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

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ABSTRACT (250 words)

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

Methods and Results- Plasma lipids (310 species) were measured on a case-cohort (n=3,779) subset from the ADVANCE trial. Weighted Cox regression was used to identify lipid species associated with future cardiovascular events and death. Multivariable models combining traditional risk factors with lipid species were developed using the Akaike information criteria. C-statistics and net reclassification indices (NRI) were calculated within a five-fold cross validation framework. Sphingolipids, phospholipids (including lyso- and ether- species), cholesteryl esters and glycerolipids were associated with future cardiovascular events and death. The addition of 7 lipids to a base model (14 traditional risk factors and medications) to predict cardiovascular events increased the c-statistic by 0.020 to 0.680 (95% CI, 0.698 - 0.702) with a corresponding categorical NRI of 5.5% (95%CI, 5.2%-5.9%) and continuous NRI of 22.7% (95%CI, 21.9%-23.5). The prediction of cardiovascular death was improved with the incorporation of 4 lipids to the base model, showing an increase in the c-statistic of 0.020 to 0.740 (95%CI, 0.738 - 0.742), a categorical NRI of 10.1% (95%CI, 9.7%-10.5%) and continuous NRI of 32.8% (95%CI, 31.7%-33.9%). The results were validated on a diabetic subcohort from the LIPID study.

Conclusion- The improvement in the prediction of cardiovascular events, above traditional risk factors, demonstrates the potential of plasma lipids as biomarkers for cardiovascular risk stratification in diabetes.

Key Words: secondary prevention, mass spectrometry, biomarker, lipidomics

Type 2 diabetes (T2D) represents a growing health burden worldwide [1]. Atherothrombotic Cardiovascular disease (CVD) is a major complication of T2D and is the leading cause of death worldwide [2-4]. The increasing incidence of T2D is placing pressure on healthcare systems. Treating and estimating the risk of CVD in those with T2D are major concerns. In order to effectively target limited health resources to those patients at highest risk, new approaches to assess risk in the T2D population are required. Different risk scores have been developed to estimate the risk of developing CVD; the Framingham risk score (FRS) [5] and the United Kingdom Prospective Diabetes Study (UKPDS) [6] are well established risk scores. However, the FRS has shown an underestimation of risk in T2D populations [7, 8], while UKPDS overestimated the risk of future cardiovascular events when applied to independent T2D cohorts [8, 9].

Traditional lipid markers (total cholesterol, low density lipoprotein cholesterol (LDL-C), triglycerides and high density lipoprotein cholesterol (HDL-C)), which are often used in risk scores, are altered in T2D 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 T2D or the related cardiovascular risk. Recent development in lipidomic technologies is providing new insight to this complex area. Plasma lipid species and classes have been found to be associated with T2D [10] and with cardiovascular disease [11]. More recently, plasma lipid species have also been associated with incident cardiovascular events [12] suggesting that these lipids may be useful biomarkers for cardiovascular risk. However, to our knowledge, there are no studies to date that have investigated the plasma lipid profile associated with cardiovascular risk in a T2D population.

We hypothesised that specific lipid species would be associated with future cardiovascular events in T2D, independent of existing risk factors. We further hypothesised that a combination of lipids and conventional risk factors will provide improved prediction of future events compared to risk factors alone. We used a high-throughput mass spectrometry platform [13] for plasma lipid profiling in a case-cohort subset from the ADVANCE (Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation) trial to identify lipid species that may predict incident

cardiovascular events defined as “major macrovascular events”, composite of “non-fatal MI, non-fatal stroke and cardiovascular death”, over a 5-year period. Our results were subsequently validated on an independent diabetic subset of the LIPID (The Long-Term Intervention with Pravastatin in Ischaemic Disease) study.

Methods

Study Populations

The ADVANCE trial was a multi-center randomised double-blinded international prospective study. The study compared the assessment of the efficacy of perindopril/indapamide (2/0.625 mg for 3 months increasing, if tolerated, to 4/1.25 mg) versus placebo and an open label evaluation of an intensive glucose lowering regimen using modified release gliclazide, with a target glycated hemoglobin (HbA1c) of 6.5% (48 mmol/mol), versus standard, guideline based glycemic control. The study was approved by the ethics committee for each participating center, and all participants provided written informed consent [14]; the Alfred Human Ethics Committee subsequently approved the current sub-study. A total of 11,140 patients were recruited with a median of 5.0 years follow-up. Samples were collected at baseline then patients underwent a six week active treatment period during which they received the fixed combination of perindopril (2mg) and indapamide (0.625mg) before randomisation. Out of 11,140 samples, 7,376 plasma samples were available from all countries involved in the ADVANCE trial except from India and China. The plasma samples were stored at -80°C for a median 8.8 years prior to analysis. The baseline data collected in the ADVANCE trial included clinical information, biochemical characteristics and demographic distribution of all participants [15].

A case-cohort study design was used (Figure 1). A sample (n=3,154) was selected at random from the 7,376 participants with available blood samples (the unenriched subcohort). This was then enriched with all those suffering cardiovascular events, renal or all-cause mortality outcomes (n=625) from the remaining 4,222 participants, to give a total 698 cardiovascular events, 355 of which were fatal (cardiovascular death, Figure 1).

The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) study (n=9,014) investigated the effect of pravastatin on death due to coronary heart disease (CHD) in patients aged from 31 to 71 years with a previous history of MI or unstable angina and baseline cholesterol levels between 4.0 to 7.0 mmol/L and fasting triglyceride <5.0 mmol/L. Patients were randomized into two groups; pravastatin (40 mg/day) or placebo, 3-36 months after an acute coronary syndrome. From the 5,991 subjects with baseline samples available, we identified 511 individuals with established T2D. T2D subjects were those who identified themselves as having diabetes or who had a fasting plasma glucose $\geq$ 7 mmol/L [16].

Lipid Extraction and Quantification

Lipids were extracted from plasma samples as described previously [17]. Briefly, Plasma (10 µL) was aliquoted into a 1.5 ml eppendorf tube and 100 µL of 1-butanol/methanol (1:1, v/v), 5 mM ammonium formate containing ISTD (Supplementary Table 1) was added. The mixture was vortexed for 10 seconds, sonicated for 60 minutes in a sonic water bath (18°C - 24°C) and then centrifuged (16,000xg, 10 min, 20°C). The supernatant was transferred into a 0.2 ml glass insert with teflon insert caps for lipidomic analysis. Lipidomic analysis of the ADVANCE and LIPID cohorts was performed by liquid chromatography electrospray ionisation tandem mass spectrometry (LC ESI-MS/MS). Details are available in the Supplementary file.

Statistical Analysis

To facilitate interpretation of the hazard ratios, the quantitative values for each lipid species were normalised to the interquartile range (IQR) for that species, prior to 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 non-event groups were used as covariates in addition to treatment allocation. Significant characteristics were age, sex, body mass index (BMI), systolic blood pressure

(SBP), HbA1c, HDL-C, estimated glomerular filtration rate (eGFR), diabetes duration, C-reactive protein (CRP), history of macrovascular disease, history of heart failure, use of antihypertensive medication, use of antiplatelet medication and exercise (Table 1). The *p*-values were corrected for multiple comparisons using the Benjamini-Hochberg method [18]. Statistical significance was determined as a corrected *p*-value of <0.05. The analyses were performed in STATA™ v10.1 (StataCorp LP, Inc., Texas, USA) using the STSELPRE procedure for case-cohort analyses.

Prior to the development of multivariable models to predict future events, a correlation minimization procedure was employed on the entire lipid dataset (log transformed values) to remove highly correlated lipids [19]. The traditional risk factors and log transformed lipid measurements were mean centered. A two stage procedure was employed to rank lipids, then build multivariable models and assess performance using the unenriched subcohort. Starting with a Cox regression base model of 14 covariates, up to 20 lipid species were added to the model in a forward selection with the aim to minimize the Akaike information criterion (AIC). This procedure was performed within a 5-fold cross-validation framework (200 repeats). Lipids were then ranked based on the average position of incorporation into these models.

Using the rank order of the 20 top lipids (from the AIC ranking) a series of models were created by the successive addition of lipids to the base covariates within a 5-fold cross validation (200 repeats). Model performance was assessed by calculating Harrell's c-statistic (using the SOMERSD command in STATA™) [20], categorical and continuous NRIs, IDI and relative IDI [21, 22]. The categorical NRI was based on 5-year risk categories of < 10%, 10-15% and > 15%. The 95% confidence intervals for each parameter were calculated.

We sought to validate our findings on a subcohort of type 2 diabetes subjects from the LIPID trial (n=511). Weighted Cox regression and Cox regression were performed to identify the association of the top ranked lipid species with future cardiovascular events and death in ADVANCE and LIPID

sub-cohorts 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, NRI (continuous and categorical), IDI and relative IDI. The covariates used in the LIPID trial analyses were age, BMI, cholesterol, HDL-C, triglycerides, current smoking, SBP, fasting glucose, atrial fibrillation, sex, stroke history, hypertension history, nature of prior acute coronary syndrome, revascularization, eGFR, dyspnea grade, angina grade, white blood cell count, peripheral vascular disease, aspirin at baseline and treatment, as have previously been used in analyses of the LIPID study [23].

Results

Baseline characteristics

Baseline characteristics, based on the outcomes status in patients in the ADVANCE study, are shown in Table 1. Those experiencing a cardiovascular event or death during the follow-up period were typically older, had a higher HbA1c and systolic blood pressure, lower HDL-C and eGFR.

Association of lipids with future cardiovascular events and death

Three out of 22 lipid classes were significantly associated with the risk of cardiovascular events (monohexosylceramide, dihexosylceramide and lysoalkylphosphatidylcholine) and two classes were associated with the risk of cardiovascular death (monohexosylceramide and dihexosylceramide), after adjustment for covariates (Figure 2). Additionally, 32 individual lipid species were significantly associated with both future cardiovascular events and death (Figure 3 and Supplementary table 2). Twenty-seven lipid species of mono- di- and trihexosylceramide, alkylphosphatidylcholine, alkenylphosphatidylcholine (containing mono unsaturated fatty acids, MUFA), lysoalkylphosphatidylcholine, and cholesteryl ester were positively associated with future cardiovascular events (Figure 3 and Supplementary table 2). While five species, containing

polyunsaturated fatty acids (PUFA), including phosphatidylcholine, alkenylphosphatidylcholine and triacylglycerol, were negatively associated with future cardiovascular events (Figure 3 and Supplementary table 2). The lipid signature associated with future cardiovascular death showed minimal differences as compared to future cardiovascular events. Thirty-one lipid species, including ceramide; mono-, di- and trihexosylceramide, sphingomyelin, alkylphosphatidylcholine, alkenylphosphatidylcholine (containing mono unsaturated fatty acids, MUFA), lysophosphatidylcholine, lysoalkylphosphatidylcholine and cholesteryl ester were positively associated with future cardiovascular death, while PC(P-36:5) was negatively associated with future cardiovascular death (Figure 3 and Supplementary table 2).

Prediction of future cardiovascular events and death

The maximum improvement of model performance (based on the c-statistic) for the prediction of future cardiovascular events was obtained by the additional of seven lipid species (PC(O-36:1) CE(18:0), PE(O-36:4), PC(28:0), LPC(20:0), PC(35:4) and LPC(18:2)) to the base model (Supplementary table 3). In contrast only four lipid species (PC(O-36:1), DG(16:0_22:5), SM(34:1) and PC(O-36:5)) were required to provide maximum improvement of the base model for cardiovascular death (Supplementary table 4). Cross-validated estimates of incremental predictive value (for future cardiovascular events) showed that the addition of 7 lipids to a model that contained the covariate risk factors improved the c-statistic by 2.0% (C-statistic =0.700, 95% CI 0.698 - 0.702), while the addition of 4 lipids to a cardiovascular death model also improved the c-statistic by 2.0% (C-statistic = 0.760, 95% CI 0.757 - 0.762). Categorical NRIs were calculated for the 5-year risk categories of < 10%, 10-15% and >15%, and improved by 5.5% (95% CI, 5.2 – 5.9) and 10.1% (95% CI, 9.7 – 10.5) for cardiovascular events and death, respectively. Continuous NRIs were 22.7% (95% CI, 21.9 – 23.5) and 32.8% (95% CI, 31.7 – 33.9) for cardiovascular events and death, respectively. IDI and relative IDI also showed corresponding improvements (Table 2, Supplementary tables 3 and 4).

