Journal of Clinical Lipidology (2019) 13, 176–185

Journal of Clinical Lipidology

The CKD plasma lipidome varies with disease severity and outcome

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KEYWORDS:
Renal disease;
Lipidomics;
Phospholipid;
Lysophospholipid;
Sphingomyelin;
Fatty acid

BACKGROUND: Various alterations in lipid metabolism have been observed in patients with chronic kidney disease (CKD).

OBJECTIVES: To determine the levels of lipid species in plasma from CKD and hemodialysis (HD) patients and test their association with CKD severity and patient outcome.

METHODS: Seventy-seven patients with CKD stage 2 to HD were grouped into classes of CKD severity at baseline and followed-up for 3.5 years for the occurrence of transition to HD or death (combined outcome). Plasma levels of phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs), sphingomyelins (SMs), and fatty acids were analyzed by flow-injection analysis coupled to tandem mass spectrometry or gas chromatography coupled with mass spectrometry. Kruskal Wallis rank tests and Cox regressions were used to analyze the association of lipids with CKD severity and the risk of combined outcome, respectively.

RESULTS: The plasma level of PCs, LPCs, and SMs was decreased in HD patients compared with nondialyzed CKD patients (all $P < .05$), whereas esterified and/or nonesterified fatty acids level did not change. Thirty-four lipids displayed significantly lower abundance in plasma of HD patients, whereas elaidic acid (C18:1ω9t) level was increased ($P < .001$). The total amount of LPCs and individual LPCs were associated with better outcome ($P < .05$). In particular, LPC 18:2 and LPC 20:3 were statistically associated with outcome in adjusted models ($P < .05$).

Harald Mischak, Joost P Schanstra, and Àngel Argilés are members of the European Uremic Toxin Work Group (EUTox) of the European Society for Artificial Organs (ESAO) endorsed by the European Renal Association—European Dialysis and Transplant Association (ERA-EDTA).

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Submitted February 13, 2018. Accepted for publication July 24, 2018.

https://doi.org/10.1016/j.jacl.2018.07.010

DISCUSSION: In HD patients, a reduction in plasma lipids is observed. Some of the alterations, namely reduced LPCs, were associated with the risk of adverse outcome. These changes could be related to metabolic dysfunctions.

Introduction

Accounting for over 70% of organic molecules, lipids constitute a major component of the human plasma.[1] Circulating lipids are mainly present in the form of sterols, glycerophospholipids, and glycerolipids transported by lipoproteins (eg, chylomicrons, low-density lipoprotein [LDL], high-density lipoprotein [HDL]). Nonesterified fatty acids (NEFAs) and lysophosphatidylcholines (LPCs) can also be found in plasma, preferably bound to albumin. It is general practice to study glycerolipids (more precisely, triacylglycerols) and LDL and HDL cholesterol levels as markers of lipid metabolism and cardiovascular risk. Dyslipidemias are frequently seen in patients with chronic kidney disease (CKD), in particular hypertriglyceridemia and low HDL levels.[2] Such changes partly explain the high risk of cardiovascular disease and mortality in these patients. However, the contribution of other plasma lipid components to the cardiovascular risk in patients with CKD has not been elucidated.

In addition to their long-known structural and energetic roles, it is now established that lipids can have major roles in signaling. This had been initially identified for steroid hormones (vitamin D, sex hormones) and has more recently been shown for others species such as lysophosphatidic acid, LPCs, leukotriene, or prostaglandins, which directly activate membrane or nuclear receptors and modulate immunity, inflammation, and atherogenic processes.[3] Indirect toxicity can also occur when lipids are modified by oxidative stress, which leads to the loss of their properties and the liberation of uremic toxins such as malondialdehyde or other advanced lipoxidation end products.[4] This suggests that changes in lipid profiles could be markers of metabolic activities and oxidative stress, which could be of interest for CKD characterization.

Metabolomic profiling of CKD patients has shown value in classifying patients with different stages of the disease[5] and in identifying metabolic species or pathways affected by the disease.[6,7] In the present study, we focused on lipid families that are phosphatidylcholines (PCs), sphingomyelins (SMs), and LPCs, which are among the most abundant circulating phospholipids, as well as fatty acids (FAs) both in their nonesterified form (NEFAs) and as a component of complex lipids (esterified FAs). Our objective was to compare the plasma lipidome of patients with different levels of renal function, to identify biological processes that could be consequences or adaptations to the reduction of renal function as well as potential mediators of disease progression.

Materials and methods

Subjects

The study population consisted of 77 patients. Fifty-two patients with CKD (range of estimated glomerular filtration rate [eGFR], 9–90 mL/min/1.73 m^2) were recruited from the Department of Nephrology, Transplantation and Dialysis of the University Hospital of Montpellier and the Public Hospital of Sète (France). Twenty-five patients on maintenance hemodialysis (HD) were recruited from the Néphrologie Dialyse Saint Guilhem dialysis unit in Sète (France). Written informed consent was obtained from all participants before the study. The study was approved by the Comité de Protection de Personnes of Montpellier and declared to the French Ministry (reference number DC-2008-417).

Blood samples were obtained from all patients, and routine analyses were performed by the hospital laboratory to quantify plasma creatinine, albumin, total proteins, C-reactive protein (CRP), hemoglobin, parathyroid hormone, urea, sodium, potassium, bicarbonate, calcium, and phosphate levels. Estimated GFR was calculated based on plasma creatinine, age, and gender using the CKD-EPI formula.[8] Urine samples were obtained from nondialyzed patients, and urinary proteins and creatinine concentrations were determined by the hospital laboratory.

Blood samples for lipidome analysis were collected during medical visits for non-HD patients or before a dialysis session for HD patients in EDTA-containing tubes. Blood was put on ice and immediately centrifuged at 4°C (10 minutes at 2000 g). Plasma was obtained and stored at −80°C until analysis.

The health status of patients was assessed after 3 years by analysis of medical records and/or by contacting patients or relatives. Event type (start or change in renal replacement therapy, death) and date were collected.

Lipidome analysis

FIA-MS/MS

Plasma PCs, LPCs, and SMs were quantified by flow-injection analysis coupled to tandem mass spectrometry (FIA-MS/MS) using AbsoluteIDQ p180 KIT plates (Biocrates Life Sciences AG, Innsbruck). Ten microliters of plasma samples, zero sample (0.01 M phosphate buffered saline solution with 40 g/L human serum albumin), and quality control solution (Dunn Labortechnik GmbH, Asbach, Germany) were pipetted into respective wells

and dried (1 hour, at room temperature (RT), under a 4 bar nitrogen stream). Derivatization was performed by addition of 20 μL 5% v/v phenylisothiocyanate reagent (20 minutes at RT). Excess liquids were removed by dehydration (30 minutes, at RT, under 3 bar nitrogen), and 300 μL of extraction solvent (HPLC grade methanol containing 5 mM ammonium acetate) were added. Samples were shaken at 450 rpm at RT for 30 minutes to extract analytes and centrifuged for 2 minutes at 500 g. Samples were diluted with 600 μL of extraction solvent and additionally 27 μL Milli-Q water. The plate was centrifuged for 2 minutes at 500 g, covered with a pierceable silicone lid and placed in the CTC PAL autosampler (Agilent Technologies, Santa Clara, CA). Ten microliters of the sample was directly injected to the Sciex API 4000 QTrap tandem mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Canada) equipped with an electrospray interface operated in MRM mode to monitor specific compounds. MS/MS spectra were acquired in positive and negative mode, and the mobile phase was 5 nM ammonium acetate Biocrates Solvent A (Biocrates Life Sciences AG, Innsbruck, Austria).

