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ORIGINAL ARTICLE

Relationships of Circulating Plasma Metabolites With the QT Interval in a Large Population Cohort

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BACKGROUND: There is a higher prevalence of heart rate corrected QT (QTc) prolongation in patients with diabetes and metabolic syndrome. QT interval genome-wide association studies have identified candidate genes for cardiac energy metabolism, and experimental studies suggest that polyunsaturated fatty acids have direct effects on ion channel function. Despite this, there has been limited study of metabolite concentration relationships with QT intervals.

METHODS: In 21 056 UK Biobank participants with same-day electrocardiograms and plasma profiling of 100 metabolites, per-metabolite regression analyses with the QTc were performed adjusting for clinically relevant variables. Participants with ischemic heart disease or heart failure were excluded. Significant metabolites ($P<5\times10^{-4}$) that replicated in an independent UK Biobank sample (N=5304), underwent Least Absolute Shrinkage and Selection Operator regression with clinical variables to identify top predictors and calculate the QTc variance explained. Two-sample Mendelian randomization and locus-level colocalization analyses were performed to test for causal relationships and shared genetic etiologies, respectively.

RESULTS: Twenty-two metabolites were associated with the QTc in main and replication regression analyses, including ketone bodies, fatty acids, glycolysis-related molecules, and amino acids. Top associations were 3-hydroxybutyrate (8.9 ms), acetone (7.9 ms), and polyunsaturated fatty acids (−7.3 ms), when comparing the highest versus lowest deciles. A combined metabolite and clinical variables Least Absolute Shrinkage and Selection Operator model significantly increased the QTc variance explained compared with the clinical-only model (11.2% versus 7.7%; $P=0.002$). There was support for a causal relationship between Linoleic acid to fatty acid ratio and the QTc, and evidence for colocalization for 15 metabolites at 7 QT loci, including *CASR* for citrate and glutamine.

CONCLUSIONS: In the largest study of metabolite-QTc relationships, we identify 22 associated metabolites and clinically relevant effect sizes, with evidence for genetic support. For the first time, we report a potentially protective effect of polyunsaturated fatty acids in humans. These metabolites may be risk factors in acquired and congenital long-QT syndrome and warrant additional investigation for arrhythmia risk stratification.

Key Words: acetones ■ fatty acids ■ heart rate ■ prevalence ■ risk factors

The QT interval is an ECG measure that primarily reflects ventricular repolarization time.[1] For every 10 ms increase in the QT interval above normal limits, it is estimated the incidence of ventricular arrhythmia increases by 5% to 7%.[2] Congenital long-QT syndrome (LQTS), is caused by pathogenic variants in genes encoding cardiac ion channel subunits (including *KCNQ1*, *KCNH2*, and *SCN5A*).[3,4] QT prolongation can also be caused by medications, abnormal electrolyte concentrations, and comorbidities (eg, ischemic heart disease), which in combination have an additive adverse effect on ventricular repolarization leading to an acquired LQTS and increased risk of malignant ventricular arrhythmia.[5,6]

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Nonstandard Abbreviations and Acronyms

GWAS	genome-wide association study
HDL	high-density lipoprotein
LASSO	Least Absolute Shrinkage and Selection Operator
LQTS	long-QT syndrome
MR	Mendelian randomization
NMR	nuclear magnetic resonance
QTc	heart rate corrected QT interval

There is increasing interest in the role of the metabolome in cardiac repolarization. A higher prevalence of QT prolongation is observed in patients with diabetes, obesity and metabolic syndrome.[5,7,8] Rare metabolic disorders (eg, Primary Carnitine Deficiency and Propionic Acidemia) are also associated with pathological QT interval changes and manifest in pediatric patients, where there are fewer potential confounding factors.[9,10] There is genetic support for the role of cardiac energy substrate utilization and insulin receptor signaling from QT interval genome-wide association studies,[11,12] and experimental evidence for a direct effect of polyunsaturated fatty acids and polyunsaturated fatty acid-analogues (eg, docosahexaenoic acid, linoleic-glycine, and linoleic-tyrosine) on cardiac potassium repolarizing currents.[13,14]

