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An altered plasma lipidome–phenome network characterizes heart failure with preserved ejection fraction

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Abstract

Aims Heart failure with preserved ejection fraction (HFpEF) is a multifactorial, multisystemic syndrome that involves alterations in lipid metabolism. This study aimed to test whether distinct plasma lipid profiles or lipid entities or both are associated with clinical and functional echocardiographic parameters in HFpEF.

Methods and results We examined the human plasma lipidome in HFpEF patients ($n = 18$) with left ventricular ejection fraction $\geq 50\%$ and N-terminal pro-brain natriuretic peptide (NT-proBNP) >125 pg/mL and control subjects ($n = 12$) using mass spectrometry-based shotgun lipidomics. The cohort included 8 women and 22 men with average age of 67.8 ± 8.6 SD. The control and disease groups were not significantly different with respect to age, body mass index, systolic and diastolic blood pressure, and waist-to-hip ratio. The disease group experienced more fatigue ($P < 0.001$), had more often coronary artery disease ($P = 0.04$), and received more medications (beta-blockers, $P < 0.001$). The disease group had significantly different levels of HFpEF-relevant parameters, including NT-proBNP ($P < 0.001$), left ventricular mass index ($P = 0.005$), left atrial volume index ($P = 0.001$), and left ventricular filling index ($P < 0.001$), and lower left ventricular end-diastolic diameter ($P = 0.014$), with no difference in left ventricular ejection fraction. Significant differences in lipid profiles between HFpEF patients and controls could not be detected, including no significant differences in abundance of circulating lipids binned by carbon chain length or by double bonds, nor at the level of individual lipid species. However, there was a striking correlation between selected lipids with smoking status that was independent of disease status, as well as between specific lipids and hyperlipidaemia [with corresponding significance of either false discovery rate (FDR) <0.1 or FDR < 0.01]. In an exploratory network analysis of correlations, we observed significantly stronger correlations within the HFpEF group between individual lipids from the cholesterol ester and phosphatidylcholine (PC) classes and clinical/echocardiographic parameters such as left atrial volume index, left ventricular end-diastolic diameters, and heart rate (FDR < 0.1). In contrast, the control group showed significantly stronger negative correlations (FDR < 0.1) between individual species from the PC and sphingomyelin classes and left ventricular mass index or systolic blood pressure.

Conclusions We did not find significant direct associations between plasma lipidomic parameters and HFpEF and therefore could not conclude that any specific lipids are biomarkers of HFpEF. The validation in larger cohort is needed to confidently conclude the absence of first-order associations.

Keywords Heart failure; HFpEF; Plasma lipids; Lipidomics; Lipid metabolism

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Introduction

Heart failure with preserved ejection fraction (HFpEF) is a commonly occurred and devastating clinical syndrome, which accounts for over a half of the estimated 64.3 million heart failure patients worldwide, with an increasing prevalence.[1] The 5 year survival rate of HFpEF patients is 69.8% and does not significantly differ from that of patients with heart failure with reduced ejection fraction (HFrEF).[2] In contrast to HFrEF, there is insufficient knowledge of HFpEF pathophysiology and lack of therapeutic options, aspects that urge to be addressed giving the poor prognosis and increasing prevalence of HFpEF.

Current data suggest that HFpEF is a multifactorial, multisystemic syndrome that comprises different clinical phenotypes.[3] Distinct HFpEF phenotypes are associated with metabolic disorders such as obesity and hyperlipidaemia, including hypertriglyceridemia or elevated low-density lipoprotein (LDL) cholesterol or both[4,5]; thus, alterations in circulating plasma lipids appear to be mediating the pathophysiological processes driving the development of HFpEF. These processes likely involve a mismatch between circulating lipid supply, cardiac fat uptake, and fatty acid metabolism resulting in the myocardial accumulation of toxic lipid intermediates [diacylglycerols (DAGs), triacylglycerols (TAGs), or ceramides (Cer)]—a process called lipotoxicity, which results in diastolic dysfunction.[6,7] In addition, cardiac metabolism can be directly disrupted by altered plasma lipid levels leading to a loss of cardiac metabolic flexibility followed by cardiac ATP depletion and functional impairment.[6] Finally, circulating lipids affect the phospholipid composition of cell membranes, including those of cardiomyocytes, thereby altering cardiomyocyte and left ventricular (LV) diastolic function.[8] Taken together, circulating lipids are centrally involved in crucial processes of HFpEF pathophysiology. Accurate and deep analyses of the circulating lipid profile in HFpEF patients may improve our understanding of relevant circulating lipid species and corresponding molecular mechanisms in the heart.

Previous studies found minor differences between healthy controls and HFpEF patients when focusing on standard clinical plasma lipid data such as TAGs, free fatty acids, or LDL cholesterol.[7,9] Beyond these results, there has been limited in-depth mass spectrometry (MS) analysis of individual molecular lipid species comparing plasma samples from control subjects and HFpEF patients. This includes studies that analysed the serum or plasma lipid profiles in selected HFpEF patients from the Aldosterone in Diastolic Heart Failure (Aldo-DHF)[10] and the Treatment of Preserved Cardiac

Function Heart Failure with an Aldosterone Antagonist (TOPCAT) cohorts[11] that investigated functional capacity, echocardiographic parameters, or cardiovascular risk. Importantly, healthy control subjects were not included in these studies. Furthermore, Lechner *et al*. studied 404 HFpEF patients from the Aldo-DHF trial and measured blood levels of various saturated fatty acids.[12] They have shown baseline blood levels of the long-chain fatty acids C14:0 and C16:0 associated with either a more pronounced cardiovascular risk profile or lower exercise capacity or both.[12] In contrast, three very-long-chain saturated fatty acids C20:0, C22:0, and C24:0 were accompanied by a lower risk phenotype.[12] Another study in this cohort demonstrated that omega-3 fatty acid blood cell membrane levels (eicosapentaenoic acid C20:5n3; docosahexaenoic acid C22:6n3) were associated with a more favourable cardiometabolic risk profile in HFpEF patients.[13] Neither study detected any significant associations between individual lipids and echocardiographic parameters of HFpEF such as E/e'.[12,13] In 433 patients from the TOPCAT trial, Javaheri *et al*. used liquid chromatography–MS (LC–MS)-based targeted sphingolipidomics to demonstrate the association of circulating ceramides Cer16:0 and Cer18:0 with death or heart failure admission in HFpEF.[14] Altogether, previous plasma lipidomic studies either focused exclusively on specific lipid classes or lacked comparisons with healthy controls.

In this study, we filled this gap by examining the human plasma lipidome in HFpEF patients and healthy control subjects using MS-based shotgun lipidomics, to test whether distinct plasma lipid profiles or lipid species are associated with clinical and functional echocardiographic parameters.

