Kidney Medicine

High-Density Lipoprotein Lipidomics and Mortality in CKD

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Rationale & Objective: Patients with chronic kidney disease (CKD) have dysfunctional high-density lipoprotein (HDL) particles that lack cardioprotective properties; altered lipid composition may be associated with these changes. To investigate HDL lipids as potential cardiovascular risk factors in CKD, we tested the associations of HDL ceramides, sphingomyelins, and phosphatidylcholines with mortality.

Study Design: We leveraged data from a longitudinal prospective cohort of participants with CKD.

Setting & Participants: We included participants aged greater than 21 years with CKD, excluding those on maintenance dialysis or with prior kidney transplant.

Exposure: HDL particles were isolated using density gradient ultracentrifugation. We quantified the relative abundance of HDL ceramides, sphingomyelins, and phosphatidylcholines via liquid chromatography tandem mass spectrometry (LC-MS/MS).

Outcomes: Our primary outcome was all-cause mortality.

Analytical Approach: We tested associations using Cox regressions adjusted for demographics, comorbid conditions, laboratory values, medication use, and highly correlated lipids with opposed effects, controlling for multiple comparisons with false discovery rates (FDR).

Results: There were 168 deaths over a median follow-up of 6.12 years (interquartile range, 3.71-9.32). After adjustment, relative abundance of HDL ceramides (HR, 1.22 per standard deviation; 95% CI, 1.06-1.39), sphingomyelins with long fatty acids (HR, 1.44; 95% CI, 1.05-1.98), and saturated and monounsaturated phosphatidylcholines (HR, 1.22; 95% CI, 1.06-1.41) were significantly associated with increased risk of all-cause mortality (FDR < 5%).

Limitations: We were unable to test associations with cardiovascular disease given limited power. HDL lipidomics may not reflect plasma lipidomics. LC-MS/MS is unable to differentiate between glucosylceramides and galactosylceramides. The cohort was comprised of research volunteers in the Seattle area with CKD.

Conclusions: Greater relative HDL abundance of 3 classes of lipids was associated with higher risk of all-cause mortality in CKD; sphingomyelins with very long fatty acids were associated with a lower risk. Altered lipid composition of HDL particles may be a novel cardiovascular risk factor in CKD.

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Patients with chronic kidney disease (CKD) experience greater risks of cardiovascular diseases and death compared with the general population.[1] Kidney disease leads to a unique pattern of dyslipidemia characterized by relatively high concentrations of triglycerides and an accumulation of small, dense low-density lipoprotein (LDL) particles; these abnormalities are generally less responsive to conventional treatments for dyslipidemia.[2-5] Patients with CKD also have dysfunctional high-density lipoprotein (HDL) particles that lack anti-inflammatory and cholesterol efflux capabilities typically appreciated in healthy individuals.[6,7] The molecular derangements underlying these deficiencies have been incompletely characterized; it is possible that changes in relative abundance of various HDL lipid molecules may be at least partially culpable for the abnormal function of HDL in CKD. Specifically, several lipid classes (including ceramides and sphingomyelins) are present on the surface of HDL and are thought to serve important signaling and regulatory roles.[8,9] It is possible that lipid alterations noted in patients with CKD contribute to cardiovascular risk, including mortality.

Recently, whole-plasma lipidomics have allowed the characterization of small lipid molecules, going beyond traditional metrics, such as lipoprotein cholesterol content and total triglycerides. These techniques have enabled quantification of individual lipids and lipid classes found in HDL particles to test their associations with health outcomes. For instance, higher levels of plasma ceramides and sphingomyelins with specific long acylated fatty acids are associated with atrial fibrillation, heart failure, and all-cause mortality in the general population.[10-12] As a first step to determine if HDL lipid composition may be a novel intervenable risk factor for cardiovascular disease in CKD, this study aimed to determine if HDL lipid composition was a risk factor for all-cause mortality in a longitudinal CKD cohort. We used targeted lipidomics to characterize the surface lipid composition of HDL particles. We hypothesized that higher concentrations of ceramides, sphingomyelins with acylated fatty acids containing 14-18

PLAIN-LANGUAGE SUMMARY

Patients with chronic kidney disease have abnormal high-density lipoprotein (HDL) particles that lack the beneficial properties associated with these particles in patients with normal kidney function. To investigate if small lipid molecules found on the surface of HDL might be associated with these changes, we tested the associations of lipid molecules found on HDL with death among patients with chronic kidney disease. We found that several lipid molecules found on the surface of HDL were associated with increased risk of death among these patients. These findings suggest that lipid molecules may be risk factors for death among patients with chronic kidney disease.

carbons and saturated and monounsaturated phosphatidylcholines would be significantly associated with all-cause mortality.

METHODS

Study Population

We used data from the Seattle Kidney Study, which began in 2004 and recruited 693 individuals with CKD from outpatient nephrology clinics in Seattle, Washington.[13] Inclusion criteria were age ≥ 18 years and a diagnosis of CKD defined by either an estimated glomerular filtration rate (eGFR) ≤ 90 mL/min/1.73 m^2 or a urinary albumin-creatinine ratio ≥ 30 mg/g. Exclusion criteria included receipt of maintenance dialysis at baseline, expectation of dialysis initiation within 3 months, prior kidney transplantation, or the inability to provide informed consent. All participants provided written informed consent, and the study protocol was approved by the Institutional Review Board at the University of Washington (approval number STUDY00001067). For this study, we excluded 192 participants without complete baseline HDL lipidomic data and 3 participants without follow-up data; our analytic cohort included 498 participants.