Despite these observations, there has been limited study of the relationships between individual circulating serum metabolite concentrations and the QT interval in humans. In 14 LQTS type-1 patients and 28 controls, an increase in the QT interval was observed following a 75 g oral glucose tolerance test (mean increase 19.8 ms and 14.2 ms, respectively)[15], demonstrating the potential for glucose to cause dynamic effects on the QT interval. In 32 male shift workers, significant correlations were reported with blood glucose, lactate, pyroglutamate, acetate, and 3-hydroxybutyrate concentrations.[16] A J-shaped relationship has previously been reported between serum concentrations of high-density lipoprotein (HDL) and the QT interval in females (N=619) in a population-based study,[17] while no association was observed in 440 male patients with primary hypercholesterolemia.[18] These findings suggest there may be sex-specific differences in metabolite-QT associations; however, the sample sizes of these studies were small and did not adjust for important confounding factors, including smoking status or dietary factors.

In this study, we aimed to establish the relationships of 100 metabolites with the QT interval in over 25 000 UK Biobank participants with internal replication, and to test whether metabolites significantly increase the variance explained of the QT interval in a prediction model. We also performed Mendelian randomization (MR) and colocalization analyses to test for causal relationships and shared genetic etiologies for observed metabolite-QT relationships. An overview of the study design is provided in Figure 1.

METHODS

Transparency and Openness Promotion Statement

Data generated by this study will be returned to the UK Biobank in accordance with researcher obligations, to be made available for further research. The UK Biobank study has approval from the National Health Service Northwest Multi-center Research Ethics Committee (ref 11/NW/0382), and all participants provided informed consent.[19]

Study Population

The UK Biobank is a prospective study of ≈500 000 individuals aged 40 to 69 years at recruitment (2006–2008), with a rich source of demographic, phenotypic (including ECGs), and genetic data.[19] In July 2023, nuclear magnetic resonance (NMR) plasma metabolite biomarkers were also made available by Nightingale Health Plc.[20]

NMR Metabolic Biomarker Data

Details of the NMR biomarker profiling platform and experimental procedures (ethylenediaminetetraacetic acid plasma sampling, NMR spectroscopy, biomarker quantification, and quality control) have been described previously.[20,21] NMR profiling of 249 metabolites is available for 274 315 UK Biobank participants sampled at recruitment, and for an additional 16 707 participants whose blood samples were obtained at a repeat visit (Table S1).[20] In addition to the quality control performed by Nightingale Health Plc, the R package ukbnmr (v2.2.1) was used to remove technical variation.[22] Each metabolite was adjusted for time between sample measurement and preparation, systematic differences between well plates and spectrometer drift, and outlying samples of nonbiological origin were removed.[22] As is standard practice in metabolomic studies, serum concentrations were log-transformed to account for rightward skew in the distribution of measurements and zero values.[20] Individuals with metabolite concentrations±4 SD from the mean were excluded, as they may reflect measurement errors (total excluded=2750). As the 249 metabolites also include lipoprotein subclasses defined by particle size that are highly correlated, lipoprotein subclasses with an absolute Spearman correlation >0.6 were randomly excluded, with 100 metabolites remaining for analysis in this study (Table S2).

Calculation of ECG Parameters

Heart rate corrected QT (QTc) intervals were calculated from ECG signals obtained during a 15-second resting phase before undergoing an exercise test.[11,23] ECG signal processing and marker annotation were performed using MATLAB, version 2018b.[24] A bandpass Butterworth filter was applied (0.5–45 Hz) for noise reduction. ECGs with fewer than 5 QRS complexes or an intrabeat correlation <0.8 were excluded. Signal averaging was implemented to further reduce noise, as previously described.[25] QRS onset and offset were obtained using

