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Mitochondrial Metabolites Predict Adverse Cardiovascular Events in Individuals with Diabetes

Brief Title: Mitochondrial Metabolites in Diabetes and Adverse CV Events

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Abstract:

Metabolic mechanisms underlying the heterogeneity of major adverse cardiovascular events (MACE) risk in individuals with type 2 diabetes mellitus (T2D) remain unclear. We hypothesized that circulating metabolites reflecting mitochondrial dysfunction predict incident MACE in T2D. Targeted mass-spectrometry profiling of 60 metabolites was performed on baseline plasma from TECOS (discovery) and EXSCEL (validation) trial biomarker substudy cohorts. A principal components analysis metabolite factor comprised of medium-chain acylcarnitines was associated with MACE in TECOS and validated in EXSCEL, with higher levels associated with higher MACE risk. Meta-analysis showed that long-chain acylcarnitines and dicarboxyliccarnitines were also associated with MACE. Metabolites remained associated with MACE in multivariate models and favorably changed with exenatide therapy. A third cohort (CATHGEN) with T2D assessed whether these metabolites improved discriminative capability multivariate for MACE; nine metabolites (medium- and long-chain acylcarnitines and one dicarboxyliccarnitine) were associated with time-to-MACE in CATHGEN. Addition of these metabolites to clinical models minimally improved the discriminative capability for MACE but did significantly down reclassify risk. Thus, metabolites reporting on dysregulated mitochondrial fatty acid oxidation are higher in individuals with T2D who experience subsequent MACE. These biomarkers may improve CV risk prediction models, be therapy responsive, and highlight emerging risk mechanisms.

Key Words: type 2 diabetes; CV events, metabolomics, mitochondria

Abbreviations:

MACE = Major Adverse Cardiac Events
TECOS = Trial Evaluating CV Outcomes with Sitagliptin
EXSCEL = Exenatide Study of CV Event Lowering
CATHGEN = Cardiac CATHeterization Genetics
T2D = Type 2 Diabetes
CV = Cardiovascular
cAD = Coronary artery disease
Introduction

Cardiovascular (CV) disease remains a primary cause of morbidity and mortality despite advancements in earlier diagnosis and treatment. Individuals with type 2 diabetes (T2D) are at excess risk of CV morbidity and mortality compared with those without T2D (1-3), however there is heterogeneity of risk that is only partially explained by clinical factors.

Cardiac metabolic machinery relies on adequate energy substrate delivery and intrinsic cardiac metabolism. The contribution of fatty acids and carbohydrates to energy expenditure, termed substrate utilization, is central to metabolic disease, and both CV disease and T2D demonstrate perturbations in metabolic homeostasis at organ-specific and systemic levels (4, 5). Emerging technologies have enabled evaluation of this metabolic machinery and have shown utility in the discovery of biomarkers associated with CV risk. Metabolomic profiling has identified elevated circulating levels of branched-chain amino acids (BCAAs) and altered arginine metabolism to be associated with coronary artery disease (CAD) (6) and dicarboxylcarnitines also appear predictive of major adverse CV events (MACE) (6-8). BCAAs and long-chain acylcarnitines are elevated in the setting of insulin-resistance and obesity (9-14), however metabolic pathways linking T2D to MACE remained inadequately understood.

CV outcome trials have demonstrated that a subset of glucose-lowering medications used for the treatment of T2D also improve CV outcomes, but understanding of the mechanisms of how these medications mitigate risk is limited (15-17). Application of metabolomic profiling may improve biologic understanding and support precision medicine approaches to ultimately improve CV outcomes for patients. Hence, we tested the hypothesis that baseline circulating metabolites reporting on mitochondrial dysfunction predict MACE in individuals with T2D. To accomplish this, we leveraged biospecimens from the Trial Evaluating CV Outcomes with Sitagliptin (TECOS) and Exenatide Study of CV Event Lowering (EXSCEL) studies, two large clinical trial cohorts with systematic capture and confirmation by central blinded adjudication of MACE; and participants with T2D from the Cardiac CATHeterization Genetics (CATHGEN) cohort to determine capability of these metabolites for predicting MACE (Figure 1).

Results

Baseline Participant Characteristics
A nested MACE case-control cohort of 996 participants in the TECOS discovery cohort was examined in the present analyses, including 498 MACE cases and 498 matched controls (Table 1). In the EXSCEL validation cohort, a similar nested MACE case-control cohort of 978 participants (487 cases, 491 controls) was included. In CATHGEN, 1330 participants with baseline T2D were included. TECOS and EXSCEL participants were older, more often male and more predominantly of self-reported white race than participants in CATHGEN. All three cohorts had a high burden of CV risk factors. In TECOS, mean BMI was lower than in EXSCEL and CATHGEN. CATHGEN participants had higher systolic blood pressure and creatinine and lower prevalence of hypertension, dyslipidemia, CAD, prior myocardial infarction (MI) and prior stroke, but higher prevalence of heart failure (HF). EXSCEL participants had the highest prevalence of peripheral arterial disease and smoking and highest mean hemoglobin A1C level (HbA1c). There were no significant differences in baseline cholesterol levels across the three cohorts. Baseline participant characteristics stratified by MACE outcome are shown in Supplemental Table 1.

Medium Chain Acylcarnitine Metabolites are Associated with Incident MACE in the TECOS and EXSCEL Clinical Trials

Unsupervised machine learning using principal components analysis (PCA) was used to reduce the correlated individual metabolites into 12 orthogonal uncorrelated metabolite factors or signatures (Supplemental Table 2). Of these, in the TECOS discovery cohort, one factor (factor 9), heavily loaded with medium-chain acylcarnitine metabolites, was associated with MACE in univariate (OR 1.26 [95% CI 1.11-1.45], false discovery rate [FDR] p=0.008) and multivariate models (OR 1.29 [95% CI 1.11-1.49], FDR p=0.009, Table 2); the OR represents the odds of experiencing an incident MACE event for every one standard deviation increase in metabolite factor levels. This metabolite factor validated for predicting incident MACE in the EXSCEL cohort in univariate (OR 1.15 [95% CI 1.02-1.30], nominal p=0.03) and multivariate analyses (OR 1.17 [95% CI 1.02-1.34], p=0.03).