HDL Isolation and Lipid Measurement

We evaluated individual the relative abundance of lipids found on the surface of HDL from the following classes: ceramides, sphingomyelins, and phosphatidylcholines. For each ceramide and sphingomyelin, we assumed the presence of the most common d(18:1) backbone; we reflect this by omitting the backbone chain (ie, sphingomyelin d(18:1)16:0, sphingomyelin with a 16-carbon, 0 double-bond fatty acid, is called sphingomyelin 16:0 throughout). The ceramides included ceramides 22:0, 24:0, and 24:1; hexosylceramides 16:0, 22:0, and 24:0; and lactosylceramide 16:0. The sphingomyelins measured included sphingomyelins with long acylated fatty acids (sphingomyelins 14:0, 16:0, and 18:0) and very long acylated fatty acids (sphingomyelins 20:0, 22:0, and 24:0). The phosphatidylcholines measured were phosphatidylcholine 28:0, 28:1, 29:0, 30:1, 30:2, 32:1, 32:2, 33:3, 34:1, 34:2, 35:4, 35:5, 36:1, 36:2, 36:3, 36:4, 36:5, 38:1, 38:2, 38:3, 38:4, 38:5, 40:5, and 40:6.

Complete details of the purification of HDL particles in this cohort have been previously published.[9] Briefly, the HDL fraction of baseline fasting ethylenediaminetetraacetic acid (EDTA) plasma (density 1.063-1.210 g/mL) was purified by a 2-step density gradient ultracentrifugation process utilizing potassium bromide. First, all lipoproteins were floated using a 1.210 g/mL potassium bromide solution and were transferred to a new tube. Second, all lipoproteins less dense than HDL were floated using 1.063 g/mL potassium bromide; the lipoproteins at the bottom of each sample after this second step were subsequently dialyzed and frozen at −80 °C before use. HDL lipids (from 10 ug HDL protein assayed by Bradford) were isolated using an organic protein precipitation method with analog internal standards, as previously described.[14]

Each sample was analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS) on a Sciex 6500. Peak areas for each endogenous lipid were normalized to the peak areas from isotope-labeled internal standards added before lipid extraction/protein precipitation with organic solvent. The internal standards used to normalize each endogenous lipid are listed in Table S1. This calculated ratio of endogenous to internal standard peak area for each lipid in each sample (peak area ratio) was then standardized to peak area ratios from calibrator samples included in each extraction batch to reduce inter-batch variability. This standardized peak area ratio is therefore a relative abundance of each lipid in the sample. Because an aliquot containing 10 μg of HDL protein (^{15}N ApoA-I) was extracted for each sample, the standardized peak area ratio is the relative abundance of each lipid per 10 μg of HDL protein.

Follow-up and Determination of All-Cause Mortality

The primary outcome was all-cause mortality. Study staff contacted participants by telephone every 6 months and in person annually. Each participant was followed until death, loss to follow-up, withdrawal of consent, or the end of administrative follow-up in December 2018, whichever came first. Study staff assessed for all-cause mortality using review of the medical records, contact with family members, and the national social security death index.

Covariates

At the initial study visit, participants provided self-reported information on their sociodemographic characteristics, medical history, medication usage, and lifestyle behaviors. Self-reported race and ethnicity were categorized as non-Hispanic White, non-Hispanic African American, Asian or Pacific Islander, American Indian or Native Alaskan, Hispanic, and other (including "African," "Armenian," "Egyptian," several European ethnicities, various

combinations of the above races and ethnicities, and several nonresponders). Serum concentrations of creatinine were measured on a Beckman-Coulter DxC autoanalyzer and are traceable to isotope dilution mass spectrometry standards. Serum cystatin C concentrations were calibrated by reconstitution of cystatin C reference material (ERM-DAY7/IFCC) per its Certificate of Analysis. Estimated GFR was calculated from serum creatinine and cystatin C using the 2021 Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI).[15] Spot urine albumin concentrations were measured by immunoturbidimetry and were indexed to urine creatinine. Additional laboratory measurements included total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) measured on the Beckman-Coulter DxC autoanalyzer; low-density lipoprotein cholesterol (LDL-C) was calculated using the typical Friedewald calculation.

Statistical Analysis

Each class of lipids (total ceramides, total sphingomyelins, long sphingomyelins, very long sphingomyelins, total phosphatidylcholines, saturated and monounsaturated phosphatidylcholines, and polyunsaturated phosphatidylcholines) was calculated as the sum of the relative amounts of constituent lipid species for each participant. Baseline characteristics of the study population were tabulated overall and by tertiles of total ceramides, given previous literature suggesting that ceramides may be associated with eGFR.[16,17] Correlation between each lipid species, and between each lipid and total HDL-C, LDL-C, total cholesterol, statin use, and use of other lipid-lowering medications were assessed using Pearson's correlation coefficients; heatmaps of each correlation coefficient were generated. Crude incidence rates of all-cause mortality were calculated across tertiles of lipid classes. For each incidence rate, a 95% confidence interval (CI) was calculated using a nonparametric bootstrap approach with 2000 replicates.[18]

Cox proportional hazards models were fit using lipid classes as the exposure and all-cause mortality (censored as above) as the outcome. We performed a series of Cox regressions with sequential adjustment for potential confounders, as in other cohorts.[10-12] Our first model was unadjusted. Model 1 adjusted for demographic factors, including age and biological sex; medical history, including diabetes, stroke, myocardial infarction, hypertension, heart failure, and waist circumference (as a surrogate for metabolic syndrome); laboratory values, including LDL-C, HDL-C, eGFR, and albuminuria; and medication use (statins and other lipid-lowering medications). In model 2 (our primary model), given high correlation between sphingomyelins and previous literature suggesting opposed risk of mortality and cardiovascular outcomes between sphingomyelins with long acylated fatty acids (associated with increased risk) and very long acylated fatty acids (associated with decreased risk), we additionally adjusted analyses considering sphingomyelins with long acylated fatty acids for sphingomyelin 22:0 and analyses considering sphingomyelins with very long acylated fatty acids for sphingomyelin 16:0. This was in an attempt to understand the "true" association of sphingomyelin classes with mortality after adjusting for other highly correlated species; this approach has been used in multiple previous studies.[10-12] We evaluated for collinearity in these models by calculating variance inflation factors. We controlled for multiple comparisons at a false discovery rate (FDR) < 5%.[19]

As a secondary analysis, we repeated our primary analyses using individual lipids as the exposure. We calculated crude incidence rates of all-cause mortality by tertiles of functional lipid classes, and evaluated associations of each functional lipid class with mortality using Cox regression with adjustment models as described above.