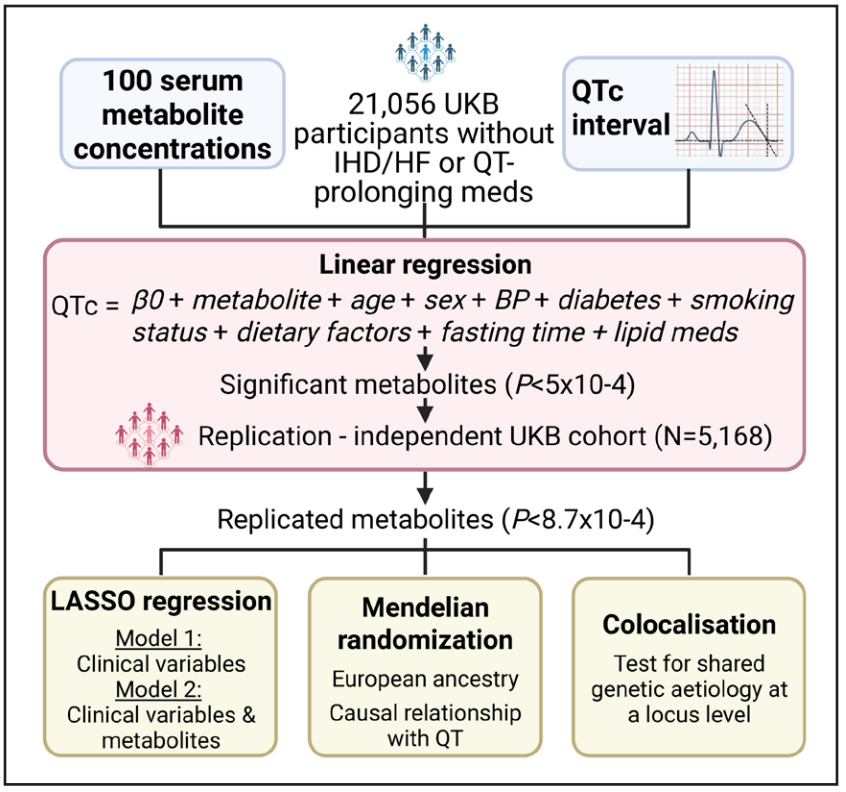

Figure 1. Study design.
Overview of study design. HF indicates heart failure; IHD indicates ischemic heart disease. Created using BioRender by Young (2025).

the Hilbert envelope method.[26] T-wave end was defined as the tangent from the minimum of the first derivative of the T-wave slope down to the isoelectric line. The QT interval was the time interval from QRS onset to T-wave end. All intervals were measured in milliseconds. Outliers were manually reviewed (≈5% of ECGs) using a graphical user interface in MATLAB. In total, QT intervals for 51 971 individuals passed quality control and the QTc was calculated using Bazett formula (QTc=QT/√RR).[27]

UK Biobank Subcohorts Included in the Analyses

In total, 23 609 UK Biobank individuals had metabolite measurements and ECG recordings taken on the same day at their recruitment visit. Participants with a diagnosis of ischemic heart disease or heart failure, at baseline or within 6 months of recruitment (identified using *International Classification of Diseases*-9 or -10 and self-reported entries), were excluded. Participants identified through the recruitment interview to be taking medication with established effects on the QT interval were also excluded (Table S3). The remaining 21 056 individuals formed the training cohort for this study.

A separate group (N=5168 after exclusions) had metabolite and QTc measurements taken at a second visit. These were not in the training cohort and formed the independent test cohort for this study.

Statistical Analyses

Relationship of Individual Metabolites With the QTc Interval

As the association of most metabolites with the QTc interval is unknown, per-metabolite linear regression analyses were performed in the training cohort. Each metabolite model was adjusted for clinically relevant covariates that were recorded at the same time point. These were age, sex, body mass index, average systolic blood pressure, smoking status, diabetes diagnosis, prescribed lipid medication, dietary factors, alcohol intake frequency, and fasting time before blood sampling. Smoking status was coded as binary (yes for current smokers and no if not smoking at the time of the visit). Dietary information was used to capture individuals with higher-than-average intake of fresh fruit (≥4 items per day), fish or meat (≥4 items of oily fish, nonoily fish, processed or fresh meat per week), and dairy (≥4 items of cheese per week or full-cream milk), which could influence circulating metabolite levels. Alcohol intake frequency was included as a categorical variable; daily, 3 to 4× per week, once or twice per week, rarely, or never.