Validation on a subcohort of the LIPID trial

A subcohort of diabetic subjects from 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 similar hazard ratios for most species to those found in ADVANCE (Figure 4).

The addition of the 7 lipids (identified in the ADVANCE cardiovascular event risk model) to the LIPID subcohort base model (21 covariates) resulted in a 2.3% increase in the c-statistic to 0.696, a categorical NRI of 11.5% and continuous NRI of 28.7%. Similarly, the incorporation of the 4 lipids (from the ADVANCE cardiovascular death risk model) to the base model to predict CVD death increased the c-Statistic by 4.3% to 0.789 and gave categorical and continuous NRIs of 10.4% and 49.9%, respectively. IDI values were also equal to, or better than, those observed in the ADVANCE cohort (Table3).

Discussion

Recent advances in liquid chromatography and mass spectrometry now enable lipidomic studies in a true epidemiological setting. Here we present the single largest lipidomic study representing over 1.1 million discrete lipid measurements across 3,779 participants of the ADVANCE trial. The power of this large dataset together with the detailed phenotyping and clinical outcomes has allowed us to identify associations between over 40 individual lipid species with future cardiovascular outcomes. Multivariable modelling demonstrated that a small number of these lipid species can significantly improve upon all other risk factors for the prediction of future cardiovascular events and death.

Sphingolipids associated with future cardiovascular events

We observed positive associations of both mono- and dihexosylceramide with the risk of future cardiovascular events. These glycosphingolipids are transported primarily by LDL (66%) [24] and their metabolism has previously been reported as a potential contributing factor in atherosclerosis progression [25]. Chatterjee *et al.* reported on the role of oxidised 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 NADPH oxidase to generate superoxide radicals, which in turn, mediate p44MAPK activation to enhance nuclear transcription factor (c-Fos) expression and stimulate the proliferation of smooth muscle cells, thereby contributing to atherosclerosis [26]. More recently, the inhibition of glycosphingolipid synthesis was shown to ameliorate atherosclerosis in both ApoE$^{-/-}$ mice and rabbits on a high fat and cholesterol diet via the oxLDL/ROS/c-Fos/smooth muscle cell cascade in addition to multiple effects of lipoprotein metabolism [27].

Phospholipids associated with future cardiovascular events

We observed a positive association with the lysoalkylphosphatidylcholine and future cardiovascular events. In addition, a number of alkylphosphatidylcholine species (PC(O)) and alkenylphosphatidylcholine (PC(P), plasmalogen) species primarily containing saturated and monounsaturated fatty acids were positively associated (Figure 3 and Supplementary table 2**).** While in contrast, phosphatidylcholine and alkenylphosphatidylcholine species containing polyunsaturated fatty acids showed a negative association 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 [28] and the unique biosynthetic pathway leading to their production. Both PC(O) and PC(P) species are synthesised by the same pathway, starting with dihydroxyacetonephosphate (DHAP) in the peroxisome. The resulting 1-O-alkyl-2-acyl-sn-glycerol is diverted to both the production of PC(O) and PE(O) species within the endoplasmic reticulum. However, while the PE(O) is subsequently desaturated to produce PE(P), the PC(O) is not, but can be de-acylated to form LPC(O). PC(P) results from either the sequential methylation PE(P) by phosphatidylethanolamine methyl treansferase (PEMT) or by the sequential action of phospholipase C and choline-phosphotransferase (Supplementary Figure 1). 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 [29, 30]. Thus, in situations of heightened oxidative stress, plasmalogens (particularly those with polyunsaturated fatty acids at the sn-2 position) are oxidised leading to an upregulation of the biosynthetic pathway, this flows into both the production of

plasmalogens (PC(P) and PE(P) that are in a continual state of flux as well as PC(O) and LPC(O) that are relatively stable and so 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 negative associations between alkyl- and alkenyl-phosphatidylcholine and phosphatidylethanolamine species in stable and unstable coronary artery disease, but did not observe the positive associations identified in these longitudinal studies [11]. However, PC(O-34:1) has previously been reported to be significantly higher in plaque as compared to plasma (a four-fold increase), while the corresponding diacyl species (PC(34:1) was not different [31], further highlighting the potential for alkylphosphatidylcholine species to accumulate in pathologic conditions.

In addition to being a marker of increase flux through the plasmalogen pathway LPC(O), also known as lyso-platelet activating factor (L-PAF), may have functional relevance to disease progression and risk of future CVE. LPC(O) are synthesized via the action of lipoprotein phospholipase A2 (Lp-PLA2), alternatively known as platelet activating factor acetylhydrolyase, on PC(O) (Supplementary Figure 1) and are considered the major precursor of platelet activating factor (PAF), a potent proinflammatory and prothrombotic signalling lipid in oxidized LDL [32]. Reducing circulating Lp-PLA2 levels in patients with acute coronary syndrome was associated with plaque regression [33] while increased levels are positively associated with the risk of CAD [34].

Fatty acids associated with future cardiovascular events

In addition to class specific associations we also observed lipid species, from multiple classes, containing long chain PUFA 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 -ethanolamine were negatively associated with T2D [10, 35] and so these observations may reflect the severity or control of diabetes, a

major risk factor for CVD, in this population. Previous studies have linked n-3 PUFA intake with traditional lipid measures and demonstrated that increased intake of n-3 PUFA reduced TG levels by 25-30% [36]. Triglycerides containing saturated fatty acids and MUFA have also been positively associated with CVD [12]. These findings may indicate an important atheroprotective effect of n-3 PUFA, as has been extensively reviewed [37, 38]. Thus the levels of n-3 PUFA in circulating lipids likely reflect both the effect of pathology associated with T2D and, potentially, a causal factor leading to increased risk of future CVE.

Lipid species as predictors of cardiovascular events and death

Cardiovascular risk scores developed for the general population have been shown to underestimate the risk of future CVD on the T2D population [39, 40]. Scores specifically designed for T2D perform better but also show limited performance [41]. 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. Importantly these same lipid species, on top of the base model (21 covariates), were able to improve risk prediction of cardiovascular events and death in the LIPID sub-cohort, thus providing independent validation of these lipid species.

In a lipidomic study of risk assessment in primary prevention, Stegemann *et al.* showed that the addition of six lipid species (selected based on the entire dataset) 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 myocardial infarction, ischemic stroke, and sudden cardiac death) by 3.74% and 14.9%, respectively. However, while the analyses were performed within a five-fold cross-validation framework, independent validation of the lipid species was not performed [12]. The seemingly differing performance of plasma lipids in terms of risk prediction in secondary prevention may relate to the rigorous phenotyping of the secondary prevention case-cohort used in this study such that, in addition to the risk factors used in the primary prevention study we also considered HbA1c, eGFR, CRP, diabetes duration, history of macrovascular disease, history of heart failure, use

of antihypertensive medication, use of antiplatelet medication and exercise as covariates in our base model.

That both studies were able to achieve improvements over traditional risk factors with relatively few lipid species highlights the potential of this approach to risk stratification in both primary and secondary prevention. That there was no overlap between the lipids selected in each study may reflect metabolic differences between the cohorts either related to their clinical status (diabetes vs non-diabetes) or to the different stages of disease progression (primary vs secondary prevention), but may also be a result of the lipids measured in each study and the statistical methods used to select optimal lipid species for model development.

From a clinical perspective it may be argued that a 2% increase in the c-statistic, albeit with a 5-10% categorical NRI, is a relatively modest improvement over the base model. However, overcoming the inherent limitations in lipidomic analyses, as discussed below, would suggest that translation of this approach into a clinical test incorporating only a few key lipid species, measured with higher accuracy and precision, would lead to improved performance.

Strengths and limitations of the study

This study represent the largest lipidomic study reported to date, incorporating over 300 lipid species in over 4,000 samples from two independent prospective clinical trials. The power of these studies has allowed us to define in detail the lipid associations with future cardiovascular events and develop multivariable models to predict future events. The size and power of this study highlights the potential of this approach to not only identify new biomarkers of disease risk but to also 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 over 300 lipid species from 22 different lipid classes and subclasses providing a broad, but still incomplete, coverage of the lipidome.

We recognize that there are many lipid species and classes not covered in this study that may show superior predictive performance. Further, the high variance associated with lipidomic measurements will lead to an underestimation of the strength of associations.

While the ADVANCE trial represents the largest cohort to undergo targeted lipidomic analysis to date, the case-cohort design resulted in a primarily Caucasian group and 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 the ADVANCE trial. Notwithstanding these differences the covariates provided, to a large extent, the same clinical phenotyping and we were able to demonstrate that the same lipid species were predictive above the clinical phenotype in both cohorts.

Conclusion

Plasma lipid species were independent predictors of future cardiovascular events and death. A small number of lipids were able to significantly improve risk stratification in a secondary prevention T2D case-cohort. The associations between individual lipid species and risk demonstrate the power of combining lipidomic analyses in epidemiological studies to inform on lipid metabolism in relation to chronic disease and highlight the need for mechanistic studies to characterise the role of individual lipid species on disease pathogenesis. These studies also raise the potential for new intervention strategies (lifestyle/drug) to modify lipid metabolism and attenuate disease progression, such as has recently been reported for plasmalogen modulation in a mouse model of atherosclerosis [42].

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Disclosures

PJM has licenced lipid biomarkers described in this manuscript to Zora Biosciences, Finland.

Figure legends

Figure 1. The ADVANCE case-cohort design.

Figure 2. The association of lipid classes and subclasses with future cardiovascular outcomes. Weighted Cox regression was performed to identify lipid classes and subclasses associated with future cardiovascular events (open diamond) and cardiovascular death (open circle). Hazard ratios were adjusted for age, sex, BMI, SBP, HbA1c, HDL-C, eGFR, diabetes duration, CRP, history of macrovascular disease, history of heart failure, use of antihypertensive medication, use of antiplatelet medication and exercise. Hazard ratios and 95% confidence intervals are shown.

Figure 3. The association of lipid species with future cardiovascular outcomes. Weighted Cox regression was performed to identify lipid species associated with future cardiovascular events (open diamond) and cardiovascular death (open circles). Hazard ratios were adjusted for age, sex, BMI, HbA1c, HDL-C, eGFR, diabetes duration, CRP, history of macrovascular disease, history of heart failure, use of antihypertensive medication, use of antiplatelet medication and exercise. Hazard ratios and 95% confidence intervals are shown for lipid species showing a significant association with either outcome. CE, 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; TG, triacylglycerol.

Figure 4. 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 CVE (Panel A) and CVD death (Panel B) were performed on the ADVANCE case-cohort (closed triangles) and the LIPID subcohort (closed diamonds). Hazard ratios and 95% confidence intervals are shown. CE, cholesteryl ester; LPC,

lysophosphatidylcholine; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; SM, sphingomyelin.

Table 1. Baseline characteristics of the ADVANCE case-cohort

Variable*	All (n=3779)	Cardiovascular events (n=698)	Non-cardiovascular events (n=3,081)	*p*-value†	Cardiovascular death (n=355)	Non-cardiovascular death (n=3,424)	*p*-value†
Continuous variables, median (1st, 3rd quartile)							
Age (years)	67 (62, 72)	70 (65, 74)	67 (61, 71)	**<0.001**	71 (66, 75)	67 (61, 71)	**<0.001**
Body mass index (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
HbA1c (%)	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**
Systolic blood pressure (mmHg)	146 (133, 160)	150 (135, 166)	145 (132, 160)	**<0.001**	149 (135, 165)	146 (133, 160)	**0.006**
Diastolic blood pressure (mmHg)	81 (74, 89)	82 (74, 89)	81 (74, 89)	0.951	81 (73, 89)	81 (74, 89)	0.235
eGFR (mL/min/1.73m^2)	71 (60, 85)	68 (55, 81)	72 (61, 86)	**<0.001**	67 (52, 79)	72 (61, 85)	**<0.001**
Diabetes duration (years)	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 (%)							

Variable*	All (n=3779)	Cardiovascular events (n=698)	Non-cardiovascular events (n=3,081)	*p*-value†	Cardiovascular death (n=355)	Non-cardiovascular death (n=3,424)	*p*-value†
Sex (male)	1471 (38.9%)	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**

* LDL, low density lipoprotein; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate.