As a sensitivity analysis, given that dialysis requirement is a major risk factor for mortality, we repeated our primary analysis with additional adjustment for time-updated dialysis requirements.

Missingness was low overall (8% for waist circumference, 6% for LDL-C levels, and ≤1% for all other covariates), and all missing covariates were multiply imputed using chained equations with the *mice* package in R.[20] The multiple analyses over imputations were combined using standard Rubin's rules to account for variability in the imputation procedure.[21]

All analyses were performed using R 4.0.2 (R Foundation for Computing).

RESULTS

Characteristics of the Study Population

Among 498 participants, the mean age was 58 (standard deviation [SD], ±14) years. A total of 161 participants (32%) were women. The mean eGFR was 45 (SD, ±24) mL/min/1.73 m^2, and the median albumin-creatinine ratio was 108 (interquartile range [IQR], 16-692) mg/g. In total 50% of participants had diabetes, 25% had a self-reported history of heart failure, and 54% were receiving statins. The mean LDL-C and HDL-C levels were 102 (SD ±44) and 42 (SD ±17) mg/dL, respectively. Compared with participants with lower relative HDL abundance of ceramides, those with higher relative abundance were more often women with fewer comorbid conditions and greater albuminuria and were less frequently taking statins (Table 1).

Correlation Between Lipid Species, Lipoproteins, and Statin Use

All lipid species were positively correlated with each other (Fig S1). The correlation was especially strong between sphingomyelins with correlation coefficients ranging from 0.73-0.97. Additionally, strong correlations were noted among phosphatidylcholines with similar numbers of carbons (eg, phosphatidylcholines 36:1 and 36:2) and

Table 1. Baseline Characteristics Overall and by Tertiles of Total Ceramides

Variable	Overall	Tertile 1 (≤ 5.23 Ceramides)	Tertile 2 (>5.23 Ceramides ≤7.52)	Tertile 3 (>7.52 Ceramides)
N	498	166	166	166
Age, y, mean (SD)	58 (14)	59 (12)	58 (14)	57 (16)
Women, n (%)	161 (32)	42 (25)	57 (34)	62 (37)
Race and ethnicity, n (%)				
Non-Hispanic White	293 (59)	110 (66)	91 (55)	92 (55)
Non-Hispanic African American	112 (22)	27 (16)	43 (26)	42 (25)
Asian or Pacific Islander	40 (8)	13 (8)	14 (8)	13 (8)
American Indian/Native Alaskan	10 (2)	2 (1)	3 (2)	5 (3)
Hispanic	24 (5)	6 (4)	8 (5)	10 (6)
Other	19 (4)	8 (5)	7 (4)	4 (2)
Estimated glomerular filtration rate, mL/min per 1.73 m^2, mean (SD)	45 (24)	45 (24)	45 (25)	45 (24)
Albumin-creatinine ratio, mg/g, median (IQR)	108 (16-692)	97 (19-574)	86 (16-630)	181 (12-1038)
Diabetes mellitus, n (%)	248 (50)	96 (58)	87 (52)	65 (39)
Myocardial infarction, n (%)	83 (17)	39 (23)	28 (17)	16 (10)
Stroke, n (%)	66 (13)	26 (16)	23 (14)	17 (10)
Congestive heart failure, n (%)	125 (25)	46 (28)	42 (25)	37 (22)
Systolic blood pressure, mmHg, mean (SD)	133 (23)	129 (22)	132 (20)	136 (25)
Diastolic blood pressure, mmHg, mean (SD)	76 (14)	74 (13)	77 (13)	77 (15)
Waist circumference, inches, mean (SD)	42 (7)	44 (7)	42 (7)	40 (7)
Current smoker, n (%)	97 (19)	29 (17)	36 (22)	32 (19)
Alcohol use, n (%)	92 (18)	23 (14)	29 (17)	40 (24)
LDL-C, mg/dL, mean (SD)	102 (44)	100 (43)	108 (42)	98 (46)
HDL-C, mg/dL, mean (SD)	42 (17)	36 (12)	42 (14)	47 (21)
Statins, n (%)	271 (54)	111 (67)	87 (52)	73 (44)
Other cholesterol medications, n (%)	35 (7)	12 (7)	13 (8)	10 (6)

Abbreviations: SD, standard deviation; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

among phosphatidylcholines with the same degree of unsaturation (eg, phosphatidylcholines 34:2 and 36:2). Notably, each lipid species was only weakly correlated with total cholesterol, HDL-C, LDL-C, and statin use (correlation coefficients ranging from -0.17 to 0.34).

Association of Lipid Classes with Risk of Mortality

We observed 168 deaths over a median follow-up time of 6.12 (IQR, 3.71-9.32) years. Over this duration of follow-up, 79 participants developed kidney failure requiring either dialysis initiation or kidney transplant. The unadjusted incidence rates (IR) of mortality were lowest among the lowest tertiles of relative HDL abundance of ceramides and sphingomyelins acylated to long fatty acids and at the highest tertile of sphingomyelins with very long acylated fatty acids (Fig 1).

When modeled continuously as standard deviations of relative abundance, total ceramides were associated with a 1.22-fold higher risk for mortality (95% CI, 1.06-1.39) after adjusting for the components of model 1. After adjusting for the components of model 1 and relative HDL abundance of sphingomyelin 22:0, sphingomyelins with long acylated fatty acids (14-18 carbons) were associated with a 1.44-fold higher risk for mortality per 1-SD increase (95% CI, 1.05-1.98). Saturated and monounsaturated phosphatidylcholines were associated with a higher risk for mortality (HR per 1-SD increase 1.22; 95% CI, 1.06-1.41) after adjustment as above (Table 2). These associations were all significant at an FDR < 5%. The *P* values for the assumption of proportional hazards were >0.05 in all models, suggesting no violation. Variance inflation factors were <5 in all models, suggesting no significant multicollinearity. Associations between lipid classes and mortality were attenuated in unadjusted models.