A Bonferroni-corrected threshold was used to account for multiple testing (*P* value [*P*]<5×10^{-4}[0.05/100]). Significant metabolites were then tested for replication in the test cohort (*P*<[0.05/number of metabolites tested]). As there are known sex differences in QTc intervals, a secondary analysis was performed to test for the presence of sex interactions with metabolite-QTc interval associations by including sex as an interaction term in the model, using the same training and test data sets and Bonferroni-corrected thresholds to declare significant findings.

LASSO Regression Model for Metabolite Feature Selection

Least Absolute Shrinkage and Selection Operator (LASSO) 10-fold cross-validation linear regression analyses using the R package glmnet (version 4.1.8) were used to develop models that explain the greatest proportion of the variance of the

QTc interval based on the input variables. LASSO regression shrinks the coefficients of less important variables toward zero, handling multicollinearity and enabling selection of the best predictors.[28] Two models were developed in the training cohort; model 1: clinically relevant covariates only, model 2: clinically relevant covariates and the replicated metabolites (combined model). The root mean squared error and adjusted R-squared were calculated to estimate the variance of QTc interval explained by each model using the R package caret (version 6.0.94). The performance of both models was than compared in the test cohort using the root mean squared error and adjusted R-squared statistics by Fisher transformation.

MR Study

MR was used to test for a causal relationship between replicated metabolites and the QT interval. First, a genome-wide association study (GWAS) of each metabolite was performed using BOLT-LMM (v2.4.1)[29] in 183 734 individuals of European ancestry without ECG data and without a prior diagnosis of ischemic heart disease or heart failure. Variants were excluded from the analysis if the minor allele frequency was <1%, or imputation quality score <0.3. BOLT-LMM software utilizes a linear mixed model and linkage disequilibrium to account for relatedness and population structure.[29] The same covariates included in the metabolite linear regression analyses were also used for the GWAS along with genotype array (UK Biobank or UK BiLEVE). For each metabolite, instrumental variables for MR were selected by linkage disequilibrium clumping of genome-wide significant variants (r^2>0.001, distance 10 000 kb) in PLINKv1.9, using the 1000 Genomes Phase III reference panel.[30,31] Only 1 variant was selected from each locus, defined as a window ±500 kb from the lead variant. The F-statistic was calculated for each instrumental variable, which is a measure of its strength of association with the exposure of interest.

For each instrumental variable, QT interval summary statistics were extracted from a previously published European ancestry GWAS meta-analysis (N=212 199).[11] There were no overlapping participants between metabolite and QT GWAS summary statistics. Causal estimates were calculated using the inverse-variance weighted multiplicative random effects method with the TwoSampleMR R package.[32] A significant result was declared if an association passed the Bonferroni-corrected threshold (P<0.05/number of metabolites tested). Sensitivity analyses including MR-Egger, weighted mode, and weighted median methods, were performed to test for bias due to pleiotropy. Heterogeneity Cochran Q statistics were calculated along with the MR-Egger intercept test. Reverse MR was also performed by testing for an association between genetically predicted QT interval and each validated serum metabolite. Heritability estimates for each metabolite were calculated using BOLT-REML under the same regression model as the main GWAS analysis.[29] The percentage variance of the metabolite explained by lead variants was calculated using the following formula:

$$\text{PVE} = \frac{\left(2 \times [\beta^2] \times \text{MAF} \times [1 - \text{MAF}]\right)}{\left(2 \times [\beta^2] \times \text{MAF}\,[1 - \text{MAF}] + \left[(\text{SE}\,(\beta))^2\right] \times 2 \times \text{N} \times \text{MAF} \times [1 - \text{MAF}]\right)}.$$