In a post-hoc exploratory meta-analysis of multivariate models for all 12 metabolite PCA factors and MACE, three PCA factors were nominally associated with MACE: the prior one identified through the discovery and validation approach (factor 9 comprised of medium-chain acylcarnitines [OR 1.22 (1.11-1.35)], p=9x10^-5)
as well as two new PCA metabolite factors which did not meet the stringent FDR cutoff for significance in TECOS alone: one comprised of medium- and long-chain acylcarnitines (factor 1, OR 1.17, [1.00-1.37], p=0.046) and one comprised of long-chain dicarboxylacylcarnitines (factor 3, OR 0.88 [0.78-0.99], p=0.04), Figure 2, Supplemental Table 3).

In exploratory analyses of these three metabolite factors with individual components of MACE, there was overall a consistent magnitude and direction of effect across the individual outcomes of non-fatal MI, non-fatal stroke and CV death (Supplemental Table 4), suggesting that these metabolites are associated with risk across heterogeneous MACE events, with the exception of factor 9 which showed strongest effects related to the atherosclerotic outcomes of non-fatal MI and non-fatal stroke.

Individual Metabolites within PCA-derived Metabolite Factors are also Associated with MACE

To determine the most significant metabolites and evaluate differences in absolute metabolite concentrations, individual metabolites heavily loaded (i.e. absolute value of factor load >0.4) within these three significant PCA factors were analyzed for association with incident MACE (22 individual metabolites) in meta-analyses combining TECOS and EXSCEL. In univariate meta-analyses, 12 individual metabolites were associated with MACE, all with higher levels in MACE cases compared with non-MACE controls: medium- and long-chain acylcarnitines (all even-chain, ranging from eight to sixteen-carbons in length) and one medium-chain dicarboxylacylcarnitine (C12-OH/C10-DC), (OR range 1.17-1.66, p<0.05, Supplemental Table 5). In multivariate meta-analyses, 10 medium- and long-chain acylcarnitines remained significant (OR range 1.20-1.57, p<0.05, Figure 3, Supplemental Table 5). The OR represents the odds of experiencing an incident MACE event for every one unit increase in the log-transformed metabolite level. These results suggest that a single metabolite is not driving the association of the metabolite factors with MACE, but instead that multiple correlated metabolites are associated individually and as a group.

Metabolites are Associated with Time-To-MACE and Improve Prediction of Clinical Outcomes in Individuals with T2D
Given the nested case-control design of the TECOS and EXSCEL cohorts, we analyzed a third cohort to enable time-to-event and incremental risk prediction analyses, namely N=1330 participants with T2D from the CATHGEN cohort (Table 1). Of these, N=664 individuals experienced MI, unstable angina, stroke or all-cause mortality in CATHGEN with a median time-to-event of 509 days (IQR 172-997 days). All 13 unique metabolites identified in either univariate or multivariate TECOS and EXSCEL meta-analysis were associated with time-to-MACE in CATHGEN in univariate models (p<0.05); nine of these metabolites (medium- and long-chain acylcarnitines and one dicarboxylacylcarnitine) remained significant in multivariate models (p<0.05, Table 3). The HR represents the odds of experiencing an incident MACE event for every one unit increase in the log-transformed metabolite level. Kaplan Meier curves by tertile of metabolite, are shown in Supplemental Figure 1. The significance of the four metabolites that did not remain significant in multivariate models (medium and long-chain acylcarnitines) were attenuated primarily by age, history of HF and creatinine.

Having converged on these nine metabolites as the most significant individual metabolites independently associated with MACE and time-to-MACE in multivariate models in three cohorts we then created a composite score of the nine metabolites by log-transforming the molar sum of these metabolites and then scaling this value. Supplemental Figure 2 demonstrates absolute values of the individual metabolites and Supplemental Figure 3 demonstrates the log-transformed, scaled-sum of the nine-metabolite score stratified by MACE in the three cohorts. Analyzed as a continuous variable in TECOS and EXSCEL, this composite metabolite score was associated with MACE in multivariate meta-analysis models (OR 1.16 [95% CI 1.06-1.29], p=0.002, Supplemental Table 6, Supplemental Figure 4). To be able to directly compare the effect size with the individual metabolites in a meaningful way, we analyzed the nine individual metabolites again after scaling their log-transformed levels. The range of the HR in multivariate meta-analysis models was (1.09-1.25). In the CATHGEN cohort the composite nine-metabolite score was also associated with time-to-MACE in continuous analyses (multivariate HR 1.26 [95% CI 1.15-1.37], p=1.5x10^-7, Supplemental Table 7). For comparison, the multivariate HR range of the scaled nine individual metabolites was (1.13 -1.25). Kaplan Meier curves by tertile of the metabolite score are shown in Figure 4. The composite score showed relative similarly magnitude of effect sizes across MACE subcomponents with perhaps slightly greater risk associated with CV death and lesser magnitude of risk for non-fatal MI (Supplemental Table 8).
We then assessed whether these nine metabolites demonstrated incremental risk prediction using receiver operator characteristic (ROC) curves. In CATHGEN, the addition of these nine metabolites was statistically significant but showed minimal effect for improving the discriminative capability for MACE over the clinical model alone (age, sex, race, history of HF, CAD, BMI, HbA1c, systolic blood pressure, creatinine, low density lipoprotein cholesterol [LDL-C] and smoking status): AUC = 0.71 (95% CI 0.68-0.74) vs. AUC=0.70 (95% CI 0.67-0.73), DeLong’s test p=0.03, Supplemental Figure 5). In reclassification analyses comparing addition of the nine individual metabolites over the clinical model alone, the overall net reclassification index (NRI) was 0.24 (p=2.4x10^{-5}), the net proportion of cases assigned to a higher risk group in the model inclusive of metabolites was 7.1% (p=0.07) and the net proportion of controls assigned to lower risk was 16.5% (p=3.3x10^{-5}). The integrated discrimination index which represents the improvement of the slopes of discrimination curves between the old and new models was 0.014 (p=1.9x10^{-5}).