GC-MS

Plasma FAs were analyzed as their corresponding fatty acid methyl ester derivatives using gas chromatography coupled with mass spectrometric (GC-MS) detection. Plasma sample (25 μL), external standard solutions,[9] blank (water), and zero sample (0.01 M phosphate buffered saline solution with 40 g/L human serum albumin) were pipetted into 2 mL vials, respectively. Methanolic HCl (100 μL) solution was added, and vials were shaken (5 minutes, 500 rpm). Methylation of FAs was allowed by resting 45 minutes at RT for NEFAs content, or by incubating 3 hour at 70°C and cooled down to RT for the total (esterified and nonesterified) fatty acid (TFA) content. Two hundred microliters of standard solution (18-methylnonadecanoic acid methyl ester in hexane, butylated hydroxytoluene in ethanol) was added and shaken for 5 minutes to extract methyl esters. After centrifugation (5 minutes, 500 rpm), 100 μL of the upper hexane phase was pipetted and dried (addition of anhydrous sodium sulfate, 20 mg). The solution was transferred to a 2 mL vial with insert and placed into a cooled CTC CombiPAL autosampler. A volume of 2 μL of solution was injected with pulsed split injection technique into a 7890GC/5795MSD instrument (Agilent Technologies, USA) to be analyzed by GC-MS with an Electron Impact ion source in SIM mode against external standards. The sample was injected under a split ratio of 10:1. The temperature of the injector was kept at 250°C. The oven temperature started at 50°C (2 minutes), risen to 200°C (3 minutes) at 25°C/min, and then to 235°C (10 minutes) with a rate of 3°C/min. The fatty acid methyl ester derivatives were separated on a DB-23 column (60 m × 250 μm × 0.25 μm) with helium as the carrier gas (200 kPa constant head column pressure). Where no external standard was available, compounds were measured semiquantitatively using spectra recorded in SCAN mode, respective ratios of characteristic ions, and the retention behavior. The semiquantification was carried out with response factors extrapolated from the nearby eluting compounds having the same number of double bonds.

Calculations

Molar concentrations were calculated with Analyst 1.4.2 Software (Applied Biosystems). Determinations were performed in a blind manner. Identity was unblinded after depositing the results in the central server of the consortium. The list of targeted molecular species and detection rates is displayed in Table S1. Total concentrations of the 5 lipid families were calculated by adding the levels of targeted molecular species from each family (16 SMs, 82 PCs, 10 LPCs, 81 NEFAs, and 81 TFAs). For each species, the relative concentration (in percentage) was calculated as the ratio of its molar concentration to the total concentration of its lipid family, multiplied by 100. A total of 36 indices were computed, which are listed in Table S2. A description of lipid annotation and isomeric species is available.[10]

Statistical analyses

Patients were grouped by CKD severity into 3 groups: CKD2-3 (eGFR $>$ 30 mL/min/1.73 m^2), CKD4-5 (eGFR $\leq$ 30 mL/min/1.73 m^2), and a group of patients receiving HD. Differences in baseline characteristics between CKD2-3, CKD4-5, and HD patients were analyzed by χ^2 tests for class variables; and for continuous variables, by ANOVA or nonparametric Kruskal Wallis rank tests, with post hoc tests adjusted for multiple comparisons using the Bonferroni correction. Differences in lipid levels between CKD2-3, CKD4-5, and HD patients were analyzed by Kruskal Wallis rank tests, where *P*-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate correction (FDR).[11] To control the risk of false positives in each analysis, FDR corrections were successively applied to lipid concentrations (239 tests), relative lipid concentrations (239 tests), and calculated indices (36 tests). Additional analyses adjusting for diabetes and the interaction diabetes × group of renal function were performed (multivariable Kruskal Wallis rank tests).

We calculated the relative abundance in non-HD patients compared with HD patients as the ratio of mean levels. We performed linear regressions on the logarithm of relative abundance to test the presence of trends related to acyl chain characteristics. Spearman rank correlations were used to detect associations between lipids and patient characteristics. The association between lipids and a combined outcome (transition to HD or death) was assessed by Cox proportional hazards models. Renal transplantation, transition to peritoneal dialysis, and loss to follow-up were considered censoring events.

Statistical analyses were performed using SAS, version 9.4, (SAS Institute, Cary, NC). Tests were performed using

a type I error of 5%. Results are given as the mean ± standard error of the mean.

Results

There were significant differences in clinical characteristics between groups of CKD severity, most of which being attributable to the differences in renal function (Table 1). Patients with early CKD stages were younger and healthier, whereas patients on HD had lower BMI. Diabetic patients were evenly distributed across groups. Compared with non-HD patients, the level of total (sum of individual molecular species) LPCs, PCs, and SMs were lower in HD patients (Table 1, Fig. 1). In contrast, there was no change in the level of total NEFAs and TFAs (Table 1).

Individual species

A total of 239 (88%) lipid species were detected in 20% of patients or more (Table S1). Among them, there were 34 compounds that were significantly associated with groups of CKD severity (FDR-adjusted $P < .05$, Table 2): 3 NEFAs (4% of targeted compounds), 3 TFAs (4%), 17 (21%) PCs, 8 (80%) LPCs, and 3 (19%) of SMs. In pairwise comparisons, all 34 species showed significantly different levels between CKD2-3 and HD patients and 29 species (85%) between CKD4-5 and HD patients. All species were reduced in the plasma of dialyzed patients, with the exception of nonesterified elaidic acid (C18:1ω9t), which was increased (Table 2). Patients with CKD2-3 and CKD4-5 showed similar plasma lipid levels, except for 2 LPCs

Table 1 Baseline characteristics by CKD groups

Characteristics	CKD2-3	CKD4-5	HD	*P*-value
Number of patients (n)	22	30	25	-
Age (y)	64.4 (54.4, 75.2)	75.9 (69.1, 79.3)	77.2 (68.2, 82.1)	.02*
Gender, female (%)	10 (45)	13 (43)	5 (20)	.1
BMI (kg/m^2)	28.5 ± 1.3	30.0 ± 1.2	25.7 ± 0.8	.02‡
eGFR (mL/min/1.76 m^2)	52.8 ± 4.0	18.6 ± 1.2	ND	<.001*
Proteinuria (g/g creatininuria)	0.1 (0, 0.2)	0.7 (0.3, 1.7)	ND	.001*
HD vintage (y)	N/A	N/A	2.5 (0.5, 3.6)	N/A
Diabetes, yes (%)	9 (41)	17 (57)	12 (48)	.5
Blood pressure (mmHg)				
SBP	142 ± 4	150 ± 4	152 ± 4	.3
DBP	73 ± 3	73 ± 2	75 ± 3	.8
Plasma levels				
Proteins (g/L)	74.7 ± 1.1	71.8 ± 1.0	63.7 ± 1.4	<.001†,‡
Albumin (g/L)	41 ± 0.6	37.3 ± 0.8	31.8 ± 0.8	<.001*,†,‡
C-reactive protein (mg/L)	1.6 (1.0, 4.4)	3.2 (1.7, 9.3)	6.6 (4.6, 21.3)	<.001†,‡
Hemoglobin (g/dL)	13.8 ± 0.3	12.9 ± 0.3	11.2 ± 0.4	<.001†,‡
PTH (pmol/L)	4.4 (3.1, 6.4)	21.5 (11.0, 30.9)	25.0 (15.6, 40.6)	<.001*,†
Urea (mmol/L)	9.3 ± 0.8	20.5 ± 1.2	21.4 ± 1.5	<.001*,†
Creatinine (μmol/L)	120 ± 7	276 ± 17	606 ± 36	<.001*,†,‡
Sodium (mmol/L)	139.8 ± 0.5	139.6 ± 0.6	136.7 ± 0.6	<.001†,‡
Potassium (mmol/L)	4.3 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	.09
Bicarbonate (mmol/L)	27.0 ± 0.6	24.3 ± 0.6	23.7 ± 0.6	.001*,†
Calcium (mmol/L)	2.35 ± 0.01	2.22 ± 0.03	2.12 ± 0.03	<.001*,†,‡
Phosphate (mmol/L)	1.10 ± 0.03	1.22 ± 0.04	1.22 ± 0.09	.3
Plasma lipids (μmol/L)				
LPCs	279 ± 14	243 ± 13	182 ± 14	<.001†,‡
PCs	2250 ± 124	2226 ± 82	1907 ± 126	.06
SMs	371 ± 16	374 ± 10	326 ± 15	.02‡
NEFAs	1133 ± 45	1273 ± 53	1230 ± 77	.3
TFAs	5016 ± 209	5103 ± 155	4731 ± 308	.5
Plasma LPCs/albumin (mol/mol)	0.44 ± 0.02	0.44 ± 0.02	0.38 ± 0.03	.3