† p-values were calculated using a Mann-Whitney U-test for continuous variables and a chi-square for dichotomous variables. Bold values are significant ($p < 0.05$).

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

Table 2. Model performance measures (95% CIs) for 5-year risk in the ADVANCE trial

Feature	c-Statistic	Categorical NRI *	Continuous NRI	IDI	Relative IDI
	Prediction of cardiovascular events				
Base model†	0.680 (0.678 - 0.682)				
Base model +7 lipids‡	0.700 (0.698 - 0.702)	0.055 (0.052 - 0.059)	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 Lipids§	0.760 (0.757 - 0.762)	0.101 (0.097 - 0.105)	0.328 (0.317 - 0.339)	0.023 (0.022 - 0.024)	0.288 (0.274 - 0.302)

* Net reclassification index, based on a categorical model of <10, 10–15, and >15% 5-years risk.

† Base model contains significant covariates in table 1.

‡ Lipids 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), LPC(18:2).

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

Table 3. Model performance measures for 5-year risk in the LIPID trial subcohort

Feature	c-Statistics	Categorical NRI *	Continuous NRI	IDI	Relative IDI
	Prediction of cardiovascular events				
Base model†	0.674				
Base model + 7 lipids‡	0.696	0.115	0.287	0.039	0.464
	Prediction of cardiovascular death				
Base model†	0.7456				
Base model + 4 lipids §	0.789	0.104	0.499	0.097	0.983

* NRI Based on a categorical model of <10, 10–15, and >15% 5-years risk.

† Base model based on: age, statin treatment arm, BMI, cholesterol, HDL-C, triglycerides at baseline, current smoking, SBP, fasting glucose, atrial fibrillation, sex, stroke history, history of hypertension, nature of prior acute coronary syndrome, revascularization, eGFR, dyspnea grade, angina grade, white blood cell count, peripheral vascular disease, aspirin.

‡ Lipids 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), PC(35:4), LPC(18:2).

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

Figure 1.

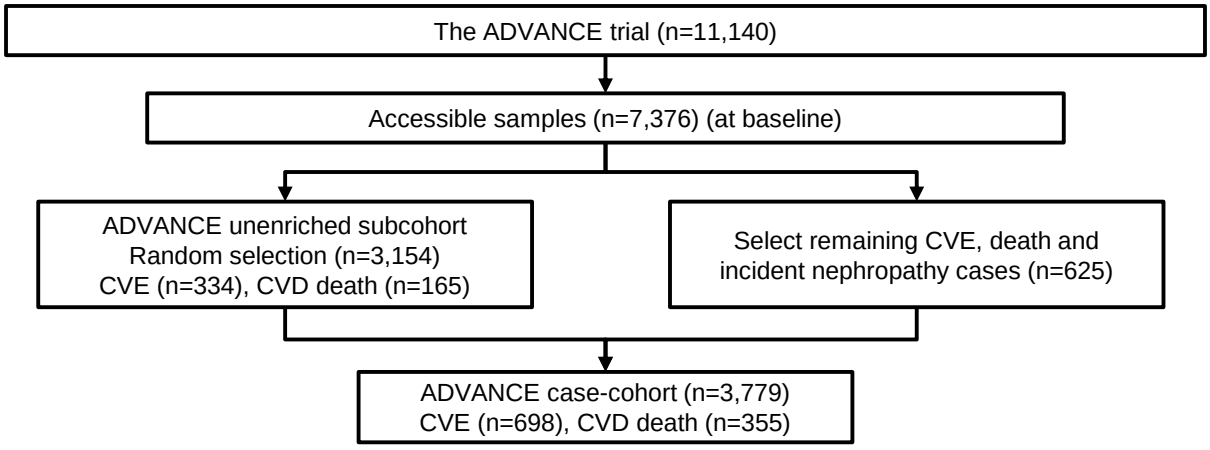

The ADVANCE trial (n=11,140)
Accessible samples (n=7,376) (at baseline)
ADVANCE unenriched subcohort
Random selection (n=3,154)
CVE (n=334), CVD death (n=165)
Select remaining CVE, death and
incident nephropathy cases (n=625)
ADVANCE case-cohort (n=3,779)
CVE (n=698), CVD death (n=355)

Figure 2.

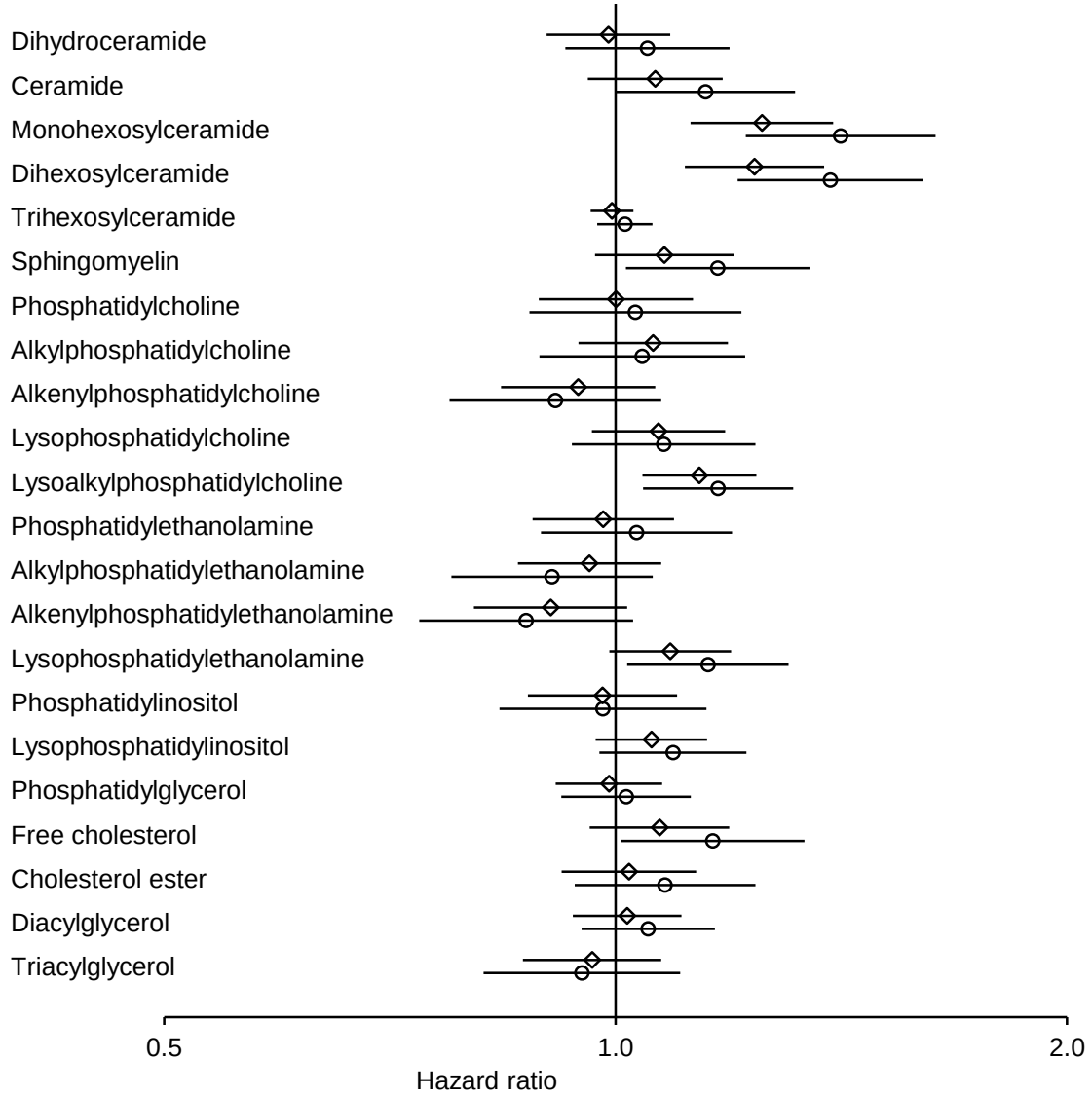

Dihydroceramide
Ceramide
Monohexosylceramide
Dihexosylceramide
Trihexosylceramide
Sphingomyelin
Phosphatidylcholine
Alkylphosphatidylcholine
Alkenylphosphatidylcholine
Lysophosphatidylcholine
Lysoalkylphosphatidylcholine
Phosphatidylethanolamine
Alkylphosphatidylethanolamine
Alkenylphosphatidylethanolamine
Lysophosphatidylethanolamine
Phosphatidylinositol
Lysophosphatidylinositol
Phosphatidylglycerol
Free cholesterol
Cholesterol ester
Diacylglycerol
Triacylglycerol
0.5
1.0
2.0
Hazard ratio

Figure 3.