Methods

Study population

Between October 2016 and November 2019, 18 HFpEF patients and 12 healthy individuals were prospectively enrolled in the German HFpEF registry.[15] Inclusion criteria for HFpEF patients were defined in accordance with the 2016 European Society of Cardiology—Heart Failure (ESC-HF) guidelines: (i) LV ejection fraction (LVEF) ≥50%; (ii) age ≥18 years; (iii) New York Heart Association (NYHA) functional class ≥II; (iv) elevated levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) >125 pg/mL; and (v) at least one additional criterion for structural heart disease or diastolic dysfunction [LV mass index (LVMI) ≥115 g/m² for men and ≥95 g/m² for

women, left atrial (LA) volume index (LAVI) >34 mL/m^2, LV filling index (E/e' mean) ≥13, and early diastolic mitral annular velocity (e' mean) <9 cm/s].[15,16] Important to note, the initial LVEF inclusion criterion of the registry was LVEF ≥ 45%.[15] To meet ESC-HF criteria, only patients with an LVEF ≥ 50% were considered in this analysis.[15] Exclusion criteria were (i) acute coronary syndrome during the past 3 months, (ii) cardiac surgery/percutaneous intervention during the past 3 months, and (iii) haemodynamic relevant pericardial disease.[15] Patients were recruited at an outpatient setting at the Charité University Hospital, Department of Internal Medicine and Cardiology, Campus Virchow Klinikum, Berlin, Germany.[15] The study complies with the Declaration of Helsinki, and the Ethics Committee of Charité University Hospital approved the research project (EA2/134/15). In addition, written informed consent was obtained from all subjects.

Echocardiography

All study participants underwent transthoracic echocardiography at rest using Philips EPIQ 7 ultrasound system (Philips Medical Systems, Andover, MA, USA).[15] In the parasternal long axis, LV end-diastolic diameter (LVEDD), end-diastolic interventricular septal thickness (IVSED), and end-diastolic posterior wall thickness (PWED) were determined.[15] To assess the LVMI, the mass of the LV was calculated from LVEDD, IVSED, and PWED and then related to the body surface area (BSA).[15] The LA end-systolic volume (LAESV) was determined using the biplane method, and by relating LAESV to BSA, LAVI was obtained.[15] The end-diastolic and end-systolic volumes (EDV and ESV) of the LV were determined biplane using Simpson's disc summation method, and subsequently, LVEF was calculated from EDV and ESV.[15] E wave and A wave were measured in pulsed-wave Doppler to evaluate the LV inflow profile.[15] Lateral and septal early diastolic mitral annulus velocities (e' lateral and e' septal) were determined in tissue Doppler, and the mean velocity (e' mean) was derived.[15] Finally, the LV filling index (E/e' mean) was calculated from the obtained parameters.[15]

Blood sampling and laboratory analysis

Venous blood samples were drawn in a sitting or lying position after a resting period of at least 5 min.[15] Samples were instantly cooled and processed. Plasma samples were stored at −80° C for further analysis. Selected laboratory parameters were directly analysed by standard methods including total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (*Table 2*). NT-proBNP was measured using an electrochemiluminescence immunoassay.

Mass spectrometry-based shotgun lipidomics

The MS-based lipidomic analysis of plasma samples was conducted in cooperation with Lipotype GmbH (Dresden, Germany), as previously described.[17] Samples were analysed by direct infusion on a QExactive mass spectrometer (Thermo Fisher Scientific, MA, USA) equipped with a TriVersa NanoMate ion source (Advion Biosciences, NY, USA).[18,19] Samples were analysed in both positive and negative ion modes, as described.[18,19] Both MS and MS–MS data were combined to monitor cholesterol ester (CE), DAG, and TAG ions as ammonium adducts; phosphatidylcholine (PC) and PC O- as acetate adducts; and PE, PE O-, and phosphatidylinositol (PI) as deprotonated anions.[18,19] MS only was used to monitor lysophosphatidylethanolamine (LPE) and LPE O- as deprotonated anions and Cer, sphingomyelin (SM), lysophosphatidylcholine (LPC), and LPC O- as acetate adducts. Data were analysed with in-house developed lipid identification software based on LipidXplorer.[18,20] Only lipid identifications with a signal-to-noise ratio >5 and a signal intensity five-fold higher than in corresponding blank samples were considered for further data analysis.[18,19] The lipid annotation consists of the first two ciphers that refer to the number of carbon atoms, while the third cipher that is separated by a colon from the first two ciphers indicates the number of double bonds, followed by the number of hydroxyl groups separated by a semicolon. For example, SM34:1;2 denotes an SM species with a total number of 34 carbon atoms, 1 double bond, and 2 hydroxyl groups in the ceramide backbone.

Statistical analysis

All statistical analyses were carried out in R Version 4.2.2. For the clinical summary table, the differences between the groups were calculated using Pearson χ^2 test in the case of categorical variables; if the number of observations was <5, Fisher's exact test was applied instead. For comparison of continuous variables, the *t*-test was used for normally distributed variables and mean values were reported, while for non-normally distributed variables, results of the Mann–Whitney *U* test and the median values were reported. The normality of the variable distribution was tested with the Shapiro–Wilk test. In the case of multiple testing, when analysing lipidomic variables, Benjamini–Hochberg (BH) false discovery rate (FDR) correction was applied.[21] The *post hoc* power calculation was performed using *pwr.t2n.test* function of the pwr R package.

The metadeconfoundR workflow,[22] developed in-house and provided as an R package, uses univariate, non-parametric statistical tests to find associations between lipidomic features and clinical parameters, then *post hoc* testing using nested linear model comparisons to check for

confounding variables, whereafter a status label is returned for each lipidomic feature. The metadeconfoundR applies ranked tests and BH FDR correction and reports standardized effect sizes.

The redundancy analysis (RDA) was performed using R vegan package with three constrained variables, the health–disease group 'Group', 'Hyperlipidaemia', and the smoking status 'Never smoker' to test how much of the lipid variance is explained in by these three variables. Subsequently, an ANOVA-like permutation test[23] was performed on RDA axis and RDA terms to assess the significance of constraints. In addition to ANOVA-like permutation test, permutational multivariate ANOVA (PERMANOVA) was performed on RDA terms.

To determine whether correlations ('second-order' associations) between lipid and echocardiographic parameters significantly differ between the clinical groups, linear regression was adapted to model differential correlations. More precisely, the relationship between two rank-transformed variables (corresponding to a Spearman correlation) was represented as a linear model between the two, with an interaction term vs. the clinical group variable (i.e. health vs. disease) to represent group-differential slope. Comparing a model with the interaction term vs. one without it under a likelihood ratio test, adjusted over the pairs of variables for multiple testing using BH FDR correction, thus constitutes a test for differential correlation that is robust to uneven group sizes.

