ventricular; NA, not applicable.
*These dependent variables were natural logarithmically transformed to satisfy the normality assumption of linear regression models. In these models e^{β} represents the fold change in Y per standard deviation increase in C16:0/C24:0. More positive global strain values signify worse LV systolic function.
†Denotes associations that retain statistical significance following a Bonferroni correction for multiple testing.

Comparison With Published Literature

To our knowledge, this is the first investigation to explore the relations between plasma ceramide concentration ratios and cardiac structure and function in a community-based sample. Circulating ceramide species are less abundant than cholesterol in plasma and have been challenging to assay precisely in the past. In recent years, the advent of liquid chromatography–mass spectrometry techniques has facilitated the more accurate measurement of several plasma ceramide molecular species. It is now increasingly recognized that distinct ceramide acyl chain lengths play important roles in biophysical properties of membranes, cell signaling, and apoptosis, with long-chain and very-long-chain ceramides (including C16:0 and C24:0) being the predominant ceramides in mammalian tissues.[17–19] C16:0 promotes programmed cell death by a cascade of events involving ceramide-mediated channels and a loss of mitochondrial outer membrane integrity that facilitate the release of cytochrome c and other proapoptotic proteins into the cytoplasm, ultimately resulting in mitochondria-induced apoptosis.[2,18] In contrast, C24:0 interferes with C16:0 ceramide-associated channel formation in the mitochondria and has predominantly antiapoptotic effects.[20] These species are also linked by intermediary metabolism enzymatic pathways.[20] For example, in cells with selective knockdown of ceramide synthetase-2 (which catalyzes the generation of very-long-chain ceramides, including C24:0) there was near elimination of C24:0 ceramide and higher C16:0 ceramide levels.[18] Furthermore, the concentration or expression of plasma ceramides and their resultant effects are modified by prevalent CVD risk burden such as diabetes mellitus and smoking.[4,5] To mitigate these influences, rather than utilizing total ceramide levels, a ceramide ratio consisting of the indexation of the long-chain ceramide species by very-long-chain ceramide species such as C16:0/24:0 ratio has emerged as a marker that provides greater information than either species alone in ceramide-related analysis.[3,4] Multiple lines of evidence support the association of alterations in concentrations of plasma ceramide species with adverse cardiovascular outcomes and mortality in patients with prior CVD[6,21–23] and in asymptomatic community-dwelling individuals.[5] These studies demonstrate the additional prognostic value of specific plasma ceramides for predicting CVD risk beyond standard CVD risk factors, including plasma lipid levels.[5] In one study of individuals with coronary artery