BMI, body mass index; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HD, hemodialysis; LPCs, lysophosphatidylcholines; N/A, not applicable; ND, not determined; NEFAs, nonesterified fatty acids; PCs, phosphatidylcholines; PTH, parathyroid hormone; SBP, systolic blood pressure; SMs, sphingomyelins; TFAs, total fatty acids.

Values are in mean ± standard error of the mean or median (interquartile range).

*CKD2-3 significantly different from CKD4-5 ($P_{adj} < .05$).

†CKD2-3 significantly different from HD ($P_{adj} < .05$).

‡CKD4-5 significantly different from HD ($P_{adj} < .05$).

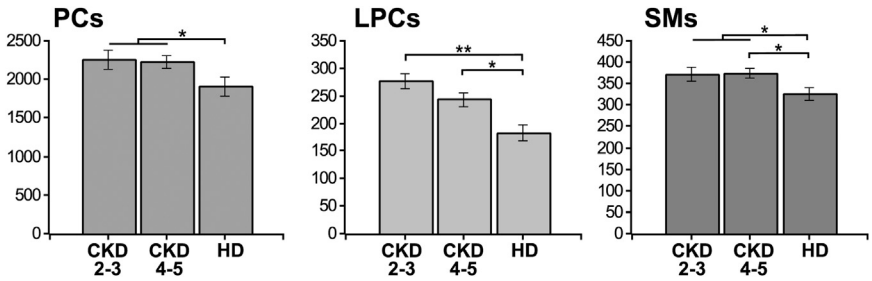

Figure 1 Mean and standard error of plasma concentrations (μmol/L) in lipid families by CKD severity. Differences across the 3 groups of CKD level and 2 groups of HD status were analyzed separately by ANOVA. *P*-values were adjusted for multiple comparisons by the Bonferroni method. $^{*}P_{adj} < .05$, $^{**}P_{adj} < .001$.CKD, chronic kidney disease. HD, hemodialysis.

(20:3 and 20:4) whose levels progressively decreased from CKD2-3 to HD patients (Table 2). Adjusting for diabetes led to similar results (data not shown).

Ratios and relative abundance

There were no clear associations between lipid characteristics (number of carbons, number of double bonds, position of the last insaturation) and the relative abundance of species (all $P > .05$). For SMs only, the abundance in non-HD patients relatively to HD patients tended to increase with the number of insaturations ($P = .08$, Fig. 2).

The analysis of lipid species in proportion to their respective lipid family showed significant differences, mainly between HD patients and non-HD patients (Table 2).The proportions of trans-FA C18:1ω9t among nonesterified and total FAs were more abundant in HD patients. In HD patients, there was a decrease in the proportion of PC34:4, SM (OH)22:1, and SM (OH)22:2. Most of these lipids were present in proportions rarely exceeding 3% of their respective lipid families (Table 2).

Association with outcome

Outcome was known for 73 (95%) patients after a median follow-up of 3.0 (IQR: 1.4, 3.3) years. There were a total of 31 events (20 deaths and 11 dialysis initiations). Increases in total LPC levels were associated with better combined outcome (Fig. 3). For an increase of 1 standard deviation (SD) of LPCs (80 μmol/L), the risk of combined outcome was reduced by 60% (hazard ratio [HR] and 95% confidence interval [CI]: 0.4 [0.3, 0.7]; $P < .001$). Separating analyses by outcome or by dialysis status led to globally concordant results, with a tendency to greater risk of adverse outcome in patients with lower LPC levels (Figs. S1 and S2).

The association between LPCs and combined outcome remained present (HR and 95% CI: 0.6 [0.3, 0.9]; $P = .04$) when accounting for age and CKD group (both $P > .05$). Adjusting on albumin rendered the association nonsignificant ($P = .08$). However, the molar ratio of LPCs/albumin was significantly associated with outcome (HR and 95% CI for a 1 SD [2 units] increase: 0.6 [0.4, 0.9]; $P = .03$). Significant associations with outcome were observed for 9 of 10 individual LPCs (C14:0, C16:0, C17:0, C18:0, C18:1, C18:2, C20:3, C20:4, C28:1; $P < .05$), two of them remaining significant after adjusting for albumin levels: LPC 18:2 (HR and 95% CI for a 1 SD [18 μmol/L] increase: 0.5 [0.3, 0.9]; $P = .02$) and LPC 20:3 (HR and 95% CI for a 1 SD [1.5 μmol/L] increase: 0.6 [0.3, 0.9]; $P = .04$).

Association with clinical features

The patterns of associations of PCs, SMs, and LPCs with clinical features were globally similar and globally the opposite of the patterns observed with NEFAs (Table 3). The total level of PCs, SMs, and LPCs increased with plasma albumin and protein level, calcemia, and phosphatemia, whereas they decreased with plasma creatinine levels. The total level of NEFAs decreased with age, eGFR, hemoglobin, bicarbonate, and sodium levels and increased with CRP and parathyroid hormone. Total LPCs and individual LPCs 18:2 and 20:3 showed the strongest correlations with many clinical parameters (Table 3).

Discussion

Lipidomics is a recent field with great potential to develop diagnostic, prognostic, or risk biomarkers, as well as to decipher changes in biological processes occurring in disease states.[12] In this study, we aimed to describe the changes in plasma lipids occurring with CKD progression using a cross-sectional design. We determined the concentration of plasma lipids from patients at different stages of CKD and patients with end-stage renal disease treated with HD and found alterations associated with patient groups. More specifically, patients on chronic HD had significantly lower plasma phospholipid levels compared with patients with early CKD, notably lower LPC levels, which were further associated with a greater risk of adverse outcome during follow-up.