Results

Patient population

Plasma samples from 18 HFpEF patients were randomly selected from the German HFpEF registry,[15] along with 12 age- and sex-matched control individuals. Patients with HFpEF had a slightly higher body mass index (BMI) and had a more frequent occurrence of arterial hypertension, coronary artery disease (CAD), atrial fibrillation, and sleep apnoea syndrome (*Table 1*). Thirteen (72.2%) HFpEF patients were in NYHA Class II and five HFpEF patients (27.8%) in NYHA Class III (*Table 1*). Beta-blockers, aldosterone antagonists, and loop diuretics were more frequently prescribed in HFpEF patients compared with controls (*Table 1*). LVEF was not significantly different between the groups (controls: 60 ± 5.4%; HFpEF: 56.4 ± 4.7%). Echocardiographic analysis revealed significantly lower LVEDD and increased LAVI, LVMI, and E/e' mean values in HFpEF patients when compared with control individuals (*Table 1*). NT-proBNP was significantly elevated in HFpEF patients (*Table 2*), whereby no differences were detected in routine plasma lipid parameters including total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides (*Table 2*).

Clinical parameters

First, we explored the relationship between different clinical and echocardiographic parameters and their association with the diagnostic group variable (HFpEF) (*Figure 1*). As expected, NYHA class correlated significantly (FDR < 0.001) with the diagnostic group variable (HFpEF). Selected parameters used for the diagnosis of HFpEF,[24] including LAVI and E/e', were significantly and positively correlated with the disease status (FDR < 0.01), while LVMI was identified as a confounder (*Figure 1*), that is to say that more variation could be explained by LAVI and E/e' parameters than by LVMI. On the other hand, LVEDD was decreased in HFpEF, trending significant at FDR < 0.1 (*Figure 1*). Other confounding variables included use of different medications, such as beta-blockers and loop diuretics, but also BMI and several clinical symptoms of the disease, such as exertional dyspnoea and fatigue (*Figure 1*). Angiotensin type 1 receptor antagonists correlated positively with LVMI independent of the group variable, with a significance estimate of *P* < 0.01. Finally, post-myocardial infarction and coronary intervention correlated positively with NYHA class, narrowly achieving significance at FDR < 0.1 (*Figure 1*).

Lipidomic parameters

We performed MS-based shotgun lipidomics in plasma samples from control subjects and HFpEF patients. Significant changes were detected in circulating lipids binned per total number of C-atoms in each lipid class between the groups for CE14, CE20, and PC38 (*Figure 2*). Further analysis of lipids binned by total number of C-atoms and clinical data showed that these differences could be attributed to the clinical covariates BMI, post-myocardial infraction, arterial hypertension, hyperlipidaemia, and statin intake rather than disease status (*Figure 3*). No significant correlation was observed with the disease status (*Figure 3*, left column). Nevertheless, a marked correlation was shown between lipid bins corresponding to different number of total C-atoms and smoking status, as well as with selected clinical parameters such as hypertension, hyperlipidaemia, statin use, and previous myocardial infarction (*Figure 3*). To assess whether saturation status of lipids is associated with the disease, we correlated the lipid species abundances binned by the number of double bonds with clinical parameters. We observed no significant correlation between disease status (HFpEF) and the abundance of lipids in each bin corresponding to a certain number of double bonds (*Figure 4*). A positive correlation between previous coronary interventions and abundance of lipids binned by the number of double bonds (degrees of saturation) did reach significance (*Figure 4*). We further assessed the relationship between the abundance of each circulating lipid and HFpEF diagnosis status while accounting for possible demographic,

Table 1 Clinical parameters in HFpEF and control

	Control (n = 12)	HFpEF (n = 18)	P value
Age (years)	65.9 ± 5.6	69 ± 0.07	0.285
Female sex, n (%)	2 (16.7)	6 (33)	0.4192
Male sex, n (%)	10 (83.3)	12 (87)	
Body mass index (kg/m^2)	25.7 ± 5	28.25 ± 4.2	0.163
Systolic blood pressure (mmHg)	139 ± 20.87	137.5 ± 28.31	0.869
Diastolic blood pressure (mmHg)	78.42 ± 12.15	83.83 ± 14.72	0.282
Waist-to-hip ratio	1.015 ± 0.08	0.98 ± 0.1	0.428
Medical history			
Hypertension, n (%)	7 (58)	16 (88)	0.083
Diabetes mellitus, n (%)	0 (0)	2 (11.1)	NA
Hyperlipidaemia, n (%)	5 (41.7)	12 (66.7)	0.328
Smoker active or ex-smoker, n (%)	5 (42)	13 (72)	0.136
Never smoker, n (%)	7 (58)	5 (27.78)	0.136
Coronary artery disease, n (%)	1 (8.3)	8 (44)	0.04*
Previous myocardial infarction, n (%)	0 (0)	6 (33.3)	NA
COPD	0 (0)	2 (11.1)	NA
Atrial fibrillation, n (%)	1 (8.3)	7 (38.89)	0.099
Sleep apnoea syndrome	0 (0)	5 (27.78)	NA
Heart failure symptoms			
NYHA Class I, n (%)	12 (100)	0 (0)	<0.001*
NYHA Class II, n (%)	0 (0)	13 (72.2)	<0.001*
NYHA Class III, n (%)	0 (0)	5 (27.78)	0.007*
Fatigue, n (%)	1 (8.3)	14 (77.78)	<0.001*
Nycturia, n (%)	8 (66.67)	13 (72.2)	1
Medication			
ACE inhibitors or ARBs, n (%)	3 (25)	6 (33.33)	0.703
Beta-blockers, n (%)	2 (16.67)	16 (88.89)	<0.001*
Calcium antagonists, n (%)	3 (25)	7 (38.89)	0.694
Aldosterone antagonists, n (%)	0 (0)	5 (27.78)	NA
Thiazide, n (%)	2 (16.67)	7 (38.89)	0.371
Loop diuretics, n (%)	0 (0)	7 (38.89)	NA
Statins, n (%)	2 (16.67)	10 (55.56)	0.057
Echocardiography			
LAVI (mL/m^2)	27.2 ± 7.2	34.55 ± 11.18	0.001*
LVEDD (mm)	51.67 ± 3.98	46.94 ± 5.94	0.014*
LVMI (g/m^2)	91.42 ± 16.04	114.05 ± 25.11	0.005*
LVEF (%)	60 ± 5.35	56.4 ± 4.73	0.13
E/e' mean	7.81 ± 1.93	12.06 ± 3.84	<0.001*

ACE, angiotensin-converting enzyme; ARBs, angiotensin receptor blockers; COPD, chronic obstructive pulmonary disease; E, early diastolic flow velocity; e', velocity of early diastolic mitral annular motion; HFpEF, heart failure with preserved ejection fraction; LAVI, left atrial volume index; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NYHA, New York Heart Association.
*P value <0.05.