Secondary Analysis: Association of Individual Lipids with Risk of Mortality

As demonstrated in Fig 2, we observed a graded higher IR of all-cause mortality across higher tertiles of ceramide 24:1 and phosphatidylcholine 30:1. We observed a graded lower IR of all-cause mortality across higher tertiles of sphingomyelin 22:0.

In unadjusted models, higher ceramide 24:1 was significantly associated with risk of all-cause mortality

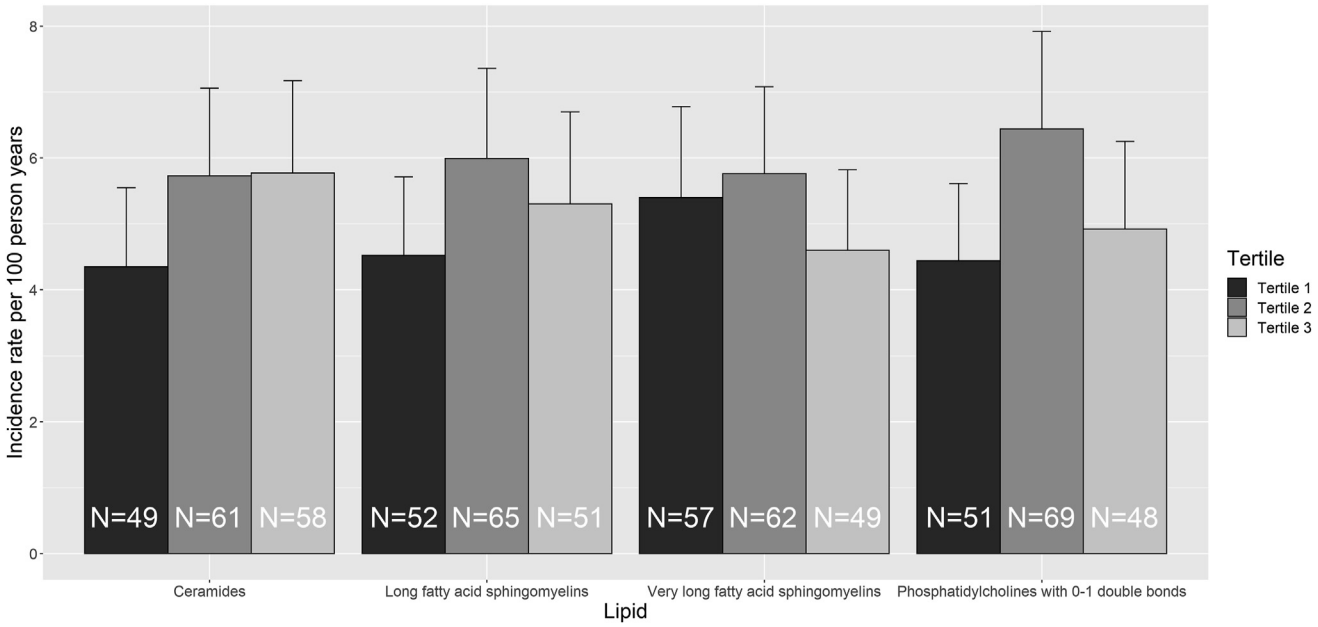

Figure 1. Unadjusted incidence rate of mortality by tertiles of various lipid classes. N indicates the number of mortality events in each tertile.

(Table 3). In model 2 (adjusted for demographics, medical history, laboratory parameters, and medication use and additionally adjusting sphingomyelins 14:0-18:0 for sphingomyelin 22:0 and sphingomyelins 20:0-24:0 for sphingomyelin 16:0), all 7 ceramides, 2 of 6 sphingomyelins, and 10 of 24 phosphatidylcholines were associated with mortality. However, only ceramides 22:0 and 24:1, hexosylceramide 16:0, sphingomyelin 16:0, and phosphatidylcholines 30:1, 34:1, and 38:2 were significantly associated with mortality at an FDR < 5%. The strongest association was for sphingomyelin 16:0 (HR for all-cause mortality per 1-SD increase 1.63; 95% CI, 1.22-2.19). The point estimates for the associations of ceramide 24:1, hexosylceramide 16:0, and phosphatidylcholines 30:1, 34:1, and 38:1 with mortality ranged from 1.19 to 1.26 (Table 3, Fig 3).

Sensitivity Analysis

In models additionally adjusting for time-updated dialysis needs, total HDL ceramides, sphingomyelins, and phosphatidylcholines remained significantly associated with risk for all-cause mortality (Table S2). All 7 ceramides and 14 of 24 phosphatidylcholines were significantly associated with mortality; however, no sphingomyelins were

Table 2. Associations Between Classes of Lipids and All-Cause Mortality in Patients with CKD (N=498)

Lipid Class	Unadjusted HR (95% CI)	Model 1 HR[e] (95% CI)	Model 2 HR[f] (95% CI)	*P* Value (Model 2)
Ceramides	1.15 (1.02-1.31)	1.22 (1.06-1.39)*	1.22 (1.06-1.39)*	0.004*
Sphingomyelins	1.05 (0.91-1.20)	1.16 (1.02-1.33)*	1.16 (1.02-1.33)*	0.03*
Long fatty acids[a]	1.10 (0.96-1.26)	1.19 (1.04-1.36)*	1.44 (1.05-1.98)*	0.02*
Very long fatty acids[b]	0.99 (0.85-1.14)	1.13 (0.99-1.30)	0.82 (0.62-1.09)	0.18
Phosphatidylcholines	1.09 (0.95-1.25)	1.19 (1.03-1.37)*	1.19 (1.03-1.37)*	0.02*
0-1 Double bonds[c]	1.10 (0.96-1.27)	1.22 (1.06-1.41)*	1.22 (1.06-1.41)*	0.006*
≥2 Double bonds[d]	1.08 (0.94-1.24)	1.17 (1.01-1.36)*	1.17 (1.01-1.36)*	0.03*