We observed a general decrease in plasma lipid levels in HD patients. Treatment with HD modifies plasma composition by removing excess water and circulating molecules. This process is mainly based on diffusion and should not be able to remove hydrophobic compounds such as lipids. This was confirmed by comparing samples taken before and directly after HD, where no change in plasma lipid concentration and distribution could be observed.[13] Nevertheless, the composition of disposable elements in contact

Table 2 Lipid levels (median and interquartile range) significantly different between CKD groups

Values	CKD2-3	CKD4-5	HD	FDR-adjusted *P*-value
Lipid concentration (μmol/L)				
C15:1w5 (NEFA)	2.8 (2.3; 3.2)	3.0 (2.6; 3.4)	2.2 (1.9; 2.7)	.03*,†
C17:2w7 (NEFA)	3.3 (2.8; 4.0)	3.7 (3.0; 4.5)	2.5 (2; 3.1)	.01*,†
C17:2w7 (TFA)	2.4 (2.2; 2.9)	2.4 (2; 3.3)	1.6 (1.4; 2.3)	.01*,†
C18:1w9t (NEFA)	1.2 (0.9; 1.6)	1.5 (1.1; 2.0)	2.0 (1.6; 3.0)	.02*,†
C21:0 (TFA)	1.1 (0.9; 1.2)	1.0 (0.9; 1.4)	0.8 (0.7; 1.0)	.03*,†
C23:0 (TFA)	10.7 (9; 13.2)	10.6 (8.2; 13.1)	7.1 (5.5; 9.0)	.01*,†
LPC a C14:0	4.8 (4.4; 5.2)	4.2 (3.8; 4.8)	0.0 (0.0; 3.9)	.005*,†
LPC a C16:0	139 (119; 147)	119 (101; 136)	92 (76; 116)	.01*,†
LPC a C16:1	4.0 (3.3; 4.5)	3.4 (2.6; 4.6)	2.6 (1.8; 3.3)	.04*
LPC a C18:0	33 (28; 46)	31 (25; 41)	22 (16; 29)	.01*,†
LPC a C18:1	35 (23; 38)	26 (21; 34)	21 (16; 24)	.02*
LPC a C18:2	46 (31; 56)	33 (29; 45)	23 (16; 33)	.009*,†
LPC a C20:3	4.4 (3.7; 5.5)	3.3 (2.5; 4.0)	2.7 (2.0; 3.0)	.004*,†,‡
LPC a C20:4	11.0 (8.0; 14.0)	7.5 (6.3; 10.0)	6.0 (4.8; 8.0)	.008*,‡
PC aa C34:4	2.4 (1.7; 2.6)	1.8 (1.4; 2.1)	1.1 (0.9; 1.7)	.009*,†
PC ae C34:2	12.0 (9.4; 14.0)	12.0 (9.8; 13.0)	8.6 (7.6; 11.0)	.02*,†
PC ae C34:3	8.6 (5.5; 9.9)	7.3 (5.7; 9.5)	5.4 (4.0; 6.5)	.02*,†
PC ae C36:3	9.0 (6.7; 11.0)	9.1 (7.3; 9.9)	6.5 (5.4; 7.8)	.02*,†
PC ae C36:4	20 (15; 24)	18 (16; 23)	14 (11; 18)	.04*,†
PC ae C36:5	15 (11; 16)	13 (11; 15)	11 (9; 13)	.04*,†
PC aa C36:6	1.3 (0.9; 1.4)	0.8 (0.6; 1.2)	0.8 (0.6; 1.0)	.04*
PC ae C38:1	6.1 (5.0; 7.9)	5.9 (5.2; 7.1)	4.3 (3.5; 5.4)	.01*,†
PC ae C38:2	10.5 (8.7; 13.0)	10.0 (9.4; 13.0)	7.5 (6.1; 9.7)	.008*,†
PC ae C38:3	6.8 (5.8; 9.6)	7.1 (6.4; 8.6)	5.1 (4.7; 6.8)	.02*,†
PC ae C40:1	2.6 (2.1; 2.8)	2.2 (2.0; 2.8)	1.8 (1.5; 2.2)	.04*,†
PC ae C40:4	2.7 (2.5; 3.7)	2.8 (2.6; 3.0)	2.3 (1.9; 2.6)	.02*,†
PC ae C42:2	0.6 (0.5; 0.8)	0.6 (0.5; 0.7)	0.5 (0.4; 0.5)	.02*,†
PC ae C42:3	0.8 (0.6; 1.1)	0.8 (0.7; 0.9)	0.6 (0.5; 0.7)	.008*,†
PC ae C42:4	1.0 (0.9; 1.2)	0.9 (0.8; 1.1)	0.8 (0.6; 0.9)	.02*,†
PC ae C44:4	0.6 (0.5; 0.7)	0.6 (0.5; 0.7)	0.5 (0.4; 0.5)	.02*,†
PC ae C44:5	2.1 (2.0; 2.4)	2.0 (1.8; 2.5)	1.8 (1.4; 2.0)	.04*,†
SM C22:3	68 (59; 82)	62 (51; 70)	49 (39; 57)	.03*
SM (OH) C22:1	13.0 (9.6; 15.0)	12.0 (9.8; 14.0)	8.8 (7.0;; 11.0)	.005*,†
SM (OH) C22:2	11.5 (9.3; 13.0)	11.0 (9.9; 14.0)	8.5 (7.2; 9.6)	.004*,†
Relative concentration (% lipid family)				
C18:1w9t, %NEFA	0.11 (0.09; 0.22)	0.12 (0.09; 0.14)	0.21 (0.14; 0.24)	.03*,†
C18:1w9t, %TFA	0.07 (0.06; 0.08)	0.07 (0.06; 0.09)	0.10 (0.09; 0.12)	.03*,†
PC aa C34:4, %PC	0.10 (08; 0.12)	0.08 (0.07; 0.10)	0.06 (0.05; 0.08)	.03*,‡
SM (OH) C22:1, %SM	3.3 (3.1; 3.5)	3.3 (3; 3.6)	2.7 (2.5; 3)	.01*,†
SM (OH) C22:2, %SM	3.2 (2.6; 3.4)	3.1 (2.9; 3.5)	2.6 (2.3; 3)	.01*,†
Computed index				
C22:1/C22:0	0.06 (0.05; 0.08)	0.08 (0.06; 0.08)	0.10 (0.09; 0.15)	.004*,†

CKD, chronic kidney disease; FDR, false discovery rate; HD, hemodialysis; LPC, lysophosphatidylcholine; NEFA, nonesterified fatty acid; PC, phosphatidylcholine; SM, sphingomyelin.

*CKD2-3 significantly different from HD ($P_{adj} < .05$).

†CKD4-5 significantly different from HD ($P_{adj} < .05$).

‡CKD2-3 significantly different from CKD4-5 ($P_{adj} < .05$).

with blood and the use of convective techniques such as hemodiafiltration might affect lipid level, notably, because of adsorption, bioincompatibility, or albumin loss in the dialyzate.[14,15] Furthermore, patients treated with HD often receive medications affecting lipid metabolism such as heparin and erythropoietin, which may be involved in the observed results.[16]

The pattern of change in most lipid species was consistent with a progressive reduction in plasma levels, from early, to moderate, and to terminal stages. This behavior, mirroring that of plasma creatinine, suggests that disease severity participates in the observed changes in lipid profile. The progression of renal failure is accompanied by changes in the interior milieu, including

