Plasma Lipidomic Profiles Improve on Traditional Risk Factors for the Prediction of Cardiovascular Events in Type 2 Diabetes Mellitus

Editorial, see p 1651

BACKGROUND: Clinical lipid measurements do not show the full complexity of the altered lipid metabolism associated with diabetes mellitus or cardiovascular disease. Lipidomics enables the assessment of hundreds of lipid species as potential markers for disease risk.

METHODS: Plasma lipid species (310) were measured by a targeted lipidomic analysis with liquid chromatography electrospray ionization–tandem mass spectrometry on a case-cohort (n=3779) subset from the ADVANCE trial (Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation). The case-cohort was 61% male with a mean age of 67 years. All participants had type 2 diabetes mellitus with ≥1 additional cardiovascular risk factors, and 35% had a history of macrovascular disease. Weighted Cox regression was used to identify lipid species associated with future cardiovascular events (nonfatal myocardial infarction, nonfatal stroke, and cardiovascular death) and cardiovascular death during a 5-year follow-up period. Multivariable models combining traditional risk factors with lipid species were optimized with the Akaike information criteria. C statistics and NRIs were calculated within a 5-fold cross-validation framework.

RESULTS: Sphingolipids, phospholipids (including lyso- and ether- species), cholesteryl esters, and glycerolipids were associated with future cardiovascular events and cardiovascular death. The addition of 7 lipid species to a base model (14 traditional risk factors and medications) to predict cardiovascular events increased the C statistic from 0.680 (95% confidence interval [CI], 0.678–0.682) to 0.700 (95% CI, 0.698–0.702; $P<0.0001$) with a corresponding continuous NRI of 0.227 (95% CI, 0.219–0.235). The prediction of cardiovascular death was improved with the incorporation of 4 lipid species into the base model, showing an increase in the C statistic from 0.740 (95% CI, 0.738–0.742) to 0.760 (95% CI, 0.757–0.762; $P<0.0001$) and a continuous net reclassification index of 0.328 (95% CI, 0.317–0.339). The results were validated in a subcohort with type 2 diabetes mellitus (n=511) from the LIPID trial (Long-Term Intervention With Pravastatin in Ischemic Disease).

CONCLUSIONS: The improvement in the prediction of cardiovascular events, above traditional risk factors, demonstrates the potential of plasma lipid species as biomarkers for cardiovascular risk stratification in diabetes mellitus.

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Clinical Perspective

What Is New?

- We have identified plasma lipid species that are associated with future cardiovascular events (32 species) and cardiovascular death (32 species).
- We have demonstrated that a small number of these plasma lipid species (4–7) can improve on current lifestyle and clinical risk factors to predict cardiovascular outcomes in individuals with type 2 diabetes mellitus who are at high risk.

What Are the Clinical Implications?

- More discriminatory risk assessment in high-risk patients is important to guide clinical intervention and to target limited health resources.
- Current biomarkers provide an incomplete view of an individual's risk for future cardiovascular events; plasma lipid species may improve current risk assessment models.
- The lipid species identified in this study may represent new therapeutic targets to modify lipid metabolism and to attenuate disease progression.

Type 2 diabetes mellitus (T2DM) represents a growing health burden worldwide.[1,2] Atherothrombotic cardiovascular disease (CVD) is a major complication of T2DM and is the leading cause of death worldwide.[3–5] The increasing incidence of T2DM is placing pressure on healthcare systems. Estimating and managing the risk of CVD in those with T2DM are major concerns. To effectively target limited health resources to those patients at highest risk, new approaches to assess risk in T2DM populations are required. Different risk scores have been developed to estimate the risk of developing CVD; the Framingham risk score[6] and the United Kingdom Prospective Diabetes Study[7] score are well-established risk scores. However, the Framingham risk score has shown an underestimation of risk in T2DM populations,[8,9] and the United Kingdom Prospective Diabetes Study score overestimated the risk of future cardiovascular events when applied to independent T2DM cohorts.[9,10]

Traditional lipid markers (total cholesterol, low-density lipoprotein [LDL] cholesterol, triglycerides, and high-density lipoprotein cholesterol [HDL-C]), which are often used in risk scores, are altered in T2DM as a result of dysfunctional lipid and lipoprotein metabolism. However, these measures alone do not explain the complexity of the altered lipid metabolism associated with T2DM or the related cardiovascular risk. Recent development in lipidomic technologies is providing new insight into this complex area. Plasma lipid species and classes/subclasses have been found to be associated with T2DM[11] and CVD.[12] More recently, plasma lipid species have also been associated with incident cardiovascular events,[13] suggesting that these lipid species may be useful biomarkers for cardiovascular risk. However, to the best of our knowledge, no studies to date have investigated the plasma lipid profile associated with cardiovascular risk in a T2DM population.

We hypothesized that specific lipid species would be associated with future cardiovascular events in T2DM independently of existing risk factors. We further hypothesized that a combination of lipid species and conventional risk factors will provide improved prediction of future events compared with risk factors alone. We used a high-throughput mass spectrometry platform[14] for plasma lipid profiling in a case-cohort subset from the ADVANCE trial (Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation) to identify lipid species that may predict incident cardiovascular events over a 5-year period. Our results were subsequently validated in an independent subset of patients with T2DM enrolled in the LIPID trial (Long-Term Intervention With Pravastatin in Ischemic Disease).

METHODS

Study Populations

The ADVANCE trial was a multicenter, randomized, double-blind, international prospective study. In a 2×2 factorial design, the study compared the efficacy of perindopril/indapamide (2/0.625 mg for 3 months, increasing if tolerated to 4/1.25 mg) versus placebo and included an open-label evaluation of an intensive glucose-lowering regimen using modified release gliclazide, with a target glycohemoglobin of 6.5% (48 mmol/mol) versus standard, guideline-based glycemic control on cardiovascular and renal outcomes. The study was approved by the ethics committee for each participating center, and all participants provided written informed consent[15]; the Alfred Human Ethics Committee subsequently approved the present substudy. A total of 11 140 patients were recruited with a median of 5.0 years of follow-up. Patients were men and women who were >55 years of age and had been diagnosed with T2DM after the age of 30 years. They had a history of CVD or ≥1 additional cardiovascular risk factors.[16] Samples were collected at baseline, and then patients underwent a 6-week active treatment period during which they received the fixed combination of perindopril (2 mg) and indapamide (0.625 mg) before randomization. Of 11 140 samples, 7376 plasma samples were available from all countries involved in the ADVANCE trial except India and China. The plasma samples were stored at −80°C for a median of 8.8 years before analysis. The baseline data collected in the ADVANCE trial included clinical information, biochemical characteristics, and demographic distribution of all participants.[17]

A case-cohort study design was used (Figure 1). A sample (n=3154) was selected at random from the 7376 participants with available blood samples (the unenriched subcohort). This sample was then enriched with all those suffering cardiovascular events, defined as major macrovascular events, a composite of nonfatal myocardial infarction (MI), nonfatal stroke, and cardiovascular death, renal outcomes, or all-cause mortality,[15] (n=625) from the remaining 4222 participants, giving a total

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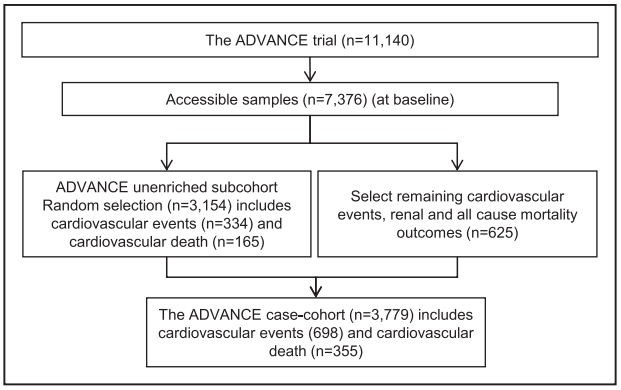

Figure 1. The ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation) case-cohort design.

of 698 cardiovascular events, defined as the first occurrence of MI, stroke, or cardiovascular death, in the follow-up period and 355 cardiovascular deaths, including death after an initial nonfatal cardiovascular event (Figure 1).

The LIPID trial (n=9014) investigated the effect of pravastatin on death caused by coronary heart disease in patients 31 to 71 years of age with a history of MI or unstable angina and baseline cholesterol levels between 4.0 and 7.0 mmol/L and fasting triglyceride <5.0 mmol/L. Patients were randomized to 2 groups, pravastatin (40 mg/d) or placebo, 3 to 36 months after an acute coronary syndrome. From the 5991 subjects with baseline samples available, we identified 511 individuals with established T2DM. Patients with T2DM were those who identified themselves as having diabetes mellitus or who had a fasting plasma glucose ≥7 mmol/L.[18] This substudy was also approved by the Alfred Human Ethics Committee.

Lipid Extraction and Quantification

Lipid species were extracted from plasma samples as described previously.[19] Briefly, plasma (10 μL) was divided into aliquots in 1.5-mL Eppendorf tubes, and 100 μL of 1-butanol/methanol (1:1, vol/vol) and 5 mmol/L ammonium formate containing internal standards (Table I in the online-only Data Supplement) were added. The mixture was vortexed for 10 seconds, sonicated for 60 minutes in a sonic water bath (18°C–24°C), and then centrifuged (16 000*g*, 10 minutes, 20°C). The supernatant was transferred into a 0.2-mL glass insert with Teflon insert caps for lipidomic analysis. Targeted lipidomic analysis of the ADVANCE and LIPID cohorts was performed by liquid chromatography electrospray ionization tandem mass spectrometry. Details are available in the online-only Data Supplement. Intra-assay (batch) and interassay (batch) coefficients of variance (%CVs) for each lipid species, based on plasma quality control samples placed every 25 participant samples, showed median %CV values of 12% and 14%, respectively, with 90% of lipid species having intra-assay and interassay %CVs <20% and 23%, respectively (Table II in the online-only Data Supplement).

Statistical Analysis

To facilitate interpretation of the hazard ratios, the quantitative values for each lipid species were normalized to the interquartile range for that species before association studies. Weighted Cox regression analyses were performed on the case-cohort to identify lipid classes, subclasses, and species associated with future cardiovascular events and death. Significant baseline characteristics between cardiovascular events and nonevent groups were used as covariates, in addition to treatment allocation. Significant characteristics were age, sex, body mass index (BMI), systolic blood pressure (SBP), glycohemoglobin, HDL-C, estimated glomerular filtration rate (eGFR), diabetes duration, C-reactive protein, history of macrovascular disease, history of heart failure, use of antihypertensive medication, use of antiplatelet medication, and exercise (Table 1). Lipid-lowering medication and total cholesterol, although not significantly associated with cardiovascular outcomes, were included as covariates in separate sensitivity analyses. Continuous and categorical covariates were analyzed with Mann-Whitney *U* tests and χ^2 tests, respectively. The *P* values (2 sided) were corrected for multiple comparisons with the Benjamini-Hochberg[20] method. Statistical significance was determined as a corrected value of $P<0.05$. The analyses were performed in STATA version 10.1 (StataCorp LP, Inc, College Station, TX) using the STSELPRE procedure for case-cohort analyses. Linear and logistic regression was used to identify associations between lipid species and cardiovascular risk factors (sex, age, BMI, and SBP) with adjustment for other risk factors and glycohemoglobin as indicated.

Principal component analysis was performed on the entire data set.[21] The stratification of the population based on sex, age, BMI, SBP, or cardiovascular outcomes across each of the principal components was determined by *t* tests. Correlation analysis using Pearson correlation coefficients was performed on the subset of lipid species that were associated with cardiovascular events and cardiovascular death.

Before the development of multivariable models to predict future events, a correlation minimization procedure was used on the entire lipid data set (log-transformed values) to remove highly correlated lipid species.[22] The traditional risk factors and log-transformed lipid measurements were mean centered. A 2-stage procedure was used to rank lipid species and then to build multivariable models and assess performance with the unenriched subcohort. Starting with a Cox regression base model of 14 covariates, we added up to 20 lipid species to the model in a forward selection with the aim of minimizing the Akaike information criterion.[23] This procedure was performed within a 5-fold cross-validation framework (200 repeats). Lipid species were then ranked on the basis of the average position of incorporation into these models. For comparison, lipid features were also ranked by the least absolute shrinkage and selection operator approach[24] within a 5-fold cross-validation framework (200 repeats).

Using the rank order of the 20 top lipid species (from the Akaike information criterion ranking), we created a series of models by the successive addition of lipid species to the base covariates within a 5-fold cross-validation (200 repeats). Model performance was assessed by calculating the Harrell C statistic (using the SOMERSD command in STATA),[25] continuous net reclassification indexes (NRIs), integrated discrimination improvement (IDI), and relative IDI.[26,27] The 95% confidence intervals (CIs) for each parameter were calculated.

We sought to validate our findings in a subcohort of participants with T2DM enrolled in the LIPID trial (n=511). Weighted

Table 1. Baseline Characteristics of the ADVANCE Case-Cohort

Variable	All (n=3779)	Cardiovascular Events (n=698)	No Cardiovascular Events (n=3081)	*P* Value*	Cardiovascular Death (n=355)	No Cardiovascular Death (n=3424)	*p* Value*
Continuous variables, median (first, third quartile)							
Age, y	67 (62, 72)	70 (65, 74)	67 (61, 71)	<0.001‡	71 (66, 75)	67 (61, 71)	<0.001‡
BMI, kg/m^2	29.4 (26.4, 32.8)	28.7 (26.1, 32.5)	29.4 (26.6, 32.9)	0.030‡	28.7 (26.1, 32.4)	29.4 (26.5, 32.9)	0.080
HbA_{1c}, %	7.2 (6.5, 8.1)	7.3 (6.5, 8.4)	7.1 (6.4, 8.1)	<0.001‡	7.5 (6.6, 8.5)	7.1 (6.4, 8.1)	<0.001‡
Glucose, mmol/L	7.9 (6.6, 9.8)	8.1 (6.6, 10.1)	7.9 (6.6, 9.7)	0.163	8.2 (6.5, 10.3)	7.9 (6.6, 9.7)	0.321
Triglycerides, mmol/L	1.70 (1.20, 2.35)	1.62 (1.20, 2.32)	1.70 (1.20, 2.36)	0.431	1.60 (1.20, 2.32)	1.70 (1.20, 2.36)	0.397
LDL cholesterol, mmol/L	3.00 (2.35, 3.70)	3.00 (2.40, 3.80)	2.99 (2.34, 3.70)	0.479	3.05 (2.40, 3.80)	2.99 (2.33, 3.70)	0.198
Total cholesterol, mmol/L	5.00 (4.30, 5.81)	5.00 (4.30, 5.80)	5.00 (4.31, 5.83)	0.278	5.04 (4.30, 5.88)	5.00 (4.30, 5.81)	0.819
HDL cholesterol, mmol/L	1.20 (1.00, 1.40)	1.10 (0.96, 1.30)	1.20 (1.00, 1.40)	<0.001‡	1.10 (1.00, 1.33)	1.20 (1.00, 1.40)	0.011‡
SBP, mm Hg	146 (133, 160)	150 (135, 166)	145 (132, 160)	<0.001‡	149 (135, 165)	146 (133, 160)	0.006‡
Diastolic blood pressure, mm Hg	81 (74, 89)	82 (74, 89)	81 (74, 89)	0.951	81 (73, 89)	81 (74, 89)	0.235
eGFR, mL·min^{-1}·1.73m^{-2}	71 (60, 85)	68 (55, 81)	72 (61, 86)	<0.001‡	67 (52, 79)	72 (61, 85)	<0.001‡
T2DM duration, y	6.0 (3.0, 11.0)	8.0 (4.0, 13.0)	6.0 (3.0, 11.0)	<0.001‡	9.0 (4.0, 15.0)	6.0 (3.0, 11.0)	<0.001‡
C-reactive protein, mg/L	1.83 (0.87, 4.09)	2.02 (0.93, 4.41)	1.79 (0.86, 4.05)	0.026‡	2.05 (1.01, 4.55)	1.80 (0.86, 4.05)	0.027‡
Dichotomous variables, n (%)							
Sex (male)	2308 (61.1)	483 (69.2)	1825 (59.2)	<0.001‡	240 (67.6)	2068 (60.4)	0.038‡
Alcohol drinker	1557 (41.2)	272 (39.0)	1285 (41.7)	0.309	125 (35.2)	1432 (41.8)	0.065
Smoker	565 (15.0)	100 (14.3)	465 (15.1)	0.637	48 (13.5)	517 (15.1)	0.464
History of macrovascular disease	1321 (35.0)	343 (49.1)	978 (31.7)	<0.001‡	187 (52.7)	1134 (33.1)	<0.001‡
History of heart failure	175 (4.6)	61 (8.7)	114 (3.7)	<0.001‡	45 (12.7)	130 (3.8)	<0.001‡
Use of antihypertensive medication	3022 (80.0)	607 (87.0)	2415 (78.4)	0.022‡	322 (90.7)	2700 (78.9)	0.018‡
Use of lipid-lowering medication	1674 (44.3)	295 (42.3)	1379 (44.8)	0.371	140 (39.4)	1534 (44.8)	0.148
Use of antiplatelet medication	1869 (49.5)	411 (58.9)	1458 (47.3)	<0.001‡	220 (62.0)	1649 (48.2)	<0.001‡
Antihypertensive treatment arm	1850 (49.0)	332 (47.6)	1518 (49.3)	0.561	154 (43.4)	1696 (49.5)	0.115
Glucose control arm	1890 (50.0)	340 (48.7)	1550 (50.3)	0.590	166 (46.8)	1724 (50.4)	0.363
Moderate or vigorous exercise†	1822 (48.2)	285 (40.8)	1537 (49.9)	0.002‡	134 (37.7)	1688 (49.3)	0.003‡

ADVANCE indicates Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation; BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; and T2DM, type 2 diabetes mellitus.

*P values were calculated with a Mann-Whitney U test for continuous variables and a χ^2 test for dichotomous variables.

†Moderate or vigorous exercise was defined as moderate and/or vigorous exercise for >15 minutes at least once weekly.

‡Significant (P<0.05).