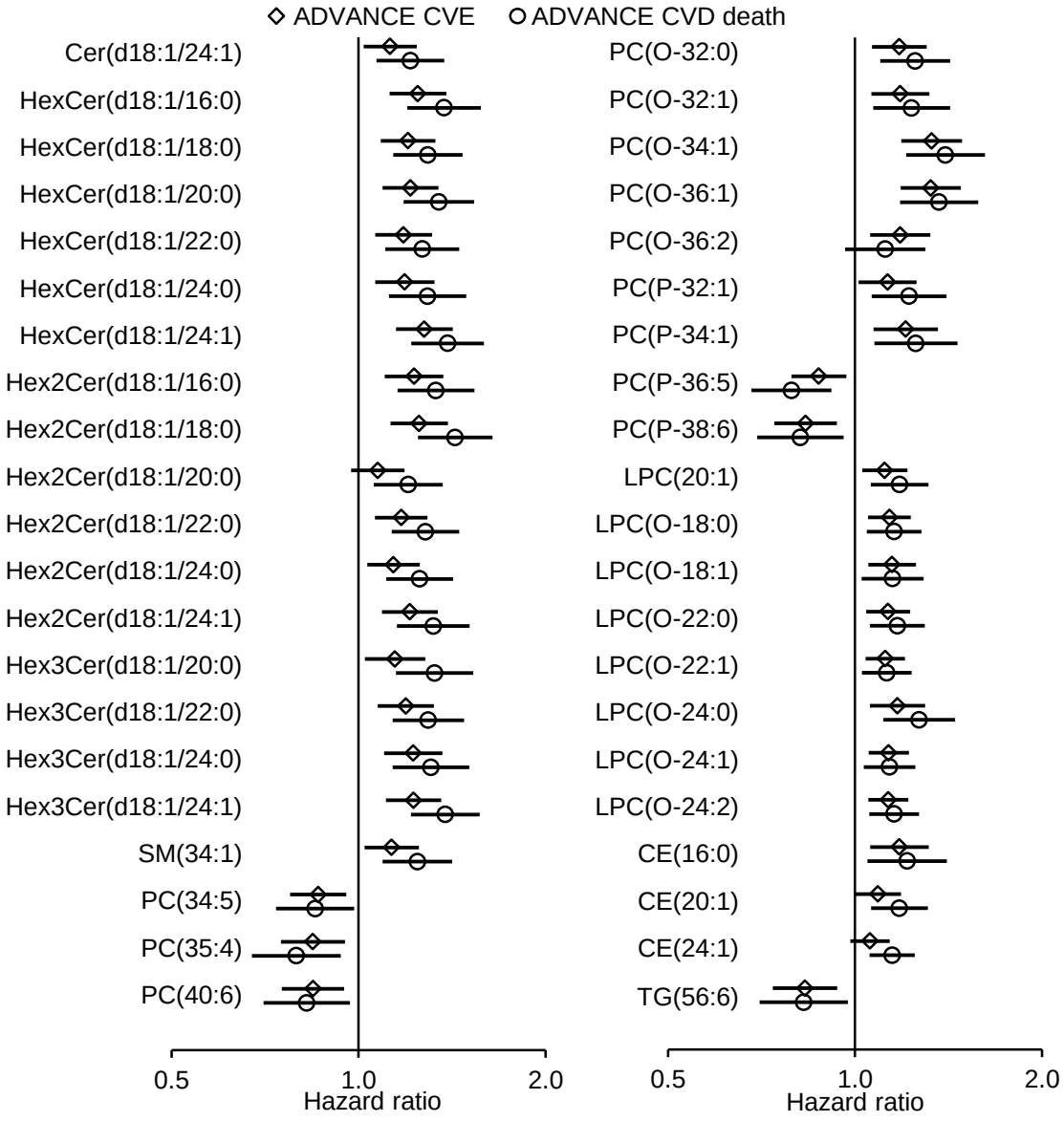
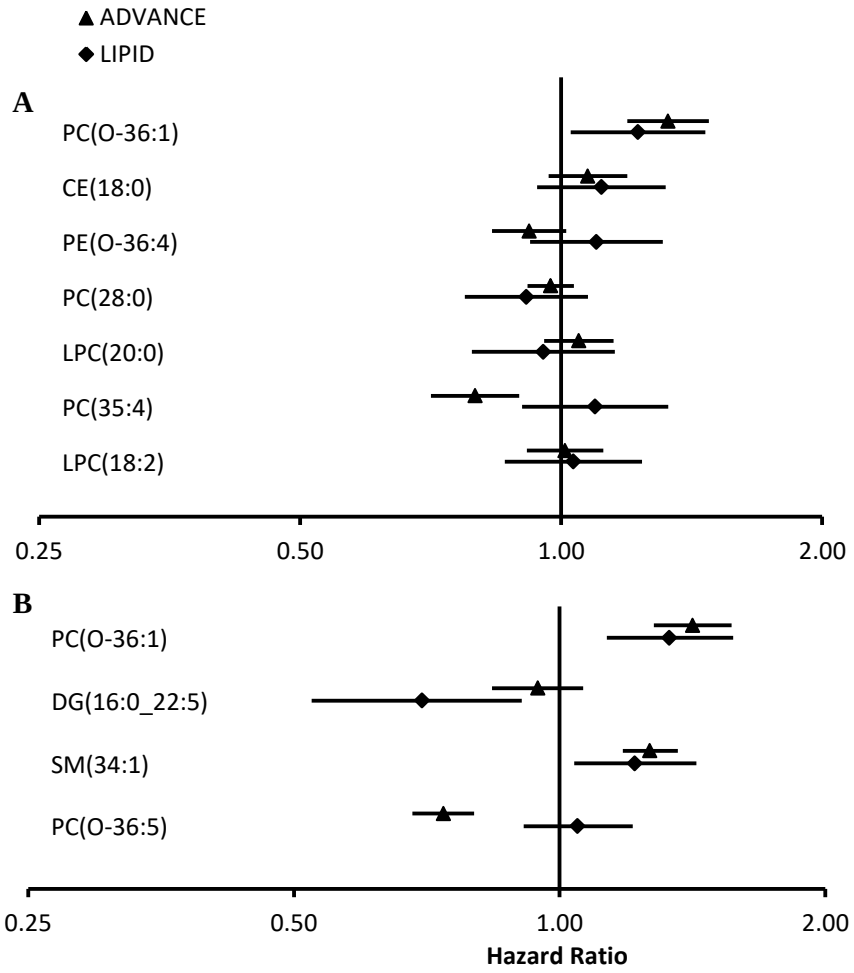

◇ ADVANCE CVE
○ ADVANCE CVD death
Cer(d18:1/24:1)
HexCer(d18:1/16:0)
HexCer(d18:1/18:0)
HexCer(d18:1/20:0)
HexCer(d18:1/22:0)
HexCer(d18:1/24:0)
HexCer(d18:1/24:1)
Hex2Cer(d18:1/16:0)
Hex2Cer(d18:1/18:0)
Hex2Cer(d18:1/20:0)
Hex2Cer(d18:1/22:0)
Hex2Cer(d18:1/24:0)
Hex2Cer(d18:1/24:1)
Hex3Cer(d18:1/20:0)
Hex3Cer(d18:1/22:0)
Hex3Cer(d18:1/24:0)
Hex3Cer(d18:1/24:1)
SM(34:1)
PC(34:5)
PC(35:4)
PC(40:6)
0.5
1.0
2.0
Hazard ratio
PC(O-32:0)
PC(O-32:1)
PC(O-34:1)
PC(O-36:1)
PC(O-36:2)
PC(P-32:1)
PC(P-34:1)
PC(P-36:5)
PC(P-38:6)
LPC(20:1)
LPC(O-18:0)
LPC(O-18:1)
LPC(O-22:0)
LPC(O-22:1)
LPC(O-24:0)
LPC(O-24:1)
LPC(O-24:2)
CE(16:0)
CE(20:1)
CE(24:1)
TG(56:6)
0.5
1.0
2.0
Hazard ratio

Figure 4.


▲ ADVANCE
◆ LIPID
A
PC(O-36:1)
CE(18:0)
PE(O-36:4)
PC(28:0)
LPC(20:0)
PC(35:4)
LPC(18:2)
0.25
0.50
1.00
2.00
B
PC(O-36:1)
DG(16:0_22:5)
SM(34:1)
PC(O-36:5)
0.25
0.50
1.00
2.00
Hazard Ratio

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Supplemental Material

Alshehry, Mundra, Barlow et al. Plasma lipidomic profiles improve upon traditional risk factors for the prediction of cardiovascular events in type 2 diabetes.

Lipid analysis

Quality control samples

Data pre-processing

Supplementary Table 1: Conditions for tandem mass spectrometry quantification of major lipid classes identified in human plasma, internal standards used and their concentration.

Supplementary Table 2: Plasma lipid species associated with future cardiovascular events and cardiovascular death adjusted for significant covariates and treatment allocation.

Supplementary Table 3: Model performance measures (95% CIs) for 5-year risk of cardiovascular events following the addition of lipid species in the ADVANCE trial.

Supplementary Table 4: Model performance measures (95% CIs) for 5-year risk of cardiovascular death following the addition of lipid species in the ADVANCE trial.

Supplementary Figure 1. Metabolic pathway of ether lipids that are altered in cardiovascular disease in a type 2 diabetes population.

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 lipids 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 in 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: 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: 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.

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

Lipid Class	Parent Ion	Fragmentation[1]	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

[1] PI, product ion; NL, neutral loss; SIM, single ion monitoring.

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

Predictors*	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
Cer(d18:0/16:0)	1.00 (0.91 - 1.09)	9.74E-01	1.06 (0.95 - 1.19)	5.74E-01
Cer(d18:0/18:0)	0.96 (0.87 - 1.06)	6.50E-01	1.02 (0.89 - 1.16)	9.07E-01
Cer(d18:0/20:0)	0.94 (0.85 - 1.04)	5.42E-01	0.99 (0.86 - 1.13)	9.43E-01
Cer(d18:0/22:0)	0.95 (0.87 - 1.05)	6.19E-01	1.00 (0.88 - 1.14)	9.96E-01
Cer(d18:0/24:0)	0.94 (0.85 - 1.04)	5.02E-01	0.98 (0.86 - 1.12)	9.07E-01
Cer(d18:0/24:1)	1.00 (0.91 - 1.10)	9.87E-01	1.06 (0.94 - 1.20)	6.08E-01
Cer(d18:1/16:0)	1.07 (0.99 - 1.16)	2.80E-01	1.10 (1.01 - 1.20)	1.32E-01
Cer(d18:1/18:0)	1.04 (0.94 - 1.15)	7.41E-01	1.10 (0.97 - 1.26)	3.92E-01
Cer(d18:1/20:0)	1.05 (0.94 - 1.16)	6.60E-01	1.13 (0.99 - 1.30)	2.32E-01
Cer(d18:1/22:0)	1.03 (0.93 - 1.15)	7.59E-01	1.10 (0.96 - 1.26)	4.24E-01
Cer(d18:1/24:0)	1.02 (0.92 - 1.14)	8.18E-01	1.09 (0.94 - 1.25)	5.38E-01
Cer(d18:1/24:1)	1.12 (1.02 - 1.24)	1.27E-01	1.22 (1.07 - 1.38)	**2.62E-02**
HexCer(d18:1/16:0)	1.25 (1.12 - 1.38)	**2.62E-03**	1.39 (1.21 - 1.60)	**1.93E-04**
HexCer(d18:1/18:0)	1.20 (1.09 - 1.33)	**1.09E-02**	1.30 (1.14 - 1.49)	**1.80E-03**
HexCer(d18:1/20:0)	1.21 (1.09 - 1.34)	**9.69E-03**	1.37 (1.20 - 1.57)	**2.20E-04**
HexCer(d18:1/22:0)	1.18 (1.06 - 1.31)	**3.00E-02**	1.30 (1.13 - 1.50)	**4.18E-03**
HexCer(d18:1/24:0)	1.19 (1.06 - 1.32)	**3.00E-02**	1.33 (1.15 - 1.54)	**2.73E-03**
HexCer(d18:1/24:1)	1.28 (1.15 - 1.42)	**5.54E-04**	1.42 (1.23 - 1.63)	**6.66E-05**
Hex2Cer(d18:1/16:0)	1.23 (1.10 - 1.37)	**9.31E-03**	1.36 (1.17 - 1.57)	**1.21E-03**
Hex2Cer(d18:1/18:0)	1.25 (1.13 - 1.39)	**2.62E-03**	1.45 (1.26 - 1.67)	**5.27E-05**
Hex2Cer(d18:1/20:0)	1.07 (0.97 - 1.19)	4.45E-01	1.21 (1.07 - 1.38)	**3.05E-02**
Hex2Cer(d18:1/22:0)	1.17 (1.06 - 1.29)	**2.88E-02**	1.30 (1.15 - 1.48)	**1.10E-03**
Hex2Cer(d18:1/24:0)	1.14 (1.03 - 1.25)	7.50E-02	1.27 (1.12 - 1.44)	**2.73E-03**
Hex2Cer(d18:1/24:1)	1.21 (1.09 - 1.34)	**1.03E-02**	1.34 (1.17 - 1.53)	**1.10E-03**
Hex3Cer(d18:1/16:0)	0.99 (0.98 - 1.01)	6.50E-01	1.00 (0.98 - 1.02)	9.96E-01
Hex3Cer(d18:1/18:0)	1.12 (1.02 - 1.25)	1.35E-01	1.19 (1.05 - 1.36)	6.81E-02
Hex3Cer(d18:1/20:0)	1.14 (1.02 - 1.28)	1.23E-01	1.31 (1.13 - 1.51)	**4.18E-03**
Hex3Cer(d18:1/22:0)	1.19 (1.07 - 1.32)	**2.37E-02**	1.31 (1.15 - 1.50)	**2.01E-03**
Hex3Cer(d18:1/24:0)	1.22 (1.10 - 1.36)	**9.41E-03**	1.33 (1.15 - 1.54)	**2.47E-03**
Hex3Cer(d18:1/24:1)	1.23 (1.11 - 1.36)	**5.06E-03**	1.39 (1.22 - 1.58)	**6.66E-05**
SM(31:1)	0.99 (0.88 - 1.11)	9.53E-01	0.91 (0.78 - 1.07)	5.46E-01
SM(32:0)	0.99 (0.89 - 1.09)	9.23E-01	1.03 (0.90 - 1.18)	8.44E-01
SM(32:1)	1.02 (0.91 - 1.15)	8.53E-01	1.04 (0.89 - 1.21)	8.44E-01
SM(32:2)	0.98 (0.86 - 1.11)	8.96E-01	0.86 (0.72 - 1.03)	3.17E-01
SM(33:1)	1.06 (0.95 - 1.18)	5.77E-01	1.08 (0.94 - 1.24)	5.49E-01
SM(34:0)	1.09 (0.98 - 1.20)	3.61E-01	1.18 (1.04 - 1.35)	8.26E-02
SM(34:1)	1.13 (1.02 - 1.25)	1.23E-01	1.24 (1.09 - 1.42)	**1.79E-02**
SM(34:2)	1.12 (0.99 - 1.26)	2.83E-01	1.14 (0.97 - 1.34)	3.19E-01
SM(34:3)	1.03 (0.92 - 1.16)	7.87E-01	0.90 (0.76 - 1.06)	4.78E-01
SM(35:1)	1.06 (0.96 - 1.18)	5.45E-01	1.10 (0.95 - 1.27)	4.78E-01
SM(35:2)	1.05 (0.93 - 1.19)	6.62E-01	0.98 (0.83 - 1.16)	9.24E-01
SM(36:1)	1.01 (0.91 - 1.13)	9.18E-01	1.09 (0.95 - 1.25)	4.86E-01