Note: Hazard ratios for mortality are per 1-SD increase in relative high-density lipoprotein cholesterol abundance of each class of lipids.
Abbreviations: HR, hazard ratios; CKD, chronic kidney disease; CI, confidence interval.
[a]Sphingomyelins with long fatty acids had acylated fatty acids containing 14-18 carbons.
[b]Sphingomyelins with very long fatty acids had acylated fatty acids containing 20-24 carbons.
[c]Saturated and monounsaturated phosphatidylcholines have 0-1 double bonds in the fatty acid chain.
[d]Polyunsaturated phosphatidylcholines have 2 or more double bonds in the fatty acid chain.
[e]Model 1 was adjusted for age, sex, waist circumference, diabetes, hypertension, smoking, alcohol, prior stroke or myocardial infarction, chronic heart failure, estimated glomerular filtration rate, log-adjusted albuminuria, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and use of antihypertensives, statins, and other cholesterol-lowering medications.
[f]Model 2 was adjusted for the components noted in model 1. In addition, long fatty acid sphingomyelins were adjusted for sphingomyelin 22:0, and very long fatty acid sphingomyelins were adjusted for sphingomyelin 16:0.
*indicates significant findings at a false discovery rate of 5%.

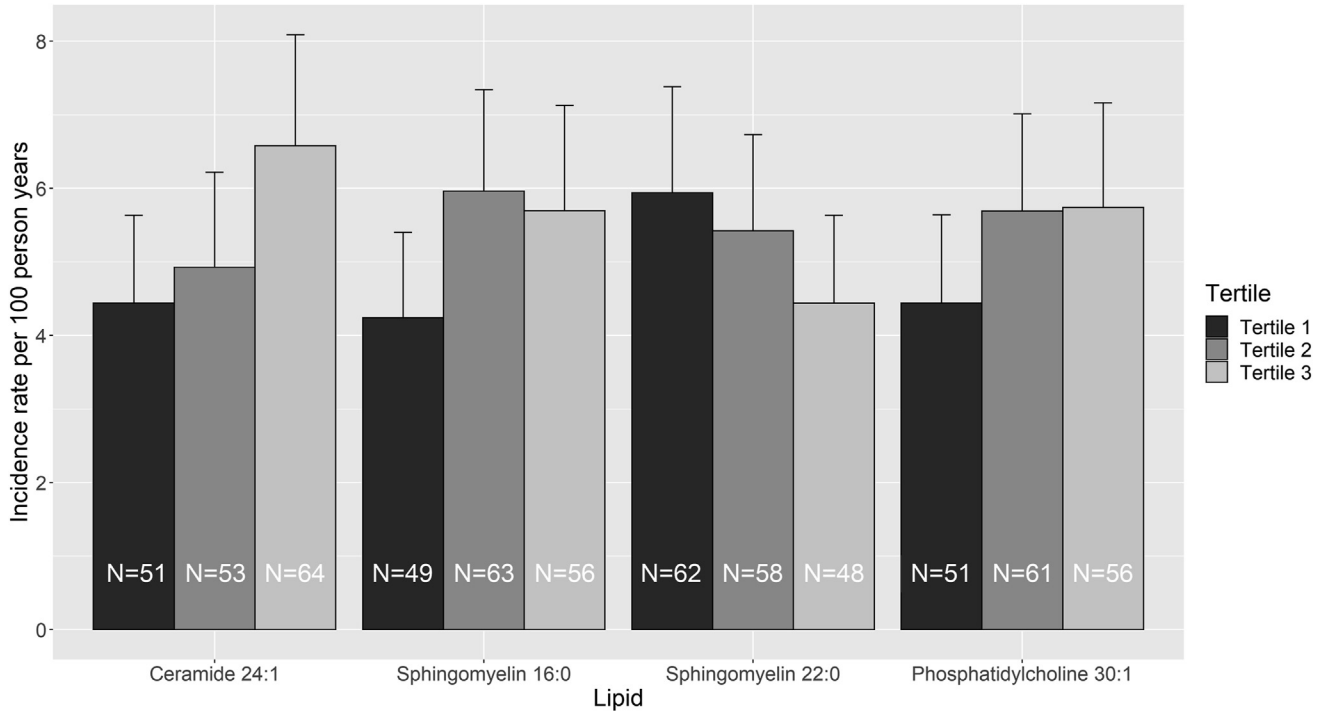

Figure 2. Unadjusted incidence rate of mortality by tertiles of various lipids. N indicates the number of mortality events in each tertile.

significantly associated with mortality at an FDR < 5% (Table S3).

DISCUSSION

In this analysis of data from a well-characterized CKD cohort, higher HDL abundance of ceramides, sphingomyelins with long acylated fatty acids, and saturated and monounsaturated phosphatidylcholines were significantly associated with increased risk for all-cause mortality; sphingomyelins with very long acylated fatty acids were associated with lower risk for all-cause mortality. It is possible that HDL lipidomic alterations may be associated with some of the functional HDL alterations observed in patients with CKD and may serve as novel mortality risk factors in this patient population.[22]

HDL consists of lipid and protein components; the lipid portion includes lipids composing the lipid monolayer (mostly phosphatidylcholines) and surface lipids predominantly contained in lipid rafts, including sphingolipids, such as ceramides, glycoceramides, and sphingomyelins.[23-25] These sphingolipids are a large class of biologically active lipids with structural, regulatory, and signaling roles.[26] Our group has previously noted positive associations between albuminuria and relative HDL abundance of total ceramides; sphingomyelins acylated to long fatty acids; ceramides 22:0 and 24:1; glucosylceramide 16:0; sphingomyelin 16:0; and phosphatidylcholines 29:0, 30:1, and 38:2.[27] Various other studies have noted associations between CKD and plasma lipid concentrations. For instance, Afshinnia et al[16] noted increased plasma concentrations of long, polyunsaturated sphingomyelins and phosphatidylcholines in patients with CKD, which they posited to be secondary to decreased β-oxidation in this population. Several additional studies have suggested that lower eGFR and greater albuminuria are associated with higher plasma concentrations of ceramides and sphingomyelins.[17,28-30]