Table 2 Laboratory parameters in HFpEF and control

|	Control (n = 12)	HFpEF (n = 18)	
NT-proBNP (pg/mL)	46.5 ± 71.71	327.5 ± 378.66	<0.001*
Total cholesterol (mg/dL)	193.83 ± 31.96	180.56 ± 34.43	0.29
LDL cholesterol (mg/dL)	106 ± 25.44	112 ± 35.24	0.949
HDL cholesterol (mg/dL)	57.5 ± 19.23	46.5 ± 10.87	0.056
Triglycerides (mg/dL)	161.5 ± 62.81	116.5 ± 88.1	0.606

HDL, high-density lipoprotein; HFpEF, heart failure with preserved ejection fraction; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-brain natriuretic peptide.
*P value <0.05.

clinical, or treatment covariates using the metadeconfoundR linear modelling framework to assess possible direct vs. indirect associations, visualized as a heatmap (*Figure 5*). Again, no significant correlations were observed with the disease status (HFpEF) (*Figure 5*), but relevant associations were found with known HFpEF risk factors, hypertension, and female sex. More specifically, abundances of various lipid species could be summarized most accurately by an overall hyperlipidaemia diagnosis and by self-reported tobacco smoking, respectively, with these two factors typically having inversed associations with measured circulating lipid abundances (*Figure 5*). Several lipid species whose abundances were increased in patients with a history of coronary interventions had an overall similar pattern of associations to

Figure 1 Interrelationships between clinical parameters and echocardiographic parameters in heart failure with preserved ejection fraction (HFpEF) analysed through metadeconfoundR modelling. The heatmap shows positive correlations in red and negative correlations in blue. Hue indicates the effect size as per the legend. '*', '**', and '***' indicate significance estimate cutoffs for deconfounded variables of 0.1, 0.01, and 0.001, respectively. The grey asterisk indicates associations that, while achieving apparent significance when analysed in isolation, nonetheless reduce to the influence of one or more of the variables for which black asterisks are shown, as per nested linear model comparisons. BMI, body mass index; DBP, diastolic blood pressure; E, early diastolic flow velocity; e', velocity of early diastolic mitral annular motion; LAVI, left atrial volume index; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NYHA, New York Heart Association; SBP, systolic blood pressure.
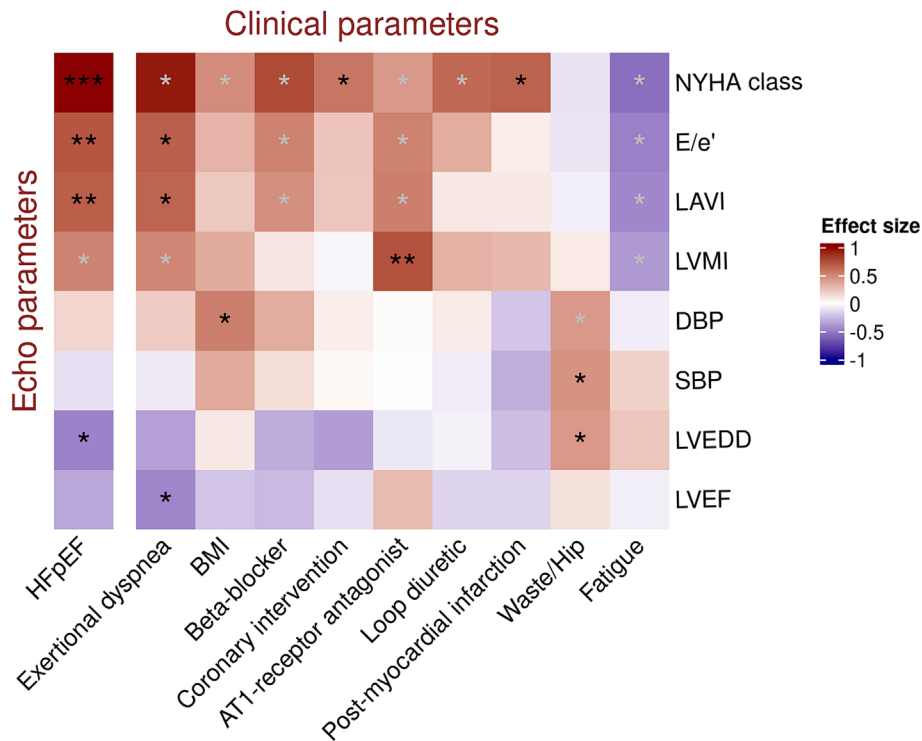

clinical and demographic variables as those lipid species, which were associated with a hyperlipidaemia diagnosis. All these reported associations achieved BH-adjusted FDR < 0.1 in our tests, which should be seen against the background of a relatively small ($N = 12 + 18$) sample size and slightly lower statistical power as shown in a *post hoc* power calculation (Supporting Information, *Figure S1*); this suggests that there likely are further associations that have not been revealed in the analysis of the present cohort.

In summary, while we could link different plasma lipid parameters measured using MS-based shotgun lipidomics to HFpEF indirectly via analysed risk factors and components/comorbidities, no direct ('first-order') association achieved significance in our present dataset, whether for individual lipid species or summary statistics through binning lipid abundances by total number of C-atoms or saturation status. On the other hand, several associations to other clinical parameters did reach significance, highlighting how a dataset of the present size still affords sufficient statistical power to conclude significance at least for effects greater in scope than

an effect size of 0.65 (absolute Cliff's delta) (Supporting Information, *Figure S1*).

We next tested for overarching relationships between clinical and lipidomic parameters using multivariate analysis (RDA; *Figure 6* and *Tables 3* and *4*). The results of these analyses were broadly consistent with the univariate analysis using metadeconfoundR as described above. In the RDA, we explored the overall variance in lipid species abundances when the variables hyperlipidaemia, smoking, and HFpEF anamnesis, respectively, were taken into account (*Table 3*). The first RDA axis explained 16.4% of the variance ($P = 0.001$), while the second RDA axis explained 8.47% of the variance ($P = 0.021$), and the third RDA axis explained 1.73% of the variance, but with no significance (*Table 3*). Smoking status (ever vs. never tobacco smoker) was significantly associated with overall lipidomic profile variability (FDR < 0.001), explaining 12.24% of overall variance, while anamnesis of hyperlipidaemia significantly explained 10.15% of overall lipidomic profile variability (FDR < 0.008). The significance of the RDA ordination was tested using ANOVA-like permuta-