Cox regression and Cox regression were performed to identify the association of the top-ranked lipid species with future cardiovascular events and death in the ADVANCE and LIPID subcohorts, respectively. To facilitate the comparison, the analyses were adjusted for age, sex, BMI, SBP, HDL-C, and eGFR only.

We then assessed the predictive performance of the selected lipid species by first computing the performance of the base model and then adding lipid species to the base model and calculating the change in model performance using the C statistic, continuous NRI, IDI, and relative IDI within a 5-fold cross-validation framework (200 repeats). The covariates used in the LIPID trial analyses were the continuous measures of age, BMI, cholesterol, HDL-C, triglycerides, SBP, fasting glucose, eGFR, and white blood cell count and the categorical measures of current smoking, dyspnea grade, angina grade, atrial fibrillation, sex, stroke history, hypertension history, MI history, revascularization, peripheral vascular disease, aspirin at baseline, and treatment, as have previously been used in analyses of the LIPID trial.[28]

RESULTS

Baseline Characteristics

Baseline characteristics that are based on the outcomes status in patients in the ADVANCE trial are shown in Table 1. Those experiencing a cardiovascular event or death during the follow-up period were typically older, had a higher glycohemoglobin and SBP and a longer duration of T2DM, were more likely to have a history of CVD, exercised less, and had lower HDL-C and eGFR.

Association of Lipid Classes/Subclasses and Species With Cardiovascular Outcomes and Risk Factors

Three of 22 lipid classes/subclasses were significantly associated with the risk of cardiovascular events (monohexosylceramide, dihexosylceramide, and lysoalkylphosphatidylcholine) and 2 subclasses were associated with the risk of cardiovascular death (monohexosylceramide and dihexosylceramide) after adjustment for covariates and correction for multiple comparisons (Figure 2). In addition, 32 individual lipid species were significantly associated with both future cardiovascular events and death (Figure 3 and Table II in the online-only Data Supplement). Twenty-seven lipid species of monohexosylceramide, dihexosylceramide, trihexosylceramide, alkylphosphatidylcholine, alkenylphosphatidylcholine (containing monounsaturated fatty acids), lysoalkylphosphatidylcholine, and cholesteryl ester were directly associated with future cardiovascular events (Figure 3 and Table II in the online-only Data Supplement). In contrast, 5 species containing polyunsaturated fatty acids (PUFAs), including phosphatidylcholine, alkenylphosphatidylcholine, and triacylglycerol, were inversely associated with future cardiovascular events (Figure 3 and Table II in the online-only Data Supplement). The lipid signature associated with future cardiovascular death showed minimal differences compared with future cardiovascular events. Thirty-one lipid species, including ceramide, monohexosylceramide, dihexosylceramide, trihexosylceramide, sphingomyelin, alkylphosphatidylcholine, alkenylphosphatidylcholine (containing monounsaturated fatty acids), lysophosphatidylcholine, lysoalkylphosphatidylcholine, and cholesteryl ester, were directly associated with future cardiovascular death, whereas 1 species of alkenylphosphatidylcholine, PC(P-36:5), was inversely associated with future cardiovascular death (Figure 3 and Table II in the online-only Data Supplement).

Regression analysis of those lipid species associated with cardiovascular outcomes with known risk factors

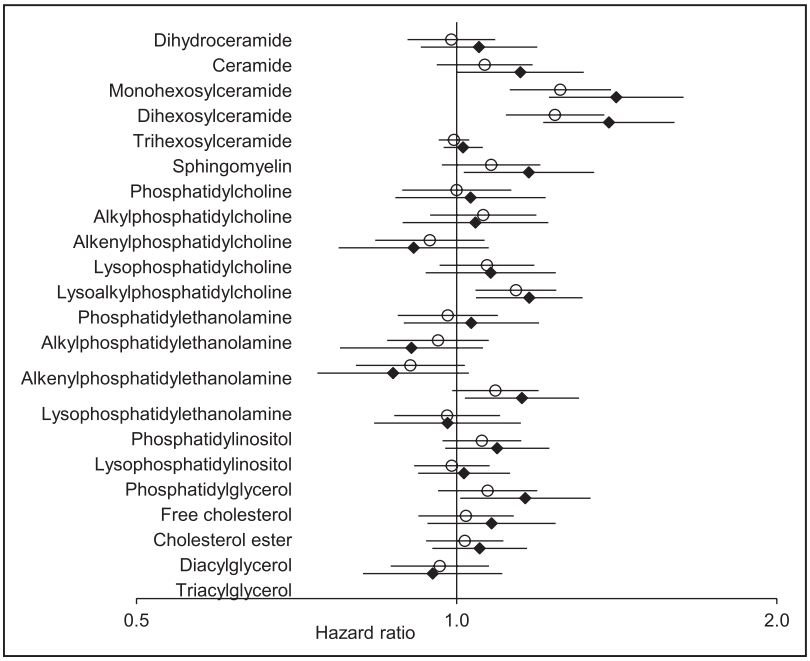

Figure 2. The association of lipid classes/subclasses with future cardiovascular outcomes.
Weighted Cox regression was performed to identify lipid classes/subclasses associated with future cardiovascular events (n=698;○) and cardiovascular death (n=355; closed diamond). Hazard ratios were adjusted for age, sex, body mass index, systolic blood pressure, glycohemoglobin, high-density lipoprotein cholesterol, estimated glomerular filtration rate, diabetes duration, C-reactive protein, history of macrovascular disease, history of heart failure, use of antihypertensive medication, use of antiplatelet medication, and exercise. The hazard ratio represents the change in outcome associated with a change in the lipid species equivalent to the interquartile range. Hazard ratios and 95% confidence intervals are shown.

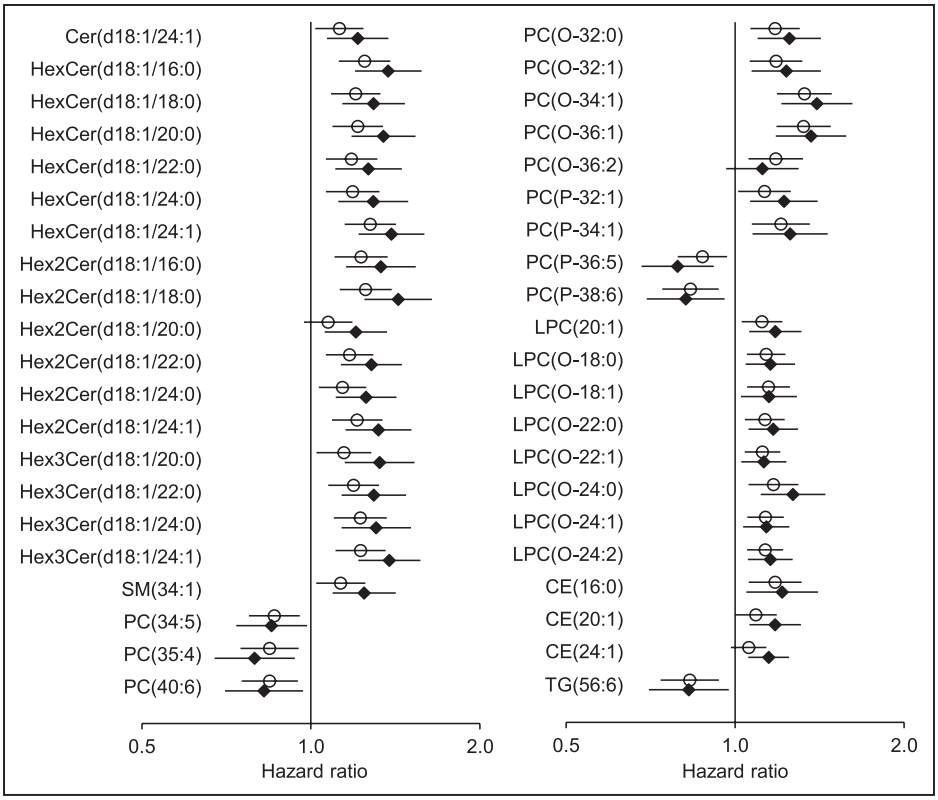

Figure 3. The association of individual lipid species with future cardiovascular outcomes.
Weighted Cox regression was performed to identify lipid species associated with future cardiovascular events (n=698; ○) and cardiovascular death (n=355; closed diamond). Hazard ratios were adjusted for age, sex, body mass index, glycohemoglobin, high-density lipoprotein cholesterol, estimated glomerular filtration rate, diabetes duration, C-reactive protein, history of macrovascular disease, history of heart failure, use of antihypertensive medication, use of antiplatelet medication, and exercise. The hazard ratio represents the change in outcome associated with a change in the lipid species equivalent to the interquartile range. Hazard ratios and 95% confidence intervals are shown for lipid species showing a significant association with either outcome. CE indicates cholesteryl ester; Cer(d18:1), ceramide; HexCer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lysophosphatidylcholine; LPC(O), lysoalkylphosphatidylcholine; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PC(P), alkenylphosphatidylcholine; SM, sphingomyelin; and TG, triacylglycerol.

for CVD (sex, age, BMI, SBP) showed a strong association for ≥1 risk factors (Table III in the online-only Data Supplement).

Sensitivity Analysis

The addition of lipid-lowering medication or total cholesterol as covariates in the regression analyses described above had no effect on the hazard ratios of those lipid species associated with cardiovascular events and cardiovascular death.

Principal Component and Correlation Analyses

The principal components PC1, PC2, and PC3 derived from the entire lipid data set explained 24.6%, 11.1%, and 8.7% of the variance. Stratification of the population on the basis of sex was observed within PC1 and PC2. Similarly, stratification of those individuals above and below the median values for age, BMI, and SBP was also observed within PC1 and PC2. In contrast, no stratification on the basis of cardiovascular outcomes was observed within PC1, PC2, or PC3 (Figure I and Table IV in the online-only Data Supplement). Correlation analysis of the 42 lipid species associated with cardiovascular outcomes showed a complex correlation structure (Figure 4).

Prediction of Future Cardiovascular Events and Death

The optimal model (based on the inflection points of the Akaike information criterion and C statistic values plotted against number of lipid species) for the prediction of future cardiovascular events was obtained by the addition of 7 lipid species, consisting of alkylphosphatidylcholine [PC(O-36:1)], cholesteryl ester [CE(18:0)], alkylphosphatidylethanolamine [PE(O-36:4)], phosphatidylcholine [PC(28:0) and PC(35:4)], and lysophosphatidylcholine [LPC(20:0) and LPC(18:2)] to the base model (Table V

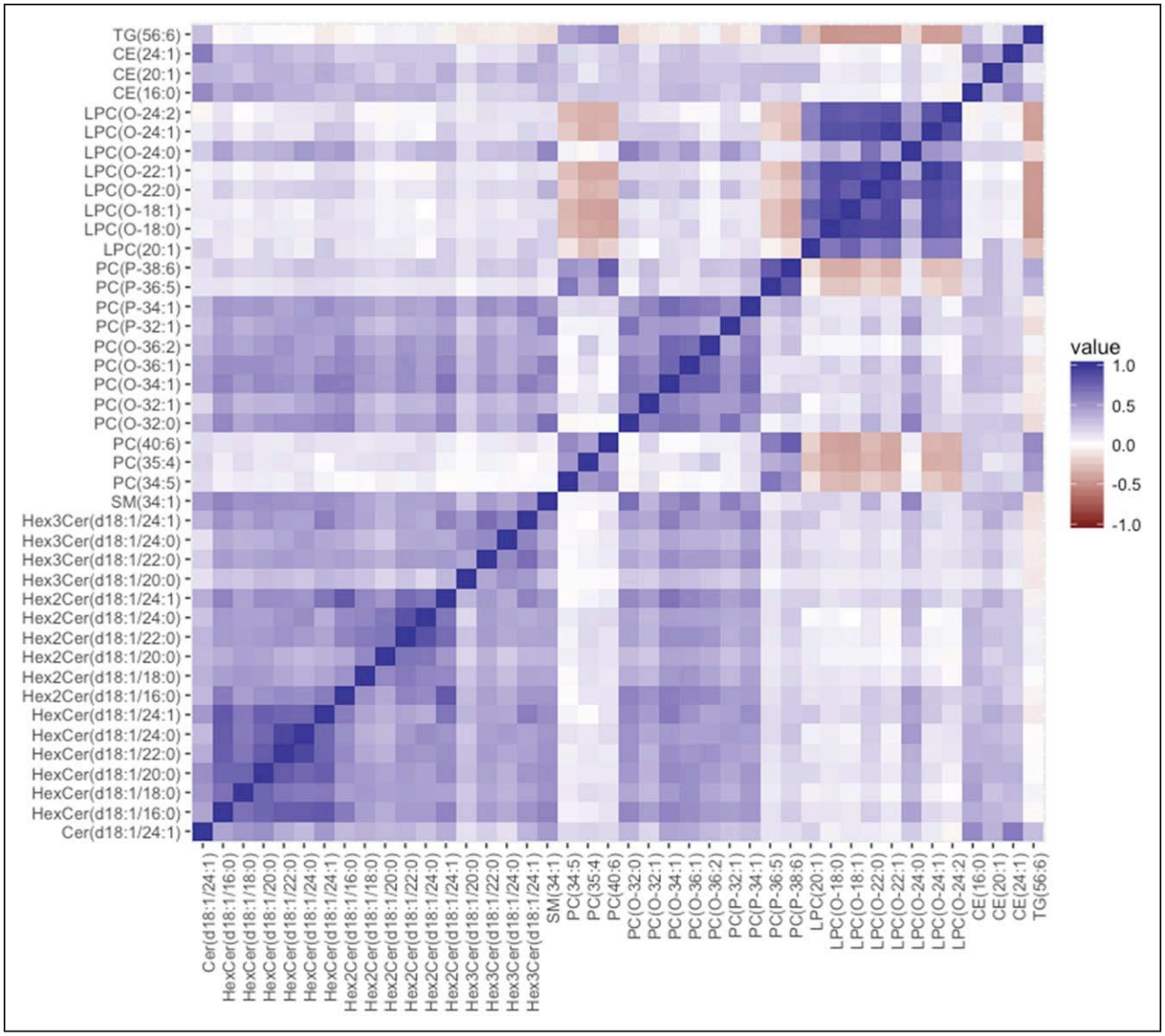

Figure 4. Correlation of lipid species associated with cardiovascular outcomes.
Pearson correlation coefficients calculated for each lipid species associated with either cardiovascular events or cardiovascular death against each other species. The correlation coefficients are presented as a heat map. CE indicates cholesteryl ester; Cer(d18:1), ceramide; HexCer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lysophosphatidylcholine; LPC(O), lysoalkylphosphatidylcholine; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PC(P), alkenylphosphatidylcholine; SM, sphingomyelin; and TG, triacylglycerol.

in the online-only Data Supplement). In contrast, only 4 lipid species—the alkylphosphatidylcholines PC(O-36:1) and PC(O-36:5); a diacylglycerol, DG(16:0_22:5); and a sphingomyelin, SM(34:1)—were required to provide the optimal model for cardiovascular death (Table VI in the online-only Data Supplement). Cross-validated estimates of incremental predictive value (for future cardiovascular events) showed that the addition of 7 lipid species to a model that contained the covariate risk factors improved the C statistic from 0.680 (95% CI, 0.678–0.682) to 0.700 (95% CI, 0.698–0.702; $P<0.0001$), whereas the addition of 4 lipid species to a cardiovascular death model also improved the C statistic from 0.740 (95% CI, 0.738–0.742) to 0.760 (95% CI, 0.757–0.762, $P<0.0001$). Continuous NRIs were 0.227 (95% CI, 0.219–0.235) and 0.328 (95% CI, 0.317–0.339) for cardiovascular events and death, respectively. IDI and relative IDI also showed corresponding improvements (Table 2 and Tables V and VI in the online-only Data Supplement). Repeating these analysis using the least absolute shrinkage and selection operator feature selection method identified 3 of the same lipid species in the top-ranked features for each model and resulted in similar but slightly inferior C statistics and NRI but slightly higher IDI values (Tables VII and VIII in the online-only Data Supplement).

Table 2. Model Performance Measures (95% CIs) for 5-Year Risk in the ADVANCE Trial

Feature	C Statistic	Continuous NRI	IDI	Relative IDI
Prediction of cardiovascular events				
Base model*	0.680 (0.678–0.682)			
Base model+7 lipid species†	0.700 (0.698–0.702)§	0.227 (0.219–0.235)	0.024 (0.023–0.024)	0.364 (0.353–0.374)
Prediction of cardiovascular death				
Base model*	0.740 (0.738–0.742)			
Base model+4 lipid species‡	0.760 (0.757–0.762)§	0.328 (0.317–0.339)	0.023 (0.022–0.024)	0.288 (0.274–0.302)

ADVANCE indicates Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation; CI, confidence interval; IDI, integrated discrimination improvement; and NRI, net reclassification index.

*Base model contains significant covariates in Table 1.

†Lipid species included in the cardiovascular events model were PC(O-36:1), CE(18:0), PE(O-36:4), PC(28:0), LPC(20:0), PC(35:4), and LPC(18:2).

‡Lipid species included in the cardiovascular death model were PC(O-36:1), DG(16:0_22:5), SM(34:1), and PC(O-36:5).

§*P* values <0.0001 relative to base model.

Validation on a Subcohort of the LIPID Trial

A subcohort of participants with T2DM enrolled in the LIPID trial (n=511) were used for validation. Cox regression of each lipid species used in the multivariable models for prediction of cardiovascular events and death, adjusting for age, sex, BMI, SBP, HDL-C, and eGFR, produced hazard ratios for most species similar to those found in the ADVANCE study (Figure II in the online-only Data Supplement). However, of the 7 species incorporated into the cardiovascular events model, PE(O-36:4) showed opposing (nonsignificant) hazard ratios, whereas PC(35:4) showed a significant inverse association in the ADVANCE subcohort but a nonsignificant hazard ratio in the LIPID subcohort. A similar situation was observed for PC(O-36:1) associations with cardiovascular death (Figure IIB in the online-only Data Supplement).