Predictors*	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
SM(36:2)	0.98 (0.87 - 1.11)	8.90E-01	0.97 (0.82 - 1.14)	8.58E-01
SM(36:3)	1.06 (0.96 - 1.18)	5.23E-01	0.98 (0.85 - 1.13)	9.17E-01
SM(38:1)	1.02 (0.92 - 1.14)	8.29E-01	1.09 (0.94 - 1.27)	5.31E-01
SM(38:2)	1.00 (0.89 - 1.12)	9.91E-01	1.07 (0.91 - 1.25)	6.53E-01
SM(39:1)	0.98 (0.88 - 1.09)	8.47E-01	0.94 (0.81 - 1.10)	6.86E-01
SM(41:1)	0.99 (0.89 - 1.10)	9.08E-01	1.01 (0.87 - 1.16)	9.84E-01
SM(41:2)	0.99 (0.88 - 1.11)	9.23E-01	0.96 (0.82 - 1.13)	8.36E-01
SM(42:1)	0.98 (0.88 - 1.08)	8.16E-01	1.06 (0.92 - 1.21)	6.81E-01
PC(28:0)	1.00 (0.94 - 1.06)	9.79E-01	1.03 (0.97 - 1.10)	5.98E-01
PC(29:0)	1.06 (0.97 - 1.17)	4.91E-01	1.13 (1.00 - 1.28)	1.87E-01
PC(30:0)	0.99 (0.89 - 1.09)	9.23E-01	1.02 (0.89 - 1.17)	8.91E-01
PC(31:0)	1.02 (0.92 - 1.14)	8.17E-01	0.99 (0.86 - 1.15)	9.69E-01
PC(31:1)	1.09 (0.97 - 1.22)	4.44E-01	1.09 (0.93 - 1.28)	5.47E-01
PC(32:0)	1.07 (0.96 - 1.18)	5.02E-01	1.18 (1.03 - 1.35)	1.04E-01
PC(32:1)	1.02 (0.91 - 1.13)	9.05E-01	1.03 (0.89 - 1.19)	8.58E-01
PC(32:2)	0.96 (0.84 - 1.09)	7.41E-01	0.95 (0.80 - 1.14)	8.28E-01
PC(32:3)	1.05 (0.94 - 1.18)	6.60E-01	0.98 (0.83 - 1.14)	8.97E-01
PC(33:0)	1.05 (0.94 - 1.17)	6.50E-01	1.00 (0.86 - 1.16)	9.88E-01
PC(33:1)	1.03 (0.92 - 1.16)	7.78E-01	0.97 (0.82 - 1.13)	8.52E-01
PC(33:2)	1.02 (0.90 - 1.15)	9.04E-01	0.99 (0.84 - 1.17)	9.69E-01
PC(33:3)	0.96 (0.85 - 1.07)	7.10E-01	0.92 (0.78 - 1.07)	5.49E-01
PC(34:0)	1.05 (0.95 - 1.16)	6.50E-01	1.14 (0.99 - 1.31)	2.32E-01
PC(34:1)	1.08 (0.97 - 1.21)	4.52E-01	1.14 (0.98 - 1.33)	2.97E-01
PC(34:2)	1.10 (0.98 - 1.23)	4.01E-01	1.17 (1.00 - 1.38)	1.87E-01
PC(34:3)	1.00 (0.89 - 1.13)	9.79E-01	0.99 (0.84 - 1.16)	9.69E-01
PC(34:4)	0.86 (0.76 - 0.98)	1.35E-01	0.81 (0.68 - 0.97)	1.04E-01
PC(34:5)	0.86 (0.78 - 0.96)	**4.77E-02**	0.84 (0.73 - 0.97)	1.04E-01
PC(35:0)	1.09 (0.98 - 1.21)	4.15E-01	1.06 (0.91 - 1.24)	6.80E-01
PC(35:1)	1.03 (0.92 - 1.16)	7.75E-01	0.97 (0.83 - 1.13)	8.58E-01
PC(35:2)	1.04 (0.92 - 1.16)	7.67E-01	0.99 (0.84 - 1.16)	9.57E-01
PC(35:3)	1.01 (0.90 - 1.14)	9.32E-01	0.96 (0.81 - 1.13)	8.37E-01
PC(35:4)	0.84 (0.75 - 0.95)	**4.97E-02**	0.78 (0.66 - 0.92)	**2.89E-02**
PC(36:0)	1.03 (0.93 - 1.15)	7.75E-01	1.10 (0.96 - 1.27)	4.43E-01
PC(36:1)	1.04 (0.94 - 1.16)	7.03E-01	1.07 (0.92 - 1.23)	6.51E-01
PC(36:2)	1.05 (0.94 - 1.19)	6.60E-01	1.08 (0.92 - 1.28)	6.00E-01
PC(36:3)	1.02 (0.90 - 1.16)	8.79E-01	1.00 (0.84 - 1.18)	9.88E-01
PC(36:4)	0.92 (0.79 - 1.07)	5.89E-01	1.02 (0.82 - 1.25)	9.53E-01
PC(36:5)	0.95 (0.86 - 1.06)	6.50E-01	0.84 (0.72 - 0.99)	1.54E-01
PC(37:4)	0.90 (0.80 - 1.01)	3.10E-01	0.82 (0.69 - 0.96)	9.88E-02
PC(37:5)	0.91 (0.82 - 1.01)	3.15E-01	0.84 (0.72 - 0.98)	1.26E-01
PC(37:6)	0.86 (0.76 - 0.96)	7.50E-02	0.85 (0.72 - 0.99)	1.60E-01
PC(38:3)	0.97 (0.86 - 1.09)	7.78E-01	0.92 (0.78 - 1.08)	5.74E-01
PC(38:4)	0.85 (0.75 - 0.95)	5.89E-02	0.83 (0.71 - 0.99)	1.50E-01
PC(38:5)	0.87 (0.77 - 0.98)	1.27E-01	0.82 (0.69 - 0.96)	1.04E-01

Predictors*	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
PC(38:6)	0.85 (0.76 - 0.96)	7.15E-02	0.83 (0.71 - 0.98)	1.19E-01
PC(38:7)	0.85 (0.75 - 0.95)	5.53E-02	0.85 (0.72 - 0.99)	1.63E-01
PC(39:5)	0.89 (0.79 - 1.00)	1.95E-01	0.80 (0.67 - 0.94)	5.39E-02
PC(39:6)	0.88 (0.78 - 0.99)	1.62E-01	0.82 (0.70 - 0.97)	1.04E-01
PC(39:7)	0.90 (0.81 - 1.00)	2.39E-01	0.91 (0.78 - 1.05)	4.48E-01
PC(40:4)	1.00 (0.90 - 1.12)	9.79E-01	1.02 (0.88 - 1.19)	8.97E-01
PC(40:5)	0.88 (0.79 - 1.00)	1.95E-01	0.82 (0.69 - 0.97)	1.19E-01
PC(40:6)	0.84 (0.75 - 0.95)	**4.25E-02**	0.82 (0.70 - 0.96)	1.03E-01
PC(40:7)	0.92 (0.82 - 1.03)	4.44E-01	0.87 (0.74 - 1.02)	2.54E-01
PC(40:8)	0.86 (0.75 - 0.98)	1.35E-01	0.82 (0.68 - 0.99)	1.60E-01
PC(O-32:0)	1.18 (1.06 - 1.30)	**2.88E-02**	1.27 (1.11 - 1.45)	**1.08E-02**
PC(O-32:1)	1.18 (1.06 - 1.32)	**3.00E-02**	1.25 (1.08 - 1.45)	**2.62E-02**
PC(O-32:2)	1.01 (0.95 - 1.07)	9.43E-01	1.03 (0.95 - 1.11)	7.53E-01
PC(O-34:1)	1.33 (1.19 - 1.49)	**1.22E-04**	1.46 (1.26 - 1.70)	**6.66E-05**
PC(O-34:2)	1.03 (0.93 - 1.14)	7.75E-01	0.96 (0.83 - 1.11)	8.36E-01
PC(O-34:3)	1.00 (0.91 - 1.11)	9.77E-01	0.96 (0.83 - 1.11)	8.33E-01
PC(O-34:4)	0.94 (0.84 - 1.04)	5.23E-01	0.85 (0.73 - 0.99)	1.60E-01
PC(O-35:4)	1.04 (0.93 - 1.17)	7.41E-01	1.01 (0.86 - 1.19)	9.69E-01
PC(O-36:0)	1.07 (0.98 - 1.16)	3.99E-01	1.15 (1.03 - 1.27)	7.97E-02
PC(O-36:1)	1.32 (1.18 - 1.48)	**1.22E-04**	1.41 (1.21 - 1.64)	**3.05E-04**
PC(O-36:2)	1.18 (1.06 - 1.32)	**3.55E-02**	1.16 (1.00 - 1.35)	1.87E-01
PC(O-36:3)	1.07 (0.96 - 1.20)	5.09E-01	1.01 (0.86 - 1.18)	9.69E-01
PC(O-36:4)	0.98 (0.88 - 1.10)	9.12E-01	0.97 (0.83 - 1.14)	8.75E-01
PC(O-36:5)	0.91 (0.83 - 1.00)	2.37E-01	0.85 (0.73 - 0.97)	1.07E-01
PC(O-38:4)	1.09 (0.97 - 1.22)	4.45E-01	1.11 (0.95 - 1.30)	4.48E-01
PC(O-38:5)	1.02 (0.91 - 1.15)	8.40E-01	0.99 (0.84 - 1.16)	9.69E-01
PC(O-40:5)	1.06 (0.95 - 1.19)	6.10E-01	1.05 (0.90 - 1.23)	7.25E-01
PC(O-40:6)	0.97 (0.87 - 1.09)	8.17E-01	0.97 (0.83 - 1.14)	8.77E-01
PC(O-40:7)	0.91 (0.81 - 1.02)	4.01E-01	0.89 (0.76 - 1.04)	4.07E-01
PC(P-30:0)	1.05 (0.95 - 1.16)	6.33E-01	1.10 (0.96 - 1.25)	4.18E-01
PC(P-32:0)	1.09 (0.98 - 1.21)	4.01E-01	1.15 (1.00 - 1.33)	1.98E-01
PC(P-32:1)	1.13 (1.01 - 1.26)	1.56E-01	1.25 (1.08 - 1.44)	**2.62E-02**
PC(P-34:1)	1.21 (1.07 - 1.36)	**3.00E-02**	1.28 (1.09 - 1.50)	**2.49E-02**
PC(P-34:2)	0.96 (0.85 - 1.09)	7.75E-01	0.94 (0.79 - 1.11)	6.81E-01
PC(P-34:3)	0.95 (0.84 - 1.07)	6.50E-01	0.88 (0.75 - 1.04)	3.46E-01
PC(P-36:2)	1.10 (0.98 - 1.23)	3.61E-01	1.03 (0.88 - 1.21)	8.52E-01
PC(P-36:4)	0.94 (0.83 - 1.05)	5.74E-01	0.90 (0.76 - 1.06)	4.78E-01
PC(P-36:5)	0.87 (0.79 - 0.97)	7.50E-02	0.79 (0.68 - 0.92)	**2.49E-02**
PC(P-38:4)	0.97 (0.86 - 1.09)	8.02E-01	0.94 (0.80 - 1.11)	7.05E-01
PC(P-38:5)	0.89 (0.79 - 1.00)	2.44E-01	0.85 (0.72 - 1.00)	1.82E-01
PC(P-38:6)	0.83 (0.74 - 0.93)	**3.00E-02**	0.81 (0.69 - 0.95)	6.81E-02
PC(P-40:5)	0.97 (0.86 - 1.09)	7.75E-01	0.91 (0.77 - 1.08)	5.49E-01
PC(P-40:6)	0.89 (0.79 - 0.99)	1.75E-01	0.84 (0.72 - 0.99)	1.53E-01
LPC(14:0)	0.99 (0.90 - 1.09)	9.23E-01	0.97 (0.85 - 1.12)	8.75E-01