Figure 2 Comparison of heart failure with preserved ejection fraction (HFpEF) and control samples with regard to lipid abundance binned by carbon chain length and analysed by classes. CE, cholesterol esters; Cer, ceramides; DAG, diacylglycerols; LPC, lysophosphatidylcholines; LPE, lysophosphatidylethanolamines; PC/PCO, phosphatidylcholines/(-ethers); PE/PEO, phosphatidylethanolamines/(-ethers); PI, phosphatidylinositols; SM, sphingomyelins; TAG, triacylglycerols.
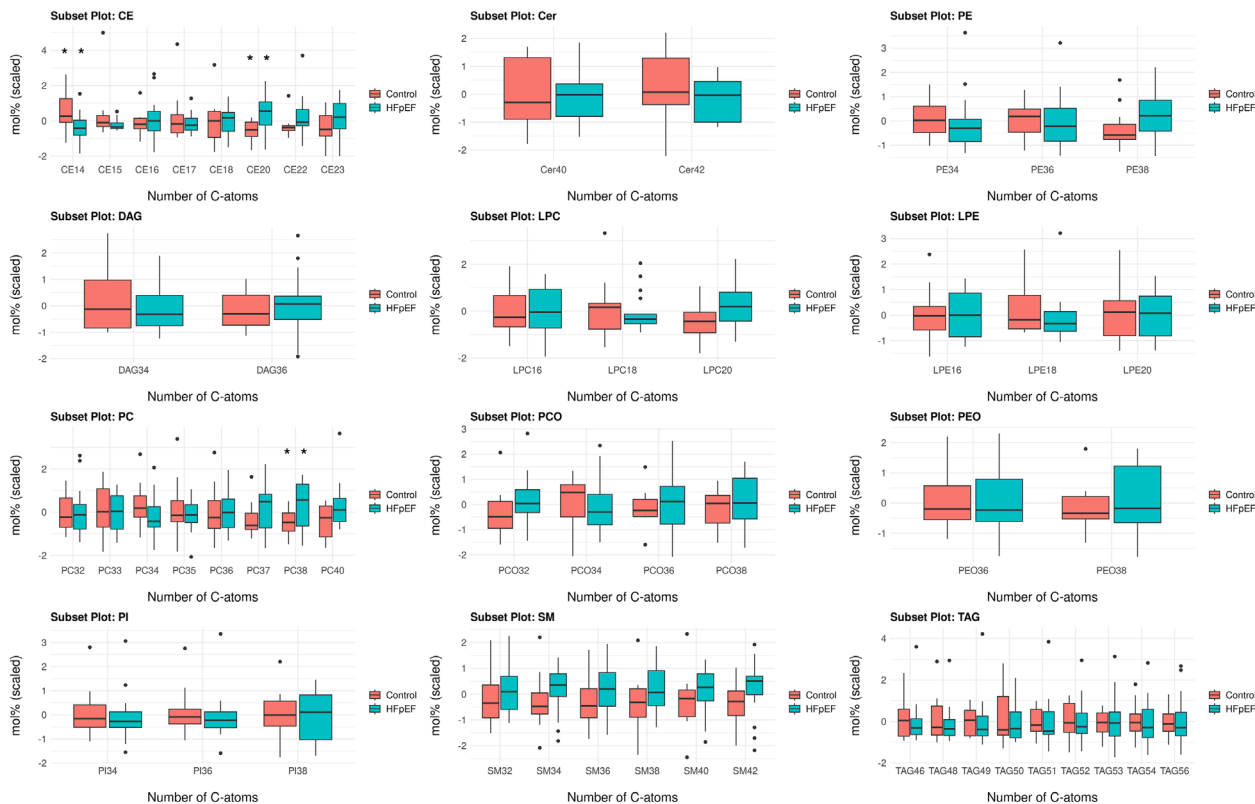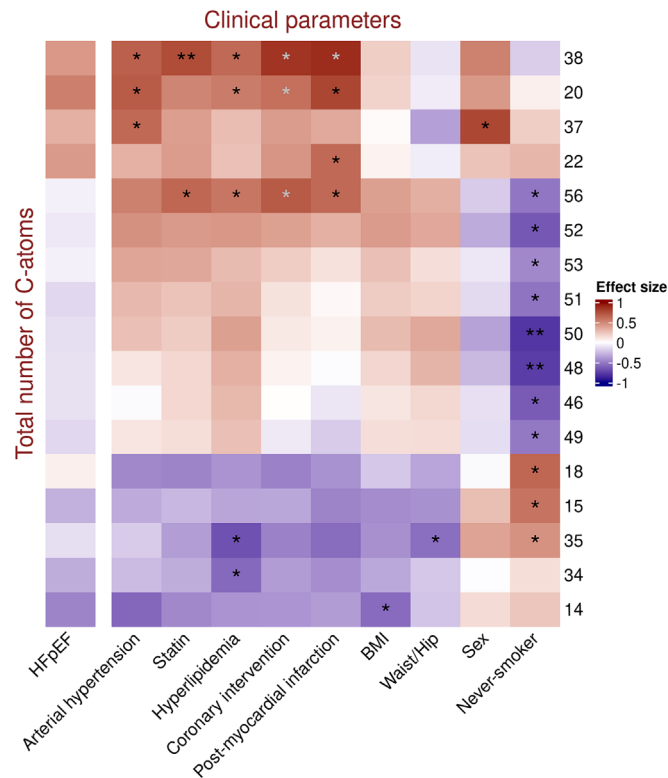

tion test[23] and PERMANOVA, which both showed consistent results pointing to hyperlipidaemia and smoking status as significant factors explaining lipidomic variability; the HFpEF status was not significant as it did not add additional explanatory power (*Table 3*). The results of the RDA multivariate analysis were replicated using another multivariate testing approach (PERMANOVA) (*Table 4*).

Differential network of correlations

In addition to 'first-order' biomarkers of HFpEF (e.g. features differentially abundant between HFpEF patients and controls), we also looked for 'second-order' biomarkers in the form of pairs of measurable parameters where the correlation between them was significantly stronger in HFpEF or in controls, respectively, allowing the representation of, for example, loss of joint regulation under disease conditions. This was done by modelling for differential correlation strength between patients and controls with regard to lipidomic and echocardiographic parameters, visualizing the resulting significant (likelihood ratio test FDR < 0.05) changes in feature coregulation as a network (*Figure 7*).

In the HFpEF group, multiple significant positive correlations were inferred (FDR < 0.1), which were absent or failed to reach significance in the control group, including positive correlations between CE23:2;0 and LVEDD (r = 0.61), PC18:1;0–20:3;0 and heart rate (b.p.m.) (r = 0.45), PC16:1;0–18:2;0 and LAVI (r = 0.52), and LPC20:4;0 and LVEF (r = 0.54) (*Figure 7*). No significant negative correlations between abundance of any lipid species and echocardiographic parameters were observed in the HFpEF group. In the control group, two clusters of significant correlations (FDR < 0.1) were apparent. The abundance of multiple PC species including PC16:0;0–22:5;0, PC16:0;0–20:5;0, PC18:0;0–18:1;0, and PC18:1;0–20:4;0 was negatively correlated with LVMI in the control group, but not in the HFpEF group (*Figure 7*). In addition, various SM species [SM34:2;2 (r = −0.77), SM36:2;2 (r = −0.51), SM38:2;2 (r = −0.61), SM40:2;2 (r = −0.61), and SM42:2;2 (r = −0.53)] and CE22:6;0 (r = −0.83) were significantly (FDR < 0.1) negatively correlated with systolic blood pressure (SBP) (*Figure 7*). Finally, PC16:0;0–16:0;0 was negatively correlated with diastolic blood pressure (DBP) and heart rate (b.p.m.) and CE14:0;0 positively correlated with LVEF (r = 0.7) in the control group (*Figure 7*). Accordingly, on the 'second-order' level of lost (or imposed)

Figure 3 Relationships between binned carbon chain length and clinical parameters in heart failure with preserved ejection fraction (HFpEF) analysed through metadeconfoundR modelling. The heatmap shows positive correlations in red and negative correlations in blue. Hue indicates the effect size as per the legend. '*', '**', and '***' indicate significance estimate cutoffs for deconfounded variables of 0.1, 0.01, and 0.001, respectively. The grey asterisk indicates associations that can be reduced to the influence of one or more of the variables for which black asterisks are shown, despite the significate effect that is achieved when analysed separately using nested linear model comparison. The chain length ranges from 14 to 56 carbon atoms, and the chains from 46 to 56 represent composite triacylglycerols. BMI, body mass index.