The addition of the 7 lipid species (identified in the ADVANCE cardiovascular event risk model) to the LIPID subcohort base model (21 covariates) resulted in an increase in the C statistic from 0.662 (95% CI, 0.661–0.662) to 0.684 (95% CI, 0.684–0.685; *P*<0.0001) and a continuous NRI of 0.297 (95% CI, 0.294–0.301). Similarly, the incorporation of the 4 lipid species (from the ADVANCE cardiovascular death risk model) to the base model to predict cardiovascular death increased the C statistic from 0.641 (95% CI, 0.637–0.645) to 0.701 (95% CI, 0.679–0.705; *P*<0.0001) and resulted in a continuous NRI of 0.481 (95% CI, 0.465–0.498). IDI values were also equal to or better than those observed in the ADVANCE cohort (Table 3).

DISCUSSION

Recent advances in liquid chromatography and mass spectrometry now enable the application of lipidomic studies in a true epidemiological setting. Here, we present the largest lipidomic study representing >1.1 million discrete lipid measurements across 3779 participants with diabetes enrolled in the ADVANCE trial. The statistical power of this large data set, together with the detailed phenotyping and clinical outcomes, has allowed us to identify associations between >40 individual lipid species with future cardiovascular outcomes. Multivariable modeling demonstrated that a small number of these lipid species can significantly improve on all other risk factors for the prediction of future cardiovascular events and death in individuals with T2DM.

Sphingolipids Associated With Future Cardiovascular Events

We observed direct associations of both monohexosylceramide and dihexosylceramide with the risk of future cardiovascular events. These glycosphingolipids are transported primarily by LDL (66%),[29] and their metabolism has previously been reported as a potential contributing factor in atherosclerosis progression.[30] Chatterjee et al[31] reported the role of oxidized LDL in the activation of lactosylceramide synthase to synthesize lactosylceramide, the major form of dihexosylceramide, in aortic smooth muscle cells. Consequently, lactosylceramide enhances the activity of nicotinamide adenine dinucleotide phosphate oxidase to generate superoxide radicals, which in turn mediate p44MAPK activation to enhance nuclear transcription factor (c-Fos) expression and to stimulate the proliferation of smooth muscle cells, thereby contributing to atherosclerosis. More recently, the inhibition of glycosphingolipid synthesis was shown to ameliorate atherosclerosis in both ApoE$^{-/-}$ mice and rabbits on a high-fat and high-cholesterol diet via the oxidized LDL/reactive oxygen species/c-Fos/smooth muscle cell cascade, in addition to multiple effects of lipoprotein metabolism.[32]

Table 3. Model Performance Measures (95% CIs) for 5-Year Risk in the LIPID Trial Subcohort

Feature	C Statistic	Continuous NRI	IDI	Relative IDI
Prediction of cardiovascular events				
Base model*	0.662 (0.661–0.662)			
Base model+7 lipid species†	0.684 (0.684–0.685)§	0.297 (0.294–0.301)	0.043 (0.043–0.044)	0.458 (0.449–0.467)
Prediction of cardiovascular death				
Base model*	0.641 (0.637–0.645)			
Base model+4 lipid species‡	0.701 (0.697–0.705)§	0.481 (0.465–0.498)	0.080 (0.075–0.084)	0.727 (0.686–0.768)

CI indicates confidence interval; IDI, integrated discrimination improvement; LIPID, Long-Term Intervention With Pravastatin in Ischemic Disease; and NRI, net reclassification index.

*Base model is based on age, statin treatment arm, body mass index, cholesterol, high-density lipoprotein cholesterol, triglycerides at baseline, current smoking, systolic blood pressure, fasting glucose, atrial fibrillation, sex, stroke history, history of hypertension, nature of prior acute coronary syndrome, revascularization, estimated glomerular filtration rate, dyspnea grade, angina grade, white blood cell count, peripheral vascular disease, and aspirin use.

†Lipid species that were included in the cardiovascular events model were PC(O-36:1), CE(18:0), PE(O-36:4), PC(28:0), LPC(20:0), and PC(35:4), LPC(18:2).

‡Lipid species that were included in the cardiovascular death model were PC(O-36:1), DG(16:0_22:5), SM(34:1), and PC(O-36:5).

§*P* values <0.0001 relative to base model.

Phospholipids Associated With Future Cardiovascular Events

We observed a direct association with the lysoalkylphosphatidylcholine and future cardiovascular events. In addition, a number of alkylphosphatidylcholine [PC(O)] species and alkenylphosphatidylcholine [PC(P); plasmalogen] species containing primarily saturated and monounsaturated fatty acids were directly associated (Figure 3 and Table II in the online-only Data Supplement). In contrast, phosphatidylcholine and alkenylphosphatidylcholine species containing PUFAs were inversely associated with future cardiovascular events. The unique and opposing sensitivity of the PC(O) and PC(P) species to future cardiovascular events may relate to the instability of the polyunsaturated PC(P) species under heightened oxidative stress[33] and the unique biosynthetic pathway leading to their production. Both PC(O) and PC(P) species are synthesized by the same pathway, starting with dihydroxyacetonephosphate in the peroxisome. The resulting 1-O-alkyl-2-acyl-sn-glycerol is diverted to the production of both PC(O) and alkylphosphatidylethanolamine [PE(O)] species within the endoplasmic reticulum. However, although the PE(O) is subsequently desaturated to produce alkylphosphatidylethanolamine [PE(P); plasmalogen], the PC(O) is not but can be deacylated to form lysoalkylphosphatidylcholine [LPC(O)]. PC(P) results from either the sequential methylation PE(P) by phosphatidylethanolamine methyl transferase or the sequential action of phospholipase C and choline-phosphotransferase (Figure III in the online-only Data Supplement). The regulatory control of this pathway is believed to be via fatty-acyl-CoA reductase 1 (Far 1) which is regulated by the membrane level of plasmalogen.[34,35] Thus, in situations of heightened oxidative stress, plasmalogens (particularly those with PUFAs at the sn-2 position) are oxidized, leading to an upregulation of the biosynthetic pathway, which flows into the production of both plasmalogens [PC(P) and PE(P)], which are in a continual state of flux, and PC(O) and LPC(O), which are relatively stable and thus accumulate within the system. These PC(O) and LPC(O) species then may represent unique biomarkers for the early detection of heightened oxidative stress associated with chronic disease.

Our previous cross-sectional studies identified inverse associations between alkylphosphatidylcholine and alkenylphosphatidylcholine and phosphatidylethanolamine species in stable and unstable coronary artery disease, but we did not observe the direct associations identified in these longitudinal studies.[12] However, PC(O-34:1) has previously been reported to be significantly higher in plaque compared with plasma (a 4-fold increase), whereas the corresponding diacyl species (PC(34:1) was not different,[36] further highlighting the potential for alkylphosphatidylcholine species to accumulate in pathological conditions.

In addition to being a marker of increase flux through the plasmalogen pathway, LPC(O), also known as lysoplatelet activating factor, may have functional relevance to disease progression and risk of future cardiovascular events. LPC(O) is synthesized via the action of lipoprotein phospholipase A2 (Lp-PLA2), alternatively known as platelet-activating factor acetylhydrolyase, on PC(O) (Figure III in the online-only Data Supplement) and is considered the major precursor of platelet activating factor, a potent proinflammatory and prothrombotic signaling lipid in oxidized LDL.[37] Reduced circulating Lp-PLA2 levels in patients with acute coronary syndrome were associated with plaque regression,[38] whereas increased levels were directly associated with the risk of coro-

nary artery disease.[39] However, a recent randomized, controlled trial of an Lp-PLA2 inhibitor, darapladib, did not result in reduced cardiovascular risk despite demonstrating a 65% reduction Lp-PLA2 activity.[40] Thus, the functional roles of Lp-PLA2 and LPC(O) in coronary artery disease remain uncertain.

Fatty Acids Associated With Future Cardiovascular Events

In a population-based study, Stegemann et al[13] identified a specific cluster of triacylglycerol species with saturated and monounsaturated acyl chains as most consistently associated with CVD, and a similar set of triacylglycerols has been associated with prevalent[11] and incident[41] diabetes mellitus. We did not observe these associations in our study in patients with T2DM, which were possibly masked by the generally elevated triglycerides associated with T2DM. However, we observed a single triglyceride [TG(56:6)] that showed a novel inverse association with future cardiovascular events. We also observed 5 other lipid species from multiple classes/subclasses containing long-chain PUFAs associated with a decreased risk of future cardiovascular events and death. It has previously been reported that PUFA-containing species of phosphatidylcholine, triacylglycerol, cholesteryl ester, lysophosphatidylcholine, and lysophosphatidylethanolamine were negatively associated with T2DM.[11,41] These observations may reflect an underlying interaction between the severity or control of T2DM in this population and cardiovascular risk. Previous studies have linked n-3 PUFA intake with traditional lipid measures and demonstrated that increased intake of n-3 PUFAs reduced triacylglycerol levels by 25% to 30%.[42] These findings may indicate an important atheroprotective effect of n-3 PUFAs, as has been extensively reviewed.[43,44]

Of the 36 lipid species that showed a direct association with cardiovascular events and death, all but one contained only saturated or monounsaturated fatty acids. The association between saturated/monounsaturated fatty acids and cardiovascular risk was also evident in the Stegemann et al[13] study. Of note, although cholesteryl esters (as a class) were not associated with cardiovascular outcomes, the saturated species CE(16:0) was directly associated with cardiovascular events in both studies, demonstrating a general agreement (at the fatty acid level) and suggesting some commonality between primary prevention and high-risk populations.

Lipid Species as Predictors of Cardiovascular Events and Death

Our initial assessment of the data structure in the ADVANCE lipidomic data set using principal component analysis highlighted the complex nature of the data set. Although sex, age, BMI, and SBP contributed to the variance represented by the principal components, cardiovascular outcomes did not appear to be contributing to this variance, highlighting the challenge in selecting lipid species for model development. Regression analysis of the subset of lipid species associated with cardiovascular outcomes with the traditional risk factors (sex, age, BMI, and SBP) showed that the sphingolipid species, which increased risk of cardiovascular events, were inversely associated with BMI. Similarly, the same lipid species were associated with female sex, further reducing risk in the male group relative to the female group. However, the same lipid species, particularly the monohexosylceramides, were also directly associated with SBP. Other lipid species such as lysoalkylphosphatidylcholine showed strong associations with age but minimal associations with other risk factors, suggesting different biological relationships for this lipid class. These differential associations suggest that there are multiple (sometimes opposing) factors that influence lipid homeostasis and thereby cardiovascular risk. The residual risk of these lipid species, after adjustment for established risk factors, including gender, age, BMI, and SBP, likely reflects other environmental and genetic factors not currently considered in cardiovascular risk. Further analyses of this and other data sets will shed new light on this residual risk and may identify therapeutic strategies to minimize such risk. Examination of the internal correlation structure of this lipid set showed a range of correlations (positive and negative) and further highlights both the complexity of the data and the redundancy of many lipid species, an important consideration for the development of multivariable prediction models.

Cardiovascular risk scores developed for the general population have been shown to underestimate the risk of future CVD in the T2DM population.[45,46] Scores specifically designed for T2DM perform better but also show limited performance.[47] In the ADVANCE study, the incorporation of 7 and 4 lipid species, on top of the traditional risk factors and medication, improved the prediction of cardiovascular events and death, respectively. The use of an alternative feature selection strategy (least absolute shrinkage and selection operator) identified a single lipid species [PC(O-36:1] as common to all models produced by either strategy, suggesting the robustness of certain lipid species and redundancy in other lipid species within these models.

Importantly, the addition of these same 7 and 4 lipid species identified in the modeling of the ADVANCE cohort to the base model (21 covariates) improved the risk prediction of cardiovascular events and death, respectively, in the LIPID subcohort, thus providing independent validation of these lipid species. Although the addition of the selected lipid species to prediction

models gave similar improvement in model performance in both the ADVANCE and LIPID data sets, not all of the lipid species showed the same associations in the LIPID subcohort when adjusted for an identical set of covariates (Figure II in the online-only Data Supplement). These differential associations may reflect the secondary prevention nature of the LIPID study, in which all participants had a prior history of CVD, whereas only 35% of the ADVANCE study participants had a history of macrovascular disease. We also note that not all of the lipid species incorporated into the multivariable models were independently associated with the outcome in question. We believe that this reflects the contribution they make to the overall model performance through their interaction with the other lipid species or covariates in the model. Analysis of these lipid species in other cohorts will help to resolve these questions.

In a study of lipid metabolites in a primary prevention cohort, Stegemann et al[13] showed that the addition of 6 lipid species (selected on the basis of the entire data set) to the conventional risk factors (used in the Framingham risk score) improved the C statistic and categorical NRI for cardiovascular events (incident fatal and nonfatal MI, ischemic stroke, and sudden cardiac death) by 3.74% and 14.9%, respectively. However, although the analyses were performed within a 5-fold cross-validation framework, independent validation of the lipid species was not performed. The differing performance of lipid species in terms of risk prediction in secondary prevention compared with primary prevention may relate to the many additional covariates used in the secondary prevention case-cohort analyses that are also associated with cardiovascular risk.

The observation that relatively few lipid species improved risk prediction over traditional risk factors both in a primary prevention cohort and in those with T2DM and increased baseline risk highlights the potential of individual lipid species identified by lipidomics to improve CVD risk stratification. That there was no overlap between the lipid species selected in each study may reflect metabolic differences between the cohorts related to either their clinical status (diabetes versus nondiabetes) or to the different stages of disease progression (primary versus secondary prevention) but may also be a result of the lipid species measured in each study and the statistical methods used to select the optimal lipid species for model development.

Strengths and Limitations of the Study

This study represent the largest lipidomic study reported to date, incorporating >300 lipid species in >4000 samples from 2 independent prospective clinical trials. The use of an independent validation cohort strengthens our findings. The statistical power provided by this "big data" approach highlights the potential of lipidomic studies not only to identify new biomarkers of disease risk but also to understand the relationship of lipid metabolism with interventions, comorbidities, and clinical outcomes.

A limitation of all lipidomic studies is that the coverage of the lipidome is incomplete. In this study, we have used a targeted approach that has enabled us to measure >300 lipid species from 22 different lipid classes/subclasses, providing a broad, but still incomplete, coverage of the lipidome. We recognize that there are many lipid species and classes/subclasses not covered in this study that may show superior predictive performance. Furthermore, the high variance associated with lipidomic measurements will lead to an underestimation of the strength of associations.

Selection of covariates for regression analysis is challenging because it is not always possible to predict interactions among covariates, lipid species, and cardiovascular events. We recognize that a large proportion of subjects (≈44%) were on lipid-lowering medication, which will influence plasma lipids in these individuals. However, lipid-lowering medication was not associated with future cardiovascular events or cardiovascular death, and when we added this as a covariate, we did not see any change in the hazard ratios for the significant lipid species. Similarly, total cholesterol was not associated with cardiovascular events and made no difference in the hazard ratios when added as a covariate (data not shown).

Although the ADVANCE study represents the largest cohort to undergo targeted lipidomic analysis to date, the case-cohort design resulted in a primarily white group, so these results may not extrapolate to all populations. We also recognize that the LIPID trial validation cohort was relatively small and that the clinical covariates were not identical to those in the ADVANCE trial. These differences notwithstanding, the covariates provided similar clinical phenotyping, and we were able to demonstrate that the same lipid species were predictive above the clinical phenotype in both cohorts.

Conclusions

Multiple lipid species were independent predictors of cardiovascular events and cardiovascular death. A small number of lipid species were able to significantly improve risk stratification among those with T2DM. The associations between individual lipid species and cardiovascular risk demonstrate the statistical power resulting from lipidomic analyses of large epidemiological studies and the potential to inform on lipid metabolism in relation to chronic disease. These results further highlight the need for mechanistic studies to characterize the role of individual lipid species in disease pathogenesis. These studies also raise the potential for new intervention strat-

egies (lifestyle/drug) to modify lipid metabolism and to attenuate disease progression, such as those that have recently been reported for plasmalogen modulation in a mouse model of atherosclerosis.[48]

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DISCLOSURES

Dr Meikle has licensed lipid biomarkers described in this article to Zora Biosciences Oy, Finland. The other authors report no conflicts.

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FOOTNOTES

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SUPPLEMENTAL MATERIAL

Plasma lipidomic profiles improve upon traditional risk factors for the prediction of cardiovascular events in type 2 diabetes.

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Lipid analysis

The lipidomic methodology used for this study was a development upon our earlier targeted methodology developed on an Agilent 1200 liquid chromatography system combined with an Applied Biosystems API 4000 Q/TRAP mass spectrometer[1]. In this study, lipidomic analysis was performed by liquid chromatography electrospray ionisation tandem mass spectrometry on an Agilent 1290 liquid chromatography system combined with an Agilent 6490 triple quadrupole mass spectrometer with a turbo-ionspray source (200°C), utilizing Mass Hunter software. Liquid chromatography was performed on a Zorbax Eclipse Plus 1.8 µm C18, 50 × 2.1 mm column (Agilent Technologies). Solvents A and B consisted of tetrahydrofuran:methanol:water in the ratio (30:20:50) and (75:20:5) respectively, both containing 10 mM ammonium formate. Columns were heated to 50°C and the auto-sampler regulated to 25°C. Lipid species (1 µL injection) were separated under gradient conditions at a flow rate of 400 µL/min. The gradient was as follows; 0% solvent B to 40% solvent B over 2.0 min, 40% solvent B to 1000% solvent B over 6.5 min, 0.5 min at 100% solvent B, a return to 0% solvent B over 0.5 min then 0.5 min at 0% solvent B prior to the next injection (total run time of 10 min).