**Pharmacologic Therapy with Exenatide Beneficially Modifies Metabolite Levels in Individuals with T2D**

Seven out of nine tested metabolites significantly changed between baseline and 12-month samples in individuals randomized to once-weekly exenatide, compared with placebo (nominal p<0.05). These included mean levels of long-chain acylcarnitines that decreased with exenatide therapy and increased in placebo randomized participants (Supplemental Figure 6, Supplemental Table 9), suggesting a beneficial effect of exenatide on these metabolites. Medium-chain acylcarnitines and dicarboxylacylcarnitines increased to a lesser extent with exenatide therapy compared with placebo. Changes in these metabolites with exenatide remained significant even after adjustment for the amount of weight loss (a known effect of glucagon-like peptide-1 receptor agonist (GLP-1 RA), Supplemental Table 9). Similar changes were seen for exenatide therapy as compared with placebo for the composite nine metabolite score (p=0.01, Supplemental Figure 5) and there was no change in the results after adjusting for amount of weight loss. Correlation between the change in the nine-metabolite summary score and change in weight loss revealed a moderate positive correlation (Pearson’s rho p=0.49).

**Discussion**
Leveraging two large international randomized clinical trials of glucose-lowering medications, for T2D for one of the largest studies of its kind, we report higher baseline levels of nine metabolites that reflect dysregulated mitochondrial fatty acid oxidation in individuals with T2D who subsequently experienced MACE compared with controls without subsequent MACE. These results support the potential clinical utility of these metabolites as biomarkers for risk prediction, given that results were robust to adjustment for covariables and were beneficially modified by exenatide. These mitochondrial metabolites, consisting primarily of medium- and long-chain acylcarnitines, may reflect subclinical impairments in mitochondrial fatty acid oxidation, identifying important molecular signatures for incident MACE in individuals with T2D. These results highlight potential metabolic biomarkers for MACE risk prediction and also highlight potential mechanisms for the beneficial effects of this class of GLP-1 RA medications which are being increasingly utilized for glycemic control and weight loss.

Mitochondrial metabolism is central to cardiac and skeletal muscle function (Figure 5). In healthy cardiac metabolism mitochondrial oxidative phosphorylation of fatty acids supplies the majority of ATP followed by carbohydrates via glycolysis (18). In a normal, healthy state myocardial metabolism has broad flexibility with the ability to utilize a range of substrates for efficient energy production. However, perturbations in energy metabolism in T2D and CV disease can contribute to worsened CV function and adverse clinical outcomes (19). In T2D, circulating free fatty acids, triglycerides and long-chain acylcarnitines are elevated and preclinical models have shown accumulation of long-chain acylcarnitines in skeletal muscle representing impaired mitochondrial fatty acid β-oxidation (12). There is also increased myocardial fatty acid uptake in T2D, altering substrate supply and impairing fatty acid β-oxidation. During ischemia, glycolysis becomes a main source of energy production, but the duration during which this can be maintained is limited and can ultimately lead to impaired contractile function secondary to intracellular buildup of acids and ions (20). Similarly in HF, decreased mitochondrial oxidative capacity results in a compensatory increase in glucose uptake for glycolysis and overall metabolic inflexibility with increased reliance on alternative substrate utilization (21).

Eight of the nine metabolites that associated with MACE in TECOS, EXSCEL and CATHGEN are medium- and long-chain acylcarnitines, which report on impaired mitochondrial fatty acid β-oxidation. Rare mitochondrial disorders of lipid metabolism caused by deficiencies in carnitine transfer enzymes are associated
with skeletal and cardiac myopathy and also display elevated levels of these metabolites (22). Acylcarnitines accumulate as a result of inefficient fatty acid oxidation from either enzymatic defects or imbalances in fatty acid oxidation to tricarboxylic acid (TCA) flux creating a bottleneck of carbon substrates (12, 23). Long-chain acylcarnitines are associated with insulin resistance as chronic overnutrition can lead to lipotoxicity and interference with insulin signaling via multiple mechanisms including incomplete β-oxidation at the mitochondrial membrane (12-14, 24). One additional metabolite associated with MACE in all three cohorts was a dicarboxylacetylcaritnine metabolite; these metabolites may similarly reflect changes in β-oxidation or may indicate changes in endoplasmic reticulum carboxylation via microsomal P450 or peroxisomal metabolism (25). The composite nine-metabolite score was not stronger than the individual metabolite analyses, this is likely related to the strong correlation of biologically grouped metabolites. When applying these metabolites to clinical prediction tools, the AUC significantly improved, though only minimally, suggesting that these metabolites report on important biology but may not improve discrimination over a clinical model alone. However, these metabolites do appear to improve down reclassification of risk (NRI), suggesting they may have some clinical utility.

Here, in one of the largest such studies, we extend and refine prior studies with a focus on participants with T2D, evaluating MACE and its subcomponents as well as changes with pharmacologic therapy. In previous work from 2023 CATHGEN participants, medium-chain acylcarnitines were identified as predictors of all-cause mortality (8). Here we find overall consistent effect sizes across MACE subcomponents, however factor 9, comprised of medium-chain acylcarnitines, may be more strongly related to atherosclerotic disease given its strongest associations were with non-fatal MI and non-fatal stroke. In 4164 participants with suspected angina recruited at University Hospitals in Norway, medium- and long-chain acylcarnitines were associated with CV death and acute MI with no effect modification according to T2D or BMI (26). These metabolites have also specifically been associated with cardioembolic stroke and stroke recurrence in the Korea University Stroke Registry and correlate with other stroke risk factors including age, atrial fibrillation, hypertension and male gender (27). In the context of cardiometabolic disease, higher levels of long-chain acylcarnitines have been identified in obese and insulin-resistant individuals compared with lean controls (11). Plasma levels of long-chain acylcarnitines are higher in HF, are higher in end-stage HF versus chronic stable
HF and are also differentially elevated in HF with reduced versus preserved ejection fraction (28, 29). In a study of 1032 Henry Ford Heart Failure Pharmacogenomic registry participants, medium- and long-chain acylcarnitines associated with ischemic etiology of HF and a prognostic metabolite profile comprised of 13 metabolites, including C18:1, added incremental risk prediction for survival over NT-proBNP over a median follow-up of almost three years (30). Similarly, in a randomized controlled trial of exercise training in ambulatory patients with HF (HF-ACTION), metabolomic profiling of 664 participants identified long-chain acylcarnitines as associated with impaired cardiorespiratory fitness (measured by peak oxygen consumption) and adverse clinical outcomes including mortality and hospitalization (31).