Although it is unknown if HDL lipidomics correlate well with the whole-plasma lipidome, given the lack of HDL-specific studies, literature evaluating whole plasma may serve as a reasonable reference. Unfortunately, few articles have assessed lipidomics as markers of outcomes specifically in patients with chronic kidney disease; fewer still have evaluated the individual lipids that we measured. Duranton et al[31] noted negative associations between lysophosphatidylcholine levels and all-cause mortality in patients with CKD, but did not note significant relationships between total sphingomyelins and mortality. However, this study was limited by a small sample size (N=77, including 49 participants with non-dialysis-requiring CKD); these authors also did not distinguish between long and very long sphingomyelins. A study of 368 incident kidney failure patients noted greater risk of mortality with higher tertiles of glucosylceramide 16:0 (adjusted HR for mortality in the highest vs lowest tertile 1.81; 95% CI, 1.02-3.22); however, the study was underpowered to detect associations between other levels of ceramides and mortality.[32] We found similar associations between hexosylceramide 16:0 and all-cause mortality in our present

Table 3. Associations Between Individual Lipids and All-Cause Mortality in Patients with CKD (N=498)

	Unadjusted		Model 1[a]		Model 2[b]	
Ceramides	**HR per SD (95% CI)**	***P* Value**	**HR per SD (95% CI)**	***P* Value**	**HR per SD (95% CI)**	***P* Value**
Ceramide 22:0	1.17 (1.04-1.31)*	0.009*	1.19 (1.04-1.37)*	0.009*	1.19 (1.04-1.37)*	0.009*
Ceramide 24:0	1.09 (0.93-1.27)	0.28	1.16 (1.01-1.33)	0.04	1.16 (1.01-1.33)	0.04
Ceramide 24:1	1.35 (1.17-1.54)*	<0.001*	1.33 (1.15-1.54)*	<0.001*	1.33 (1.15-1.54)*	<0.001*
Hexosylceramide 16:0	1.16 (1.01-1.34)	0.03	1.26 (1.08-1.48)*	0.003*	1.26 (1.08-1.48)*	0.003*
Hexosylceramide 22:0	1.10 (0.97-1.25)	0.14	1.15 (1.00-1.32)	0.04	1.15 (1.00-1.32)	0.04
Hexosylceramide 24:0	1.07 (0.93-1.24)	0.32	1.19 (1.02-1.39)	0.02	1.19 (1.02-1.39)	0.02
Lactosylceramide 16:0	1.11 (0.97-1.27)	0.13	1.19 (1.02-1.40)	0.03	1.19 (1.02-1.40)	0.03
Sphingomyelins						
Long sphingomyelins						
Sphingomyelin 14:0	1.08 (0.94-1.24)	0.29	1.16 (1.01-1.33)	0.03	1.24 (0.95-1.62)	0.12
Sphingomyelin 16:0	1.18 (1.03-1.36)	0.02	1.24 (1.08-1.44)*	0.003*	1.63 (1.22-2.19)*	0.001*
Sphingomyelin 18:0	1.04 (0.90-1.20)	0.60	1.14 (0.98-1.33)	0.09	1.14 (0.88-1.47)	0.32
Very long sphingomyelins						
Sphingomyelin 20:0	0.98 (0.85-1.14)	0.80	1.13 (0.97-1.32)	0.11	0.80 (0.60-1.06)	0.12
Sphingomyelin 22:0	0.97 (0.83-1.12)	0.64	1.11 (0.96-1.28)	0.15	0.75 (0.57-0.99)	0.04
Sphingomyelin 24:0	1.01 (0.88-1.17)	0.85	1.14 (1.00-1.31)	0.05	0.84 (0.63-1.11)	0.21
Phosphatidylcholines						
0-1 Double bonds						
Phosphatidylcholine 28:1	0.98 (0.84-1.15)	0.83	1.10 (0.91-1.32)	0.32	1.10 (0.91-1.32)	0.32
Phosphatidylcholine 28:0	1.08 (0.94-1.24)	0.26	1.15 (1.01-1.32)	0.04	1.15 (1.01-1.32)	0.04
Phosphatidylcholine 29:0	1.05 (0.91-1.21)	0.47	1.13 (0.97-1.30)	0.12	1.13 (0.97-1.3)	0.12
Phosphatidylcholine 30:1	1.18 (1.04-1.35)*	0.01*	1.25 (1.08-1.44)*	0.003*	1.25 (1.08-1.44)*	0.003*
Phosphatidylcholine 32:1	1.11 (0.95-1.28)	0.19	1.21 (1.02-1.43)	0.03	1.21 (1.02-1.43)	0.03
Phosphatidylcholine 34:1	1.21 (1.05-1.40)*	0.009*	1.26 (1.08-1.47)*	0.003*	1.26 (1.08-1.47)*	0.003*
Phosphatidylcholine 36:1	1.08 (0.94-1.24)	0.30	1.19 (1.02-1.38)	0.03	1.19 (1.02-1.38)	0.03
Phosphatidylcholine 38:1	1.03 (0.89-1.19)	0.72	1.14 (1.00-1.31)	0.05	1.14 (1.00-1.31)	0.05
≥2 Double bonds						
Phosphatidylcholine 30:2	1.10 (0.96-1.27)	0.16	1.14 (0.98-1.33)	0.08	1.14 (0.98-1.33)	0.08
Phosphatidylcholine 32:2	0.98 (0.83-1.15)	0.80	1.24 (1.03-1.50)	0.03	1.24 (1.03-1.50)	0.03
Phosphatidylcholine 33:3	1.03 (0.90-1.19)	0.65	1.11 (0.95-1.29)	0.18	1.11 (0.95-1.29)	0.18
Phosphatidylcholine 34:2	1.10 (0.95-1.26)	0.20	1.18 (1.01-1.39)	0.04	1.18 (1.01-1.39)	0.04
Phosphatidylcholine 35:5	1.15 (1.01-1.30)	0.04	1.19 (1.04-1.37)	0.01	1.19 (1.04-1.37)	0.01
Phosphatidylcholine 35:4	1.06 (0.94-1.21)	0.33	1.12 (0.98-1.29)	0.10	1.12 (0.98-1.29)	0.10
Phosphatidylcholine 36:5	0.95 (0.80-1.12)	0.51	1.05 (0.89-1.25)	0.54	1.05 (0.89-1.25)	0.54
Phosphatidylcholine 36:4	1.12 (0.97-1.29)	0.14	1.12 (0.95-1.31)	0.19	1.12 (0.95-1.31)	0.19
Phosphatidylcholine 36:3	1.11 (0.98-1.25)	0.11	1.13 (0.99-1.30)	0.07	1.13 (0.99-1.30)	0.07
Phosphatidylcholine 36:2	1.08 (0.94-1.24)	0.29	1.19 (1.02-1.39)	0.02	1.19 (1.02-1.39)	0.02
Phosphatidylcholine 38:5	1.10 (0.95-1.28)	0.20	1.14 (0.96-1.34)	0.13	1.14 (0.96-1.34)	0.13
Phosphatidylcholine 38:4	1.07 (0.92-1.24)	0.40	1.09 (0.92-1.29)	0.30	1.09 (0.92-1.29)	0.30
Phosphatidylcholine 38:3	1.02 (0.89-1.18)	0.76	1.11 (0.95-1.29)	0.18	1.11 (0.95-1.29)	0.18
Phosphatidylcholine 38:2	1.18 (1.04-1.34)*	0.01*	1.24 (1.08-1.44)*	0.003*	1.24 (1.08-1.44)*	0.003*
Phosphatidylcholine 40:6	1.00 (0.85-1.17)	0.96	1.06 (0.89-1.27)	0.50	1.06 (0.89-1.27)	0.50
Phosphatidylcholine 40:5	1.05 (0.91-1.21)	0.53	1.18 (1.00-1.4)	0.05	1.18 (1.00-1.4)	0.05