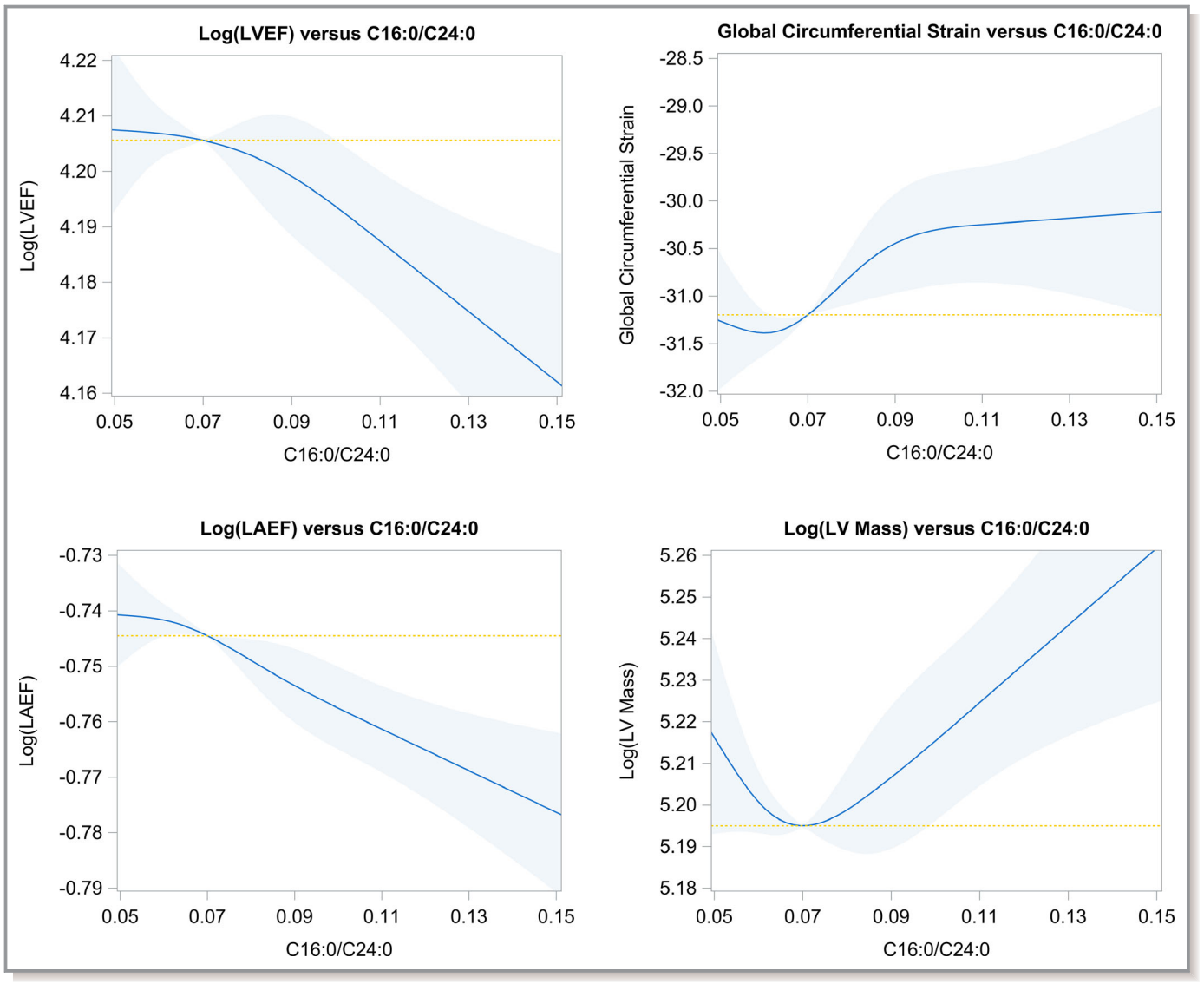

Figure. Restricted cubic splines plots showing the relations between ceramide ratio and LVEF, LAEF, global circumferential strain, and LV mass. Shaded areas represent the 95% CIs. The reference value in each panel is set to the median C16:0/C24:0 ratio of 0.07. The yellow hashed line serves as a reference line of "no association." LAEF indicates left atrial emptying fraction; LV, left ventricular; LVEF, left ventricular ejection fraction.

disease, there were more pronounced differences in circulating ceramide profiles than in the standard lipid markers when subjects who died during follow-up were compared with those who survived.[4] Elevated plasma total ceramide levels have also been reported to be associated with New York Heart Association functional classes in individuals with HF and reduced ejection fraction.[24] Similarly, in patients with HF, LV assist device placement reduced the detectable level of total ceramides present in the myocardium.[25] In the CHS, a lower plasma level of the 24:0 fatty acid, lignoceric acid, was also associated with a higher risk of HF, which is consistent with data from experimental studies.[6] Similarly, in the FHS, we previously reported that a higher level of C16:0 in relation to C24:0 was associated with an increased risk of incident HF. The present investigation is consistent with these findings and importantly offers additional insight into underlying mechanisms by demonstrating an association of higher circulating ceramide ratio (C16:0/C24:0) with adverse cardiac structural and functional alterations. Antecedent subclinical impairment in LV strain by speckle-tracking echocardiography, LVEF, LV mass, and LA function have been widely established as strong independent predictors of future HF, CVD, and all-cause mortality.[7,13,14] In the present study, there was no association between C16:0/C24:0 and E/e′ (a surrogate for LV diastolic dysfunction). The reason for this observed lack of association with E/e′ is not entirely clear but may be partly related to the

narrow distribution of E/e′ ratio in our relatively healthy sample with a median E/e′ of 6.6 (which is well below thresholds for LV diastolic dysfunction).[26] Although there was no association of the ceramide ratio with E/e′, we observed statistically strong associations between C16:0/C24:0 and higher LAV_{es} and lower LAEF—a sensitive marker for subclinical LA remodeling that may identify individuals at high risk for HF independent of LVEF.[27,28] Furthermore, we observed associations between C16:0/C24:0 and GCS but not with GLS. It is conceivable that distinct aspects of myocardial deformation may have differential associations with various biomarkers of CVD risk.[14] Our observation of an association with GCS but not with GLS may suggest potential involvement of mesocardial more than endocardial myocardial function, a speculative premise that warrants further investigation.