Colocalization Analyses

To test for shared causal variants between the QT interval and metabolite at a locus level, metabolite-QT colocalization analyses were performed using the R package Hypothesis Prioritization for Multitrait Colocalization.[33] Hypothesis Prioritization for Multitrait Colocalization uses a Bayesian divisive clustering algorithm to identify traits that colocalize at distinct causal variants in a genomic region, using variant-level trait-specific coefficients and corresponding standard errors. Support for colocalization was declared if the posterior probability for a shared causal variant at a shared associated genomic region was >0.75. Multitrait colocalization was also used to identify shared causal variants at these loci across multiple traits using European ancestry GWAS summary statistics for type 2 diabetes,[34] metabolic syndrome,[35] coronary artery disease,[36] and heart failure.[37]

RESULTS

In total, 21 056 UK Biobank individuals were included in the training cohort with plasma metabolite measurements and ECG data obtained during their recruitment visit (Table). The median age was 58 years (interquartile range, 50–63) and the median QTc was 396 ms (interquartile range, 381–411). An independent set of 5168 individuals with metabolite and ECG measurements from their second UK Biobank visit, were used as the test cohort. Demographic information for each cohort (Table) were broadly similar, however as blood sampling for test group was performed at a second visit, they were older (median age 62 [interquartile range, 56–66]) and a greater proportion of individuals

Table. Comparison of UK Biobank Individuals in Training Versus Test Cohorts

	Training cohort	Test cohort
Sample size	21 056	5168
Sex % female	11 192 (53.2%)	2676 (51.8%)
Age, y	58 (50–63)	62 (56–66)
Body mass index, kg/m^2	26.6 (24.1–29.4)	26 (23.8–28.7)
Systolic blood pressure, mm Hg	136 (124.5–148.5)	137 (125.5–149.5)
QTc	396 (381–411)	394 (380–410)
Diabetes	691 (3.3%)	170 (3.4%)
Lipid lowering medication	2743 (13.0%)	869 (16.8%)
Current smoker	1782 (8.5%)	218 (4.2%)
Fresh fruit >3/d	3157 (15.0%)	735 (14.2%)
Fish/meat >3/d	1474 (7%)	346 (6.7%)
Dairy >4/d	3925 (18.6%)	983 (18.5%)
Daily alcohol intake	4297 (20.4%)	922 (17.8%)
Fasting time	4 (3–5)	4 (3–5)

Summary statistics are supplied as absolute numbers with percentages (%) or median values with interquartile range. kg indicates kilogram; m^2, meter squared; mmHg, millimeter of mercury; ms, millisecond; QTc, heart rate corrected QT interval.

were taking lipid lowering medication (17.9% verses 14.2%).

Per-metabolite Associations With the QTc Interval

In the per-metabolite linear regression model, 57 of the 100 tested metabolites had significant associations with the QTc interval after covariates adjustment ($P<5\times10^{-4}$; Table S4; Figure 2). For 14 metabolites, individuals in the top decile of the population metabolite distribution had an absolute QTc difference >5 ms compared with the bottom decile (Figure 3; Table S5). These included ketone bodies; 3-hydroxybutyrate (8.9 ms) and acetone (7.9 ms), and the ratios of polyunsaturated fatty acid (−7.3 ms), omega-6 fatty acid (−6.9 ms) and linoleic acid (−6.7 ms) to total fatty acids. Modifying the definition of a diagnosis of diabetes to also include any participant taking diabetic medication, increased the number of participants with this diagnosis by 9 (691 in total). This had no significant impact on the linear regression findings (Table S6).

Of the 57 significant metabolites, 22 were replicated ($P<8.7\times10^{-4}$ [0.05/57]) in the test cohort with concordant directions of effect. There was also suggestive support for a further 7 metabolites ($8.7\times10^{-4}<P<0.05$; Table S7). Replicated metabolites included amino acid (glutamine, glycine), ketone bodies, glycoprotein acetyls, glycolysis-related metabolites (citrate, glucose) and fatty acids (Table S8). Out of the 63 tested apolipoprotein, cholesterol, triglyceride and phospholipid metabolites, only cholesterol esters percentage in large LDL and phospholipid percentage in large HDL were significant. No association was observed for HDL ($P=0.56$) and there was no support for a nonlinear relationship when testing quartiles of the HDL distribution in men and women separately.