The mass spectrometer was operated in dynamic/scheduled multiple reaction monitoring (dMRM) mode. There were 310 unique lipid species measured together with 15 stable isotope or non-physiological lipid standards (Supplementary Table 1 and 2). Mass spectrometer voltages used for the acquisition of data were; fragmentor voltage, 380 V and cell accelerator voltage, 5 V. The collision energy voltage was set individually for each lipid class and subclass and is listed in Supplementary Table 1. Acquisition windows were set to between 0.7 and 1.76 min depending on the chromatographic properties of the lipid. Further, there were several sets of isobaric lipid species which shared the same nominal parent ion mass and also give rise to the same product ions. Specifically, for isobaric species of PC, PC(O) and PC(P) the parent and product ions (m/z 184) the same. As a result a single MRM transition was used to measure the corresponding species within each subclass, using an increased MRM window time (22 combinations). Additionally there were eight occurrences of isobaric PE, PE(O) and PE(P) lipid species, representing the neutral loss of 141 Da, which were similarly combined into a single dMRM transition. Analysis of triacylglycerols was based on single ion monitoring. To

perform this analysis in the dynamic/scheduled multiple reaction monitoring (dMRM) mode both Q1 and Q3 were set to the $[M+NH_4]^+$ values for each triacylglycerol species and the collision energy was reduced to 5 V to minimise collision induced dissociation.

While most lipid classes and subclasses have similar response factors for lipid species within the class, some classes show greater variation in response factors between species. Consequently, correction factors were applied for some lipid classes as we have described earlier[1] but now adjusted for the Agilent mass spectrometer. Diacyl- and triacylglycerol (DG and TG): Fragmentation of the ammoniated adducts of DG and TG leads to the loss of ammonia and a fatty acid. In this context it is important to recognize that for species which contain more than one of the same fatty acid, the loss of that fatty acid will result in an enhanced signal, as it is the end product from two competing pathways. Consequently, where we used an MRM transition that corresponded to the loss of a fatty acid that was present more than once, we divided by the number of times that fatty acid was present. While we recognize that the response factor for different species of TG varied substantially, the lack of suitable standards precluded the determination of suitable response factors for each TG species.

Cholesteryl ester (CE): Response factors were determined with seven commercially available species and used to create a formula to extrapolate for all CE chain lengths and double bonds. Saturated species were characterized by the following relationship: $y = 0.1486x - 1.5917$, where y is the response factor relative to the CE 18:0 d 6 internal standard and x is the carbon chain length. For monounsaturated species, the response factor was multiplied by 1.84 and for polyunsaturated species by 6.0. Phosphatidylinositol (PI): A single response factor was calculated for all PI species to account for the use of the PE 17:0/17:0 as the internal standard for this lipid class. A nine point standard curve was created using commercially available PI 32:0 and subsequently spiked into solvent containing a fixed concentration of PE 17:0/17:0. The standard curve resulted in a linear response and indicated a response factor of 1.44 for phosphatidylinositol species relative to phosphatidylethanolamine standard. Other lipid species were not corrected.

Quality Control Samples

Two types of quality control samples were utilized in this study. Plasma from six healthy volunteers was pooled and split into multiple aliquots. We refer to these samples as plasma quality control (PQC) samples. These samples are then subjected to extraction and LC-MS analysis alongside samples from the study to provide a measure of analytical variability across the study as a whole. Additionally we utilized identical lipid extracts, which were prepared by pooling the lipid extracts from multiple PQC samples using this mixture to prepare multiple aliquots which were referred to as technical quality control (TQC) samples. Analysis of these samples captures only the variation associated with the LC-MS performance. Within the analytical process every twenty-five plasma samples a PQC and TQC were included.

Data pre-processing

In this study, samples were run in multiple batches. An extraction batch consisted 500 plasma samples, 22 PQC, 24 TQC and 11 blank samples (resulting in 8 batches). Two batches were run consecutively between cleaning of the mass spectrometer. A median centering approach was used for correction of the batch effect. The median PQC concentration of each lipid for each batch was used as a reference point to align the samples with the entire cohort. The alignment was performed by calculating a correction factor to adjust the concentration of each PQC lipid in each batch to the median value for all batches.

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Supplementary Table 1: Conditions for tandem mass spectrometry analysis of lipid species.

Lipid class/subclass	Parent Ion	Fragmentation*	Number of features	Internal standard	Internal standard (pmol)	Collision Energy (V)
Dihydroceramide (Cer(d18:0))	$[M+H]^+$	NL, 18 Da	6	Cer(d18:0/8:0)	50	21
Ceramide (Cer(d18:1))	$[M+H]^+$	PI, m/z 264.3	6	Cer(d18:1/17:0)	100	29
Monohexocylceramide (HexCer)	$[M+H]^+$	PI, m/z 264.3	6	Glucosylceramide 16:0 d3	50	33
Dihexosylceramide (Hex2Cer)	$[M+H]^+$	PI, m/z 264.3	6	Lactosylceramide 16:0 d3	50	53
Trihexosylceramide (Hex3Cer)	$[M+H]^+$	PI, m/z 264.3	6	Hex3Cer(17:0)	50	57
Sphingomyelin (SM)	$[M+H]^+$	PI, m/z 184.1	20	SM(d18:1/12:0)	200	25
Phosphatidylcholine (PC)	$[M+H]^+$	PI, m/z 184.1	46	PC(13:0/13:0)	100	21
Alkylphosphatidylcholine (PC(O))	$[M+H]^+$	PI, m/z 184.1	19	PC(13:0/13:0)	100	21
Alkenylphosphatidylcholine (PC(P))	$[M+H]^+$	PI, m/z 184.1	14	PC(13:0/13:0)	100	21
Lysophosphatidylcholine (LPC)	$[M+H]^+$	PI, m/z 184.1	22	LPC(13:0)	100	21
Lysoalkylphosphatidylcholine (LPC(O))	$[M+H]^+$	PI, m/z 104.1	10	LPC(13:0)	100	21
Phosphatidylethanolamine (PE)	$[M+H]^+$	NL, 141 Da	21	PE(17:0/17:0)	100	17
Alkylphosphatidylethanolamine (PE(O))	$[M+H]^+$	NL, 141 Da	12	PE(17:0/17:0)	100	17
Alkenylphosphatidylethanolamine (PE(P))	$[M+H]^+$	NL, 141 Da	11	PE(17:0/17:0)	100	17
Lysophosphatidylethanolamine (LPE)	$[M+H]^+$	NL, 141 Da	6	LPE(14:0)	100	17
Phosphatidylinositol (PI)	$[M+NH_4]^+$	NL, 277 Da	16	PE(17:0/17:0)	100	17
Lysophosphatidylinositol (LPI)	$[M+NH_4]^+$	NL, 277 Da	4	PE(17:0/17:0)	100	17
Phosphatidylglycerol (PG)	$[M+NH_4]^+$	NL, 189 Da	3	PG(17:0/17:0)	100	21
Cholesterol ester (CE)	$[M+NH_4]^+$	PI, m/z 369.3	26	CE(18:0)-d6	1000	10
Free cholesterol (COH)	$[M-H_2O]^+$	PI, m/z 161.2	1	COH-d7	10000	23
Diacylglycerol (DG)	$[M+NH_4]^+$	NL, NH_3 + fatty acid	24	DG(15:0/15:0)	200	21
Triacylglycerol (TG)	$[M+NH_4]^+$	SIM	25	TG(17:0/17:0/17:0)	100	5

* PI, product ion; NL, neutral loss; SIM, single ion monitoring.

Supplementary Table 2: Associations of plasma lipid species with cardiovascular events and cardiovascular death.