Discussion

We investigated the plasma lipidome of HFpEF patients (*n* = 18) and controls (*n* = 12) using MS-based shotgun lipidomics, to test for potential 'first-order' biomarkers by analysing between-group differences in individual lipid features and for 'second-order' biomarkers by detecting significantly different correlations when comparing the correlations between lipid species and echocardiographic parameters.
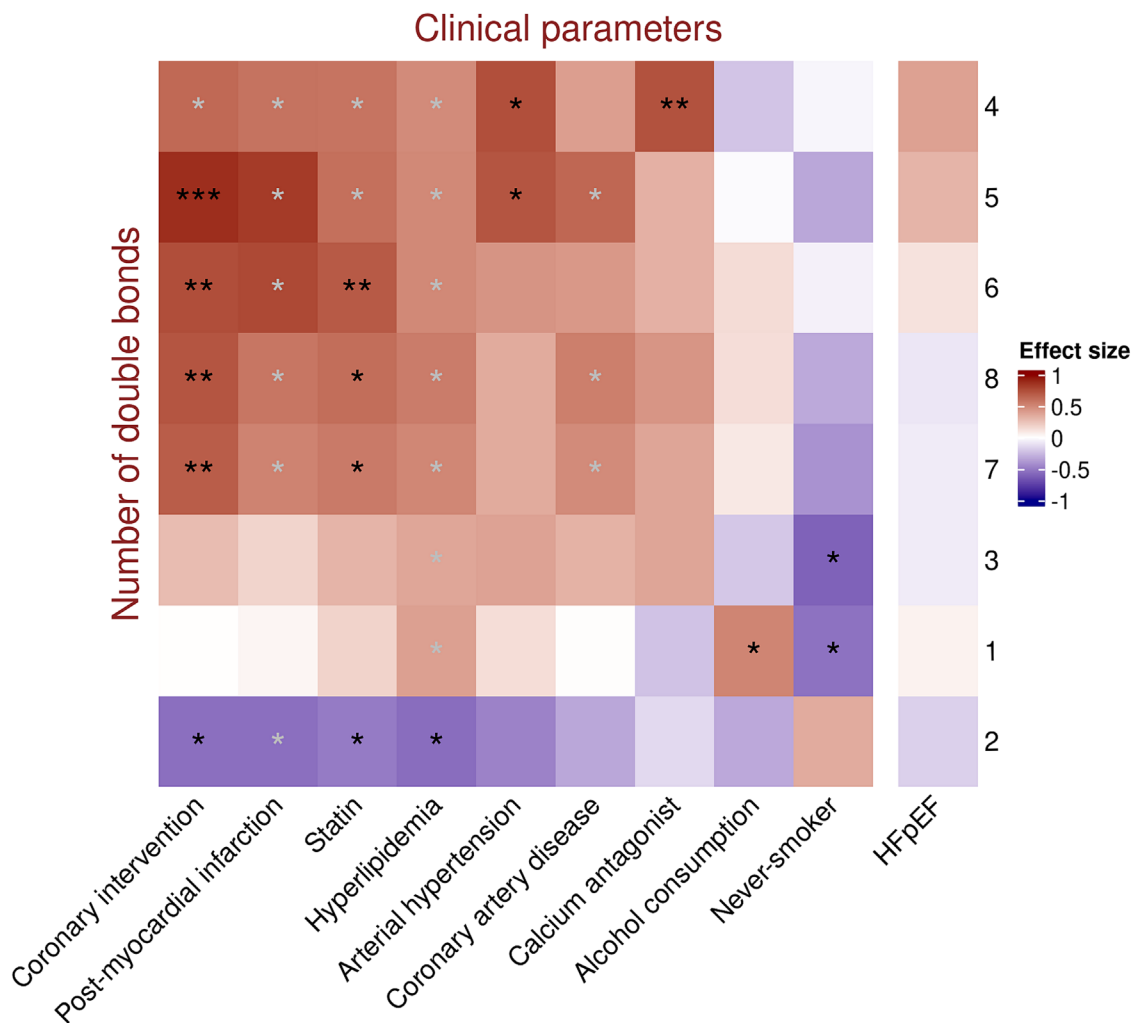
While HFpEF patients and controls did not differ significantly at the level of individual lipid species or lipids binned by carbon chain length and number of double bonds, that is, saturation degree, many features of the lipid profiles did correlate significantly with risk factors of HFpEF, hyperlipidaemia, and tobacco smoking. In an exploratory network analysis

coregulation, we observe more of a possible lipidomic signature than we can on the 'first order' of significantly differential abundance.

of significantly different correlations between groups, we observed significantly stronger correlations in HFpEF patients than controls between abundances of individual lipids from the CE and PC classes and echocardiographic parameters (e. g. LAVI, LVEDD, and b.p.m.). In contrast, the control group showed significantly stronger negative correlations between abundance of individual species from the PC and SM classes with LVMI or SBP.

As previously mentioned, little data are available on MS-based plasma or serum lipidome analyses in HFpEF; this includes metabolomic analyses demonstrating the regulation of plasma/serum acylcarnitines in HFpEF patients.[9,25,26] Javaheri *et al*. investigated the serum abundance of sphingolipids in 433 patients from the TOPCAT trial showing that circulating ceramides Cer16:0 and Cer18:0 are associated with death or heart failure admission in HFpEF.[14] Furthermore, Zordoky *et al*. identified lower serum concentrations of PC and SM species in HFpEF patients compared with non-heart failure controls.[26] However, we detected only a tendency towards lower abundance of selected PC species in our HFpEF cohort and could not identify any significant dif-

Figure 4 Interrelationships between binned number of double bonds and clinical parameters in heart failure with preserved ejection fraction (HFpEF) analysed through metadeconfoundR modelling. The heatmap shows positive correlations in red and negative correlations in blue. Hue indicates the effect size as per the legend. '*', '**', and '***' indicate significance estimate cutoffs for deconfounded variables of 0.1, 0.01, and 0.001, respectively. The grey asterisk indicates associations whose significant effect that is achieved when analysed in isolation can be reduced to the influence of one or more of the variables for which black asterisks are shown, as per nested linear model comparison.


ferences in circulating lipid patterns including when binning by carbon chain length, degree of (un)saturation, and individual lipid species between HFpEF patients and controls. The causes of this discrepancy likely include differences in sample size as well as in variation of HFpEF phenotypes of the investigated cohorts. Considering the disagreement among these results and taking into account recently published study results on examining the relationship between plasma metabolome/lipidome changes and cardiac function parameters in HFpEF, future plasma lipidome analyses should be cautiously interpreted, at least from a mechanistic point of view.[25] Although we found no significant differences between the control and disease groups, we found a strikingly significant correlation between smoking status and individual

plasma lipid species independent of disease status. In particular, 'never-smoker' anamnesis was significantly correlated with decreased abundances of multiple TAGs. These data are in line with previously published studies consistently reporting increased TAGs in smokers, reflecting an overall adverse cardiovascular risk profile in these individuals.[27,28]