Prior studies have suggested that these metabolites are modifiable by exercise and pharmacologic therapy. For example, in the HF-ACTION trial, long-chain acylcarnitines levels were found to be beneficially modifiable with exercise (i.e. decrease), but in analyses stratified by T2D, these metabolites decreased to a lesser extent with exercise in patients with T2D (32). Further, stronger associations of these metabolites with mortality and hospitalization were seen in participants with T2D compared with those without T2D (32). Interestingly, metabolomic profiling in 234 participants from the DEFINE-HF trial which randomized patients with HF with reduced ejection fraction to 12 weeks of the sodium-glucose co-transporter-2 inhibitor (SGLT2i) dapagliflozin vs. placebo found that medium-chain acylcarnitines and ketone-related metabolites increased with SGLT2i (33). Increases in short- and medium-chain acylcarnitines without an increase in long-chain acylcarnitines may suggest an overall increased in fatty acid oxidation secondary to metabolic reprogramming from SGLT2i therapy. In DEFINE-HF participants, increases in long-chain acylcarnitines and dicarboxylacylcarnitines were associated with intermediate outcomes including quality of life and NT-proBNP, regardless of SGLT2i therapy. In exploratory analyses presented here, we found that medium- and long-chain acylcarnitines and dicarboxylacylcarnitines were favorably modified by exenatide, i.e. they increased to a lesser extent in individuals randomized to exenatide as compared with placebo (medium-chain acylcarnitines and dicarboxylacylcarnitine), or decreased in exenatide-randomized while increasing in placebo randomized individuals (long-chain acylcarnitines). Importantly, change in weight loss with exenatide therapy did not fully explain this association, suggesting alternative direct or indirect effects of exenatide therapy. As GLP-1 RA have gained FDA approval and increased clinical utility for glycemic control and weight management, the
metabolic findings here may identify mechanisms of beneficial class effects that could be translated to future patient care. Given that these metabolites are modifiable by lifestyle and pharmacologic interventions, if they are ultimately demonstrated to be in causal pathways, they could serve to identify potential pharmacologic targets and possibly to identify individuals for personalized therapies.

There are several strengths to our approach. First, we utilized samples from two robust CV outcomes clinical trials of participants with T2D with centrally adjudicated outcomes blinded by treatment assignment as well as a third validation cohort of participants with T2D undergoing evaluation for ischemic heart disease. A large number of biologically relevant metabolites were accurately measured with the addition of internal standards enabling absolute quantification. We used a discovery approach with careful adjustment for multiple comparisons of metabolites factors and inclusion of two validation cohorts. Finally, we applied these findings to demonstrate their potential clinical utility by assessing incremental risk predictive capabilities, and showed that they are modified by GLP-1 RA therapy. However, important study limitations should be noted. As the majority of participants in these studies have obesity and all have T2D, differential findings in levels of these metabolites and impact on MACE outcomes or treatment effects by BMI and diabetes status could not be thoroughly assessed. Non-alcoholic fatty liver disease (NAFLD) is another relevant comorbid condition that has reported effects on MACE and is likely to be present in these populations, but was unable to be determined in these cohorts. Importantly, we cannot determine the tissue source of medium- and long-chain acylcarnitines in this study, which could include myocardial or skeletal origin. The liver is not a high energy consumptive organ (compared to ATP utilization in cardiac and skeletal muscle) and therefore is not known to be a source of circulating acylcarnitine levels. Though we hypothesize these findings are due to inefficient fatty acid oxidation, increases in TCA flux or changes in enzymatic expression could also be contributing and were not able to be analyzed here. Further, while we conducted careful multivariate adjustments, other emerging biomarkers of MACE risk were not assessed. Finally, while we adjusted for a measure of glycemia (HbA1c), we were not able to adjust for other potential clinical risk factors including insulin resistance, physical activity or diet.

In three cohorts of individuals with T2D, with a total of 1649 MACE events analyzed, we demonstrate herein the power of applying robust metabolomic profiling technologies to clinical trials of T2D glucose-lowering medications to identify relevant biology and potential clinical utility of related circulating biomarkers reporting on
dysregulated mitochondrial metabolism. As all participants in the present study had T2D and we adjusted for HbA1c levels, these findings suggest additional potential influence of impaired mitochondrial efficiency at the molecular level that associates with future CV events. Extending prior observations, the present results confirm associations between selected metabolites reflecting mitochondrial dysfunction and risk for atherosclerotic and thrombotic CV disease complications, adding to literature describing the importance of acylcarnitines in CV disease, and further, highlight potential mechanisms of effect of GLP-1 RA medications. Incorporation of measurement of these metabolites for individuals with T2D may aid in risk stratification and ultimately identification of novel therapeutic targets to limit the burden of MACE in this population. Taken together, elevated levels of medium- and long-chain acylcarnitines may reflect abnormalities in biologic pathways of energy utilization in either myocardium, peripheral skeletal muscle or both. In the present work, higher levels of medium- and long-chain acylcarnitines in individuals with T2D may reflect subclinical metabolic inflexibility and inefficient mitochondrial fatty acid oxidation, but are modifiable by GLP-1 RA therapy. Further work is warranted to determine the clinical applicability of these findings to CV risk prediction and role of these biomarkers in specific types of CV disease as well as to evaluate whether these findings are generalizable to other GLP-1 RA medications.

Methods

Study Populations

TECOS Clinical Trial Biomarker Substudy. The discovery cohort consisted of participants from the placebo arm of the Trial Evaluating CV Outcomes with Sitagliptin (TECOS) (34). TECOS included 14,671 participants in total, with 7,226 in the placebo arm. Briefly, TECOS was a randomized, placebo-controlled cardiovascular outcomes trial of sitagliptin, a dipeptidyl peptidase 4 (DPP-4) inhibitor, in individuals with T2D and established atherosclerotic CV disease. Randomization occurred between December 2008 and July 2012, and the trial concluded in March 2015. Participants at baseline were at least 50 years of age with a glycated HbA1c between 6.5 and 8.0%, and were followed for a median of 3.0 years. The primary outcome was time to the first event of the composite of unstable angina, non-fatal MI, non-fatal stroke or CV death. A nested matched MACE case-
control subset of all TECOS placebo-arm participants with available baseline peripheral blood samples who experienced incident MACE were identified (N=498) and 1:1 controls were selected from the placebo group, matched on history of HF, CAD, BMI, HbA1c, creatinine, LDL-C, fasting status and left ventricular ejection fraction.