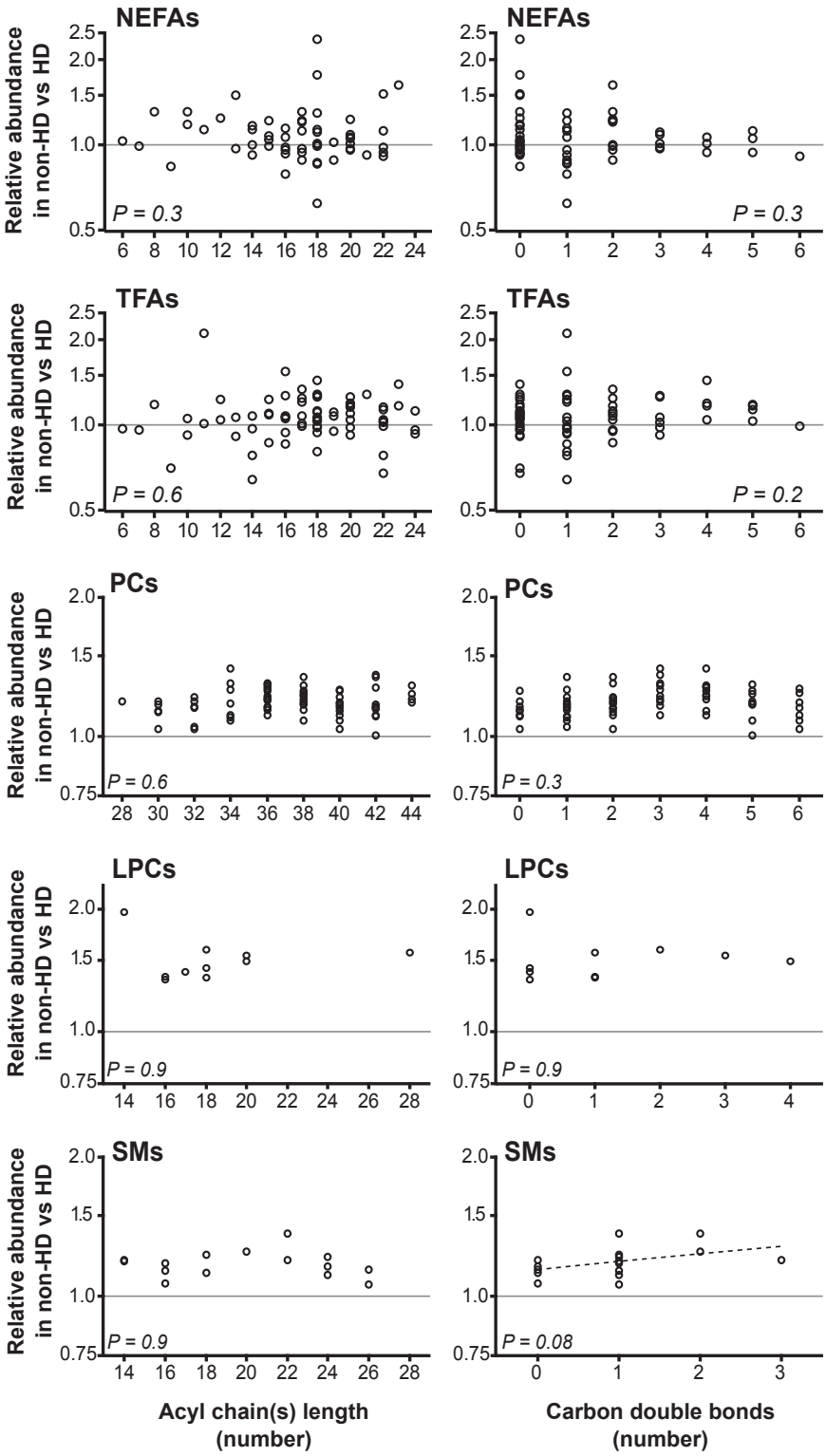

Figure 2 Association of lipid characteristic with the relative abundance in non-HD compared with HD patients. For each lipid, the ratio of non-HD level to HD level is displayed. Linear regressions were used to test for associations between lipid characteristics and relative abundance, but there were no statistically significant associations. HD, hemodialysis; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; NEFA, nonesterified fatty acid; TFA, total fatty acid.

a decrease in plasma albumin possibly indicating malnutrition. Low albumin levels per se, although the preferred transporter for LPCs or FAs, do not appear to influence plasma LPCs levels.[17] In addition, long-term interventions or weight loss do not affect lipidomic profiles,[18,19] suggesting that nutrition may participate in the observed changes but is unlikely to be the only cause of lipid alterations.

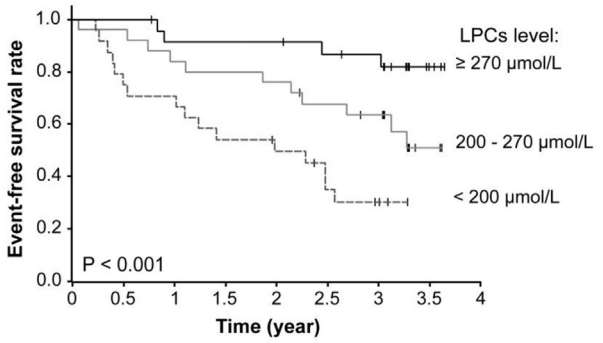

Figure 3 Kaplan-Meier survival curves for the combined outcome (dialysis or mortality) by tertile of LPCs. LPCs, lysophosphatidylcholines.

In the present study, there was a decrease in most LPCs in HD patients compared with earlier CKD stages and a tendency to lower levels than healthy controls from other studies.[1,2] Lower LPCs have previously been associated with acute kidney injury, CKD, sepsis, and adverse outcome.[20–26] Other studies, however, reported no change or increased levels of LPCs in patients with renal diseases.[27,28] LPCs act on G protein–coupled receptors and display proatherogenic activities mainly, although antimicrobial, antioxidative, and antiatherogenic effects have also been reported.[29] LPCs are derived from PCs by enzymatic activities of lecithin:cholesterol acyl transferase (LCAT), phospholipase A_2, and endothelial lipase.[29] In CKD patients, both LCAT deficiency and increased phospholipase A_2 activity have been reported.[13,30] This suggests that in renal patients, LCAT has a major role in LPC production, or alternatively that increased LPC clearance leads to low LPC levels. In contrast to a previous study,[20] we observed a lower molar ratio of plasma LPCs/PCs in HD patients, suggesting that when PCs were less abundant, the production of LPCs further decreased. Interestingly, in sepsis animal models, therapeutic LPC improved bacterial elimination.[31] In the present study and others, LPCs correlated positively with albumin and/or negatively with CRP,[24,26,32] pointing inflammation as a possible cause or consequence of low LPCs.

Using follow-up data, we observed that low LPCs, and more particularly LPC 18:2 and 20:3, were associated with the occurrence of adverse outcome (transition to HD or death). Interestingly, this is the first time that LPC 20:3 is associated with transition to HD and mortality. In previous studies, low LPC 18:2 has been associated with CKD progression,[25] and low LPC 18:2 or 20:3 with heart failure, metabolic syndrome, and hepatic cancer.[33-35] Interestingly, we observed that although the molar ratio LPCs/albumin did not change significantly across groups of patients, it remained associated with a better outcome. This suggests that LPCs are associated with the risk of mortality independently of albumin levels, which is a strong predictor of mortality tightly associated with LPC levels. Assessing plasma LPCs, LPC 18:2 and/or LPC 20:3 could be of prognostic utility, provided that it demonstrates added value to established risk markers.

Compared with healthy controls,[1,2,36] the present population of CKD and HD patients tended to have higher PC and SM plasma levels. Studies comparing patients with healthy controls consistently reported increased plasma SM levels and phospholipid levels (consisting mainly of PCs and SMs) in CKD or HD.[28,37,38] Concerning plasma PCs, 2 studies reported significant reductions in PC levels in HD patients, whereas another observed a tendency to increased levels in patients.[27,37,38] Nonetheless, compared with CKD patients, we observed reductions in plasma PC

Table 3 Correlations between lipid families and patient characteristics

Spearman ρ	NEFAs	SMs	PCs	LPCs	LPC a C20:3	LPC a C18:2
Clinical characteristics						
Age	−0.17	−0.04	−0.07	**−0.30**	**−0.30**	**−0.28**
eGFR	−0.14	0.16	0.20	**0.49**	**0.56**	**0.48**
BMI	−0.05	0.11	**0.34**	0.14	**0.25**	0.11
Plasmatic levels						
Albumin	−0.01	0.16	0.20	**0.50**	**0.60**	**0.52**
Hemoglobin	**−0.26**	0.11	0.11	**0.44**	**0.39**	**0.42**
Calcium	0.02	**0.23**	**0.34**	**0.42**	**0.52**	**0.40**
Bicarbonate	**−0.30**	−0.08	−0.15	**0.38**	**0.47**	**0.37**
Sodium	**−0.29**	0.15	0.02	**0.37**	**0.24**	**0.39**
Proteins	−0.08	**0.28**	**0.24**	**0.37**	**0.41**	**0.29**
Phosphate	0.04	**0.27**	0.22	−0.02	−0.13	−0.12
Potassium	−0.09	−0.07	−0.08	−0.12	−0.16	−0.19
CRP	0.16	0.07	−0.04	**−0.35**	**−0.30**	**−0.36**
PTH	0.22	−0.05	−0.15	**−0.36**	**−0.44**	**−0.36**
Urea	0.11	−0.13	−0.17	**−0.37**	**−0.46**	**−0.33**
Creatinine	0.14	−0.20	**−0.22**	**−0.45**	**−0.54**	**−0.45**

CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PTH, parathyroid hormone; NEFA, nonesterified fatty acid.