Next, we visualized a network of significantly different correlations between the groups with regard to correlations between individual plasma lipid species and echocardiographic parameters. Here, we identified distinct significant correlations between individual lipid species and selected echocardiographic parameters that depended on HFpEF diagnosis. In the context of HFpEF, a significant correlation between abundance of PC16:1;0–18:2;0 and LAVI was detected in the

Figure 5 Interrelationships between abundance of lipid species and clinical parameters in heart failure with preserved ejection fraction (HFpEF) analysed through metadeconfoundR modelling. The heatmap shows positive correlations in red and negative correlations in blue. Hue indicates the effect size as per the legend. '*', '**', and '***' indicate significance estimate cutoffs for deconfounded variables of 0.1, 0.01, and 0.001, respectively. The grey asterisk indicates associations whose significant effect that is achieved when analysed in isolation can be reduced to the influence of one or more of the variables for which black asterisks are shown, as per nested linear model comparison. The first two ciphers of the lipid species indicate the length of the carbon chain. The third cipher separated by colon (:) indicates the number of double bonds, and the fourth cipher separated by semicolon (;) indicates the number of hydroxyl groups in that chain. In case there are two chains in the lipid species, this order is repeated one more time for the second carbon chain. CE, cholesterol esters; DAG, diacylglycerols; LPC, lysophosphatidylcholines; PC/PCO, phosphatidylcholines/(-ethers); PE, phosphatidylethanolamines; SM, sphingomyelins; TAG, triacylglycerols.
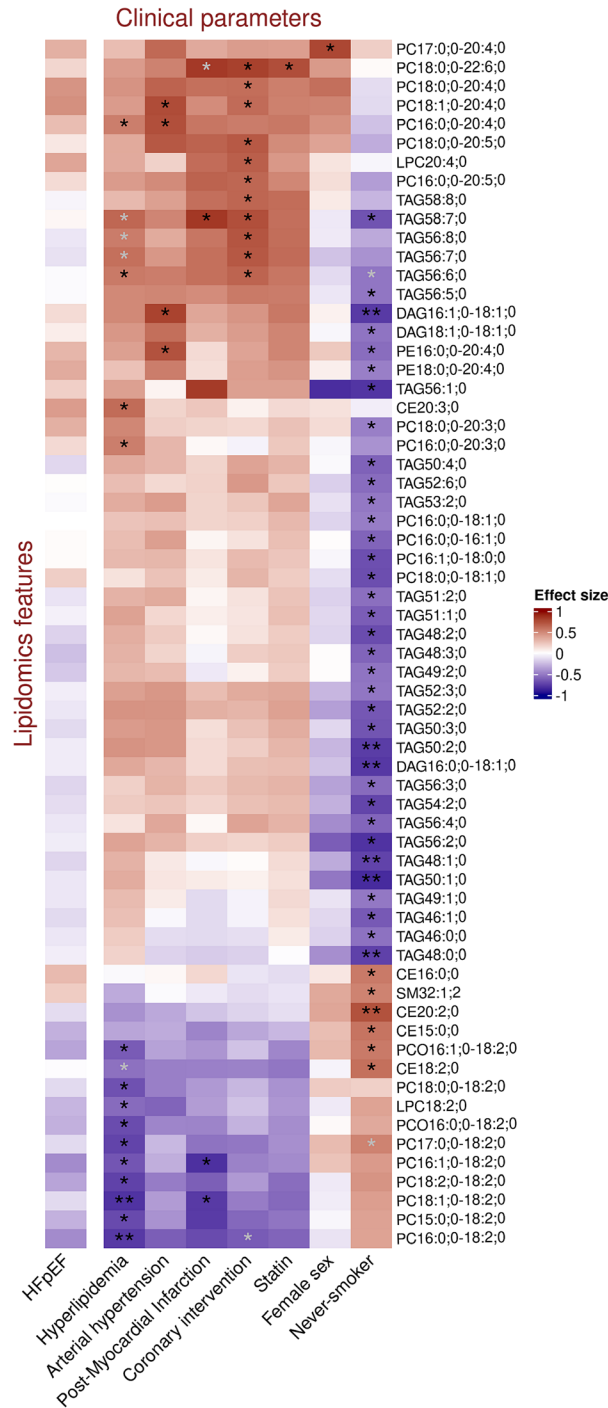
Figure 6 Multivariate analysis reveals smoking and hyperlipidaemia directly connected to lipidomic composition rather than through their associated heart failure with preserved ejection fraction (HFpEF) pathology. Multivariate analysis (RDA) for overarching relationships between clinical and lipidomic parameters was performed with constrained variables: *Group* (HFpEF), *Hyperlipidaemia*, and *Never Smoker* smoking status showing that smoking status and hyperlipidaemia are significantly associated with the overall lipidomic profile ($P < 0.001$ and $P < 0.008$, respectively), while HFpEF status did not show significance in the lipidomic profile variability.
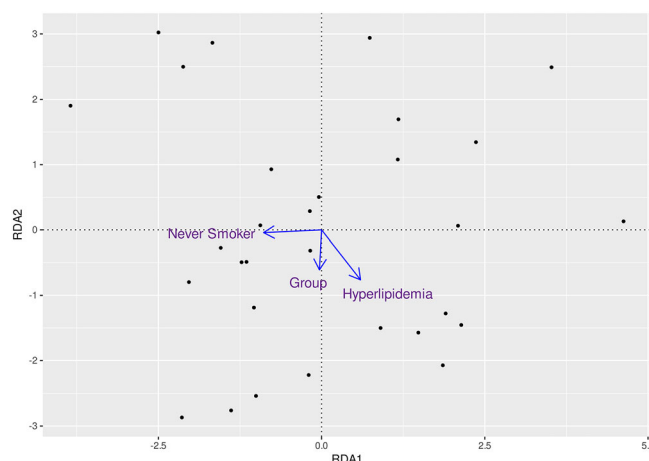
Table 3 RDA

	RDA1	RDA2	RDA
Group	−0.04115	−0.58040	−0.8133
Hyperlipidaemia	0.60361	−0.76810	0.2137
Never smoker	−0.88266	−0.06352	0.4657

ANOVA by axis

	df	Variance	F	P value
RDA1	1	16.441	4.9503	0.001***
RDA2	1	8.477	2.5523	0.021*
RDA3	1	1.733	0.5217	0.927

ANOVA by terms

	df	Variance	F	P value
Group	1	4.252	1.2804	0.222
Hyperlipidaemia	1	10.152	3.0567	0.008**
Never smoker	1	12.246	3.6872	0.001***

RDA, redundancy analysis.
*P value <0.05.
**P value <0.01.
***P value <0.001.