Predictors*	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
LPC(15:0)	1.07 (0.97 - 1.18)	5.01E-01	1.00 (0.88 - 1.15)	9.85E-01
LPC(16:0)	1.06 (0.96 - 1.17)	5.45E-01	1.08 (0.94 - 1.24)	5.49E-01
LPC(16:1)	1.06 (0.96 - 1.18)	5.02E-01	0.99 (0.85 - 1.14)	9.38E-01
LPC(17:0)	1.06 (0.97 - 1.16)	5.02E-01	1.01 (0.89 - 1.15)	9.26E-01
LPC(17:1)	1.06 (0.96 - 1.17)	5.45E-01	0.99 (0.86 - 1.14)	9.69E-01
LPC(18:0)	1.06 (0.96 - 1.16)	5.23E-01	1.07 (0.94 - 1.22)	5.75E-01
LPC(18:1)	1.11 (1.01 - 1.22)	1.71E-01	1.09 (0.95 - 1.25)	4.78E-01
LPC(18:2)	1.05 (0.95 - 1.17)	6.15E-01	1.05 (0.91 - 1.22)	7.03E-01
LPC(18:3)	1.01 (0.91 - 1.13)	9.35E-01	0.98 (0.84 - 1.14)	9.19E-01
LPC(20:0)	1.04 (0.95 - 1.15)	6.60E-01	1.08 (0.96 - 1.22)	4.78E-01
LPC(20:1)	1.12 (1.03 - 1.21)	7.50E-02	1.18 (1.06 - 1.31)	**3.05E-02**
LPC(20:2)	1.10 (1.01 - 1.20)	1.71E-01	1.15 (1.02 - 1.28)	1.04E-01
LPC(20:3)	0.98 (0.89 - 1.09)	8.96E-01	0.95 (0.82 - 1.10)	7.25E-01
LPC(20:4)	0.95 (0.85 - 1.05)	6.10E-01	0.95 (0.82 - 1.10)	7.05E-01
LPC(20:5)	0.96 (0.87 - 1.06)	6.62E-01	0.90 (0.79 - 1.04)	4.07E-01
LPC(22:0)	1.02 (0.92 - 1.13)	8.57E-01	1.08 (0.95 - 1.23)	5.34E-01
LPC(22:1)	1.03 (0.99 - 1.08)	4.30E-01	1.06 (1.01 - 1.12)	8.54E-02
LPC(22:5)	0.98 (0.90 - 1.07)	8.47E-01	0.95 (0.83 - 1.07)	6.53E-01
LPC(22:6)	0.95 (0.86 - 1.04)	5.57E-01	0.92 (0.81 - 1.06)	5.34E-01
LPC(24:0)	0.97 (0.88 - 1.08)	8.00E-01	1.07 (0.93 - 1.22)	6.00E-01
LPC(26:0)	1.00 (0.91 - 1.11)	9.79E-01	1.08 (0.95 - 1.23)	5.34E-01
LPC(O-16:0)	1.10 (1.02 - 1.19)	1.03E-01	1.11 (1.00 - 1.23)	1.87E-01
LPC(O-18:0)	1.14 (1.05 - 1.23)	**2.96E-02**	1.16 (1.05 - 1.29)	**3.69E-02**
LPC(O-18:1)	1.15 (1.05 - 1.25)	**3.00E-02**	1.16 (1.03 - 1.31)	8.26E-02
LPC(O-20:0)	1.01 (0.92 - 1.12)	9.19E-01	1.11 (0.98 - 1.26)	3.21E-01
LPC(O-20:1)	1.12 (1.02 - 1.23)	1.23E-01	1.14 (1.01 - 1.29)	1.60E-01
LPC(O-22:0)	1.13 (1.04 - 1.23)	**3.55E-02**	1.18 (1.06 - 1.31)	**2.49E-02**
LPC(O-22:1)	1.12 (1.04 - 1.20)	**3.13E-02**	1.14 (1.04 - 1.25)	5.20E-02
LPC(O-24:0)	1.17 (1.06 - 1.30)	**3.18E-02**	1.32 (1.15 - 1.51)	**2.38E-03**
LPC(O-24:1)	1.13 (1.05 - 1.22)	**2.37E-02**	1.16 (1.05 - 1.28)	**2.62E-02**
LPC(O-24:2)	1.13 (1.05 - 1.22)	**2.37E-02**	1.17 (1.07 - 1.29)	**1.63E-02**
PE(32:0)	1.02 (0.92 - 1.13)	8.17E-01	1.09 (0.95 - 1.24)	4.84E-01
PE(32:1)	1.02 (0.94 - 1.11)	7.79E-01	1.02 (0.91 - 1.14)	8.80E-01
PE(34:1)	1.04 (0.95 - 1.14)	6.50E-01	1.08 (0.95 - 1.22)	4.93E-01
PE(34:2)	1.07 (0.97 - 1.17)	4.77E-01	1.12 (0.99 - 1.27)	2.52E-01
PE(34:3)	1.03 (0.94 - 1.14)	7.67E-01	1.04 (0.91 - 1.19)	7.76E-01
PE(35:1)	1.00 (0.91 - 1.10)	9.79E-01	0.99 (0.86 - 1.13)	9.26E-01
PE(35:2)	1.05 (0.95 - 1.17)	6.24E-01	1.06 (0.92 - 1.22)	6.53E-01
PE(36:0)	1.02 (0.91 - 1.14)	8.79E-01	1.00 (0.86 - 1.16)	9.89E-01
PE(36:1)	1.03 (0.95 - 1.12)	7.41E-01	1.07 (0.96 - 1.19)	5.34E-01
PE(36:2)	1.05 (0.96 - 1.15)	5.77E-01	1.11 (0.98 - 1.25)	3.19E-01
PE(36:3)	1.05 (0.95 - 1.16)	6.15E-01	1.08 (0.94 - 1.23)	5.49E-01
PE(36:4)	0.97 (0.88 - 1.08)	7.78E-01	1.02 (0.88 - 1.17)	9.26E-01
PE(36:5)	0.98 (0.89 - 1.07)	7.78E-01	0.96 (0.85 - 1.09)	7.76E-01

Predictors*	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
PE(38:3)	0.98 (0.89 - 1.09)	8.96E-01	1.00 (0.87 - 1.15)	9.89E-01
PE(38:4)	0.95 (0.85 - 1.06)	6.32E-01	1.00 (0.87 - 1.16)	9.88E-01
PE(38:5)	0.95 (0.85 - 1.05)	6.11E-01	0.94 (0.81 - 1.09)	6.80E-01
PE(38:6)	0.92 (0.83 - 1.03)	4.44E-01	0.97 (0.84 - 1.13)	8.77E-01
PE(40:4)	0.95 (0.87 - 1.04)	5.12E-01	1.00 (0.88 - 1.12)	9.84E-01
PE(40:5)	0.95 (0.87 - 1.04)	5.48E-01	0.98 (0.86 - 1.11)	8.75E-01
PE(40:6)	0.88 (0.79 - 0.99)	1.72E-01	0.94 (0.81 - 1.10)	6.80E-01
PE(40:7)	0.95 (0.86 - 1.05)	6.06E-01	0.94 (0.82 - 1.08)	6.17E-01
PE(O-34:1)	1.05 (0.95 - 1.17)	6.15E-01	1.03 (0.90 - 1.19)	8.44E-01
PE(O-34:2)	1.01 (0.92 - 1.12)	9.08E-01	0.95 (0.82 - 1.09)	6.80E-01
PE(O-36:2)	1.07 (0.96 - 1.18)	5.02E-01	1.07 (0.93 - 1.23)	6.17E-01
PE(O-36:3)	1.03 (0.93 - 1.14)	7.78E-01	0.99 (0.86 - 1.14)	9.69E-01
PE(O-36:4)	0.97 (0.88 - 1.07)	7.78E-01	0.91 (0.79 - 1.05)	4.78E-01
PE(O-36:5)	0.93 (0.85 - 1.02)	4.33E-01	0.90 (0.79 - 1.03)	3.32E-01
PE(O-36:6)	0.88 (0.80 - 0.98)	1.23E-01	0.81 (0.69 - 0.94)	**4.38E-02**
PE(O-38:4)	0.99 (0.89 - 1.11)	9.74E-01	0.97 (0.83 - 1.12)	8.48E-01
PE(O-38:5)	0.96 (0.86 - 1.06)	6.62E-01	0.89 (0.76 - 1.04)	3.51E-01
PE(O-40:5)	0.97 (0.86 - 1.09)	7.78E-01	0.96 (0.82 - 1.12)	8.42E-01
PE(O-40:6)	0.89 (0.80 - 0.99)	1.75E-01	0.86 (0.73 - 1.00)	1.75E-01
PE(O-40:7)	0.92 (0.81 - 1.03)	4.32E-01	0.90 (0.77 - 1.06)	4.78E-01
PE(P-34:1)	1.01 (0.91 - 1.13)	9.32E-01	1.04 (0.89 - 1.20)	8.44E-01
PE(P-34:2)	1.01 (0.91 - 1.12)	9.23E-01	0.97 (0.84 - 1.13)	8.80E-01
PE(P-36:1)	1.09 (0.98 - 1.21)	4.01E-01	1.08 (0.93 - 1.24)	5.86E-01
PE(P-36:2)	1.07 (0.96 - 1.20)	5.01E-01	1.05 (0.91 - 1.22)	7.39E-01
PE(P-36:4)	0.91 (0.81 - 1.01)	3.07E-01	0.88 (0.76 - 1.03)	3.19E-01
PE(P-38:4)	0.91 (0.81 - 1.02)	3.40E-01	0.86 (0.73 - 1.01)	2.31E-01
PE(P-38:5)	0.89 (0.79 - 1.00)	2.02E-01	0.85 (0.72 - 1.00)	1.87E-01
PE(P-38:6)	0.86 (0.77 - 0.98)	1.23E-01	0.84 (0.71 - 1.00)	1.75E-01
PE(P-40:4)	0.97 (0.88 - 1.08)	8.02E-01	1.03 (0.89 - 1.18)	8.75E-01
PE(P-40:5)	0.93 (0.82 - 1.05)	5.02E-01	0.91 (0.77 - 1.07)	5.31E-01
PE(P-40:6)	0.88 (0.79 - 0.99)	1.75E-01	0.85 (0.72 - 1.00)	1.83E-01
LPE(16:0)	1.08 (0.98 - 1.18)	3.61E-01	1.15 (1.02 - 1.30)	1.10E-01
LPE(18:0)	1.04 (0.95 - 1.13)	6.62E-01	1.09 (0.98 - 1.22)	3.15E-01
LPE(18:1)	1.11 (1.01 - 1.21)	1.47E-01	1.13 (1.00 - 1.27)	1.87E-01
LPE(18:2)	1.12 (1.02 - 1.23)	1.27E-01	1.15 (1.02 - 1.31)	1.32E-01
LPE(20:4)	1.04 (0.94 - 1.16)	7.03E-01	1.06 (0.92 - 1.22)	6.81E-01
LPE(22:6)	0.96 (0.86 - 1.06)	6.99E-01	0.97 (0.85 - 1.11)	8.51E-01
PI(32:0)	1.00 (0.91 - 1.09)	9.79E-01	1.03 (0.91 - 1.16)	8.44E-01
PI(32:1)	1.05 (0.96 - 1.14)	5.77E-01	1.04 (0.93 - 1.17)	7.23E-01
PI(34:0)	0.96 (0.86 - 1.07)	7.10E-01	0.96 (0.82 - 1.11)	8.02E-01
PI(34:1)	1.07 (0.97 - 1.17)	5.01E-01	1.08 (0.94 - 1.23)	5.47E-01
PI(36:1)	1.12 (1.02 - 1.24)	1.35E-01	1.10 (0.96 - 1.27)	4.18E-01
PI(36:2)	1.07 (0.96 - 1.19)	5.02E-01	1.11 (0.96 - 1.29)	4.20E-01
PI(36:3)	1.06 (0.95 - 1.18)	6.13E-01	1.03 (0.89 - 1.20)	8.52E-01