Coefficients with $P < .05$ are shown in bold.

and SM levels in the subgroup treated with HD, suggesting that correcting some of the uremia-linked disturbances by dialysis participates in their normalization. PCs cannot be removed by HD suggesting that other mechanisms including changes in diet and metabolism should explain plasma impoverishment.[27,37] Interestingly, lower PCs have been associated with progression to renal failure in CKD patients,[39] but this was not observed in the present study.

Compared with healthy controls,[1,2,36] we observed that CKD and HD patients tended to have similar NEFA and TFA levels. This is in contrast with previous studies reporting significant elevations in NEFAs or TFAs in HD patients.[27,28] Interestingly, there was a decrease in the plasma level of various odd-chain FAs in HD patients. Odd-chain FAs are underrepresented FAs (only about 1% of FAs) that were originally thought to directly reflect dietary intake. Although this can be true for some FAs (ie, C15:0 and dairy fat), human synthesis of odd-chain FAs by α-oxidation has been shown to occur.[40] This pathway could be inhibited in HD patients, hence explaining the plasma impoverishment.

In the present study, the lipid species that are commented may represent pools of isomers that cannot be identified by the used technology. Still, using this setting, we succeeded in giving insight into the consequences of CKD onto lipid metabolism and the association of plasma lipid depletion with adverse outcome. We identified a limited number of species with interesting associations with CKD severity and outcome. The pools of isomers related to these species may have significant biological effects and deserve further study. The influence of circulating albumin and lipoprotein levels on lipids levels, bioavailability, and biological activity should also be addressed. In the present study, patients were not asked to fast before sampling; however, it has been shown that the level of SMs, PCs, and LPCs was only moderately affected by food intake.[41] Results from our study and two other studies performing lipidome analyses of patients at different stages of CKD did not overlap well, possibly due to differences in tested populations and study design, as well as a lack of standardization of lipidomics methods.[2,42] Finally, it should be reminded that the study is observational, and therefore results should only be interpreted as associations and not as cause-and-effect relationships.

In conclusion, analyzing the plasma lipidome allowed the identification of patterns of change that followed the course of disease severity and others that were specific to HD patients. Using a combination of cross-sectional and longitudinal data, we found that LPC levels were correlated with many aspects of the disease and furthermore with the risk of transition to end-stage renal disease or death.

Acknowledgments

Financial disclosure: This work was supported by the EuroTransBio Grant ETB-2006-016 to the Urosysteomics consortium. Part of the results of this work has been presented at the 54th annual meeting of the ERA-EDTA in Madrid, 2017, as a poster.

Authors' contribution: All authors contributed substantially to the completion of this project and have read and approved the final manuscript. Research idea and study design were provided by H.M. and A.A. Data acquisition was done by N.G., F.D., U.L., K.M.W., M.-F.S., and A.A. Statistical analysis was done by F.D. and A.A. Data analysis and interpretation were done by F.D., J.L., N.G., A.A., J.-S.S.-B., and J.P.S.

Data sharing statement: Deidentified individual participant data underlying the results presented in the manuscript will be archived by the corresponding author and accessible up to 5 years following article publication. Proposals including a protocol and statistical analysis plan should be sent for evaluation to argiles@rd-n.org. Data transfer, handling, and use should be defined by contract and comply with the French Data Protection Authority (CNIL) recommendations.

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Appendix

Table S1 List of targeted lipids and detection rates by lipid family

Family	Species	Detection rate (%)
A. Nonesterified fatty acids (NEFAs)		
NEFA	C6:0	100
NEFA	C7:0	100
NEFA	C8:0	100
NEFA	C9:0	100
NEFA	C10:0	100
NEFA	9Me-C10:0	31
NEFA	C11:0	30
NEFA	C11:1ω5	42
NEFA	C12:0	100
NEFA	C13:0	58
NEFA	12Me-C13:0	81
NEFA	C13:2ω3	13
NEFA	C14:0	100
NEFA	13Me-C14:0	96
NEFA	12Me-C14:0	92
NEFA	C14:1ω5	77
NEFA	C14:1ω9	18
NEFA	C14:1ω7	8
NEFA	C15:0	100
NEFA	14Me-C15:0	99
NEFA	C15:1ω5	99
NEFA	C15:2ω9	99
NEFA	C16:0	100
NEFA	15Me-C16:0	100
NEFA	14Me-C16:0	99
NEFA	C16:1ω9	99
NEFA	C16:1ω7	99
NEFA	C16:1ω13	6
NEFA	C16:1ω11	12
NEFA	C16:1ω5	47
NEFA	C17:0	99
NEFA	15Me-C17:0	99
NEFA	C17:1ω7	1
NEFA	C17:1ω9	94
NEFA	C17:1ω5	97
NEFA	C17:2ω9	99
NEFA	C17:2ω7	99
NEFA	C17:2ω6	91
NEFA	C18:0	100
NEFA	8Me-C18:0	44
NEFA	17Me-C18:0	57
NEFA	2Me-C18:0	62
NEFA	C18:1ω9t	88
NEFA	C18:1ω9	99
NEFA	C18:1ω7	99
NEFA	C18:1ω11	82
NEFA	C18:1ω6	19
NEFA	C18:2ω6t	1
NEFA	C18:2ω6	99
NEFA	C18:3ω6	91
NEFA	C18:3ω3	86
NEFA	C18:4ω3	1
NEFA	C19:0	99

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Table S1 (*continued*)

Family	Species	Detection rate (%)
NEFA	C19:2ω6	86
NEFA	C19:2ω3	0
NEFA	C20:0	99
NEFA	C20:1ω9	95
NEFA	C20:2ω6	97
NEFA	C20:2ω3	58
NEFA	C20:3ω9	66
NEFA	C20:3ω6	99
NEFA	C20:3ω3	4
NEFA	C20:4ω6	99
NEFA	C20:4ω3	52
NEFA	C20:5ω3 (EPA)	99
NEFA	C20:5ω6	1
NEFA	C21:0	97
NEFA	C22:0	17
NEFA	21Me-C22:0	21
NEFA	C22:1ω9	3
NEFA	C22:2ω6	0
NEFA	C22:3ω3	75
NEFA	C22:4ω6	97
NEFA	C22:5ω3	99
NEFA	C22:5ω6 (2E)	96
NEFA	C22:6ω3 (DHA)	99
NEFA	C23:0	0
NEFA	C23:2ω9	61
NEFA	C24:0	3
NEFA	C24:1ω9	0
NEFA	C24:2ω3	0
B. Total fatty acids (TFAs)		
TFA	C6:0	100
TFA	C7:0	100
TFA	C8:0	100
TFA	C9:0	100
TFA	C10:0	100
TFA	9Me-C10:0	45
TFA	C11:0	58
TFA	C11:1ω5	40
TFA	C12:0	100
TFA	C13:0	94
TFA	12Me-C13:0	84
TFA	C13:2ω3	17
TFA	C14:0	100
TFA	13Me-C14:0	100
TFA	12Me-C14:0	100
TFA	C14:1ω5	100
TFA	C14:1ω9	69
TFA	C14:1ω7	52
TFA	C15:0	100
TFA	14Me-C15:0	100
TFA	C15:1ω5	100
TFA	C15:2ω9	100
TFA	C16:0	100
TFA	15Me-C16:0	100
TFA	14Me-C16:0	99
TFA	C16:1ω9	100
TFA	C16:1ω7	100
TFA	C16:1ω13	84