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
Cer(d18:0/16:0)	51 (24)	26	26	1.00 (0.91 - 1.09)	9.74E-01	1.06 (0.95 - 1.19)	5.67E-01
Cer(d18:0/18:0)	180 (43)	8	11	0.96 (0.87 - 1.06)	6.50E-01	1.03 (0.90 - 1.17)	8.34E-01
Cer(d18:0/20:0)	46 (24)	17	23	0.94 (0.85 - 1.04)	5.42E-01	1.00 (0.87 - 1.14)	9.74E-01
Cer(d18:0/22:0)	206 (114)	17	21	0.95 (0.87 - 1.05)	6.19E-01	1.00 (0.88 - 1.14)	9.79E-01
Cer(d18:0/24:0)	213 (132)	18	22	0.94 (0.85 - 1.04)	5.02E-01	0.98 (0.86 - 1.12)	8.65E-01
Cer(d18:0/24:1)	2,380 (1,101)	21	23	1.00 (0.91 - 1.10)	9.87E-01	1.06 (0.93 - 1.20)	6.44E-01
Cer(d18:1/16:0)	371 (133)	8	11	1.07 (0.99 - 1.16)	2.80E-01	1.10 (1.01 - 1.20)	1.24E-01
Cer(d18:1/18:0)	146 (73)	9	9	1.04 (0.94 - 1.15)	7.41E-01	1.11 (0.97 - 1.26)	3.53E-01
Cer(d18:1/20:0)	121 (56)	9	9	1.05 (0.94 - 1.16)	6.60E-01	1.13 (0.99 - 1.29)	2.29E-01
Cer(d18:1/22:0)	974 (425)	8	8	1.03 (0.93 - 1.15)	7.59E-01	1.10 (0.96 - 1.26)	4.51E-01
Cer(d18:1/24:0)	3,413 (1,427)	8	10	1.02 (0.92 - 1.14)	8.18E-01	1.07 (0.93 - 1.24)	5.98E-01
Cer(d18:1/24:1)	1,293 (563)	6	9	1.12 (1.02 - 1.24)	1.27E-01	1.21 (1.07 - 1.37)	**3.15E-02**
HexCer(d18:1/16:0)	913 (413)	13	13	1.25 (1.12 - 1.38)	**2.62E-03**	1.37 (1.20 - 1.57)	**4.02E-04**
HexCer(d18:1/18:0)	128 (68)	21	21	1.20 (1.09 - 1.33)	**1.09E-02**	1.29 (1.14 - 1.47)	**2.72E-03**
HexCer(d18:1/20:0)	117 (60)	14	15	1.21 (1.09 - 1.34)	**9.69E-03**	1.35 (1.18 - 1.54)	**4.32E-04**
HexCer(d18:1/22:0)	1,007 (494)	11	10	1.18 (1.06 - 1.31)	**3.00E-02**	1.27 (1.10 - 1.45)	**1.22E-02**
HexCer(d18:1/24:0)	1,323 (647)	9	11	1.19 (1.06 - 1.32)	**3.00E-02**	1.29 (1.12 - 1.49)	**8.37E-03**
HexCer(d18:1/24:1)	1,021 (541)	8	10	1.28 (1.15 - 1.42)	**5.54E-04**	1.39 (1.22 - 1.59)	**1.66E-04**
Hex2Cer(d18:1/16:0)	4,659 (1,725)	13	10	1.23 (1.10 - 1.37)	**9.31E-03**	1.33 (1.15 - 1.54)	**2.72E-03**
Hex2Cer(d18:1/18:0)	112 (57)	14	24	1.25 (1.13 - 1.39)	**2.62E-03**	1.43 (1.25 - 1.64)	**1.16E-04**
Hex2Cer(d18:1/20:0)	119 (65)	26	28	1.07 (0.97 - 1.19)	4.45E-01	1.20 (1.06 - 1.37)	**4.72E-02**
Hex2Cer(d18:1/22:0)	367 (169)	13	15	1.17 (1.06 - 1.29)	**2.88E-02**	1.28 (1.13 - 1.45)	**2.72E-03**
Hex2Cer(d18:1/24:0)	378 (166)	10	13	1.14 (1.03 - 1.25)	7.50E-02	1.25 (1.11 - 1.42)	**7.27E-03**
Hex2Cer(d18:1/24:1)	1,251 (590)	12	11	1.21 (1.09 - 1.34)	**1.03E-02**	1.32 (1.15 - 1.51)	**2.14E-03**
Hex3Cer(d18:1/16:0)	954 (459)	12	11	0.99 (0.98 - 1.01)	6.50E-01	1.00 (0.98 - 1.02)	9.80E-01
Hex3Cer(d18:1/18:0)	143 (85)	30	29	1.12 (1.02 - 1.25)	1.35E-01	1.18 (1.03 - 1.34)	1.00E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
Hex3Cer(d18:1/20:0)	68 (54)	40	38	1.14 (1.02 - 1.28)	1.23E-01	1.33 (1.15 - 1.53)	**3.00E-03**
Hex3Cer(d18:1/22:0)	168 (88)	18	20	1.19 (1.07 - 1.32)	**2.37E-02**	1.29 (1.13 - 1.48)	**3.03E-03**
Hex3Cer(d18:1/24:0)	168 (85)	21	22	1.22 (1.10 - 1.36)	**9.41E-03**	1.31 (1.13 - 1.51)	**4.76E-03**
Hex3Cer(d18:1/24:1)	366 (192)	18	16	1.23 (1.11 - 1.36)	**5.06E-03**	1.38 (1.21 - 1.57)	**1.16E-04**
SM(31:1)	303 (165)	5	5	0.99 (0.88 - 1.11)	9.53E-01	0.91 (0.77 - 1.06)	5.12E-01
SM(32:0)	372 (195)	14	13	0.99 (0.89 - 1.09)	9.23E-01	1.04 (0.91 - 1.19)	8.07E-01
SM(32:1)	10,766 (4,647)	9	9	1.02 (0.91 - 1.15)	8.53E-01	1.04 (0.89 - 1.21)	8.16E-01
SM(32:2)	783 (376)	4	5	0.98 (0.86 - 1.11)	8.96E-01	0.84 (0.71 - 1.01)	2.20E-01
SM(33:1)	7,387 (3,347)	18	16	1.06 (0.95 - 1.18)	5.77E-01	1.08 (0.94 - 1.24)	5.59E-01
SM(34:0)	5,079 (2,257)	22	24	1.09 (0.98 - 1.20)	3.61E-01	1.18 (1.04 - 1.35)	9.43E-02
SM(34:1)	121,073 (47,126)	22	24	1.13 (1.02 - 1.25)	1.23E-01	1.24 (1.09 - 1.42)	**1.46E-02**
SM(34:2)	17,077 (5,636)	5	5	1.12 (0.99 - 1.26)	2.83E-01	1.10 (0.94 - 1.29)	5.20E-01
SM(34:3)	101 (49)	4	7	1.03 (0.92 - 1.16)	7.87E-01	0.86 (0.73 - 1.02)	2.48E-01
SM(35:1)	3,201 (1,534)	20	20	1.06 (0.96 - 1.18)	5.45E-01	1.10 (0.96 - 1.27)	4.41E-01
SM(35:2)	493 (216)	8	8	1.05 (0.93 - 1.19)	6.62E-01	0.96 (0.82 - 1.13)	8.08E-01
SM(36:1)	32,929 (14,096)	21	24	1.01 (0.91 - 1.13)	9.18E-01	1.10 (0.96 - 1.27)	4.15E-01
SM(36:2)	10,043 (4,040)	6	6	0.98 (0.87 - 1.11)	8.90E-01	0.96 (0.81 - 1.13)	8.08E-01
SM(36:3)	791 (395)	6	5	1.06 (0.96 - 1.18)	5.23E-01	0.94 (0.82 - 1.09)	6.78E-01
SM(38:1)	49,311 (16,198)	14	15	1.02 (0.92 - 1.14)	8.29E-01	1.09 (0.94 - 1.26)	5.40E-01
SM(38:2)	38,765 (12,339)	9	13	1.00 (0.89 - 1.12)	9.91E-01	1.06 (0.91 - 1.24)	6.92E-01
SM(39:1)	6,201 (2,677)	19	22	0.98 (0.88 - 1.09)	8.47E-01	0.94 (0.81 - 1.09)	6.44E-01
SM(41:1)	11,812 (5,065)	23	26	0.99 (0.89 - 1.10)	9.08E-01	1.00 (0.87 - 1.16)	9.79E-01
SM(41:2)	5,439 (2,308)	18	15	0.99 (0.88 - 1.11)	9.23E-01	0.95 (0.81 - 1.11)	7.74E-01
SM(42:1)	7,799 (3,392)	21	25	0.98 (0.88 - 1.08)	8.16E-01	1.05 (0.92 - 1.21)	7.01E-01
PC(28:0)	123 (129)	12	11	1.00 (0.94 - 1.06)	9.79E-01	1.04 (0.98 - 1.11)	5.11E-01
PC(29:0)	114 (64)	17	21	1.06 (0.97 - 1.17)	4.91E-01	1.14 (1.01 - 1.29)	1.44E-01
PC(30:0)	1,673 (1,198)	15	20	0.99 (0.89 - 1.09)	9.23E-01	1.03 (0.90 - 1.18)	8.16E-01
PC(31:0)	496 (364)	14	17	1.02 (0.92 - 1.14)	8.17E-01	1.00 (0.86 - 1.15)	9.91E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
PC(31:1)	720 (331)	12	13	1.09 (0.97 - 1.22)	4.44E-01	1.09 (0.93 - 1.27)	5.59E-01
PC(32:0)	7,676 (3,127)	18	23	1.07 (0.96 - 1.18)	5.02E-01	1.18 (1.03 - 1.35)	1.00E-01
PC(32:1)	15,074 (8,391)	7	9	1.02 (0.91 - 1.13)	9.05E-01	1.03 (0.89 - 1.19)	8.28E-01
PC(32:2)	4,076 (1,668)	8	7	0.96 (0.84 - 1.09)	7.41E-01	0.95 (0.80 - 1.13)	7.91E-01
PC(32:3)	223 (108)	6	7	1.05 (0.94 - 1.18)	6.60E-01	0.94 (0.81 - 1.10)	7.01E-01
PC(33:0)	736 (369)	11	15	1.05 (0.94 - 1.17)	6.50E-01	0.99 (0.85 - 1.15)	9.52E-01
PC(33:1)	2,399 (1,258)	12	9	1.03 (0.92 - 1.16)	7.78E-01	0.96 (0.82 - 1.13)	8.16E-01
PC(33:2)	1,375 (750)	8	8	1.02 (0.90 - 1.15)	9.04E-01	0.98 (0.84 - 1.15)	9.06E-01
PC(33:3)	47 (31)	9	9	0.96 (0.85 - 1.07)	7.10E-01	0.91 (0.78 - 1.07)	5.59E-01
PC(34:0)	1,800 (753)	17	21	1.05 (0.95 - 1.16)	6.50E-01	1.14 (0.99 - 1.30)	2.29E-01
PC(34:1)	128,319 (51,915)	8	9	1.08 (0.97 - 1.21)	4.52E-01	1.13 (0.97 - 1.31)	3.60E-01
PC(34:2)	190,871 (59,967)	8	8	1.10 (0.98 - 1.23)	4.01E-01	1.14 (0.98 - 1.34)	2.85E-01
PC(34:3)	7,830 (4,253)	5	7	1.00 (0.89 - 1.13)	9.79E-01	0.97 (0.83 - 1.14)	8.51E-01
PC(34:4)	578 (378)	9	9	0.86 (0.76 - 0.98)	1.35E-01	0.82 (0.69 - 0.97)	1.17E-01
PC(34:5)	49 (53)	8	8	0.86 (0.78 - 0.96)	**4.77E-02**	0.85 (0.74 - 0.98)	1.37E-01
PC(35:0)	93 (46)	16	19	1.09 (0.98 - 1.21)	4.15E-01	1.05 (0.91 - 1.22)	7.49E-01
PC(35:1)	3,003 (1,375)	11	10	1.03 (0.92 - 1.16)	7.75E-01	0.96 (0.82 - 1.12)	8.08E-01
PC(35:2)	5,376 (2,503)	6	8	1.04 (0.92 - 1.16)	7.67E-01	0.97 (0.83 - 1.14)	8.51E-01
PC(35:3)	710 (301)	8	9	1.01 (0.90 - 1.14)	9.32E-01	0.95 (0.81 - 1.12)	7.91E-01
PC(35:4)	493 (282)	8	9	0.84 (0.75 - 0.95)	**4.97E-02**	0.79 (0.67 - 0.94)	5.36E-02
PC(36:0)	99 (42)	22	20	1.03 (0.93 - 1.15)	7.75E-01	1.10 (0.96 - 1.26)	4.43E-01
PC(36:1)	22,860 (10,253)	12	13	1.04 (0.94 - 1.16)	7.03E-01	1.06 (0.92 - 1.23)	6.73E-01
PC(36:2)	117,746 (42,379)	7	7	1.05 (0.94 - 1.19)	6.60E-01	1.06 (0.90 - 1.24)	7.64E-01
PC(36:3)	84,851 (33,640)	6	8	1.02 (0.90 - 1.16)	8.79E-01	0.97 (0.82 - 1.15)	8.65E-01
PC(36:4)	55,223 (56,205)	7	9	0.92 (0.79 - 1.07)	5.89E-01	1.02 (0.83 - 1.26)	8.98E-01
PC(36:5)	844 (9,600)	7	8	0.95 (0.86 - 1.06)	6.50E-01	0.85 (0.73 - 0.99)	1.73E-01
PC(37:4)	2,186 (1,129)	10	10	0.90 (0.80 - 1.01)	3.10E-01	0.82 (0.70 - 0.97)	1.05E-01
PC(37:5)	493 (331)	8	11	0.91 (0.82 - 1.01)	3.15E-01	0.84 (0.73 - 0.98)	1.27E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
PC(37:6)	199 (166)	8	11	0.86 (0.76 - 0.96)	7.50E-02	0.86 (0.73 - 1.01)	2.20E-01
PC(38:3)	15,315 (7,724)	8	8	0.97 (0.86 - 1.09)	7.78E-01	0.91 (0.78 - 1.07)	5.40E-01
PC(38:4)	39,459 (22,117)	6	9	0.85 (0.75 - 0.95)	5.89E-02	0.85 (0.72 - 1.00)	1.82E-01
PC(38:5)	26,895 (14,246)	8	7	0.87 (0.77 - 0.98)	1.27E-01	0.82 (0.69 - 0.96)	1.00E-01
PC(38:6)	27,236 (15,583)	8	10	0.85 (0.76 - 0.96)	7.15E-02	0.84 (0.71 - 0.98)	1.38E-01
PC(38:7)	2,087 (1,455)	17	20	0.85 (0.75 - 0.95)	5.53E-02	0.86 (0.73 - 1.00)	2.11E-01
PC(39:5)	291 (173)	10	9	0.89 (0.79 - 1.00)	1.95E-01	0.79 (0.67 - 0.94)	5.36E-02
PC(39:6)	520 (392)	8	10	0.88 (0.78 - 0.99)	1.62E-01	0.83 (0.70 - 0.97)	1.08E-01
PC(39:7)	20 (17)	13	13	0.90 (0.81 - 1.00)	2.39E-01	0.91 (0.79 - 1.06)	4.98E-01
PC(40:4)	1,381 (821)	10	9	1.00 (0.90 - 1.12)	9.79E-01	1.02 (0.88 - 1.18)	8.73E-01
PC(40:5)	4,047 (2,345)	6	7	0.88 (0.79 - 1.00)	1.95E-01	0.82 (0.70 - 0.97)	1.17E-01
PC(40:6)	9,620 (6,334)	9	8	0.84 (0.75 - 0.95)	**4.25E-02**	0.82 (0.70 - 0.97)	1.05E-01
PC(40:7)	1,363 (942)	10	9	0.92 (0.82 - 1.03)	4.44E-01	0.86 (0.73 - 1.01)	2.23E-01
PC(40:8)	271 (200)	7	10	0.86 (0.75 - 0.98)	1.35E-01	0.83 (0.69 - 1.00)	1.81E-01
PC(O-32:0)	1,195 (474)	14	22	1.18 (1.06 - 1.30)	**2.88E-02**	1.25 (1.10 - 1.42)	**1.25E-02**
PC(O-32:1)	194 (79)	10	10	1.18 (1.06 - 1.32)	**3.00E-02**	1.23 (1.07 - 1.42)	**4.32E-02**
PC(O-32:2)	14 (11)	17	20	1.01 (0.95 - 1.07)	9.43E-01	1.02 (0.94 - 1.10)	8.08E-01
PC(O-34:1)	1,809 (628)	8	9	1.33 (1.19 - 1.49)	**1.22E-04**	1.40 (1.21 - 1.62)	**4.03E-04**
PC(O-34:2)	1,490 (806)	8	13	1.03 (0.93 - 1.14)	7.75E-01	0.94 (0.82 - 1.08)	6.44E-01
PC(O-34:3)	45 (27)	15	19	1.00 (0.91 - 1.11)	9.77E-01	0.94 (0.81 - 1.08)	6.44E-01
PC(O-34:4)	64 (37)	13	10	0.94 (0.84 - 1.04)	5.23E-01	0.83 (0.71 - 0.97)	1.00E-01
PC(O-35:4)	734 (423)	12	15	1.04 (0.93 - 1.17)	7.41E-01	0.99 (0.84 - 1.16)	9.57E-01
PC(O-36:0)	14 (06)	15	21	1.07 (0.98 - 1.16)	3.99E-01	1.14 (1.03 - 1.26)	9.43E-02
PC(O-36:1)	173 (70)	12	10	1.32 (1.18 - 1.48)	**1.22E-04**	1.37 (1.18 - 1.58)	**1.06E-03**
PC(O-36:2)	683 (284)	7	8	1.18 (1.06 - 1.32)	**3.55E-02**	1.12 (0.96 - 1.30)	3.83E-01
PC(O-36:3)	2,772 (1,088)	7	8	1.07 (0.96 - 1.20)	5.09E-01	0.98 (0.84 - 1.14)	8.73E-01
PC(O-36:4)	5,762 (2,683)	9	9	0.98 (0.88 - 1.10)	9.12E-01	0.97 (0.83 - 1.13)	8.16E-01
PC(O-36:5)	344 (267)	11	10	0.91 (0.83 - 1.00)	2.37E-01	0.84 (0.73 - 0.97)	1.00E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
PC(O-38:4)	1,853 (922)	9	9	1.09 (0.97 - 1.22)	4.45E-01	1.10 (0.94 - 1.28)	5.20E-01
PC(O-38:5)	5,402 (2,538)	12	11	1.02 (0.91 - 1.15)	8.40E-01	0.97 (0.83 - 1.14)	8.51E-01
PC(O-40:5)	523 (251)	6	9	1.06 (0.95 - 1.19)	6.10E-01	1.04 (0.89 - 1.20)	8.16E-01
PC(O-40:6)	467 (249)	8	8	0.97 (0.87 - 1.09)	8.17E-01	0.97 (0.83 - 1.14)	8.39E-01
PC(O-40:7)	672 (413)	10	10	0.91 (0.81 - 1.02)	4.01E-01	0.89 (0.76 - 1.04)	4.05E-01
PC(P-30:0)	55 (31)	18	20	1.05 (0.95 - 1.16)	6.33E-01	1.11 (0.98 - 1.27)	3.13E-01
PC(P-32:0)	890 (365)	16	21	1.09 (0.98 - 1.21)	4.01E-01	1.16 (1.01 - 1.34)	1.69E-01
PC(P-32:1)	95 (44)	24	24	1.13 (1.01 - 1.26)	1.56E-01	1.22 (1.06 - 1.40)	**4.64E-02**
PC(P-34:1)	1,045 (417)	8	8	1.21 (1.07 - 1.36)	**3.00E-02**	1.25 (1.07 - 1.46)	**4.34E-02**
PC(P-34:2)	2,278 (1,093)	9	8	0.96 (0.85 - 1.09)	7.75E-01	0.92 (0.78 - 1.08)	5.94E-01
PC(P-34:3)	213 (114)	18	20	0.95 (0.84 - 1.07)	6.50E-01	0.87 (0.74 - 1.03)	2.85E-01
PC(P-36:2)	634 (306)	9	9	1.10 (0.98 - 1.23)	3.61E-01	1.01 (0.86 - 1.18)	9.70E-01
PC(P-36:4)	3,796 (1,879)	9	9	0.94 (0.83 - 1.05)	5.74E-01	0.90 (0.77 - 1.06)	4.63E-01
PC(P-36:5)	257 (208)	8	9	0.87 (0.79 - 0.97)	7.50E-02	0.79 (0.68 - 0.92)	**2.52E-02**
PC(P-38:4)	821 (461)	8	9	0.97 (0.86 - 1.09)	8.02E-01	0.95 (0.81 - 1.11)	7.67E-01
PC(P-38:5)	2,307 (1,218)	9	8	0.89 (0.79 - 1.00)	2.44E-01	0.84 (0.72 - 0.99)	1.78E-01
PC(P-38:6)	646 (417)	8	9	0.83 (0.74 - 0.93)	**3.00E-02**	0.82 (0.70 - 0.96)	9.43E-02
PC(P-40:5)	472 (248)	8	11	0.97 (0.86 - 1.09)	7.75E-01	0.91 (0.77 - 1.07)	5.35E-01
PC(P-40:6)	173 (117)	7	10	0.89 (0.79 - 0.99)	1.75E-01	0.85 (0.73 - 0.99)	1.73E-01
LPC(14:0)	1,137 (632)	5	4	0.99 (0.90 - 1.09)	9.23E-01	0.98 (0.86 - 1.12)	8.73E-01
LPC(15:0)	566 (316)	6	5	1.07 (0.97 - 1.18)	5.01E-01	1.00 (0.87 - 1.14)	9.94E-01
LPC(16:0)	57,497 (32,559)	5	7	1.06 (0.96 - 1.17)	5.45E-01	1.08 (0.94 - 1.23)	5.59E-01
LPC(16:1)	1,838 (987)	4	5	1.06 (0.96 - 1.18)	5.02E-01	0.97 (0.84 - 1.12)	8.16E-01
LPC(17:0)	939 (639)	7	5	1.06 (0.97 - 1.16)	5.02E-01	1.01 (0.89 - 1.14)	9.70E-01
LPC(17:1)	164 (88)	4	6	1.06 (0.96 - 1.17)	5.45E-01	0.98 (0.86 - 1.13)	8.98E-01
LPC(18:0)	19,126 (12,394)	12	8	1.06 (0.96 - 1.16)	5.23E-01	1.07 (0.94 - 1.21)	5.73E-01
LPC(18:1)	10,723 (5,363)	3	6	1.11 (1.01 - 1.22)	1.71E-01	1.08 (0.94 - 1.23)	5.59E-01
LPC(18:2)	12,626 (5,685)	3	5	1.05 (0.95 - 1.17)	6.15E-01	1.04 (0.90 - 1.20)	7.91E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
LPC(18:3)	358 (255)	11	14	1.01 (0.91 - 1.13)	9.35E-01	0.97 (0.84 - 1.13)	8.35E-01
LPC(20:0)	65 (38)	16	13	1.04 (0.95 - 1.15)	6.60E-01	1.09 (0.96 - 1.23)	4.41E-01
LPC(20:1)	163 (113)	6	7	1.12 (1.03 - 1.21)	7.50E-02	1.18 (1.06 - 1.31)	**2.92E-02**
LPC(20:2)	193 (110)	5	8	1.10 (1.01 - 1.20)	1.71E-01	1.15 (1.03 - 1.29)	9.43E-02
LPC(20:3)	1,350 (706)	5	8	0.98 (0.89 - 1.09)	8.96E-01	0.95 (0.83 - 1.10)	7.71E-01
LPC(20:4)	3,000 (1,599)	5	7	0.95 (0.85 - 1.05)	6.10E-01	0.96 (0.83 - 1.11)	8.08E-01
LPC(20:5)	549 (454)	4	5	0.96 (0.87 - 1.06)	6.62E-01	0.90 (0.78 - 1.03)	3.60E-01
LPC(22:0)	20 (10)	21	18	1.02 (0.92 - 1.13)	8.57E-01	1.08 (0.95 - 1.23)	5.23E-01
LPC(22:1)	14 (10)	10	10	1.03 (0.99 - 1.08)	4.30E-01	1.07 (1.02 - 1.12)	6.92E-02
LPC(22:5)	307 (168)	6	10	0.98 (0.90 - 1.07)	8.47E-01	0.95 (0.84 - 1.08)	6.78E-01
LPC(22:6)	865 (479)	4	8	0.95 (0.86 - 1.04)	5.57E-01	0.93 (0.82 - 1.06)	5.59E-01
LPC(24:0)	39 (17)	20	21	0.97 (0.88 - 1.08)	8.00E-01	1.08 (0.94 - 1.23)	5.59E-01
LPC(26:0)	10 (05)	28	29	1.00 (0.91 - 1.11)	9.79E-01	1.09 (0.95 - 1.24)	4.84E-01
LPC(O-16:0)	410 (472)	9	9	1.10 (1.02 - 1.19)	1.03E-01	1.10 (0.99 - 1.22)	2.29E-01