EXSCEL Clinical Trial Biomarker Substudy. The validation cohort consisted of participants from EXSCEL, a randomized, placebo-controlled trial of once-weekly exenatide, a GLP-1 RA (35). Overall EXSCEL included 14,752 adult participants with T2D, with HbA1c levels between 6.5 to 10.0%, approximately 70% of whom had cardiovascular disease. Randomization occurred between June 2010 and September 2015. The trial concluded in May 2017 with participants followed for a median of 3.2 years. The EXSCEL primary outcome was time to the first event of the composite of non-fatal MI, non-fatal stroke or CV death (MACE). Overall, 978 participants (487 cases and 491 controls) were identified and used for metabolomic profiling included in the present analyses.

CATHGEN Cohort. The Catheterization Genetics (CATHGEN) study includes 9334 patients who underwent cardiac catheterization at Duke University Medical Center (Durham, NC) between January 2001 and December 2010 (36). For the present analyses, 1330 participants with a diagnosis of T2D at the time of study enrollment and available metabolomics data were included. Demographics and comorbidities were collected through medical record review at study enrollment, and yearly follow-up was conducted for events and vital status. These data were supplemented with review of electronic health records. Clinical outcomes were determined using International Classification of Diseases, Ninth Revision (ICD-9) and Tenth Revision (ICD-10) codes ≥30 days from study enrollment and Social Security Death Index (SSDI) and National Death Index (NDI) data. A composite outcome of unstable angina, non-fatal MI, non-fatal stroke and all-cause mortality (MACE) was defined and used in time-to-event analyses. To exclude procedural-related events, incident events were defined starting at 30 days after date of index catheterization and study enrollment.

Metabolomic Profiling
Tandem flow injection mass spectrometry was used to quantify 60 metabolites (45 acylcarnitines and 15 amino acids) on frozen plasma samples that were previously unthawed. Samples were collected under a standardized study protocol. Baseline metabolites measured in 996 TECOS samples, 978 EXSCEL samples and 1330 CATHGEN samples were used in the present analyses. As described previously, proteins were removed by precipitation (6). Acylcarnitines and amino acids were esterified with hot acidic methanol and n-butanol, respectively. Mass spectrometry was performed with a Xevo TQD instrument (Waters Corp., Milford, Massachusetts). Internal standards were added to enable quantitative assessment of metabolites (4); CVs have been previously reported (6). Metabolomic assays were performed by the Metabolomics Core Laboratory at the Duke Molecular Physiology Institute. Staff from the Core Laboratory were blinded to clinical characteristics and outcomes of participant samples.

**Statistics**

Metabolites with >25% of values below lower limits of detection (LOD) were excluded from analyses (one metabolite: C7-DC acylcarnitine); metabolite values below LOD were analyzed as “0”. Given high collinearity between metabolites residing in shared biologic pathways, PCA with varimax rotation was used for dimensionality reduction in the TECOS discovery cohort, resulting in orthogonal factors composed of a weighted sum of correlated metabolites. PCA factors with an eigenvalue >1 were retained (Kaiser criterion). PCA weights for each metabolite within each factor as created in the TECOS study population were projected onto each individual in the EXSCEL cohort to create the same metabolite factors. All metabolites were log-transformed prior to analyses.

For the matched sample set in the TECOS discovery cohort, conditional logistic regression of PCA factors was used to test association with MACE events in both univariate and multivariate models using FDR p<0.1. Multivariate models in TECOS included relevant covariates for which participants were not matched: age, sex, race, systolic blood pressure and smoking status. PCA metabolite factors significant after adjustment for multiple comparisons in TECOS were then assessed for association with MACE in the EXSCEL validation cohort using both univariate and multivariate logistic regression; nominal validation was considered at p<0.05.
Multivariate models in EXSCEL were adjusted for age, sex, race, history of HF, CAD, BMI, HbA1c, systolic blood pressure, creatinine, LDL-C and smoking status. Median imputation was used for missing LDL-C values.

Post-hoc exploratory meta-analyses of multivariate models from TECOS and EXSCEL were then performed using the meta package in R; metabolite factors with nominal p<0.05 were identified. Analyses were also conducted for subcomponents of MACE (non-fatal MI, non-fatal stroke, CV death) and meta-analyzed. Sensitivity analyses of individual metabolites within PCA factors significant in meta-analyses with an absolute factor loading (>0.4) was also performed using meta-analyses, to determine the most important individual metabolites (p<0.05).

Given that the case:control study design of the TECOS and EXSCEL cohorts precluded time-to-event analyses, we used the CATHGEN cohort to determine if identified significant individual metabolites from the TECOS/EXSCEL analyses were associated with time-to-MACE. Specifically, we used an accelerated failure time (AFT) parametric model based on a Weibull distribution to estimate the relationship between time-to-MACE event and significant metabolites. We used ROC curves with AUC analyses to assess the incremental value of metabolites significant in the AFT analyses to predict MACE on top of a clinical model (age, sex, race, history of HF, CAD, BMI, HbA1c, systolic blood pressure, creatinine, LDL-C and smoking status). Net reclassification index (NRI) and integrated discrimination improvement (IDI) analyses were used to evaluate the improvement in prognostic discrimination from adding significant metabolites (37, 38). Median imputation was used for missing HbA1c, creatinine and LDL-C values. Significance was considered at p<0.05.

A composite score was then created from significant metabolites. Given the variable magnitude in absolute value of metabolites, metabolites were summed, log-transformed, and then scaled for analyses. In CATHGEN, C12-OH/C10-DC had 15% over values below lower limits of quantification; these values were median imputed for composite metabolite score calculations. This composite sum was then tested in univariate and multivariate models for MACE using conditional logistic regression followed by meta-analysis in TECOS and EXSCEL and using AFT models in CATHGEN. Composite metabolite scores were tested by as continuous variables in each cohort. The composite metabolite score was also used to test outcomes in the MACE subcomponents non-fatal MI, non-stroke and CV death in TECOS and EXCSEL and meta-analyzed with the score treated as a continuous variable.
To assess whether metabolite levels can be modified by GLP-1 RA, we tested the change in metabolite levels between baseline and 12-month plasma samples from 973 EXSCEL participants using Mann-Whitney-Wilcoxon tests for significant individual metabolites. Only baseline placebo-arm participant biospecimens from the TECOS trial were available for the present analyses, therefore change in metabolite level with sitagliptin could not be tested. For these exploratory analyses significance was considered at nominal p<0.05. To determine whether change in metabolite with exenatide was explained by weight loss with exenatide, we performed a linear mixed model with random intercepts for participants, including an interaction term between Timepoint and treatment, and adjusting for change in weight. The composite metabolite score was also used to test for interactions with exenatide treatment using linear mixed models. Finally, the correlation between change in metabolite score and change in weight was tested.