Predictors*	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
PI(36:4)	0.97 (0.86 - 1.08)	7.75E-01	0.96 (0.82 - 1.12)	8.36E-01
PI(38:2)	1.06 (0.95 - 1.17)	6.10E-01	1.07 (0.93 - 1.24)	5.91E-01
PI(38:3)	0.96 (0.85 - 1.07)	7.03E-01	0.92 (0.78 - 1.08)	5.74E-01
PI(38:4)	0.92 (0.82 - 1.04)	5.01E-01	0.93 (0.79 - 1.10)	6.51E-01
PI(38:5)	1.00 (0.91 - 1.11)	9.79E-01	0.94 (0.81 - 1.09)	6.53E-01
PI(38:6)	0.96 (0.87 - 1.06)	6.60E-01	0.99 (0.86 - 1.13)	9.32E-01
PI(40:4)	0.97 (0.87 - 1.07)	7.41E-01	1.00 (0.87 - 1.15)	9.88E-01
PI(40:5)	0.93 (0.83 - 1.03)	4.45E-01	0.90 (0.78 - 1.05)	4.49E-01
PI(40:6)	0.93 (0.84 - 1.03)	4.45E-01	0.93 (0.80 - 1.07)	5.74E-01
LPI(18:0)	1.05 (0.96 - 1.14)	5.70E-01	1.07 (0.96 - 1.20)	4.86E-01
LPI(18:1)	1.12 (1.03 - 1.21)	7.50E-02	1.16 (1.04 - 1.29)	6.81E-02
LPI(18:2)	1.07 (0.97 - 1.17)	4.77E-01	1.13 (1.00 - 1.28)	1.79E-01
LPI(20:4)	0.99 (0.89 - 1.10)	9.32E-01	1.02 (0.89 - 1.17)	8.91E-01
PG(34:1)	1.00 (0.95 - 1.05)	9.74E-01	1.01 (0.95 - 1.07)	9.08E-01
PG(36:1)	1.00 (0.91 - 1.10)	9.74E-01	1.06 (0.94 - 1.21)	6.00E-01
PG(36:2)	0.96 (0.87 - 1.06)	6.99E-01	0.97 (0.85 - 1.11)	8.52E-01
Cholesterol	1.07 (0.96 - 1.19)	5.02E-01	1.16 (1.01 - 1.34)	1.60E-01
CE(14:0)	1.04 (0.94 - 1.15)	7.03E-01	1.06 (0.92 - 1.22)	6.81E-01
CE(15:0)	1.08 (0.97 - 1.19)	4.45E-01	1.05 (0.91 - 1.21)	7.05E-01
CE(16:0)	1.18 (1.06 - 1.31)	**3.46E-02**	1.24 (1.06 - 1.43)	**4.86E-02**
CE(16:1)	1.02 (0.94 - 1.11)	7.87E-01	1.03 (0.92 - 1.16)	8.37E-01
CE(16:2)	1.04 (0.95 - 1.13)	6.99E-01	1.04 (0.92 - 1.18)	7.25E-01
CE(17:0)	1.07 (0.97 - 1.18)	5.01E-01	1.06 (0.92 - 1.21)	6.77E-01
CE(17:1)	1.12 (1.01 - 1.24)	1.82E-01	1.18 (1.02 - 1.36)	1.10E-01
CE(18:0)	1.14 (1.02 - 1.26)	1.27E-01	1.19 (1.03 - 1.37)	1.10E-01
CE(18:1)	1.12 (1.01 - 1.24)	1.82E-01	1.18 (1.02 - 1.36)	1.10E-01
CE(18:2)	1.08 (0.98 - 1.20)	3.85E-01	1.16 (1.01 - 1.32)	1.40E-01
CE(18:3)	1.00 (0.91 - 1.10)	9.87E-01	1.03 (0.91 - 1.17)	8.44E-01
CE(20:1)	1.09 (1.00 - 1.19)	2.13E-01	1.18 (1.06 - 1.31)	**2.62E-02**
CE(20:2)	1.10 (0.99 - 1.22)	2.80E-01	1.19 (1.03 - 1.37)	9.88E-02
CE(20:3)	1.03 (0.92 - 1.15)	7.75E-01	1.04 (0.90 - 1.22)	8.02E-01
CE(20:4)	0.97 (0.87 - 1.08)	7.75E-01	1.02 (0.89 - 1.17)	8.91E-01
CE(20:5)	0.92 (0.83 - 1.01)	3.00E-01	0.91 (0.80 - 1.04)	4.48E-01
CE(22:0)	1.05 (0.97 - 1.13)	5.02E-01	1.11 (1.02 - 1.21)	8.26E-02
CE(22:1)	1.01 (0.96 - 1.08)	8.12E-01	1.07 (0.99 - 1.15)	2.42E-01
CE(22:4)	1.06 (0.95 - 1.17)	6.11E-01	1.14 (0.99 - 1.31)	2.26E-01
CE(22:5)	0.99 (0.89 - 1.11)	9.74E-01	1.02 (0.88 - 1.18)	9.06E-01
CE(22:6)	0.89 (0.80 - 1.00)	2.04E-01	0.93 (0.80 - 1.08)	6.00E-01
CE(24:0)	1.02 (0.96 - 1.09)	7.41E-01	1.07 (1.00 - 1.13)	1.60E-01
CE(24:1)	1.06 (0.98 - 1.14)	4.19E-01	1.14 (1.05 - 1.24)	**2.49E-02**
CE(24:4)	1.08 (0.97 - 1.19)	4.43E-01	1.15 (1.01 - 1.32)	1.52E-01
CE(24:5)	1.03 (0.94 - 1.13)	7.67E-01	1.07 (0.95 - 1.22)	5.47E-01
CE(24:6)	0.94 (0.86 - 1.02)	4.01E-01	0.94 (0.84 - 1.06)	5.92E-01
DG(14:0_16:0)	1.03 (0.97 - 1.09)	6.15E-01	1.07 (0.99 - 1.15)	2.30E-01

Predictors*	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
DG(14:0_18:1)	1.00 (0.92 - 1.09)	9.79E-01	1.00 (0.89 - 1.13)	9.88E-01
DG(14:0_18:2)	0.97 (0.88 - 1.06)	7.48E-01	0.96 (0.85 - 1.10)	8.20E-01
DG(16:0_16:0)	1.04 (0.98 - 1.09)	4.77E-01	1.08 (1.01 - 1.16)	1.46E-01
DG(16:0_18:0)	1.04 (0.98 - 1.10)	5.02E-01	1.06 (0.98 - 1.14)	3.59E-01
DG(16:0_18:1)	1.02 (0.94 - 1.11)	8.17E-01	1.05 (0.95 - 1.17)	6.00E-01
DG(16:0_18:2)	0.99 (0.91 - 1.08)	9.48E-01	1.02 (0.91 - 1.13)	9.06E-01
DG(16:0_20:3)	0.93 (0.85 - 1.02)	4.01E-01	0.91 (0.79 - 1.04)	4.11E-01
DG(16:0_20:4)	0.94 (0.86 - 1.02)	4.44E-01	0.95 (0.84 - 1.07)	6.37E-01
DG(16:0_22:5)	0.97 (0.89 - 1.07)	7.71E-01	0.98 (0.86 - 1.11)	8.75E-01
DG(16:0_22:6)	0.92 (0.85 - 1.00)	2.37E-01	0.94 (0.84 - 1.05)	5.74E-01
DG(16:1_18:0)	0.99 (0.92 - 1.08)	9.56E-01	0.97 (0.86 - 1.09)	8.28E-01
DG(16:1_18:1)	1.01 (0.92 - 1.10)	9.54E-01	0.99 (0.87 - 1.12)	9.36E-01
DG(18:0_18:0)	0.93 (0.84 - 1.03)	4.52E-01	0.92 (0.80 - 1.06)	5.47E-01
DG(18:0_18:1)	1.03 (0.96 - 1.11)	6.62E-01	1.05 (0.96 - 1.15)	5.74E-01
DG(18:0_18:2)	1.01 (0.93 - 1.10)	8.96E-01	1.03 (0.94 - 1.13)	7.54E-01
DG(18:0_20:4)	0.90 (0.82 - 0.99)	1.72E-01	0.95 (0.83 - 1.07)	6.46E-01
DG(18:1_18:1)	1.02 (0.94 - 1.11)	7.75E-01	1.05 (0.95 - 1.16)	6.08E-01
DG(18:1_18:2)	0.99 (0.90 - 1.08)	9.23E-01	1.01 (0.90 - 1.14)	9.22E-01
DG(18:1_18:3)	0.97 (0.88 - 1.06)	7.10E-01	0.98 (0.86 - 1.10)	8.58E-01
DG(18:1_20:0)	1.00 (0.92 - 1.09)	9.87E-01	0.99 (0.88 - 1.12)	9.69E-01
DG(18:1_20:3)	0.92 (0.83 - 1.01)	2.81E-01	0.89 (0.77 - 1.02)	2.78E-01
DG(18:1_20:4)	0.92 (0.84 - 1.02)	3.61E-01	0.93 (0.81 - 1.06)	5.54E-01
DG(18:2_18:2)	0.97 (0.90 - 1.05)	7.27E-01	0.98 (0.88 - 1.09)	8.75E-01
TG(48:0)	0.99 (0.90 - 1.09)	9.56E-01	0.98 (0.86 - 1.13)	9.24E-01
TG(48:1)	0.95 (0.86 - 1.06)	6.62E-01	0.93 (0.80 - 1.09)	6.31E-01
TG(48:2)	0.96 (0.88 - 1.06)	7.03E-01	0.94 (0.82 - 1.08)	6.51E-01
TG(48:3)	0.97 (0.89 - 1.05)	7.03E-01	0.95 (0.84 - 1.08)	6.88E-01
TG(49:1)	0.95 (0.85 - 1.07)	6.62E-01	0.89 (0.75 - 1.04)	3.92E-01
TG(50:0)	0.99 (0.90 - 1.09)	9.23E-01	0.98 (0.85 - 1.12)	8.75E-01
TG(50:1)	0.99 (0.89 - 1.11)	9.43E-01	0.99 (0.84 - 1.15)	9.43E-01
TG(50:2)	0.96 (0.86 - 1.07)	7.10E-01	0.93 (0.79 - 1.09)	6.08E-01
TG(50:3)	0.93 (0.84 - 1.04)	5.02E-01	0.88 (0.75 - 1.04)	3.50E-01
TG(50:4)	0.93 (0.85 - 1.03)	4.77E-01	0.88 (0.76 - 1.02)	2.97E-01
TG(51:1)	0.96 (0.86 - 1.07)	7.10E-01	0.91 (0.78 - 1.08)	5.49E-01
TG(51:2)	0.94 (0.84 - 1.06)	6.15E-01	0.88 (0.75 - 1.04)	3.50E-01
TG(52:1)	0.97 (0.88 - 1.07)	7.71E-01	0.96 (0.84 - 1.11)	8.11E-01
TG(52:2)	1.00 (0.90 - 1.12)	9.74E-01	1.00 (0.86 - 1.17)	9.88E-01
TG(52:3)	0.98 (0.88 - 1.10)	9.08E-01	0.99 (0.85 - 1.15)	9.66E-01
TG(52:4)	0.95 (0.85 - 1.06)	6.19E-01	0.94 (0.81 - 1.09)	6.80E-01
TG(53:2)	0.97 (0.87 - 1.08)	7.67E-01	0.91 (0.79 - 1.06)	5.34E-01
TG(54:0)	0.99 (0.90 - 1.09)	9.23E-01	0.96 (0.83 - 1.09)	7.39E-01
TG(54:1)	0.99 (0.93 - 1.05)	8.47E-01	0.99 (0.90 - 1.08)	8.97E-01
TG(54:2)	1.00 (0.91 - 1.09)	9.74E-01	1.00 (0.88 - 1.13)	9.95E-01
TG(54:3)	1.05 (0.95 - 1.15)	6.19E-01	1.05 (0.92 - 1.20)	6.81E-01

Predictors*	Cardiovascular events† (cases/non-cases, 698/3,081)		Cardiovascular death‡ (cases/non-cases, 355/3,424)	
	HR (95% CI)§	p-value‖	HR (95% CI)§	p-value‖
TG(54:4)	0.97 (0.87 - 1.07)	7.59E-01	0.95 (0.82 - 1.10)	7.46E-01
TG(54:5)	0.93 (0.84 - 1.03)	4.52E-01	0.93 (0.80 - 1.07)	5.86E-01
TG(54:6)	0.90 (0.81 - 1.00)	1.86E-01	0.87 (0.75 - 1.01)	2.38E-01
TG(56:6)	0.83 (0.74 - 0.94)	**3.00E-02**	0.81 (0.68 - 0.96)	8.54E-02

* 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: Performance of models to predict cardiovascular events.