Note: Hazard ratios for mortality are per 1-SD increase in relative high-density lipoprotein cholesterol abundance of each class of lipids.
Abbreviations: HR, hazard ratios; SD, standard deviation; CI, confidence interval.
[a]Model 1 was adjusted for age, sex, waist circumference, diabetes, hypertension, smoking, alcohol, prior stroke or myocardial infarction, chronic heart failure, estimated glomerular filtration rate, log-adjusted albuminuria, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and use of antihypertensives, statins, and other cholesterol-lowering medications.
[b]Model 2 was adjusted for the components noted in model 1. In addition, sphingomyelins 14:0-18:0 were adjusted for sphingomyelin 22:0, and sphingomyelins 20:0-24:0 were adjusted for sphingomyelin 16:0.
*indicates significant findings at a false discovery rate of 5%.

analyses; however, because we modeled hexosylceramide continuously, our effect sizes are not directly comparable. Similarly, although hexosylceramide 16:0 in our analyses likely represents glucosylceramide 16:0, this is not certain. Sphingomyelin 22:0 has been shown to be negatively associated with mortality in patients with type 1 diabetes, although the mean eGFR of participants was notably higher than in our present analyses (81 ± 26 vs

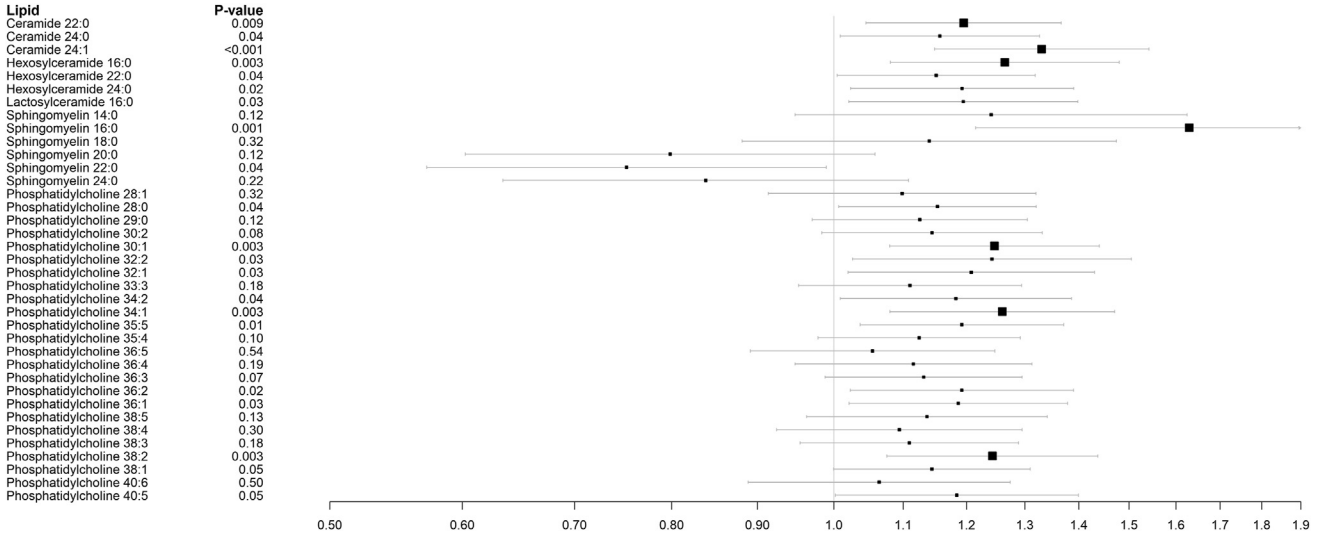

Figure 3. Association of lipids with all-cause mortality. Hazard ratios (95% confidence intervals) for all-cause mortality per 1-SD increase in individual lipid species is reported. Larger box size indicates significance at an FDR < 0.05. Adjusted for age, sex, DM, HTN, stroke, CHF, smoking, alcohol, eGFR, albuminuria, HDL, LDL, and use of antihypertensives, statins, and other cholesterol-lowering medications. Additionally, sphingomyelins 14:0-18:0 were adjusted for sphingomyelin 22:0, and sphingomyelins 20:0-24:0 were adjusted for sphingomyelin 18:0. Abbreviations: HR, hazard ratio; SD, standard deviation; FDR, false discovery rate; DM, diabetes mellitus; HTN, hypertension; CHF, chronic heart failure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

45 ± 26 mL/min/1.73 m^2).[33] Sphingomyelins acylated to very long-chain fatty acids were associated with lower mortality risk in our study. In our secondary analysis, sphingomyelins 20:0 and 22:0 were negatively associated with mortality, although these associations were not significant at an FDR < 5%.