**Study Approval**

All study participants in TECOS, EXSCEL and CATHGEN gave written informed consent for participation in the parent study and for use of their stored biospecimens for future use. The institutional review board (IRB) for each site approved the primary studies and the Duke IRB approved this biomarker substudy.

**Data Availability**

The datasets generated for these analyses will be shared on reasonable request to the corresponding author for review by the EXSCEL and TECOS publications committees and the CATHGEN steering committee.

**Author Contributions:**


**Acknowledgements**

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trial was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. The parent EXSCEL trial was funded by Amylin Pharmaceuticals, Inc., a wholly owned subsidiary of AstraZeneca. The authors would like to thank Cindy M. Westerhout for assistance in design and selection of the TECOS case and control samples.

References


Figure 1. Overview of study design and statistical methods in cohorts with T2D and metabolomic profiling

Previous CVD defined as a history of major coronary artery disease, ischemic cerebrovascular disease or atherosclerotic peripheral arterial disease. T2D, Type 2 Diabetes Mellitus; TECOS, Trial Evaluating CV Outcomes with Sitagliptin; EXSCEL, Exenatide Study of CV Event Lowering; CATHGEN, Cardiac CATHeterization Genetics; CVD, cardiovascular disease; CAD, coronary artery disease; MI, myocardial infarction, HbA1C, hemoglobin A1C; MACE, Major Adverse Cardiac Events; ROC, receiving operator characteristic; AUC, area under the curve; NRI, net reclassification index; IDI, integrated discrimination index.

<table>
<thead>
<tr>
<th>T2D cohorts with targeted metabolomic profiling</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TECOS</strong> Discovery cohort</td>
</tr>
<tr>
<td>• 14,671 with T2D enrolled in randomized trial of DPP-4 inhibitor sitagliptin</td>
</tr>
<tr>
<td>• 7,226 placebo arm participants</td>
</tr>
<tr>
<td>• 29.3% female</td>
</tr>
<tr>
<td>• Mean age 65.5 years</td>
</tr>
<tr>
<td>• Mean HbA1C 7.2%</td>
</tr>
<tr>
<td>• 100% with previous CVD</td>
</tr>
<tr>
<td><strong>EXSCEL</strong> Validation cohort</td>
</tr>
<tr>
<td>• 14,752 with T2D enrolled in randomized trial of GLP-1 receptor agonist exenatide</td>
</tr>
<tr>
<td>• 38.0% female</td>
</tr>
<tr>
<td>• Median age 62.0 years</td>
</tr>
<tr>
<td>• Median HbA1C 8.0%</td>
</tr>
<tr>
<td>• 73.1% with previous CVD</td>
</tr>
<tr>
<td><strong>CATHGEN</strong> Validation cohort</td>
</tr>
<tr>
<td>• 9,334 participants enrolled at time of cardiac catheterization</td>
</tr>
<tr>
<td>• 37.8% female</td>
</tr>
<tr>
<td>• Mean age 62.0 years</td>
</tr>
<tr>
<td>• 1,616 with T2D</td>
</tr>
<tr>
<td>• 46.7% with previous CVD</td>
</tr>
</tbody>
</table>

Metabolomic profiling of 45 acylcarnitines and 15 amino acids

996 with metabolomics data → 498 MACE cases / 498 controls
• 4-point MACE: CV death, unstable angina, non-fatal MI and non-fatal stroke
  • Median follow-up 3.0 years

978 with metabolomics data → 487 MACE cases / 491 controls
• 3-point MACE: CV death, non-fatal MI and non-fatal stroke
  • Median follow-up 3.2 years

1330 with metabolomics data → 664 MACE cases / 666 controls
• 4-point MACE: All-cause mortality as a surrogate for CV death, unstable angina, MI and stroke
  • Median follow-up 4.8 years

Biomarker discovery and meta-analysis for MACE

• PCA metabolite factors tested → Validation of metabolite factors
  
  **Table 2**

  • Multivariate meta-analysis of metabolite factors
    • Figure 2, Supplemental Table 3

  • Meta-analysis of individual metabolites from significant factors
    Figure 3, Supplemental Table 5

Medium- and long-chain acylcarnitines as biomarkers for MACE

• Composite metabolite score
  Figure 4, Supplemental Table 6-7

• Assessment of change in metabolite with exenatide therapy
  Supplemental Figure 6, Supplemental Table 9

• Time-to-MACE event analysis
  Table 3, Supplemental Figure 1

  • Significant metabolites tested for incremental risk prediction using ROC curves, AUC, NRI & IDI
  Supplemental Figure 5
Figure 2. Meta-analysis of PCA metabolite factors for association with MACE in the combined TECOS and EXSCEL cohorts

Forest plot shows multivariate odds ratio for meta-analysis in TECOS and EXSCEL for association between PCA metabolites factors with MACE. Factor 1 is comprised of medium- and long-chain acylcarnitines, Factor 3 is comprised of long-chain dicarboxylacylcarnitines and Factor 9 is comprised of medium chain acylcarnitines (p<0.05).
Figure 3. Multivariate models for individual metabolites heavily loaded on PCA factors significantly associated with MACE in meta-analysis

Forest plot shows multivariate meta-analysis of individual metabolites heavily loaded on Factors 1, 3 and 9 in TECOS and EXSCEL. Nine significant metabolites are shown in bold: C8, C10:1, C12-OH/C10/DC, C12:1, C12, C14:1, C14:2, C16:2, C16:1. MCACs, Medium-chain acylcarnitines; LCACs, Long-chain acylcarnitines; LCDAs, Long-chain dicarboxylic acylcarnitines.
Figure 4. Kaplan-Meier curve for MACE in CATHGEN by tertile of nine-metabolite score