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Table S1 (*continued*)

Family	Species	Detection rate (%)
TFA	C16:1ω11	42
TFA	C16:1ω5	81
TFA	C17:0	100
TFA	15Me-C17:0	100
TFA	C17:1ω7	1
TFA	C17:1ω9	100
TFA	C17:1ω5	96
TFA	C17:2ω9	100
TFA	C17:2ω7	100
TFA	C17:2ω6	70
TFA	C18:0	100
TFA	8Me-C18:0	39
TFA	17Me-C18:0	100
TFA	2Me-C18:0	99
TFA	C18:1ω9t	100
TFA	C18:1ω9	100
TFA	C18:1ω7	100
TFA	C18:1ω11	99
TFA	C18:1ω6	65
TFA	C18:2ω6t	8
TFA	C18:2ω6	100
TFA	C18:3ω6	100
TFA	C18:3ω3	100
TFA	C18:4ω3	68
TFA	C19:0	100
TFA	C19:2ω6	97
TFA	C19:2ω3	100
TFA	C20:0	100
TFA	C20:1ω9	100
TFA	C20:2ω6	100
TFA	C20:2ω3	100
TFA	C20:3ω9	99
TFA	C20:3ω6	100
TFA	C20:3ω3	94
TFA	C20:4ω6	100
TFA	C20:4ω3	97
TFA	C20:5ω3 (EPA)	100
TFA	C20:5ω6	68
TFA	C21:0	100
TFA	C22:0	100
TFA	21Me-C22:0	43
TFA	C22:1ω9	100
TFA	C22:2ω6	4
TFA	C22:3ω3	100
TFA	C22:4ω6	100
TFA	C22:5ω3	100
TFA	C22:5ω6 (2E)	100
TFA	C22:6ω3 (DHA)	100
TFA	C23:0	100
TFA	C23:2ω9	40
TFA	C24:0	99
TFA	C24:1ω9	100
TFA	C24:2ω3	96
C. Lysophosphatidylcholines (LPCs) and phosphatidylcholines (PCs)		
LPC (PC)	lysoPC a C6:0	10
LPC (PC)	lysoPC a C14:0	71
LPC (PC)	lysoPC a C16:0	100

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Table S1 (*continued*)

Family	Species	Detection rate (%)
LPC (PC)	lysoPC a C16:1	100
LPC (PC)	lysoPC a C17:0	100
LPC (PC)	lysoPC a C18:0	100
LPC (PC)	lysoPC a C18:1	100
LPC (PC)	lysoPC a C18:2	100
LPC (PC)	lysoPC a C20:3	96
LPC (PC)	lysoPC a C20:4	100
LPC (PC)	lysoPC a C24:0	5
LPC (PC)	lysoPC a C26:0	0
LPC (PC)	lysoPC a C26:1	0
LPC (PC)	lysoPC a C28:0	1
LPC (PC)	lysoPC a C28:1	77
PC	PC aa C24:0	1
PC	PC aa C26:0	0
PC	PC aa C28:1	100
PC	PC aa C30:0	100
PC	PC aa C30:2	100
PC	PC aa C32:0	100
PC	PC aa C32:1	100
PC	PC aa C32:2	100
PC	PC aa C32:3	100
PC	PC aa C34:1	100
PC	PC aa C34:2	100
PC	PC aa C34:3	100
PC	PC aa C34:4	100
PC	PC aa C36:0	100
PC	PC aa C36:1	100
PC	PC aa C36:2	100
PC	PC aa C36:3	100
PC	PC aa C36:4	100
PC	PC aa C36:5	100
PC	PC aa C36:6	100
PC	PC aa C38:0	100
PC	PC aa C38:1	100
PC	PC aa C38:3	100
PC	PC aa C38:4	100
PC	PC aa C38:5	100
PC	PC aa C38:6	100
PC	PC aa C40:1	70
PC	PC aa C40:2	100
PC	PC aa C40:3	100
PC	PC aa C40:4	100
PC	PC aa C40:5	100
PC	PC aa C40:6	100
PC	PC aa C42:0	100
PC	PC aa C42:1	100
PC	PC aa C42:2	100
PC	PC aa C42:4	100
PC	PC aa C42:5	100
PC	PC aa C42:6	90
PC	PC ae C30:0	100
PC	PC ae C30:1	100
PC	PC ae C30:2	100
PC	PC ae C32:1	100
PC	PC ae C32:2	100
PC	PC ae C34:0	100
PC	PC ae C34:1	100

(*continued on next page*)

Table S1 (*continued*)

Family	Species	Detection rate (%)
PC	PC ae C34:2	100
PC	PC ae C34:3	100
PC	PC ae C36:0	100
PC	PC ae C36:1	100
PC	PC ae C36:2	100
PC	PC ae C36:3	100
PC	PC ae C36:4	100
PC	PC ae C36:5	100
PC	PC ae C38:0	100
PC	PC ae C38:1	100
PC	PC ae C38:2	100
PC	PC ae C38:3	100
PC	PC ae C38:4	100
PC	PC ae C38:5	100
PC	PC ae C38:6	100
PC	PC ae C40:0	16
PC	PC ae C40:1	100
PC	PC ae C40:2	100
PC	PC ae C40:3	100
PC	PC ae C40:4	100
PC	PC ae C40:5	100
PC	PC ae C40:6	100
PC	PC ae C42:0	66
PC	PC ae C42:1	100
PC	PC ae C42:2	100
PC	PC ae C42:3	100
PC	PC ae C42:4	100
PC	PC ae C42:5	100
PC	PC ae C44:3	100
PC	PC ae C44:4	100
PC	PC ae C44:5	100
PC	PC ae C44:6	100
D. Sphingomyelins (SMs)		
SM	SM C14:0	100
SM	SM C16:0	100
SM	SM C16:1	100
SM	SM C18:0	100
SM	SM C18:1	100
SM	SM C20:2	100
SM	SM C22:3	100
SM	SM C24:0	100
SM	SM C24:1	100
SM	SM C26:0	100
SM	SM C26:1	100
SM	SM (OH) C14:1	100
SM	SM (OH) C16:1	100
SM	SM (OH) C22:1	100
SM	SM (OH) C22:2	100
SM	SM (OH) C24:1	100