Potential Mechanisms

There are several plausible mechanisms that support a relation between the circulating C16:0/C24:0 and subclinical cardiac remodeling and dysfunction. Sphingosine-1-phosphate, a signaling molecule formed by the phosphorylation of sphingosine, is interconvertible with ceramides.[29] Sphingosine-1-phosphate can function to inhibit apoptosis and is also reported to suppress the proapoptotic effects of ceramides.[29,30] In porcine models, administration of sphingosine-1-phosphate receptor agonist reduced post–myocardial infarction LV remodeling and improved LV systolic function.[31] Sphingosine-1-phosphate is also a constituent of high-density lipoprotein and a contributor to many of the beneficial effects of high-density lipoprotein on the heart and circulatory system.[32]

Animal and human studies have shown that total ceramides, and C16:0 in particular, are related to markers of inflammation (like interleukin-6), insulin resistance, and diabetes mellitus through activation of inflammatory mediators such as tumor necrosis factor-α as well as adiponectin receptor–associated ceramidase activity.[33,34] In a dietary study, consumption of a Western diet increased total ceramide content in myocardium with normal geometry and LV hypertrophy.[35]Aging, smoking, and prevalent CVD are also associated with higher ceramide ratio (C16:0/C24:0) in the community.[5] Sphingosine, which is the main constituent of the ceramide backbone, has been reported to mediate the negative inotropic effects of tumor necrosis factor-α in adult mammalian cardiomyocytes.[36] In in vitro and murine studies, exogenous cell-permeable ceramide can induce cardiomyocyte apoptosis and the inhibition of serine palmitoyl–coenzyme A transferase—which is vital for ceramide biosynthesis—mitigated atherosclerosis development.[36,37] Overall, these findings suggest that ceramides may play a contributory role in the cardiometabolic dysfunction that precedes cardiac remodeling and overt HF.

Strengths and Limitations

The large, well-characterized community-based sample; standardized measurement of echocardiographic measurements; and contemporaneous assessment of ceramides with liquid chromatography–mass spectrometry–based state-of-the art assays strengthen our investigation. Our study sample was mainly composed of middle-aged to elderly white men and women of predominantly European ancestry; therefore, the generalizability of our findings to other age or racial groups is uncertain. Our cross-sectional study design precludes any causal inferences.

Conclusions

In our cross-sectional investigation of a moderate-sized community-based sample, we observed that a higher ceramide ratio (C16:0/C24:0) was associated with potentially unfavorable subclinical cardiac structural and functional alterations, which may represent an important underlying pathophysiological mechanism linking circulating ceramide species to HF risk in the community.

Sources of Funding

This work was supported by grants P20 HL113444 P30 DK020579, from the National Institutes of Health, and contracts N01-HL25195 and HHSN268201500001I (Dr Vasan) from the National Heart, Lung, and Blood Institute and National Institutes of Health grants HL080124, HL071039, HL077447, HL107385, 1R01HL126136, 5R01HL107385, 1R01HL60040, 1RO1HL70100, R01HL131532, and R01HL134168. Dr McManus's time was supported by grants R01HL126911, R01HL 137734, R01HL137794, R01HL13660, and R01HL141434, also from the National Heart, Lung, and Blood Institute. Dr Peterson's time was supported by grants from the NIH R34 HL138253-01, 1R01AG060499-01, and a grant from the Barnes Jewish Hospital Foundation.

Disclosures

Dr Vasan is supported in part by the Evans Medical Foundation and the Jay and Louis Coffman Endowment from the Department of Medicine, Boston University School of Medicine. Dr Cheng reports receiving consulting fees from Zogenix, unrelated to the submitted work. Dr McManus has received research support from Apple Computer, Bristol-Myers Squibb, Boehringher-Ingelheim, Pfizer, Samsung, Philips Healthcare, and Biotronik; has received consultancy fees from Bristol-Myers Squibb, Pfizer, Flexcon, Boston Biomedical Associates, and Samsung; and has inventor equity in Mobile Sense Technologies, Inc (CT).

Dr Mitchell is the president of Cardiovascular Engineering, Inc, a company that designs and manufactures devices that measure vascular stiffness. Dr Peterson serves as a consultant to and receives honoraria and grant support from Novartis and Servier. Dr Peterson consults for Radius Pharmaceuticals, unrelated to the submitted work. A patent application for use of the ceramide biomarkers is pending (Drs Peterson, Duncan, Vasan, and Xanthakis).

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SUPPLEMENTAL MATERIAL

Figure S1. Least squared mean plots per tertile of C16/24 ratio for: Left ventricular (LV) global circumferential strain; LV mass; Left atrial emptying fraction; LV ejection fraction Models were adjusted for the following covariates: age, sex, heart rate, height, weight, diabetes, systolic blood pressure, antihypertensive medication use, current smoking, estimated glomerular filtration rate, and total/HDL cholesterol ratio.

Per tertile of C16:0/24:0 ratio there was a linear trend towards worse GCS and lower LAEF. In contrast, there was a non-linear trend between LVM and C16:0/24:0. A propensity towards a trend was observed (albeit non-significant) of lower LVEF per tertile of C16:0/24:0 ratio.