Kaplan-Meier curve for MACE in CATHGEN by tertile of the nine-metabolite score (C8, C10:1, C12-OH/C10/DC, C12:1, C12, C14:1, C14:2, C16:2, C16:1). Events were defined starting at 30 days after date of index catheterization and study enrollment to avoid procedural related events. 664 individuals suffered MACE in CATHGEN with a median time-to-event of 509 days.
Medium- and long-chain fatty acids are transported across the plasma membrane by fatty acid transporters and then converted into their acyl-CoA and cross the outer mitochondrial membranes. The inner mitochondrial membrane is impermeable to acyl-coAs and carnitine palmitoyl transferase I is required to esterify the acyl-CoA plus carnitine into acylcarnitines. Inside the inner mitochondrial membrane carnitine acyltransferase is reverse esterified back to an acyl-CoA to undergo β-oxidation with carbon chain removal until an acetyl-CoA remains to enter the TCA cycle, and ultimately the electron transport chain for ATP energy production. In states of impaired mitochondrial fatty acid β-oxidation and metabolic inflexibility, plasma levels of medium- and long-chain acylcarnitines levels may increase. Based on the present analyses in T2D, increased levels of medium- and long-chain acylcarnitines associate with incident MACE. The working hypothesis for the data presented here is that inefficient fatty acid oxidation in the mitochondria contribute to the bottle neck of substrate utilization and build-up of circulating levels of acylcarnitines. However, the tissue source is not clear and alternatively, accumulations in these metabolites could also reflect increased mitochondrial fatty acid flux with incomplete fatty acid oxidation. CPT I, carnitine palmitoyltransferase I; CPT II, carnitine palmitoyltransferase II; TCA, tricarboxylic acid; ATP, adenosine triphosphate.

Figure 5. Mitochondrial Markers of Dysregulated Fatty Acid Oxidation Associate with MACE in T2D

Mitochondrial Markers of Dysregulated Fatty Acid Oxidation Associate with MACE in T2D
### Table 1. Baseline Characteristics by Study

<table>
<thead>
<tr>
<th></th>
<th>TECOS</th>
<th>EXSCEL</th>
<th>CATHGEN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>996</td>
<td>978</td>
<td>1330</td>
</tr>
<tr>
<td><strong>Age (mean [SD], years)</strong></td>
<td>66.19 (8.09)</td>
<td>65.29 (8.49)</td>
<td>60.50 (11.50)</td>
</tr>
<tr>
<td><strong>Sex, male (%)</strong></td>
<td>727 (73.0)</td>
<td>739 (75.6)</td>
<td>749 (56.3)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, N (%)</td>
<td>784 (78.7)</td>
<td>847 (86.6)</td>
<td>811 (61.0)</td>
</tr>
<tr>
<td>Black, N (%)</td>
<td>29 (2.9)</td>
<td>18 (1.8)</td>
<td>413 (31.1)</td>
</tr>
<tr>
<td>Other, N (%)</td>
<td>183 (18.4)</td>
<td>113 (11.6)</td>
<td>106 (8.0)</td>
</tr>
<tr>
<td><strong>Body-mass index (mean [SD], kg/m²)</strong></td>
<td>31.30 (5.91)</td>
<td>33.35 (6.04)</td>
<td>32.89 (7.97)</td>
</tr>
<tr>
<td><strong>Systolic BP (mean [SD], mmHg)</strong></td>
<td>135.54 (17.62)</td>
<td>134.84 (16.06)</td>
<td>149.46 (25.92)</td>
</tr>
<tr>
<td><strong>Hypertension, N (%)</strong></td>
<td>884 (88.8)</td>
<td>889 (90.9)</td>
<td>1048 (78.8)</td>
</tr>
<tr>
<td><strong>Dyslipidemia, N (%)</strong></td>
<td>793 (79.6)</td>
<td>852 (87.1)</td>
<td>937 (70.5)</td>
</tr>
<tr>
<td><strong>CAD, N (%)</strong></td>
<td>808 (81.1)</td>
<td>750 (76.7)</td>
<td>919 (69.5)</td>
</tr>
<tr>
<td><strong>MI, N (%)</strong></td>
<td>484 (48.6)</td>
<td>501 (51.2)</td>
<td>370 (27.8)</td>
</tr>
<tr>
<td><strong>HF, N (%)</strong></td>
<td>290 (29.1)</td>
<td>277 (28.3)</td>
<td>475 (36.5)</td>
</tr>
<tr>
<td><strong>Cerebrovascular disease, N (%)</strong></td>
<td>253 (25.4)</td>
<td>230 (23.5)</td>
<td>141 (10.6)</td>
</tr>
<tr>
<td><strong>PAD, N (%)</strong></td>
<td>131 (13.2)</td>
<td>200 (20.4)</td>
<td>132 (9.9)</td>
</tr>
<tr>
<td><strong>Smoking, N (%)</strong></td>
<td>114 (11.4)</td>
<td>548 (56.0)</td>
<td>647 (48.6)</td>
</tr>
<tr>
<td><strong>HbA1c% (mean [SD])</strong></td>
<td>7.28 (0.58)</td>
<td>8.16 (0.94)</td>
<td>7.71 (1.67)</td>
</tr>
<tr>
<td><strong>Creatinine (mean [SD], mg/dL)</strong></td>
<td>1.03 (0.28)</td>
<td>1.09 (0.30)</td>
<td>1.41 (1.50)</td>
</tr>
<tr>
<td><strong>Total Cholesterol (mean [SD], mg/dL)</strong></td>
<td>166.76 (46.51)</td>
<td>177.32 (215.35)</td>
<td>179.70 (59.38)</td>
</tr>
<tr>
<td><strong>LDL-C (mean [SD], mg/dL)</strong></td>
<td>92.66 (40.39)</td>
<td>91.52 (42.31)</td>
<td>92.13 (30.33)</td>
</tr>
<tr>
<td><strong>HDL-C (mean [SD], mg/dL)</strong></td>
<td>42.46 (12.39)</td>
<td>44.22 (40.58)</td>
<td>44.03 (14.10)</td>
</tr>
</tbody>
</table>

Data presented as mean (standard deviation) or N (%). BP, blood pressure; CAD, coronary artery disease; MI, myocardial infarction; HF, heart failure; PAD, peripheral arterial disease; HbA1c, hemoglobin A1C; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol. Smoking variable definitions: TECOS (Current Smoker=1; Not Current Smoker=0); EXSCEL and CATHGEN (Current or Former Smoker =1; Never=0)
Table 2. Principal components analysis (PCA) metabolite factors and their association with MACE in TECOS discovery cohort and EXSCEL validation cohort