Table S2 List of indices computed from lipidomic results

Index type	Index
Sum	Sum of NEFAs
Sum	Sum of TFAs
Sum	Sum of LPCs
Sum	Sum of PCs
Sum	Sum of SMs
Sum	Sum of saturated NEFAs
Sum	Sum of saturated TFAs
Sum	Sum of monoinsaturated NEFAs
Sum	Sum of monoinsaturated TFAs
Sum	Sum of polyinsaturated NEFAs
Sum	Sum of polyinsaturated TFAs
Sum	Sum of ω6 polyinsaturated NEFAs
Sum	Sum of ω6 polyinsaturated TFAs
Sum	Sum of ω3 polyinsaturated NEFAs
Sum	Sum of ω3 polyinsaturated TFAs
Ratio	Sum of C14:1/C14:0 (TFAs)
Ratio	Sum of C15:1/C15:0 (TFAs)
Ratio	Sum of C16:1/C16:0 (TFAs)
Ratio	Sum of C17:1/C17:0 (TFAs)
Ratio	Sum of C18:1/C18:0 (TFAs)
Ratio	Sum of C20:1/C20:0 (TFAs)
Ratio	Sum of C22:1/C22:0 (TFAs)
Ratio	Sum of C24:1/C24:0 (TFAs)
Ratio	Sum of SMs/sum of PCs
Ratio	Sum of LPCs/sum of PCs
Ratio	Sum of LPCs/albumin
Ratio	LPC a C14:0/albumin
Ratio	LPC a C16:0/albumin
Ratio	LPC a C16:1/albumin
Ratio	LPC a C17:0/albumin
Ratio	LPC a C18:0/albumin
Ratio	LPC a C18:1/albumin
Ratio	LPC a C18:2/albumin
Ratio	LPC a C20:3/albumin
Ratio	LPC a C20:4/albumin
Ratio	LPC a C28:1/albumin

LPC, lysophosphatidylcholine; NEFA, nonesterified fatty acid; PC, phosphatidylcholine; SM, sphingomyelin; TFAs, total fatty acids.

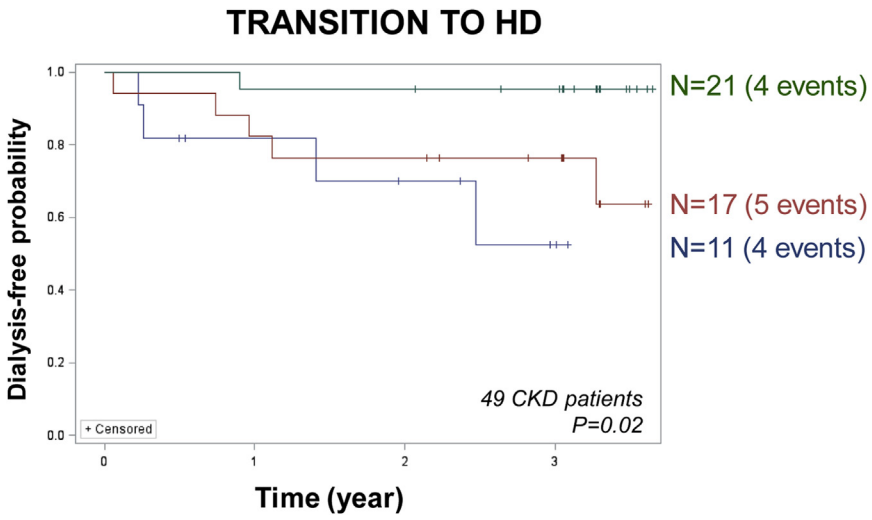

Figure S1 Association between LPC levels and the risk of transition to hemodialysis. There were 49 CKD patients at risk of progressing to hemodialysis. Patients were grouped by levels of LPC: <200 μmol/L (blue), 200–270 μmol/L (red), and ≥ 270 μmol/L (green). Kaplan-Meier survival curves and log-rank test *P*-value are displayed. CKD, chronic kidney disease; HD, hemodialysis; LPC, lysophosphatidylcholine.

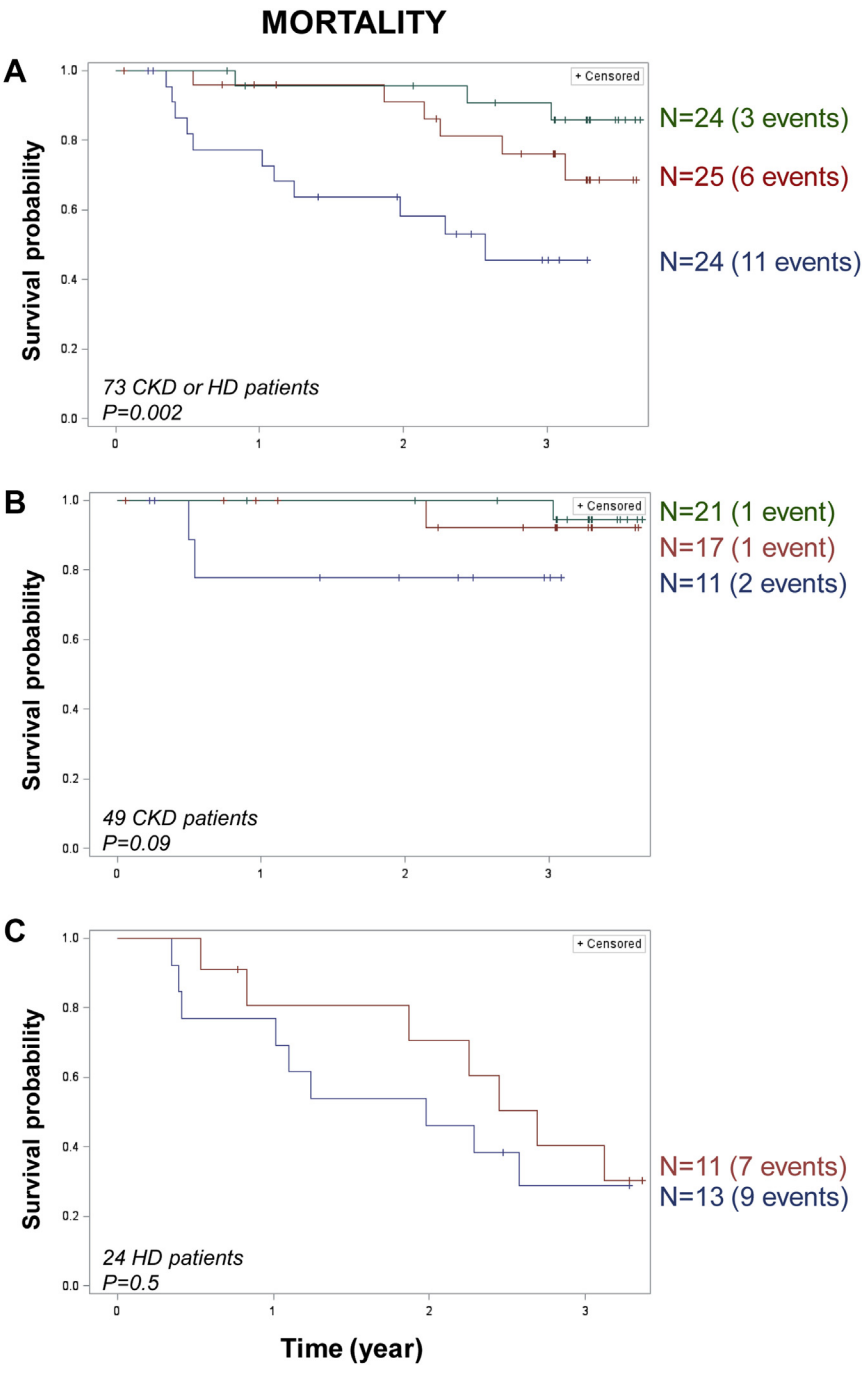

Figure S2 Association between LPC levels and the risk of death. There were 24 HD patients and 49 CKD patients at risk of dying, analyzed together (panel A) and separately (panels B and C). Patients were grouped by levels of LPC: <200 μmol/L (blue), 200–270 μmol/L (red), and ≥ 270 μmol/L (green). Kaplan-Meier survival curves and log-rank tests *P*-values are displayed. CKD, chronic kidney disease; LPC, lysophosphatidylcholine.