Associations between whole-plasma lipidomics and all-cause mortality have been better investigated in the general population compared to the limited number of studies in CKD. In a study of 4,612 participants in the Cardiovascular Health Study, sphingomyelin 16:0 and ceramide 16:0 were significantly associated with all-cause mortality, and sphingomyelin 20:0, 22:0, and 24:0, and ceramides 22:0 and 24:0 were negatively associated with mortality.[12] We also demonstrated significant associations between sphingomyelin 16:0 and mortality; our associations between sphingomyelins 20:0, 22:0, and 24:0 were similar to these findings but did not reach statistical significance. In a pooled cohort of 2,642 participants in the Framingham Heart Study and 3,134 participants in the Study of Health in Pomerania, ceramides 22:0 and 24:0 were also negatively associated with all-cause mortality.[34] We did not demonstrate significant associations between these ceramides and mortality in our population, although our data focused on HDL (vs whole plasma). However, these data may suggest different HDL biology in patients with CKD.

Compared with sphingolipids, the associations between phosphatidylcholines (found primarily in the lipid monolayer of HDL) and all-cause mortality have been less well-demonstrated in general. However, an analysis of 3,316 participants in the Ludwigshafen Risk and Cardiovascular Health Study noted positive associations between plasma phosphatidylcholines 30:1, 34:1, and 38:2 and mortality.[35] These same 3 phosphatidylcholines were also significantly associated with mortality in our analyses. Similarly, Mundra et al[36] recently investigated associations between phosphatidylcholines and cardiovascular death, noting that saturated and monounsaturated phosphatidylcholines seemed to be associated with increased risk, whereas longer, polyunsaturated phosphatidylcholines were associated with decreased risk. We found that saturated and monounsaturated phosphatidylcholines were associated with increased mortality risk, possibly supporting these findings.

The biologic mechanisms underlying associations of sphingolipids and phosphatidylcholines with mortality are incompletely understood, with most prior work focusing on sphingolipids. Sphingolipids have been implicated in multiple biologic pathways of inflammation and oxidative stress, apoptosis, and atherosclerosis, which may increase mortality risk.[37-42] There is increasing evidence from human fibroblast cell lines as well as yeast, worm, and fly models that sphingolipids are involved in senescence and lifespan regulation via dephosphorylation of retinoblastoma protein and direct activation of serine/threonine protein phosphatases, both contributing to cell-cycle arrest.[43-45] The associations of phosphatidylcholines with mortality remain mechanistically unclear. However, saturated and monounsaturated phosphatidylcholines may be markers of diet and physical activity rather than truly biologically active molecules.[46,47] Alternatively, these

saturated and monounsaturated phosphatidylcholines may have pro-inflammatory effects compared to polyunsaturated phosphatidylcholines.[35] It is similarly unclear why long and very long sphingomyelins are positively and negatively associated, respectively, with adverse outcomes. Sphingomyelins with very long acylated fatty acids may impart different membrane properties than sphingomyelins with long acylated fatty acids.[48,49] Future work investigating these differences may be indicated. It may also be important to understand patient-level characteristics (including modifiable risk factors and medication use patterns) associated with differential lipid signatures because these may identify future therapeutic targets.

This study has several notable strengths. We leveraged data from a well-characterized CKD cohort including various stages of CKD. Our targeted approach for lipid quantification with use of internal standards allowed more accurate assessments than nontargeted methods. However, this study does have several limitations. We were unable to test associations of lipids with cardiovascular events given limited power. Second, we measured a small number of lipid species compared with the entire lipidomic composition of HDL, and it is unclear whether HDL lipidomics reflect whole-plasma lipidomics either in measurement or mechanistic function. Third, all lipid concentrations were relative to an internal calibrator, and the validity of our results are contingent on the inter-run consistency of amounts of calibrator present. Our approach used LC-MS/MS, which cannot determine the number of carbons in a particular chain. All measured lipids contained 2 chains, and we were unable to determine the chain lengths for phosphatidylcholines. We assumed the most common d(18:1) backbone for sphingolipids; although uncommon, it is possible that different backbones existed. Similarly, LC-MS/MS cannot differentiate between glucosylceramides and galactosylceramides; given this ambiguity, we reported simply hexosylceramides, which may limit comparisons to other work. We analyzed a mix of HDL-2 and HDL-3 rather than subtypes alone. All comorbid conditions were self-reported, possibly causing under-reporting, and we lacked data on severity. We were further unable to exclude participants with active malignancy or chronic diseases which may have limited life expectancy. We lacked healthy controls and a validation cohort. The cohort was composed of research volunteers in the Seattle area, limiting generalizability to other CKD populations. Finally, the technical difficulty of isolating HDL may limit applicability.

In conclusion, we noted significant associations between higher HDL abundance of ceramides, long acylated chain sphingomyelins, saturated and monounsaturated phosphatidylcholines, ceramides 22:0 and 24:1, hexosylceramide 16:0, sphingomyelin 16:0, and phosphatidylcholines 30:1, 34:1, and 38:2 and all-cause mortality in patients with CKD. It is possible that HDL lipidomics may serve a role as novel contributors to mortality in patients with CKD. Future studies are needed to investigate possible associations between HDL lipids and functional deficits that may contribute to the disproportionate risk of mortality in patients with CKD.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1: Correlation of Individual Lipid Species, Lipoproteins, and Statin Use at Baseline.

Table S1: Individual Lipid Precursors, Fragments, and Internal Standards Used for Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Analysis.

Table S2: Associations Between Classes of Lipids and All-Cause Mortality in Patients With CKD and Additionally Adjusted for Time-Updated Dialysis Requirements.

Table S3: Associations Between Individual Lipids and All-Cause Mortality in Patients With CKD and Additionally Adjusted for Time-Updated Dialysis Requirements.

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