<table>
<thead>
<tr>
<th>Factor</th>
<th>Factor Name</th>
<th>Metabolites†</th>
<th>TECOS</th>
<th>EXSCEL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td>1</td>
<td>Medium- and long-chain acylcarnitines</td>
<td>C6, C8, C10:1, C10, C12:1, C12, C14:2, C14:1, C14, C16:2, C16:1</td>
<td>1.07 (0.93-1.23)</td>
<td>1.09 (0.92-1.28)</td>
</tr>
<tr>
<td>2</td>
<td>Long-chain acylcarnitines and short-chain dicarboxylicarnitines</td>
<td>C16, C18:2, C18:1, C18, C20:4, C4-DC/C4-DC, C5-OH/C3-DC, Ser</td>
<td>1.44 (1.05-1.98)</td>
<td>1.48 (1.06-2.07)</td>
</tr>
<tr>
<td>3</td>
<td>Long-chain dicarboxylicarnitines</td>
<td>C20, C22, C12-OH/C10-DC, C16-OH/C14-DC, C18:1-DC, C18:1-OH/C16:1-DC, C18-OH/C16-DC, C20-OH/C18-DC</td>
<td>0.94 (0.82-1.07)</td>
<td>0.91 (0.79-1.05)</td>
</tr>
<tr>
<td>4</td>
<td>Branched chain amino acids</td>
<td>Leu/Ile, Met, Orn, Phe, Tyr, Val</td>
<td>1.01 (0.88-1.15)</td>
<td>0.95 (0.82-1.10)</td>
</tr>
<tr>
<td>5</td>
<td>Medium- and long-chain dicarboxylicarnitines</td>
<td>C2, C4-OH, C12:1, C6-DC/C8-OH, C8:1-DC, C8:1-OH/C6:1-DC, C10-OH/C8-DC, C12-OH/C10-DC, C14:1-OH, C14-OH/C12-DC, C16:1-OH/C14:1-DC</td>
<td>1.16 (1.02-1.33)</td>
<td>1.12 (0.97-1.30)</td>
</tr>
<tr>
<td>6</td>
<td>Amino acids and C5-DC</td>
<td>C5-DC, Arg, Cit, Orn</td>
<td>1.02 (0.90-1.17)</td>
<td>0.98 (0.85-1.13)</td>
</tr>
<tr>
<td>7</td>
<td>Amino acids</td>
<td>Ala, Gly, Pro, Ser</td>
<td>0.90 (0.79-1.03)</td>
<td>0.89 (0.76-1.02)</td>
</tr>
<tr>
<td>8</td>
<td>C3-C5 and short-chain dicarboxylicarnitines</td>
<td>C3, C4/C4, C5, C5-DC, C5-OH/C3-DC</td>
<td>1.01 (0.89-1.15)</td>
<td>1.02 (0.89-1.18)</td>
</tr>
<tr>
<td>9</td>
<td>Medium-chain acylcarnitines</td>
<td>C8:1, C10:3, C10:2, C12:1</td>
<td>1.26 (1.11-1.45)***</td>
<td>1.29 (1.11-1.49)***</td>
</tr>
<tr>
<td>10</td>
<td>C5:1 and Medium-chain dicarboxylicarnitines</td>
<td>C5:1, C8:1-OH/C6:1-DC</td>
<td>1.18 (1.04-1.34)</td>
<td>1.11 (0.97-1.28)</td>
</tr>
<tr>
<td>11</td>
<td>Glutamate/Glutamic Acid and Arginine</td>
<td>Glx, Arg</td>
<td>0.93 (0.82-1.05)</td>
<td>0.97 (0.84-1.11)</td>
</tr>
<tr>
<td>12</td>
<td>C18:2-OH</td>
<td>C18:2-OH</td>
<td>0.99 (0.87-1.11)</td>
<td>1.00 (0.88-1.15)</td>
</tr>
</tbody>
</table>

Multivariate models in TECOS include relevant covariates for which participants were not matched: age, gender, race, systolic blood pressure and smoking status. Multivariate models in EXSCEL were adjusted for age, gender, race history of heart failure, coronary artery disease, body-mass index, hemoglobin A1C, systolic blood pressure, creatinine, low density lipoprotein cholesterol and smoking status. FDR adjusted p-values <0.1 were considered statistically significant in the discovery cohort. †Metabolites within the PCA factor with an absolute value of load on the factor >0.4, *p<0.1, **p<0.05, ***p<0.01. FDR, false discovery rate.
Table 3. Accelerated failure time model for association of individual metabolites with time-to-MACE in the CATHGEN cohort.

| Metabolite* | Univariate |  |  |  |  |  |  |  |
|-------------|------------|---|---|---|---|---|---|
|             | HR (95% CI) | p  | HR (95% CI) | p  |
| C10:1       | 2.07 (1.76-2.44) | 2.2x10^{-18} | 1.57 (1.31-1.89) | 1.5x10^{-6} |
| C10         | 1.22 (1.10-1.36) | 1.3x10^{-4} | 1.09 (0.98-1.21) | 0.1 |
| C12         | 1.69 (1.48-1.93) | 4.7x10^{-15} | 1.40 (1.21-1.63) | 9.5x10^{-6} |
| C14:2       | 1.28 (1.14-1.44) | 4.4x10^{-5} | 1.13 (1.02-1.25) | 0.02 |
| C14:1       | 1.57 (1.36-1.81) | 9.5x10^{-10} | 1.34 (1.16-1.55) | 1.0x10^{-4} |
| C14         | 1.13 (1.02-1.25) | 0.02 | 1.05 (0.95-1.17) | 0.3 |
| C16:1       | 1.33 (1.15-1.54) | 1.5x10^{-4} | 1.24 (1.07-1.43) | 0.005 |
| C12:1       | 1.90 (1.62-2.23) | 4.8x10^{-15} | 1.45 (1.22-1.73) | 3.0x10^{-6} |
| C8:1        | 1.41 (1.22-1.63) | 3.9x10^{-6} | 1.07 (0.91-1.26) | 0.4 |
| C10:2       | 1.23 (1.12-1.34) | 1.9x10^{-5} | 1.07 (0.97-1.18) | 0.2 |
| C8          | 1.58 (1.38-1.81) | 5.7x10^{-11} | 1.30 (1.13-1.51) | 4.1x10^{-4} |
| C16:2       | 1.18 (1.09-1.27) | 1.9x10^{-5} | 1.12 (1.04-1.21) | 0.003 |
| C12-OH/C10-DC | 1.17 (1.11-1.24) | 4.4x10^{-9} | 1.08 (1.02-1.14) | 0.008 |

The 13 metabolites identified in TECOS and EXSCEL were associated with time-to-MACE in CATHGEN in univariate models and nine of these metabolites remained significant in multivariate models. *All listed metabolites are acylcarnitines.