A significant sex-metabolite interaction was observed for 4 metabolites in the training cohort; glycine ($P=1.1\times10^{-7}$), glycoprotein acetyls ($P=9.5\times10^{-7}$), linoleic acid to total fatty acid ratio ($P=3.7\times10^{-3}$) and monosaturated fatty acid to total fatty acid ratio ($P=1.5\times10^{-4}$). However, none of these replicated in the test cohort (all interaction $P>0.0125$).

Variable Selection for Enhancing QTc Interval Prediction

To select the most important predictors for the QTc interval, 10-fold cross-validated LASSO linear regression models was constructed using the training data set. For model 1 (clinically relevant covariates only), age, sex, body mass index, systolic blood pressure and use of lipid lowering medication were retained in the model. The root mean squared error was 22.3 and adjusted R-squared was 0.103. In model 2 (clinically relevant covariates and the 22 replicated metabolites), ketone bodies (3-hydroxybutyrate, acetone), amino acids (glutamine, glycine), glycolysis-related metabolites (glucose, lactate), fatty acids (linoleic, polyunsaturated and saturated fatty acids) and lipoprotein subclasses (percentage of phospholipids in large HDL) were retained in the model along with the same clinical covariates as in model 1 (Table S9). For model 2, the root mean squared error was smaller (21.8) and the adjusted R-squared was 0.136, which is statistically significant improvement compared with model 1 ($P=2.04\times10^{-8}$). When applying these models in the independent test cohort, a similar improvement was observed; model 1 adjusted R-squared=0.077, model 2=0.112. Therefore, in the test data set, model 2 explained a statistically significant additional 3.5% of QTc variance ($P=0.002$), representing a 44.5% increase in the total variance explained compared with model 1.

MR Study

Lead variants from each metabolite GWAS in 184 140 UK Biobank individuals of European ancestry without ECG data or a prior diagnosis of ischemic heart disease or heart failure, were used as instrumental variables. The F-statistic was >10 for all instrumental variables indicating the level of weak instrumental bias is likely small. Only the 22 replicated metabolites were included (Table S10). Heritability estimates were 4.5% to 33.6% for the included metabolites and the percentage variance explained by lead variants was 0.4% to 9.1% (Table S11).

In the primary analysis using the inverse-variance weighted random effects method, only linoleic acid to total fatty acid ratio was significant after correction for multiple testing ($P=5.5\times10^{-5}$; Table S11). Sensitivity analyses (MR-Egger, weighted mode and weighted median analyses) were also significant with concordant directions of effect and a nonsignificant MR-Egger intercept P value. Leave-one-out tests demonstrated that the association for linoleic acid to total fatty acid ratio was driven by a single variant on chromosome 11 (rs174528; Figure S1). No significant associations were observed in reverse-MR analyses (Table S11).

Colocalization Analyses Between Replicated Metabolites and the QT Interval

To test for shared causal variants between the 22 replicated metabolites and the QT interval at individual genomic regions, colocalization analyses were performed using Hypothesis Prioritization for Multitrait Colocalization.[33] At 7 QT loci, 15 metabolites had support for colocalization (posterior probability >0.75; Table S12, Figure S2A through S2J). At 1 locus, 12 metabolites (citrate, mono- and polyunsaturated fatty acids) colocalized. Candidate genes at this locus are *FADS1* (Fatty acid desaturase 1) and *FADS2* (which