LPC(O-18:0)	141 (181)	7	8	1.14 (1.05 - 1.23)	**2.96E-02**	1.16 (1.04 - 1.28)	**4.77E-02**
LPC(O-18:1)	281 (357)	9	10	1.15 (1.05 - 1.25)	**3.00E-02**	1.15 (1.02 - 1.29)	1.02E-01
LPC(O-20:0)	2,280 (215)	10	8	1.01 (0.92 - 1.12)	9.19E-01	1.13 (0.99 - 1.28)	2.23E-01
LPC(O-20:1)	17 (10)	11	11	1.12 (1.02 - 1.23)	1.23E-01	1.14 (1.00 - 1.29)	1.78E-01
LPC(O-22:0)	67 (39)	11	10	1.13 (1.04 - 1.23)	**3.55E-02**	1.17 (1.06 - 1.29)	**2.92E-02**
LPC(O-22:1)	26 (29)	7	8	1.12 (1.04 - 1.20)	**3.13E-02**	1.13 (1.03 - 1.23)	9.43E-02
LPC(O-24:0)	52 (21)	14	14	1.17 (1.06 - 1.30)	**3.18E-02**	1.27 (1.11 - 1.45)	**8.37E-03**
LPC(O-24:1)	62 (45)	6	6	1.13 (1.05 - 1.22)	**2.37E-02**	1.14 (1.03 - 1.25)	7.10E-02
LPC(O-24:2)	16 (16)	8	10	1.13 (1.05 - 1.22)	**2.37E-02**	1.16 (1.05 - 1.27)	**2.83E-02**
PE(32:0)	46 (24)	22	22	1.02 (0.92 - 1.13)	8.17E-01	1.09 (0.96 - 1.25)	4.41E-01
PE(32:1)	106 (121)	16	19	1.02 (0.94 - 1.11)	7.79E-01	1.01 (0.91 - 1.13)	8.85E-01
PE(34:1)	1,197 (990)	8	14	1.04 (0.95 - 1.14)	6.50E-01	1.07 (0.95 - 1.21)	5.59E-01
PE(34:2)	2,102 (1,719)	10	17	1.07 (0.97 - 1.17)	4.77E-01	1.10 (0.97 - 1.24)	3.83E-01
PE(34:3)	133 (132)	16	22	1.03 (0.94 - 1.14)	7.67E-01	1.03 (0.90 - 1.17)	8.16E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
PE(35:1)	71 (50)	15	20	1.00 (0.91 - 1.10)	9.79E-01	0.98 (0.86 - 1.12)	8.98E-01
PE(35:2)	119 (91)	17	21	1.05 (0.95 - 1.17)	6.24E-01	1.04 (0.91 - 1.20)	7.84E-01
PE(36:0)	26 (09)	24	22	1.02 (0.91 - 1.14)	8.79E-01	0.98 (0.85 - 1.14)	9.01E-01
PE(36:1)	906 (716)	12	11	1.03 (0.95 - 1.12)	7.41E-01	1.06 (0.95 - 1.18)	5.59E-01
PE(36:2)	4,605 (3,614)	11	15	1.05 (0.96 - 1.15)	5.77E-01	1.09 (0.96 - 1.23)	4.36E-01
PE(36:3)	1,732 (1,432)	17	21	1.05 (0.95 - 1.16)	6.15E-01	1.06 (0.93 - 1.21)	6.44E-01
PE(36:4)	2,094 (1,793)	15	21	0.97 (0.88 - 1.08)	7.78E-01	1.02 (0.89 - 1.17)	8.85E-01
PE(36:5)	142 (148)	21	24	0.98 (0.89 - 1.07)	7.78E-01	0.96 (0.85 - 1.09)	7.81E-01
PE(38:3)	641 (548)	11	15	0.98 (0.89 - 1.09)	8.96E-01	0.99 (0.87 - 1.14)	9.70E-01
PE(38:4)	5,704 (4,559)	9	16	0.95 (0.85 - 1.06)	6.32E-01	1.01 (0.87 - 1.16)	9.70E-01
PE(38:5)	2,556 (2,085)	14	20	0.95 (0.85 - 1.05)	6.11E-01	0.94 (0.81 - 1.09)	6.91E-01
PE(38:6)	3,694 (3,386)	13	18	0.92 (0.83 - 1.03)	4.44E-01	0.98 (0.85 - 1.13)	8.65E-01
PE(40:4)	148 (148)	9	15	0.95 (0.87 - 1.04)	5.12E-01	1.01 (0.89 - 1.13)	9.70E-01
PE(40:5)	143 (155)	13	19	0.95 (0.87 - 1.04)	5.48E-01	0.99 (0.87 - 1.11)	8.98E-01
PE(40:6)	2,174 (2,151)	12	14	0.88 (0.79 - 0.99)	1.72E-01	0.95 (0.81 - 1.10)	7.23E-01
PE(40:7)	437 (442)	19	28	0.95 (0.86 - 1.05)	6.06E-01	0.94 (0.82 - 1.08)	6.41E-01
PE(O-34:1)	109 (60)	16	19	1.05 (0.95 - 1.17)	6.15E-01	1.02 (0.89 - 1.18)	8.51E-01
PE(O-34:2)	94 (69)	14	21	1.01 (0.92 - 1.12)	9.08E-01	0.93 (0.81 - 1.06)	5.59E-01
PE(O-36:2)	73 (44)	18	20	1.07 (0.96 - 1.18)	5.02E-01	1.04 (0.91 - 1.20)	7.84E-01
PE(O-36:3)	84 (67)	18	24	1.03 (0.93 - 1.14)	7.78E-01	0.97 (0.85 - 1.12)	8.46E-01
PE(O-36:4)	462 (332)	15	21	0.97 (0.88 - 1.07)	7.78E-01	0.90 (0.78 - 1.04)	4.36E-01
PE(O-36:5)	31 (36)	15	27	0.93 (0.85 - 1.02)	4.33E-01	0.90 (0.79 - 1.03)	3.42E-01
PE(O-36:6)	35 (38)	25	28	0.88 (0.80 - 0.98)	1.23E-01	0.81 (0.70 - 0.94)	5.36E-02
PE(O-38:4)	360 (239)	12	18	0.99 (0.89 - 1.11)	9.74E-01	0.96 (0.83 - 1.12)	8.16E-01
PE(O-38:5)	381 (273)	14	22	0.96 (0.86 - 1.06)	6.62E-01	0.88 (0.76 - 1.03)	3.27E-01
PE(O-40:5)	133 (92)	18	18	0.97 (0.86 - 1.09)	7.78E-01	0.97 (0.83 - 1.13)	8.16E-01
PE(O-40:6)	247 (137)	16	24	0.89 (0.80 - 0.99)	1.75E-01	0.86 (0.74 - 1.00)	2.13E-01
PE(O-40:7)	148 (118)	21	24	0.92 (0.81 - 1.03)	4.32E-01	0.90 (0.76 - 1.05)	4.51E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
PE(P-34:1)	68 (38)	17	20	1.01 (0.91 - 1.13)	9.32E-01	1.03 (0.89 - 1.20)	8.34E-01
PE(P-34:2)	171 (110)	19	23	1.01 (0.91 - 1.12)	9.23E-01	0.96 (0.83 - 1.12)	8.08E-01
PE(P-36:1)	54 (31)	16	17	1.09 (0.98 - 1.21)	4.01E-01	1.08 (0.93 - 1.24)	5.81E-01
PE(P-36:2)	211 (131)	11	18	1.07 (0.96 - 1.20)	5.01E-01	1.04 (0.90 - 1.20)	8.08E-01
PE(P-36:4)	397 (287)	17	25	0.91 (0.81 - 1.01)	3.07E-01	0.89 (0.77 - 1.04)	3.76E-01
PE(P-38:4)	470 (328)	12	19	0.91 (0.81 - 1.02)	3.40E-01	0.87 (0.74 - 1.02)	2.69E-01
PE(P-38:5)	741 (515)	18	21	0.89 (0.79 - 1.00)	2.02E-01	0.85 (0.72 - 1.01)	2.17E-01
PE(P-38:6)	372 (278)	16	22	0.86 (0.77 - 0.98)	1.23E-01	0.85 (0.72 - 1.01)	2.17E-01
PE(P-40:4)	47 (33)	16	19	0.97 (0.88 - 1.08)	8.02E-01	1.04 (0.91 - 1.19)	8.07E-01
PE(P-40:5)	218 (145)	12	18	0.93 (0.82 - 1.05)	5.02E-01	0.91 (0.77 - 1.08)	5.59E-01
PE(P-40:6)	201 (146)	14	20	0.88 (0.79 - 0.99)	1.75E-01	0.86 (0.73 - 1.01)	2.23E-01
LPE(16:0)	1,769 (1,182)	9	7	1.08 (0.98 - 1.18)	3.61E-01	1.15 (1.02 - 1.30)	1.07E-01
LPE(18:0)	2,313 (1,596)	16	13	1.04 (0.95 - 1.13)	6.62E-01	1.10 (0.99 - 1.23)	2.29E-01
LPE(18:1)	1,240 (669)	5	5	1.11 (1.01 - 1.21)	1.47E-01	1.12 (0.99 - 1.27)	2.17E-01
LPE(18:2)	1,736 (965)	4	5	1.12 (1.02 - 1.23)	1.27E-01	1.13 (0.99 - 1.28)	2.17E-01
LPE(20:4)	1,044 (462)	5	6	1.04 (0.94 - 1.16)	7.03E-01	1.06 (0.92 - 1.21)	6.78E-01
LPE(22:6)	864 (411)	5	5	0.96 (0.86 - 1.06)	6.99E-01	0.97 (0.85 - 1.11)	8.16E-01
PI(32:0)	86 (91)	10	10	1.00 (0.91 - 1.09)	9.79E-01	1.04 (0.92 - 1.17)	7.91E-01
PI(32:1)	212 (262)	8	10	1.05 (0.96 - 1.14)	5.77E-01	1.04 (0.93 - 1.17)	7.66E-01
PI(34:0)	29 (13)	26	31	0.96 (0.86 - 1.07)	7.10E-01	0.97 (0.83 - 1.12)	8.16E-01
PI(34:1)	1,344 (958)	8	9	1.07 (0.97 - 1.17)	5.01E-01	1.07 (0.94 - 1.23)	5.59E-01
PI(36:1)	1,196 (700)	11	11	1.12 (1.02 - 1.24)	1.35E-01	1.08 (0.94 - 1.24)	5.59E-01
PI(36:2)	3,405 (1,748)	7	9	1.07 (0.96 - 1.19)	5.02E-01	1.08 (0.93 - 1.25)	5.75E-01
PI(36:3)	724 (426)	7	12	1.06 (0.95 - 1.18)	6.13E-01	1.01 (0.87 - 1.17)	9.70E-01
PI(36:4)	1,058 (694)	6	13	0.97 (0.86 - 1.08)	7.75E-01	0.96 (0.83 - 1.13)	8.16E-01
PI(38:2)	178 (94)	11	14	1.06 (0.95 - 1.17)	6.10E-01	1.05 (0.92 - 1.21)	7.03E-01
PI(38:3)	1,940 (1,066)	7	13	0.96 (0.85 - 1.07)	7.03E-01	0.91 (0.78 - 1.07)	5.54E-01
PI(38:4)	9,501 (5,933)	7	10	0.92 (0.82 - 1.04)	5.01E-01	0.94 (0.80 - 1.11)	7.13E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
PI(38:5)	689 (447)	8	13	1.00 (0.91 - 1.11)	9.79E-01	0.92 (0.80 - 1.07)	5.59E-01
PI(38:6)	204 (162)	8	10	0.96 (0.87 - 1.06)	6.60E-01	0.99 (0.87 - 1.13)	9.21E-01
PI(40:4)	168 (100)	14	14	0.97 (0.87 - 1.07)	7.41E-01	1.00 (0.87 - 1.15)	9.92E-01
PI(40:5)	523 (362)	7	10	0.93 (0.83 - 1.03)	4.45E-01	0.90 (0.78 - 1.05)	4.40E-01
PI(40:6)	499 (385)	8	12	0.93 (0.84 - 1.03)	4.45E-01	0.93 (0.81 - 1.08)	6.27E-01
LPI(18:0)	163 (315)	17	18	1.05 (0.96 - 1.14)	5.70E-01	1.08 (0.96 - 1.21)	4.68E-01
LPI(18:1)	72 (50)	9	13	1.12 (1.03 - 1.21)	7.50E-02	1.15 (1.03 - 1.29)	9.38E-02
LPI(18:2)	91 (49)	8	8	1.07 (0.97 - 1.17)	4.77E-01	1.13 (1.00 - 1.28)	1.93E-01
LPI(20:4)	177 (81)	7	8	0.99 (0.89 - 1.10)	9.32E-01	1.03 (0.90 - 1.18)	8.28E-01
PG(34:1)	71 (49)	15	21	1.00 (0.95 - 1.05)	9.74E-01	1.01 (0.95 - 1.07)	8.51E-01
PG(36:1)	75 (59)	24	26	1.00 (0.91 - 1.10)	9.74E-01	1.06 (0.94 - 1.20)	6.13E-01
PG(36:2)	72 (52)	22	25	0.96 (0.87 - 1.06)	6.99E-01	0.96 (0.84 - 1.10)	7.76E-01
Cholesterol	1,801,861 (629,374)	15	13	1.07 (0.96 - 1.19)	5.02E-01	1.15 (1.00 - 1.32)	1.89E-01
CE(14:0)	13,383 (10,335)	11	13	1.04 (0.94 - 1.15)	7.03E-01	1.06 (0.92 - 1.22)	6.89E-01
CE(15:0)	5,468 (3,599)	6	16	1.08 (0.97 - 1.19)	4.45E-01	1.06 (0.92 - 1.22)	6.92E-01
CE(16:0)	235,482 (80,474)	11	13	1.18 (1.06 - 1.31)	**3.46E-02**	1.21 (1.05 - 1.41)	8.13E-02
CE(16:1)	192,417 (177,310)	11	14	1.02 (0.94 - 1.11)	7.87E-01	1.03 (0.92 - 1.16)	8.16E-01
CE(16:2)	12,689 (9,756)	13	17	1.04 (0.95 - 1.13)	6.99E-01	1.03 (0.91 - 1.17)	8.08E-01
CE(17:0)	6,820 (3,957)	11	16	1.07 (0.97 - 1.18)	5.01E-01	1.06 (0.92 - 1.21)	6.78E-01
CE(17:1)	1,240,574 (521,637)	11	16	1.12 (1.01 - 1.24)	1.82E-01	1.18 (1.02 - 1.35)	1.17E-01
CE(18:0)	27,627 (13,115)	11	12	1.14 (1.02 - 1.26)	1.27E-01	1.18 (1.02 - 1.36)	1.24E-01
CE(18:1)	1,240,574 (521,637)	12	15	1.12 (1.01 - 1.24)	1.82E-01	1.18 (1.02 - 1.35)	1.17E-01
CE(18:2)	8,970,151 (4,184,389)	13	14	1.08 (0.98 - 1.20)	3.85E-01	1.15 (1.01 - 1.30)	1.73E-01
CE(18:3)	618,447 (518,584)	13	15	1.00 (0.91 - 1.10)	9.87E-01	1.03 (0.90 - 1.16)	8.34E-01
CE(20:1)	1,235 (755)	19	20	1.09 (1.00 - 1.19)	2.13E-01	1.18 (1.06 - 1.31)	**2.83E-02**
CE(20:2)	5,748 (2,731)	15	18	1.10 (0.99 - 1.22)	2.80E-01	1.19 (1.04 - 1.37)	9.43E-02
CE(20:3)	289,098 (179,301)	11	13	1.03 (0.92 - 1.15)	7.75E-01	1.03 (0.89 - 1.20)	8.30E-01
CE(20:4)	2,961,993 (2,209,480)	11	14	0.97 (0.87 - 1.08)	7.75E-01	1.03 (0.90 - 1.18)	8.30E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
CE(20:5)	1,447,735 (1,541,089)	13	15	0.92 (0.83 - 1.01)	3.00E-01	0.91 (0.80 - 1.05)	4.61E-01
CE(22:0)	377 (315)	37	37	1.05 (0.97 - 1.13)	5.02E-01	1.11 (1.02 - 1.21)	9.43E-02
CE(22:1)	966 (807)	30	31	1.01 (0.96 - 1.08)	8.12E-01	1.07 (1.00 - 1.15)	2.06E-01
CE(22:4)	7,088 (4,491)	14	15	1.06 (0.95 - 1.17)	6.11E-01	1.14 (0.99 - 1.31)	2.17E-01
CE(22:5)	46,666 (37,635)	15	15	0.99 (0.89 - 1.11)	9.74E-01	1.02 (0.88 - 1.18)	8.78E-01
CE(22:6)	751,845 (714,719)	12	16	0.89 (0.80 - 1.00)	2.04E-01	0.94 (0.81 - 1.09)	6.73E-01
CE(24:0)	574 (422)	20	24	1.02 (0.96 - 1.09)	7.41E-01	1.07 (1.00 - 1.13)	1.69E-01
CE(24:1)	774 (562)	24	27	1.06 (0.98 - 1.14)	4.19E-01	1.15 (1.06 - 1.25)	**1.83E-02**
CE(24:4)	578 (419)	18	23	1.08 (0.97 - 1.19)	4.43E-01	1.17 (1.03 - 1.32)	1.00E-01
CE(24:5)	634 (538)	21	21	1.03 (0.94 - 1.13)	7.67E-01	1.06 (0.94 - 1.20)	6.00E-01
CE(24:6)	1,241 (1,290)	13	22	0.94 (0.86 - 1.02)	4.01E-01	0.94 (0.83 - 1.06)	5.59E-01
DG(14:0_16:0)	690 (352)	14	18	1.03 (0.97 - 1.09)	6.15E-01	1.07 (0.99 - 1.14)	2.51E-01
DG(14:0_18:1)	1,078 (856)	10	13	1.00 (0.92 - 1.09)	9.79E-01	1.00 (0.89 - 1.12)	9.92E-01
DG(14:0_18:2)	393 (323)	13	17	0.97 (0.88 - 1.06)	7.48E-01	0.97 (0.85 - 1.10)	8.08E-01
DG(16:0_16:0)	14,099 (6,247)	13	14	1.04 (0.98 - 1.09)	4.77E-01	1.07 (1.00 - 1.15)	1.78E-01
DG(16:0_18:0)	14,826 (3,044)	14	16	1.04 (0.98 - 1.10)	5.02E-01	1.05 (0.98 - 1.13)	4.51E-01
DG(16:0_18:1)	12,144 (8,625)	7	12	1.02 (0.94 - 1.11)	8.17E-01	1.05 (0.95 - 1.17)	6.29E-01
DG(16:0_18:2)	5,036 (3,440)	9	17	0.99 (0.91 - 1.08)	9.48E-01	1.01 (0.91 - 1.13)	8.98E-01
DG(16:0_20:3)	246 (206)	16	19	0.93 (0.85 - 1.02)	4.01E-01	0.91 (0.80 - 1.05)	4.51E-01
DG(16:0_20:4)	442 (412)	15	18	0.94 (0.86 - 1.02)	4.44E-01	0.96 (0.85 - 1.07)	6.92E-01
DG(16:0_22:5)	213 (179)	27	28	0.97 (0.89 - 1.07)	7.71E-01	0.98 (0.86 - 1.11)	8.65E-01
DG(16:0_22:6)	244 (291)	20	29	0.92 (0.85 - 1.00)	2.37E-01	0.95 (0.86 - 1.06)	6.71E-01
DG(16:1_18:0)	192 (163)	19	18	0.99 (0.92 - 1.08)	9.56E-01	0.96 (0.86 - 1.08)	7.81E-01
DG(16:1_18:1)	2,912 (2,265)	11	14	1.01 (0.92 - 1.10)	9.54E-01	0.98 (0.86 - 1.11)	8.51E-01
DG(18:0_18:0)	13,594 (2,801)	15	16	0.93 (0.84 - 1.03)	4.52E-01	0.93 (0.81 - 1.07)	5.59E-01
DG(18:0_18:1)	1,947 (1,365)	11	10	1.03 (0.96 - 1.11)	6.62E-01	1.04 (0.95 - 1.15)	6.31E-01
DG(18:0_18:2)	1,104 (768)	8	13	1.01 (0.93 - 1.10)	8.96E-01	1.02 (0.93 - 1.13)	8.16E-01
DG(18:0_20:4)	251 (172)	14	22	0.90 (0.82 - 0.99)	1.72E-01	0.97 (0.86 - 1.09)	7.91E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
DG(18:1_18:1)	22,129 (14,954)	13	12	1.02 (0.94 - 1.11)	7.75E-01	1.04 (0.94 - 1.15)	6.78E-01
DG(18:1_18:2)	8,421 (5,893)	14	13	0.99 (0.90 - 1.08)	9.23E-01	1.00 (0.89 - 1.13)	9.79E-01
DG(18:1_18:3)	1,202 (879)	14	14	0.97 (0.88 - 1.06)	7.10E-01	0.97 (0.86 - 1.10)	8.08E-01
DG(18:1_20:0)	326 (90)	18	14	1.00 (0.92 - 1.09)	9.87E-01	0.99 (0.88 - 1.12)	9.70E-01
DG(18:1_20:3)	518 (430)	17	16	0.92 (0.83 - 1.01)	2.81E-01	0.89 (0.78 - 1.02)	2.85E-01
DG(18:1_20:4)	1,211 (1,108)	17	19	0.92 (0.84 - 1.02)	3.61E-01	0.93 (0.82 - 1.06)	5.59E-01
DG(18:2_18:2)	2,735 (2,386)	14	14	0.97 (0.90 - 1.05)	7.27E-01	0.97 (0.87 - 1.09)	8.16E-01
TG(48:0)	3,446 (3,597)	14	18	0.99 (0.90 - 1.09)	9.56E-01	0.99 (0.87 - 1.13)	9.21E-01
TG(48:1)	10,920 (11,170)	11	15	0.95 (0.86 - 1.06)	6.62E-01	0.94 (0.81 - 1.10)	6.92E-01
TG(48:2)	8,179 (7,465)	13	16	0.96 (0.88 - 1.06)	7.03E-01	0.95 (0.83 - 1.09)	6.92E-01
TG(48:3)	2,642 (2,188)	13	18	0.97 (0.89 - 1.05)	7.03E-01	0.95 (0.84 - 1.08)	7.04E-01
TG(49:1)	1,488 (1,357)	14	17	0.95 (0.85 - 1.07)	6.62E-01	0.89 (0.76 - 1.05)	4.36E-01
TG(50:0)	5,319 (4,246)	12	19	0.99 (0.90 - 1.09)	9.23E-01	0.97 (0.85 - 1.11)	8.39E-01
TG(50:1)	31,250 (21,300)	11	17	0.99 (0.89 - 1.11)	9.43E-01	1.00 (0.85 - 1.16)	9.79E-01
TG(50:2)	39,942 (25,057)	14	17	0.96 (0.86 - 1.07)	7.10E-01	0.93 (0.80 - 1.09)	6.44E-01
TG(50:3)	19,468 (13,378)	14	17	0.93 (0.84 - 1.04)	5.02E-01	0.89 (0.76 - 1.03)	3.58E-01
TG(50:4)	4,461 (3,836)	12	17	0.93 (0.85 - 1.03)	4.77E-01	0.88 (0.76 - 1.02)	2.78E-01
TG(51:1)	2,761 (2,188)	13	16	0.96 (0.86 - 1.07)	7.10E-01	0.92 (0.78 - 1.08)	5.73E-01
TG(51:2)	4,720 (2,993)	12	18	0.94 (0.84 - 1.06)	6.15E-01	0.89 (0.76 - 1.04)	4.04E-01
TG(52:1)	15,053 (12,157)	12	17	0.97 (0.88 - 1.07)	7.71E-01	0.96 (0.84 - 1.11)	8.08E-01
TG(52:2)	105,342 (48,171)	10	16	1.00 (0.90 - 1.12)	9.74E-01	0.99 (0.85 - 1.16)	9.74E-01
TG(52:3)	63,613 (31,901)	14	17	0.98 (0.88 - 1.10)	9.08E-01	0.98 (0.85 - 1.14)	8.78E-01
TG(52:4)	39,342 (27,173)	11	17	0.95 (0.85 - 1.06)	6.19E-01	0.93 (0.81 - 1.08)	6.29E-01
TG(53:2)	2,897 (1,752)	15	18	0.97 (0.87 - 1.08)	7.67E-01	0.91 (0.78 - 1.06)	5.08E-01
TG(54:0)	1,025 (295)	16	25	0.99 (0.90 - 1.09)	9.23E-01	0.96 (0.84 - 1.09)	7.81E-01
TG(54:1)	2,455 (2,048)	12	18	0.99 (0.93 - 1.05)	8.47E-01	0.99 (0.90 - 1.08)	8.69E-01
TG(54:2)	13,209 (8,120)	9	16	1.00 (0.91 - 1.09)	9.74E-01	1.00 (0.88 - 1.13)	9.92E-01
TG(54:3)	26,431 (13,187)	11	20	1.05 (0.95 - 1.15)	6.19E-01	1.04 (0.91 - 1.18)	7.84E-01