Table 4 PERMANOVA, permutations 9999

Formula = Hellinger ~ group + hyperlipidaemia + never smoker					
	df	Sum of squares	R^2	F	P value
Group	1	0.004949	0.03113	1.1551	0.302
Hyperlipidaemia	1	0.019076	0.12000	4.4525	0.003**
Never smoker	1	0.023551	0.14815	5.4969	0.001***
Residual	26	0.111393	0.70072		
Total	29	0.158969	1.00000		

PERMANOVA, permutational multivariate ANOVA.
**P value <0.01.
***P value <0.001.

HFpEF group. As a consequence of chronically elevated LV filling pressure, an increased LAVI is a diagnostic criterion for HFpEF.[24] As PCs are critical structural components of mammalian cell membranes, and their alterations affect cell membrane integrity,[29] their increased plasma levels, associated with enlarged LA volumes, may reflect alterations in cell membranes and adverse LA remodelling occurring during HFpEF.[30] Recent plasma lipidomic data from the PREDIMED study (Prevencion con Dieta Mediterranea) revealed a multi-lipid score associated with an increased risk for atrial fibrillation.[31] Interestingly, distinct PC species were components of this multi-lipid score.[31] Because the occurrence of atrial fibrillation is closely related to adverse LA remodelling, these results may suggest a link between plasma lipid patterns, including altered PC levels, and electrical/morphological LA alterations. The network further identified an inverse correlation of abundance of various plasma SM species with SBP in the control group. This is consistent with previously published studies showing an inverse correlation between abundance of individual SM species and incident ischaemic stroke, as well as with incident heart failure, both of which are cardiovascular diseases closely related to changes in SBP.[32,33] The absence of significance of this relationship in the HFpEF group indicates that the (patho) physiological links between plasma lipid changes and clinical parameters are highly differentiated depending on the disease status of an individual.

Our study is limited due to a small sample size that did not allow us to analyse the lipidomic dataset with respect to different HFpEF subphenotypes, which is likely to be relevant

Figure 7 A network of disease-differential correlations interconnects clinical/echocardiographic parameters with lipidomic species in heart failure (HF) with preserved ejection fraction (EF). Networks shown are those with Benjamini–Hochberg false discovery rate <0.05 for significantly differential correlations. The network shows all nodes connected by edges achieving significance (full line) $P < 0.1$, as well as the non-significant (dashed line) edges further connecting this set of nodes indirectly. BPM, heart beats per minute; CE, cholesterol esters; DBP, diastolic blood pressure; E: early diastolic flow velocity; e': velocity of early diastolic mitral annular motion; LAVI, left atrial volume index; LPC, lysophosphatidylcholines; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; PC/PCO, phosphatidylcholines/(-ethers); PE, phosphatidyl-ethanolamines; SBP, systolic blood pressure; SM, sphingomyelins.
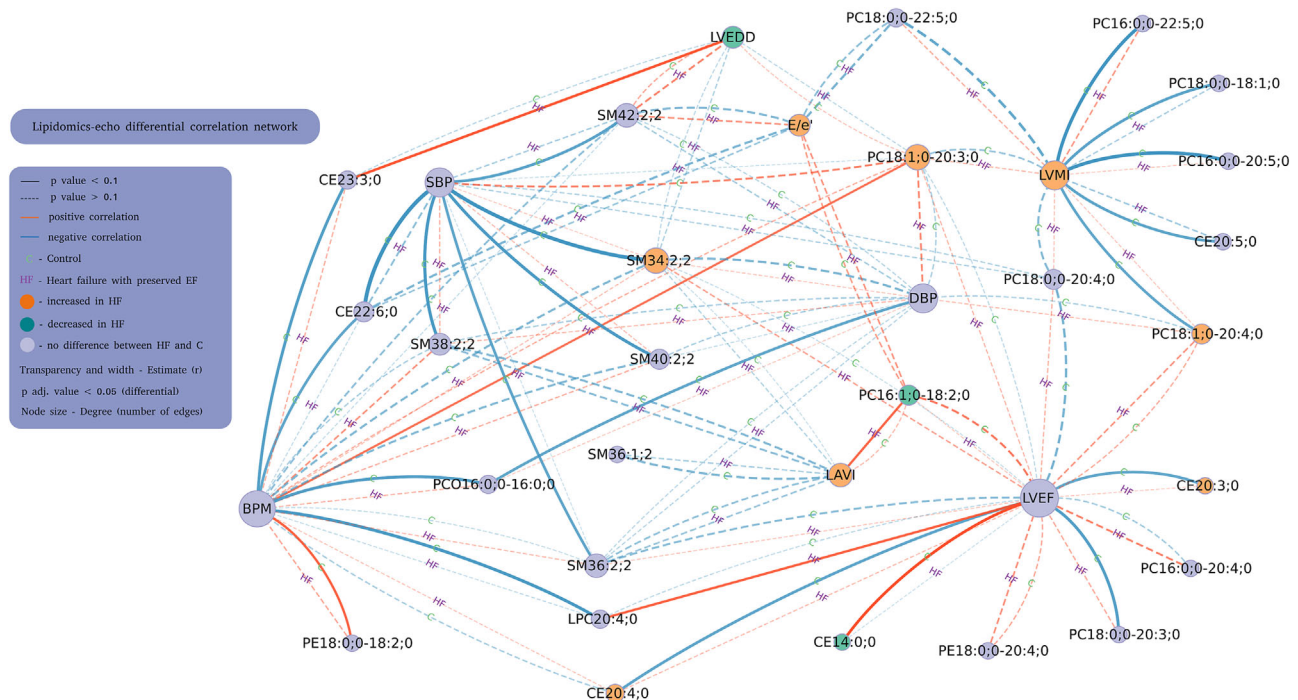
and should be performed in future studies and in larger cohorts. In addition, we used conservative, non-parametric tests that are less powerful in detecting associations at a small sample size; however due to conservative nature of these tests, the detected associations can be concluded with greater confidence.

In summary, we could not conclude significant direct associations between plasma lipidomic parameters and HFpEF and therefore could not detect specific lipids as biomarkers of HFpEF, but we show links between lipidomic parameters and some of the symptoms/covariates and risk factors of the disease. Going instead to 'second-order' correlations, in a hypothesis-generating network analysis of correlations, we identified significant differences between distinct lipid species and selected echocardiographic parameters in HFpEF providing the basis for further clinical and mechanistic studies. Despite the small sample size, the lack of significant direct correlations between plasma lipidomic features and HFpEF diagnosis in this study may point towards complex, non-linear relationships between circulating lipids and alterations of systemic/cardiac lipid metabolism during progression of this syndrome.

Acknowledgements

Conflict of interest

C.K. is an employee of Lipotype GmbH, Dresden, Germany. All other authors have declared that no conflict of interest exists.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. A post-hoc power calculation. Observed significant ($P < 0.1$) effect sizes against calculated post-hoc power for each of the observed effect sizes represented by black dots.

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