Model	Feature*	C-Statistics	NRI Categorical†	NRI Continuous	IDI	Relative IDI
Base model	Base model	0.680 (0.678 - 0.682)				
Model 1	PC(O-36:1)	0.690 (0.688 - 0.692)	0.027 (0.023 - 0.030)	0.186 (0.178 - 0.193)	0.010 (0.010 - 0.011)	0.160 (0.155 - 0.166)
Model 2	CE(18:0)	0.688 (0.687 - 0.690)	0.019 (0.016 - 0.023)	0.174 (0.166 - 0.181)	0.010 (0.010 - 0.011)	0.161 (0.155 - 0.167)
Model 3	PE(O-36:4)	0.689 (0.687 - 0.691)	0.024 (0.020 - 0.028)	0.183 (0.175 - 0.190)	0.012 (0.011 - 0.012)	0.182 (0.175 - 0.189)
Model 4	PC(28:0)	0.694 (0.692 - 0.696)	0.039 (0.035 - 0.043)	0.220 (0.212 - 0.228)	0.017 (0.016 - 0.017)	0.260 (0.252 - 0.268)
Model 5	LPC(20:0)	0.693 (0.691 - 0.695)	0.035 (0.031 - 0.039)	0.205 (0.198 - 0.213)	0.017 (0.016 - 0.017)	0.261 (0.252 - 0.269)
Model 6	PC(35:4)	0.698 (0.696 - 0.699)	0.047 (0.043 - 0.051)	0.247 (0.239 - 0.255)	0.021 (0.021 - 0.022)	0.332 (0.322 - 0.341)
Model 7‡	**LPC(18:2)**	**0.700 (0.698 - 0.702)**	**0.055 (0.052 - 0.059)**	**0.227 (0.219 - 0.235)**	**0.024 (0.023 - 0.024)**	**0.364 (0.353 - 0.374)**
Model 8	PE(32:0)	0.699 (0.697 - 0.701)	0.058 (0.054 - 0.062)	0.228 (0.220 - 0.236)	0.024 (0.024 - 0.025)	0.375 (0.365 - 0.386)
Model 9	PC(34:5)	0.698 (0.697 - 0.700)	0.055 (0.051 - 0.059)	0.250 (0.243 - 0.258)	0.027 (0.026 - 0.028)	0.420 (0.409 - 0.431)
Model 10	TG(54:0)	0.698 (0.696 - 0.699)	0.055 (0.051 - 0.059)	0.253 (0.245 - 0.261)	0.029 (0.028 - 0.029)	0.443 (0.432 - 0.454)
Model 11	CE(24:1)	0.697 (0.695 - 0.699)	0.058 (0.054 - 0.062)	0.227 (0.220 - 0.235)	0.029 (0.028 - 0.030)	0.447 (0.436 - 0.458)
Model 12	LPC(20:4)	0.696 (0.694 - 0.698)	0.055 (0.051 - 0.059)	0.219 (0.212 - 0.227)	0.029 (0.028 - 0.030)	0.450 (0.438 - 0.461)
Model 13	CE(22:0)	0.699 (0.697 - 0.701)	0.059 (0.054 - 0.063)	0.255 (0.247 - 0.263)	0.032 (0.032 - 0.033)	0.501 (0.489 - 0.513)
Model 14	SM(34:2)	0.698 (0.696 - 0.700)	0.057 (0.053 - 0.062)	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)	0.697 (0.695 - 0.699)	0.050 (0.046 - 0.054)	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)	0.699 (0.697 - 0.701)	0.049 (0.045 - 0.053)	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)	0.700 (0.698 - 0.701)	0.049 (0.045 - 0.053)	0.249 (0.241 - 0.257)	0.038 (0.037 - 0.039)	0.583 (0.570 - 0.596)
Model 18	PC(32:2)	0.702 (0.700 - 0.704)	0.047 (0.042 - 0.052)	0.256 (0.249 - 0.264)	0.041 (0.040 - 0.042)	0.635 (0.622 - 0.649)
Model 19	SM(34:1)	0.701 (0.700 - 0.703)	0.046 (0.041 - 0.051)	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)	0.702 (0.700 - 0.704)	0.047 (0.043 - 0.052)	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.

† Net reclassification index, based on a categorical model of <10, 10–15, and >15% 5-years risk.

‡ Denotes the optimal model with seven lipid features.

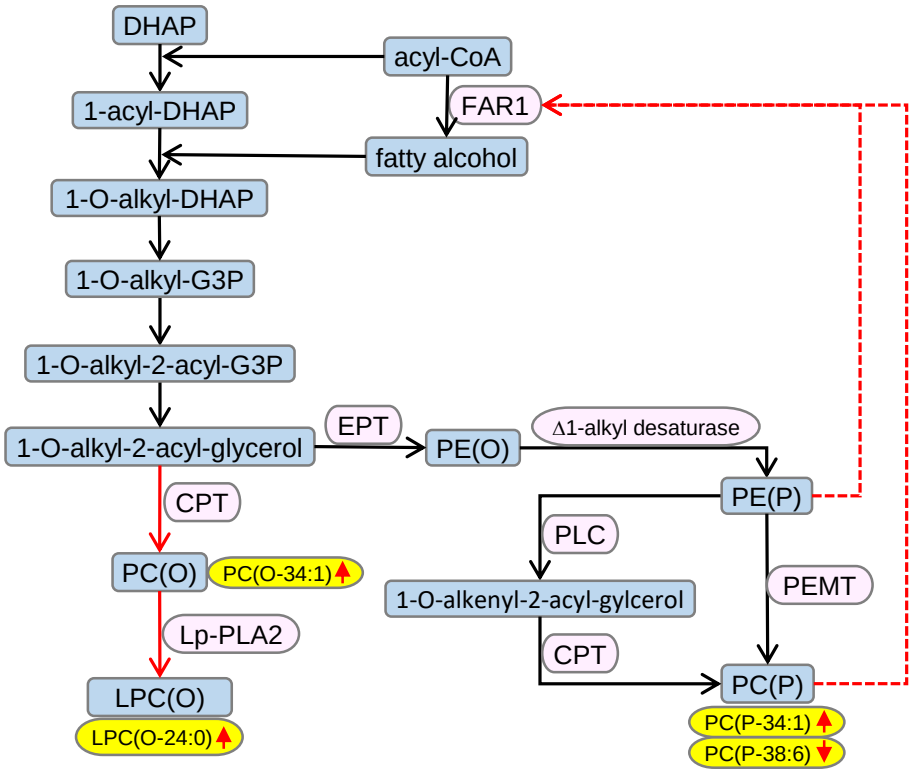
Supplementary Table 4: Performance of models to predict cardiovascular death.

Model	Feature*	C-Statistics	NRI Categorical†	NRI Continuous	IDI	Relative IDI
Base model†	Base model	0.740 (0.738 - 0.742)				
Model 1	PC(O-36:1)	0.748 (0.745 - 0.750)	0.071 (0.067 - 0.075)	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)	0.746 (0.744 - 0.749)	0.065 (0.061 - 0.069)	0.229 (0.220 - 0.239)	0.015 (0.014 - 0.016)	0.192 (0.183 - 0.202)
Model 3	SM(34:1)	0.749 (0.746 - 0.751)	0.069 (0.065 - 0.073)	0.249 (0.239 - 0.259)	0.017 (0.016 - 0.018)	0.214 (0.203 - 0.225)
Model 4‡	**PC(O-36:5)**	**0.760 (0.757 - 0.762)**	**0.101 (0.097 - 0.105)**	**0.328 (0.317 - 0.339)**	**0.023 (0.022 - 0.024)**	**0.288 (0.274 - 0.302)**
Model 5	PI(32:0)	0.757 (0.755 - 0.760)	0.103 (0.099 - 0.108)	0.326 (0.315 - 0.336)	0.023 (0.022 - 0.024)	0.294 (0.280 - 0.308)
Model 6	SM(41:2)	0.760 (0.758 - 0.762)	0.112 (0.107 - 0.117)	0.376 (0.365 - 0.386)	0.031 (0.030 - 0.032)	0.399 (0.382 - 0.416)
Model 7	CE(22:4)	0.758 (0.756 - 0.760)	0.113 (0.109 - 0.118)	0.360 (0.349 - 0.370)	0.031 (0.030 - 0.033)	0.404 (0.388 - 0.421)
Model 8	LPE(18:1)	0.757 (0.755 - 0.760)	0.110 (0.105 - 0.114)	0.364 (0.353 - 0.374)	0.033 (0.032 - 0.034)	0.425 (0.408 - 0.441)
Model 9	LPC(14:0)	0.757 (0.755 - 0.759)	0.108 (0.103 - 0.113)	0.350 (0.340 - 0.361)	0.033 (0.031 - 0.034)	0.418 (0.401 - 0.435)
Model 10	PC(O-32:1)	0.757 (0.754 - 0.759)	0.122 (0.117 - 0.127)	0.390 (0.380 - 0.400)	0.035 (0.033 - 0.036)	0.443 (0.425 - 0.461)
Model 11	LPC(18:2)	0.758 (0.756 - 0.761)	0.119 (0.114 - 0.124)	0.419 (0.409 - 0.429)	0.038 (0.036 - 0.040)	0.489 (0.469 - 0.509)
Model 12	PE(O-36:4)	0.760 (0.757 - 0.762)	0.121 (0.116 - 0.126)	0.422 (0.411 - 0.432)	0.040 (0.038 - 0.041)	0.508 (0.488 - 0.529)
Model 13	PC(O-36:3)	0.763 (0.761 - 0.765)	0.110 (0.105 - 0.115)	0.423 (0.413 - 0.433)	0.040 (0.039 - 0.042)	0.516 (0.495 - 0.537)
Model 14	LPI(20:4)	0.767 (0.764 - 0.769)	0.102 (0.097 - 0.107)	0.421 (0.411 - 0.431)	0.042 (0.040 - 0.044)	0.537 (0.515 - 0.559)
Model 15	PC(38:4)	0.775 (0.772 - 0.777)	0.112 (0.108 - 0.117)	0.422 (0.412 - 0.432)	0.049 (0.047 - 0.051)	0.628 (0.604 - 0.652)
Model 16	TG(54:0)	0.775 (0.773 - 0.777)	0.109 (0.104 - 0.113)	0.399 (0.389 - 0.409)	0.051 (0.049 - 0.053)	0.653 (0.629 - 0.678)
Model 17	PC(34:5)	0.774 (0.772 - 0.776)	0.108 (0.103 - 0.113)	0.404 (0.394 - 0.414)	0.053 (0.051 - 0.055)	0.672 (0.647 - 0.697)
Model 18	PC(35:4)	0.773 (0.771 - 0.775)	0.105 (0.100 - 0.110)	0.394 (0.384 - 0.404)	0.053 (0.051 - 0.055)	0.675 (0.650 - 0.700)
Model 19	PC(34:2)	0.778 (0.775 - 0.780)	0.099 (0.094 - 0.105)	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)	0.778 (0.776 - 0.780)	0.103 (0.098 - 0.108)	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.

† Net reclassification index, based on a categorical model of <10, 10–15, and >15% 5-years risk.

‡ Denotes the optimal model with four lipid features.



Supplementary Figure 1: Metabolic pathway of ether lipids 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.

Reference

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