Predictor*	Concentration	Intra-assay	Inter-assay	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	pmol/mL (IQR)	%CV	%CV	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
TG(54:4)	24,081 (14,361)	15	20	0.97 (0.87 - 1.07)	7.59E-01	0.95 (0.82 - 1.10)	7.32E-01
TG(54:5)	14,431 (9,801)	15	21	0.93 (0.84 - 1.03)	4.52E-01	0.93 (0.81 - 1.07)	5.75E-01
TG(54:6)	8,090 (6,665)	13	19	0.90 (0.81 - 1.00)	1.86E-01	0.88 (0.76 - 1.02)	2.56E-01
TG(56:6)	8,128 (5,723)	15	19	0.83 (0.74 - 0.94)	**3.00E-02**	0.83 (0.70 - 0.97)	1.17E-01

* CE, cholesteryl ester; Cer(d18:0), dihydroceramide; Cer(d18:1), ceramide; COH, free cholesterol; DG, diacylglycerol; HexCer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lysophosphatidylcholine; LPC(O), lysoalkylphosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PC(P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE(O), alkylphosphatidylethanolamine; PE(P), alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SM, sphingomyelin; TG, triacylglycerol.

† Weighted Cox regression of lipid species against cardiovascular events adjusted for age, sex, BMI, SBP, HbA1c, HDL-C, eGFR, diabetes duration, CRP, history of macrovascular disease, heart failure, antihypertensive treatment, antiplatelet treatment, and exercise (moderate or vigorous).

‡ Weighted Cox regression of lipid species against cardiovascular death adjusted for age, sex, BMI, SBP, HbA1c, HDL-c, eGFR, diabetes duration, history of CRP, macrovascular disease, HF, usage of antihypertensive treatment, current antiplatelet, and exercise (moderate or vigorous).

§ HR= Hazard ratio, (95% CI) = 95% of confidence interval.

‖ p-values were corrected for multiple comparisons using the Benjamini-Hochberg method; $p<0.05$ considered statistically significant and is shown in bold.

Supplementary Table 3: Association of lipid species with cardiovascular risk factors.

Lipid species*	AGE†		BMI‡		SBP§		Gender‖	
	beta coefficient	p-value#	beta coefficient	p-value#	beta coefficient	p-value#	IQR odds ratio	p-value#
Cer(d18:1/24:1)	-0.13	3.66E-01	0.23	3.12E-02	2.94	4.85E-12	1.23	4.18E-07
HexCer(d18:1/16:0)	0.33	2.04E-02	-0.48	1.06E-05	2.7	3.54E-09	1.46	2.83E-18
HexCer(d18:1/18:0)	0.02	8.79E-01	-0.19	8.06E-02	2.59	4.40E-09	1.8	5.66E-39
HexCer(d18:1/20:0)	0.11	4.41E-01	-0.49	4.32E-06	2.93	6.72E-11	1.56	3.14E-24
HexCer(d18:1/22:0)	0.16	3.05E-01	-0.7	1.26E-10	3.38	1.10E-13	1.31	3.00E-10
HexCer(d18:1/24:0)	0.13	4.08E-01	-1.05	1.46E-22	3.5	1.04E-13	1.12	1.18E-02
HexCer(d18:1/24:1)	0.52	1.14E-04	-0.53	1.10E-06	2.22	1.32E-06	1.31	4.05E-10
Hex2Cer(d18:1/16:0)	0.48	5.89E-04	-0.41	1.75E-04	1.13	1.83E-02	1.26	2.29E-07
Hex2Cer(d18:1/18:0)	0.06	6.52E-01	0.12	3.17E-01	1.52	1.07E-03	1.49	1.48E-19
Hex2Cer(d18:1/20:0)	-0.07	6.22E-01	0.05	6.21E-01	0.68	1.40E-01	1.42	1.98E-17
Hex2Cer(d18:1/22:0)	-0.06	6.52E-01	-0.27	9.42E-03	1.04	1.83E-02	1.38	5.72E-15
Hex2Cer(d18:1/24:0)	-0.35	6.19E-03	-0.56	2.14E-08	1.34	1.82E-03	1.18	5.23E-05
Hex2Cer(d18:1/24:1)	0.28	5.11E-02	-0.49	6.00E-06	1.51	1.03E-03	1.25	2.62E-07
Hex3Cer(d18:1/20:0)	0.64	9.49E-06	-0.34	3.61E-03	0.24	6.80E-01	1.61	3.14E-24
Hex3Cer(d18:1/22:0)	0.6	8.25E-06	-0.68	2.58E-10	1.75	1.43E-04	1.82	2.55E-38
Hex3Cer(d18:1/24:0)	0.15	3.10E-01	-1.02	6.52E-22	1.4	2.73E-03	1.44	9.16E-17
Hex3Cer(d18:1/24:1)	0.65	1.11E-06	-0.68	1.54E-10	1.29	4.81E-03	1.8	2.55E-38
SM(34:1)	0.22	1.17E-01	-0.35	6.73E-04	2.91	2.51E-11	1.58	2.21E-26
PC(34:5)	-0.17	1.38E-01	-0.07	4.13E-01	0.2	6.52E-01	1.18	8.25E-07
PC(35:4)	-0.31	2.68E-02	-0.1	3.85E-01	0	9.98E-01	1.39	1.49E-14
PC(40:6)	0.08	5.96E-01	0.06	5.66E-01	-0.3	5.98E-01	1.19	2.74E-05
PC(O-32:0)	0.7	1.12E-07	-0.26	1.43E-02	2.31	2.23E-07	1.24	4.56E-07
PC(O-32:1)	0.77	3.71E-09	-0.14	2.18E-01	0.41	4.37E-01	1.28	6.71E-09
PC(O-34:1)	0.71	1.92E-07	-0.44	6.57E-05	1.72	2.28E-04	1.42	2.42E-15
PC(O-36:1)	0.56	4.97E-05	-0.12	3.17E-01	2.06	8.42E-06	1.31	1.11E-09
PC(O-36:2)	0.32	2.28E-02	-0.47	1.06E-05	1.85	5.26E-05	1.33	4.64E-11
PC(P-32:1)	0.69	4.93E-07	-0.04	6.98E-01	2.32	5.20E-07	1.21	1.00E-05

Lipid species*	AGE†		BMI‡		SBP§		Gender‖	
	beta coefficient	p-value#	beta coefficient	p-value#	beta coefficient	p-value#	IQR odds ratio	p-value#
PC(P-34:1)	0.77	7.64E-09	-0.56	2.72E-07	2.11	3.31E-06	1.48	4.26E-19
PC(P-36:5)	0.15	2.32E-01	-0.07	4.26E-01	-0.02	9.87E-01	0.89	2.05E-03
PC(P-38:6)	0.12	4.08E-01	-0.38	3.56E-04	0.32	5.79E-01	1.17	2.34E-04
LPC(20:1)	0.42	1.63E-04	-0.47	1.60E-07	0.91	1.83E-02	1.14	1.39E-04
LPC(O-18:0)	0.54	1.01E-06	-0.07	4.26E-01	0.12	7.96E-01	1.1	6.17E-03
LPC(O-18:1)	0.49	4.97E-05	-0.1	3.52E-01	0.23	6.52E-01	1.16	1.13E-04
LPC(O-22:0)	0.74	5.15E-11	-0.41	5.53E-06	0.46	2.80E-01	1.08	2.04E-02
LPC(O-22:1)	0.73	1.23E-12	-0.07	3.94E-01	-0.51	1.75E-01	1.1	2.36E-03
LPC(O-24:0)	0.86	1.80E-10	-0.84	1.95E-15	1.78	1.06E-04	1.09	4.09E-02
LPC(O-24:1)	0.78	1.12E-13	-0.08	3.94E-01	-0.46	2.35E-01	1.07	2.93E-02
LPC(O-24:2)	0.7	4.86E-12	-0.38	3.06E-06	-0.17	6.80E-01	1.11	1.55E-03
CE(16:0)	-0.63	3.24E-06	0.47	1.30E-05	2.56	2.43E-08	1.24	8.11E-07
CE(20:1)	-0.18	1.34E-01	-0.18	4.26E-02	1.78	1.41E-06	1.19	2.03E-06
CE(24:1)	-0.6	4.25E-10	0.22	4.71E-03	1.65	5.20E-07	1.04	1.78E-01
TG(56:6)	-0.88	1.80E-10	0.21	6.69E-02	-0.07	9.25E-01	0.89	6.17E-03

* CE, cholesteryl ester; Cer(d18:1), ceramide; HexCer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lysophosphatidylcholine; PC, LPC(O), lysoalkylphosphatidylcholine; phosphatidylcholine; PC(O), alkylphosphatidylcholine; SM, sphingomyelin; TG, triacylglycerol.

† Regression of lipid species against age adjusted for BMI, SBP, gender and HbA1c.

‡ Regression of lipid species against BMI adjusted for age, SBP, gender and HbA1c.

§ Regression of lipid species against SBP adjusted for age, BMI, gender and HbA1c.

‖ Regression of lipid species against gender adjusted for age, BMI, SBP and HbA1c.

P-values were corrected for multiple comparisons by the method of Benjamini Hochberg, p<1.00E-05 are highlighted in red, 1.00E-05<p<0.05 are highlighted in orange.

Supplementary Table 4: Stratification of covariates and cardiovascular outcomes by principal component analysis[*].

Stratification characteristic	All lipid species (310) [†]		
	PC1[‡]	PC2	PC3
age	**1.07E-09**	**1.48E-04**	7.19E-01
BMI	**8.29E-16**	**6.36E-07**	2.24E-01
sex	**5.97E-15**	**2.12E-12**	4.80E-01
SBP	**8.69E-06**	1.21E-01	3.07E-01
CVD events	8.42E-01	5.89E-01	8.78E-02
CVD death	6.26E-01	5.99E-01	2.41E-01

[*] Principal component analyses was performed on all plasma lipid species using z-scores of log transformed data. The significance of the separation of the population based on gender, cardiovascular outcomes or above vs. below the median values for age, BMI and SBP, within each of the principal components was calculated using Student's t-tests. P-values are reported, $p<0.05$ are shown in bold.
[†] PCA performed using all lipid species.
[‡] PC = principal component

Supplementary Table 5: Performance of models to predict cardiovascular events.

Model	Feature*	AIC†	c-Statistic	Continuous NRI	IDI	Relative IDI
Base model	Base model	3863	0.680 (0.678 - 0.682)			
Model 1	PC(O-36:1)	3848	0.690 (0.688 - 0.692)	0.186 (0.178 - 0.193)	0.010 (0.010 - 0.011)	0.160 (0.155 - 0.166)
Model 2	CE(18:0)	3850	0.688 (0.687 - 0.690)	0.174 (0.166 - 0.181)	0.010 (0.010 - 0.011)	0.161 (0.155 - 0.167)
Model 3	PE(O-36:4)	3849	0.689 (0.687 - 0.691)	0.183 (0.175 - 0.190)	0.012 (0.011 - 0.012)	0.182 (0.175 - 0.189)
Model 4	PC(28:0)	3841	0.694 (0.692 - 0.696)	0.220 (0.212 - 0.228)	0.017 (0.016 - 0.017)	0.260 (0.252 - 0.268)
Model 5	LPC(20:0)	3843	0.693 (0.691 - 0.695)	0.205 (0.198 - 0.213)	0.017 (0.016 - 0.017)	0.261 (0.252 - 0.269)
Model 6	PC(35:4)	3837	0.698 (0.696 - 0.699)	0.247 (0.239 - 0.255)	0.021 (0.021 - 0.022)	0.332 (0.322 - 0.341)
Model 7‡	**LPC(18:2)**	**3835**	**0.700 (0.698 - 0.702)**	**0.227 (0.219 - 0.235)**	**0.024 (0.023 - 0.024)**	**0.364 (0.353 - 0.374)**
Model 8	PE(32:0)	3836	0.699 (0.697 - 0.701)	0.228 (0.220 - 0.236)	0.024 (0.024 - 0.025)	0.375 (0.365 - 0.386)
Model 9	PC(34:5)	3835	0.698 (0.697 - 0.700)	0.250 (0.243 - 0.258)	0.027 (0.026 - 0.028)	0.420 (0.409 - 0.431)
Model 10	TG(54:0)	3835	0.698 (0.696 - 0.699)	0.253 (0.245 - 0.261)	0.029 (0.028 - 0.029)	0.443 (0.432 - 0.454)
Model 11	CE(24:1)	3836	0.697 (0.695 - 0.699)	0.227 (0.220 - 0.235)	0.029 (0.028 - 0.030)	0.447 (0.436 - 0.458)
Model 12	LPC(20:4)	3838	0.696 (0.694 - 0.698)	0.219 (0.212 - 0.227)	0.029 (0.028 - 0.030)	0.450 (0.438 - 0.461)
Model 13	CE(22:0)	3835	0.699 (0.697 - 0.701)	0.255 (0.247 - 0.263)	0.032 (0.032 - 0.033)	0.501 (0.489 - 0.513)
Model 14	SM(34:2)	3836	0.698 (0.696 - 0.700)	0.243 (0.235 - 0.251)	0.032 (0.032 - 0.033)	0.500 (0.488 - 0.512)
Model 15	DG(14:0_16:0)	3837	0.697 (0.695 - 0.699)	0.240 (0.232 - 0.248)	0.033 (0.032 - 0.034)	0.507 (0.495 - 0.519)
Model 16	Hex3Cer(d18:1/24:0)	3834	0.699 (0.697 - 0.701)	0.266 (0.258 - 0.274)	0.036 (0.035 - 0.037)	0.560 (0.547 - 0.573)
Model 17	DG(18:0_18:0)	3834	0.700 (0.698 - 0.701)	0.249 (0.241 - 0.257)	0.038 (0.037 - 0.039)	0.583 (0.570 - 0.596)
Model 18	PC(32:2)	3830	0.702 (0.700 - 0.704)	0.256 (0.249 - 0.264)	0.041 (0.040 - 0.042)	0.635 (0.622 - 0.649)
Model 19	SM(34:1)	3830	0.701 (0.700 - 0.703)	0.260 (0.252 - 0.267)	0.043 (0.042 - 0.044)	0.660 (0.646 - 0.674)
Model 20	DG(16:0_22:5)	3831	0.702 (0.700 - 0.704)	0.262 (0.254 - 0.269)	0.043 (0.042 - 0.044)	0.662 (0.647 - 0.676)

* Denotes the lipid species added to the features in the preceding model. CE, cholesteryl ester; DG, diacylglycerol; Hex3Cer, trihexosylceramide; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PE,phosphatidylethanolamine; PE(O), alkylphosphatidylethanolamine; SM, sphingomyelin.
† Figure represents average AIC of all models with those lipid features.
‡ Denotes the optimal model with seven lipid features.

Supplementary Table 6: Performance of models to predict cardiovascular death.

Model	Feature*	AIC†	c-Statistic	Continuous NRI	IDI	Relative IDI
Base model	Base model	1880	0.740 (0.738 - 0.742)			
Model 1	PC(O-36:1)	1867	0.748 (0.745 - 0.750)	0.227 (0.217 - 0.236)	0.015 (0.014 - 0.015)	0.187 (0.178 - 0.196)
Model 2	DG(16:0_22:5)	1869	0.746 (0.744 - 0.749)	0.229 (0.220 - 0.239)	0.015 (0.014 - 0.016)	0.192 (0.183 - 0.202)
Model 3	SM(34:1)	1867	0.749 (0.746 - 0.751)	0.249 (0.239 - 0.259)	0.017 (0.016 - 0.018)	0.214 (0.203 - 0.225)
Model 4‡	**PC(O-36:5)**	**1859**	**0.760 (0.757 - 0.762)**	**0.328 (0.317 - 0.339)**	**0.023 (0.022 - 0.024)**	**0.288 (0.274 - 0.302)**
Model 5	PI(32:0)	1860	0.757 (0.755 - 0.760)	0.326 (0.315 - 0.336)	0.023 (0.022 - 0.024)	0.294 (0.280 - 0.308)
Model 6	SM(41:2)	1854	0.760 (0.758 - 0.762)	0.376 (0.365 - 0.386)	0.031 (0.030 - 0.032)	0.399 (0.382 - 0.416)
Model 7	CE(22:4)	1856	0.758 (0.756 - 0.760)	0.360 (0.349 - 0.370)	0.031 (0.030 - 0.033)	0.404 (0.388 - 0.421)
Model 8	LPE(18:1)	1856	0.757 (0.755 - 0.760)	0.364 (0.353 - 0.374)	0.033 (0.032 - 0.034)	0.425 (0.408 - 0.441)
Model 9	LPC(14:0)	1858	0.757 (0.755 - 0.759)	0.350 (0.340 - 0.361)	0.033 (0.031 - 0.034)	0.418 (0.401 - 0.435)
Model 10	PC(O-32:1)	1857	0.757 (0.754 - 0.759)	0.390 (0.380 - 0.400)	0.035 (0.033 - 0.036)	0.443 (0.425 - 0.461)
Model 11	LPC(18:2)	1855	0.758 (0.756 - 0.761)	0.419 (0.409 - 0.429)	0.038 (0.036 - 0.040)	0.489 (0.469 - 0.509)
Model 12	PE(O-36:4)	1856	0.760 (0.757 - 0.762)	0.422 (0.411 - 0.432)	0.040 (0.038 - 0.041)	0.508 (0.488 - 0.529)
Model 13	PC(O-36:3)	1855	0.763 (0.761 - 0.765)	0.423 (0.413 - 0.433)	0.040 (0.039 - 0.042)	0.516 (0.495 - 0.537)
Model 14	LPI(20:4)	1853	0.767 (0.764 - 0.769)	0.421 (0.411 - 0.431)	0.042 (0.040 - 0.044)	0.537 (0.515 - 0.559)
Model 15	PC(38:4)	1848	0.775 (0.772 - 0.777)	0.422 (0.412 - 0.432)	0.049 (0.047 - 0.051)	0.628 (0.604 - 0.652)
Model 16	TG(54:0)	1849	0.775 (0.773 - 0.777)	0.399 (0.389 - 0.409)	0.051 (0.049 - 0.053)	0.653 (0.629 - 0.678)
Model 17	PC(34:5)	1850	0.774 (0.772 - 0.776)	0.404 (0.394 - 0.414)	0.053 (0.051 - 0.055)	0.672 (0.647 - 0.697)
Model 18	PC(35:4)	1852	0.773 (0.771 - 0.775)	0.394 (0.384 - 0.404)	0.053 (0.051 - 0.055)	0.675 (0.650 - 0.700)
Model 19	PC(34:2)	1848	0.778 (0.775 - 0.780)	0.429 (0.419 - 0.440)	0.058 (0.056 - 0.060)	0.747 (0.721 - 0.774)
Model 20	HexCer(d18:1/22:0)	1847	0.778 (0.776 - 0.780)	0.441 (0.430 - 0.451)	0.059 (0.057 - 0.061)	0.758 (0.732 - 0.785)

* Denotes the lipid species added to the features in the preceding model. CE, cholesteryl ester; DG, diacylglycerol; Hex3Cer, trihexosylceramide; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PE, phosphatidylethanolamine; PE(O), alkylphosphatidylethanolamine; SM, sphingomyelin.
† Figure represents average AIC of all models with those lipid features.
‡ Denotes the optimal model with four lipid features.

Supplementary Table 7: Feature inclusion frequency using LASSO based feature selection*.

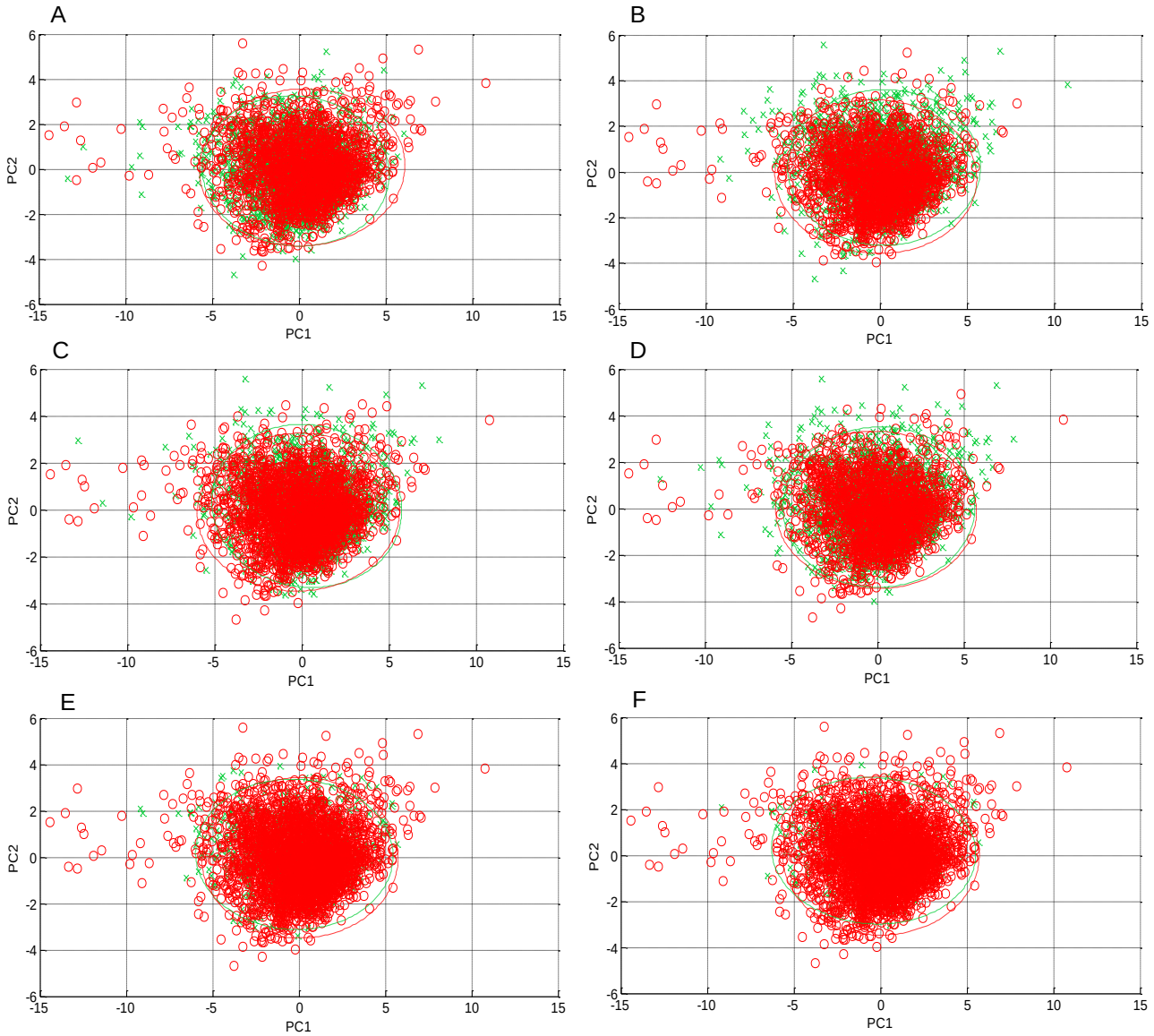
Rank No	Cardiovascular events		Cardiovascular death	
	Lipid Name	Selection Frequency (%)	Lipid Name	Selection Frequency (%)
1	PC(34:5)	89.7%	PC(36:5)	86.3%
2	PC(O-36:1)	64.9%	PC(34:5)	76.6%
3	HexCer(d18:1/18:0)	54.4%	CE(22:1)	46.8%
4	PI(36:1)	43.7%	PC(O-36:1)	33.9%
5	TG(52:4)	41.0%	CE(22:4)	32.5%
6	PC(36:5)	40.8%	HexCer(d18:1/18:0)	32.2%
7	CE(22:4)	32.2%	SM(34:1)	21.3%
8	LPC(O-22:1)	30.5%	LPE(18:1)	18.9%
9	Hex2Cer(d18:1/18:0)	29.7%	Hex2Cer(d18:1/20:0)	16.7%
10	LPC(16:1)	24.4%	HexCer(d18:1/22:0)	16.0%
11	PE(P-36:2)	23.1%	PC(O-36:5)	14.2%
12	PI(34:0)	22.7%	CE(24:1)	13.8%
13	LPE(18:1)	22.2%	Cer(d18:0/16:0)	13.4%
14	Hex3Cer(d18:1/24:0)	20.6%	Hex2Cer(d18:1/24:1)	12.2%
15	Hex2Cer(d18:1/24:1)	19.5%	PC(O-32:1)	12.1%
16	TG(48:3)	18.8%	TG(52:4)	12.0%
17	PC(38:4)	15.1%	PC(35:4)	11.9%
18	PC(35:4)	13.8%	Hex2Cer(d18:1/18:0)	11.3%
19	Hex3Cer(d18:1/22:0)	12.0%	Hex3Cer(d18:1/16:0)	10.7%
20	CE(18:0)	12.0%	Hex3Cer(d18:1/20:0)	10.6%

* LASSO based model development to predict cardiovascular events or cardiovascular death was performed within a 5-fold cross-validation framework (200 repeats). Lipid features were ranked by the frequency of their incorporation in the resulting 100 models. Lipid species also selected in the final predictive models by the AIC minimisation approach (Figure 5) are highlighted.

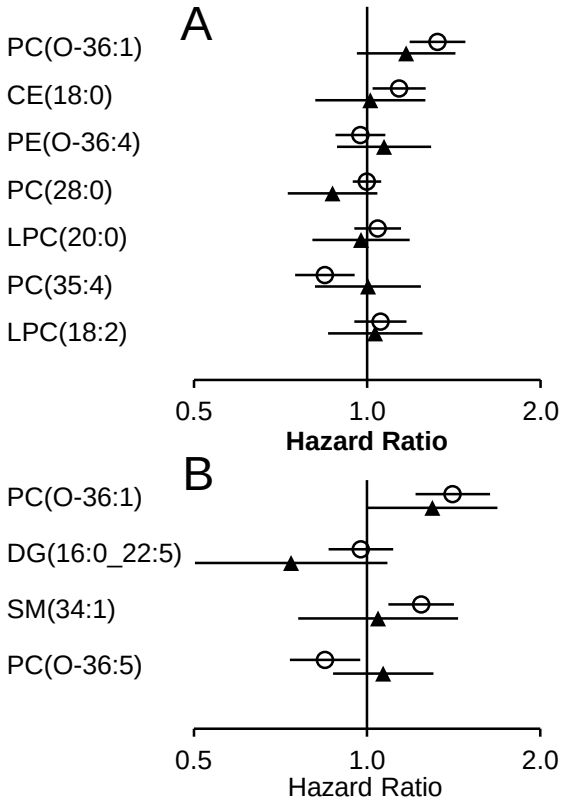
Supplementary Table 8: Model performance measures (95% CIs) for 5-year risk in the ADVANCE trial using LASSO based feature selection

Feature	c-Statistic	Continuous NRI	IDI	Relative IDI
	Prediction of cardiovascular events			
Base model*	0.674 (0.672-0.675)			
Base model +3 lipid species†	0.687 (0.685-0.689)§	0.160 (0.154-0.166)	0.047 (0.047-0.048)	0.718 (0.706-0.730)
Base model +6 lipid species†	0.690 (0.688-0.691)§	0.171 (0.164-0.177)	0.053 (0.052-0.054)	0.801 (0.789-0.814)
	Prediction of cardiovascular death			
Base model*	0.741 (0.738-0.743)			
Base model + 3 Lipid species‡	0.747(0.745-0.749)§	0.298 (0.288-0.308)	0.017 (0.016-0.018)	0.214 (0.204-0.224)
Base model + 6 Lipid species‡	0.749(0.747-0.751)§	0.294 (0.284-0.304)	0.029 (0.028-0.030)	0.369 (0.354-0.383)

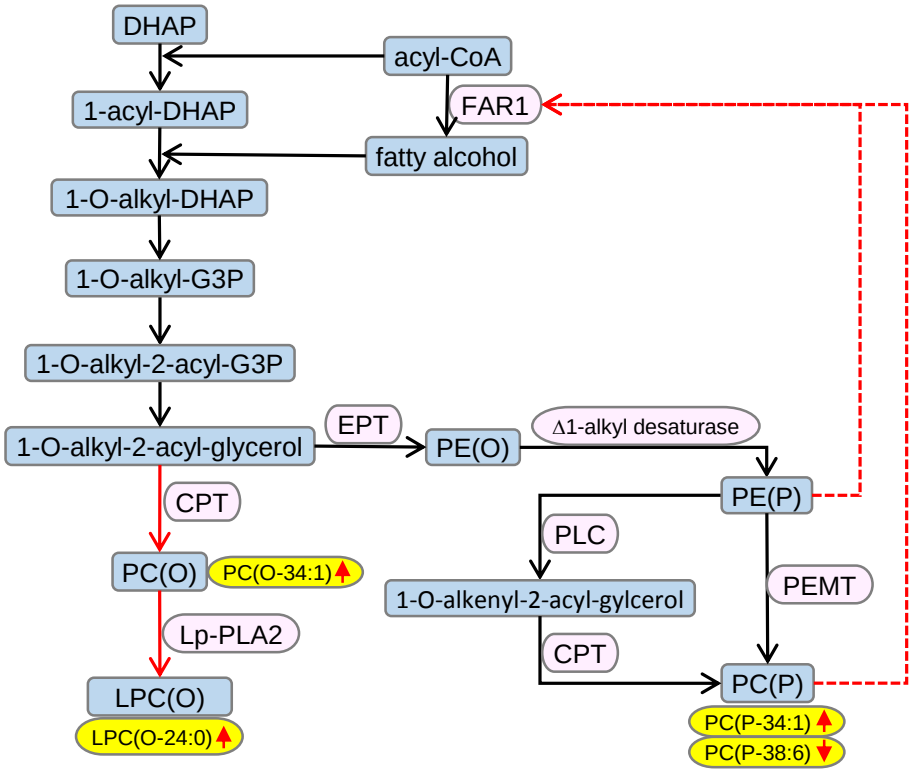
* Base model contains significant covariates in Table 1.
† Lipid ranks for the cardiovascular events model were: PC(34:5), PC(O-36:1), HexCer(d18:1/18:0), PI(36:1), TG(52:4), PC(36:5).
‡ Lipid ranks for the cardiovascular death model were: PC(36:5), PC(34:5), CE(22:1), PC(O-36:1) , CE(22:4), HexCer(d18:1/18:0).
§ p-values <0.0001 relative to base model.



Supplementary Figure 1. Principal component analysis of plasma lipid species. PCA was performed on all plasma lipid species using z-scores of log transformed data. PC1 is plotted against PC2 for all participants showing stratification above (green cross) or below (red circle) the median for age (A), BMI (B), SBP (D), as female (green cross) and male (red circle) for gender (C) and stratified as cases (green cross) or controls (red circle) for cardiovascular events (E) cardiovascular death (F).



Supplementary Figure 2. Association between lipid species and cardiovascular outcomes in the ADVANCE and LIPID cohorts. Weighted Cox regression analyses, adjusted for age, sex, BMI, SBP, HDL-C and eGFR, of lipid species incorporated into the risk models for cardiovascular events (Panel A) and cardiovascular death (Panel B) were performed on the ADVANCE case-cohort (open circles) and Cox regression analyses using the same covariates were performed on the LIPID subcohort (closed triangles). Hazard ratios and 95% confidence intervals are shown. The hazard ratio represents the change in outcome associated with a change in the lipid species equivalent to the interquartile range. CE, cholesteryl ester; DG, diacylglycerol; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PE(O), alkylphosphatidylethanolamine; SM, sphingomyelin.



Supplementary Figure 3: Metabolic pathway of ether lipid species associated with cardiovascular events and cardiovascular death. Partial lipid metabolic pathway of alkyl- and alkenylphospholipids showing lipid metabolites (blue boxes), enzymes (pink boxes) and associations seen for alky-, alkenyl- and lysoalkylphosphatidylcholine and alkylphosphatidylethanolamine species with cardiovascular events and death (yellow boxes). The dashed red arrow represents the negative feedback regulation of plasmalogen synthesis. Metabolite abbreviations: Δ1 alkyl-desaturase, plasmanylethanolamine desaturase; DHAP, dihydroxyacetone phosphate; G3P, glycerol-3-phosphate; LPC(O), lysoalkylphosphatidylcholine; PC(O), alkylphosphatidylcholine; PC(P), alkenylphosphatidylcholine; PE(O), alkylphosphatidylethanolamine; PC(P), alkenylphosphatidylethanolamine; Enzyme abbreviations: CPT, choline-phosphotransferase; EPT, ethanolamine-phosphotransferase; FAR1, fatty acyl-CoA reductase 1; Lp-PLA2, lipoprotein phospholipase A2; PEMT, phosphatidylethanolamine N-methyltransferase; PLC, phospholipase C.