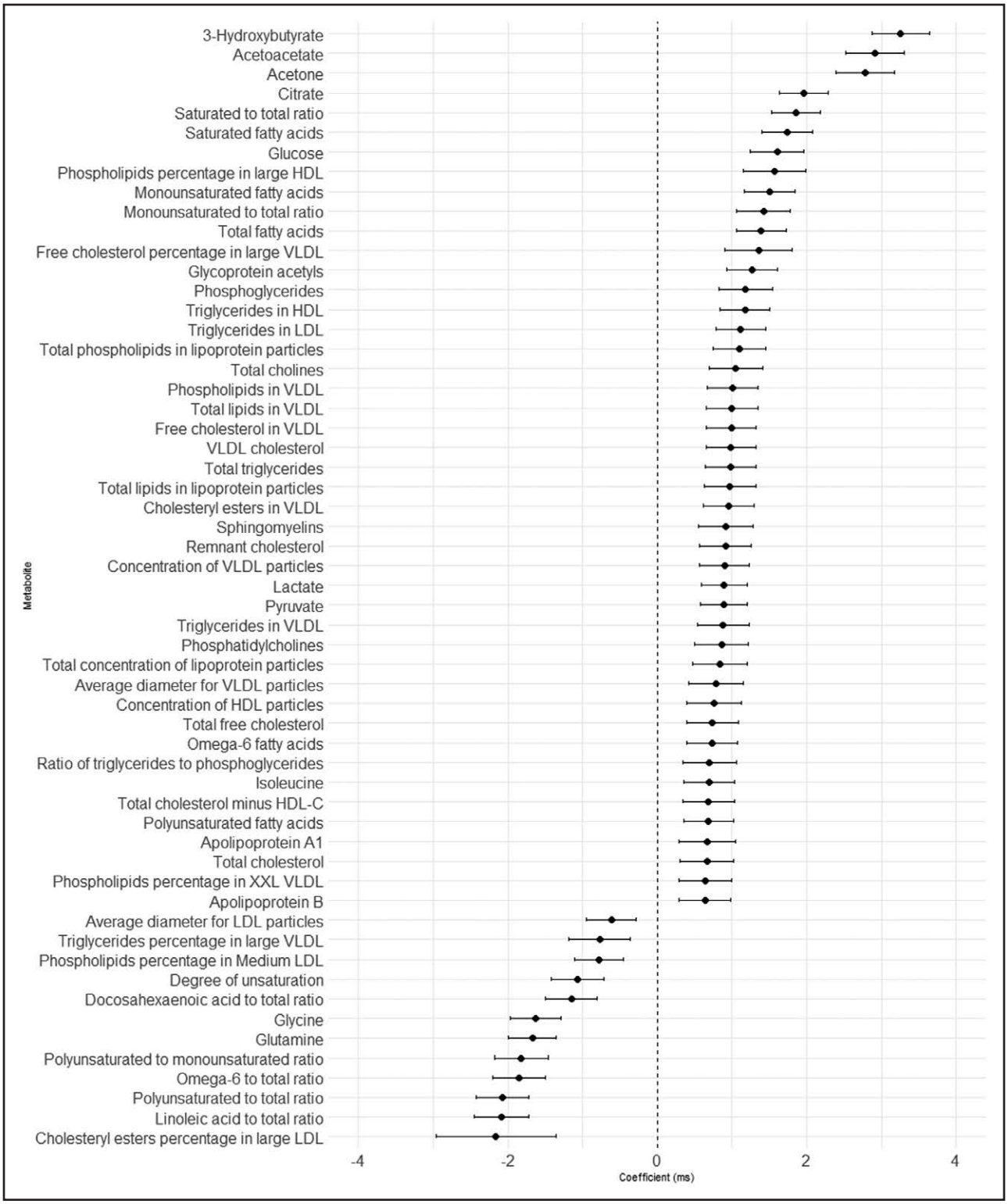

Figure 2. Multivariate linear regression results for each metabolite with a significant association with the heart rate corrected QT (QTc) interval in the training cohort.

X axis; coefficient for each metabolite in millisecond, per SD increase in metabolite concentration. *Y* axis; metabolite. Significant associations were defined as *P* values less than the Bonferroni-corrected threshold (0.05/100, $P<5\times10^{-4}$). Error bars indicate 95% CIs. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and VLDL, very low-density lipoprotein.

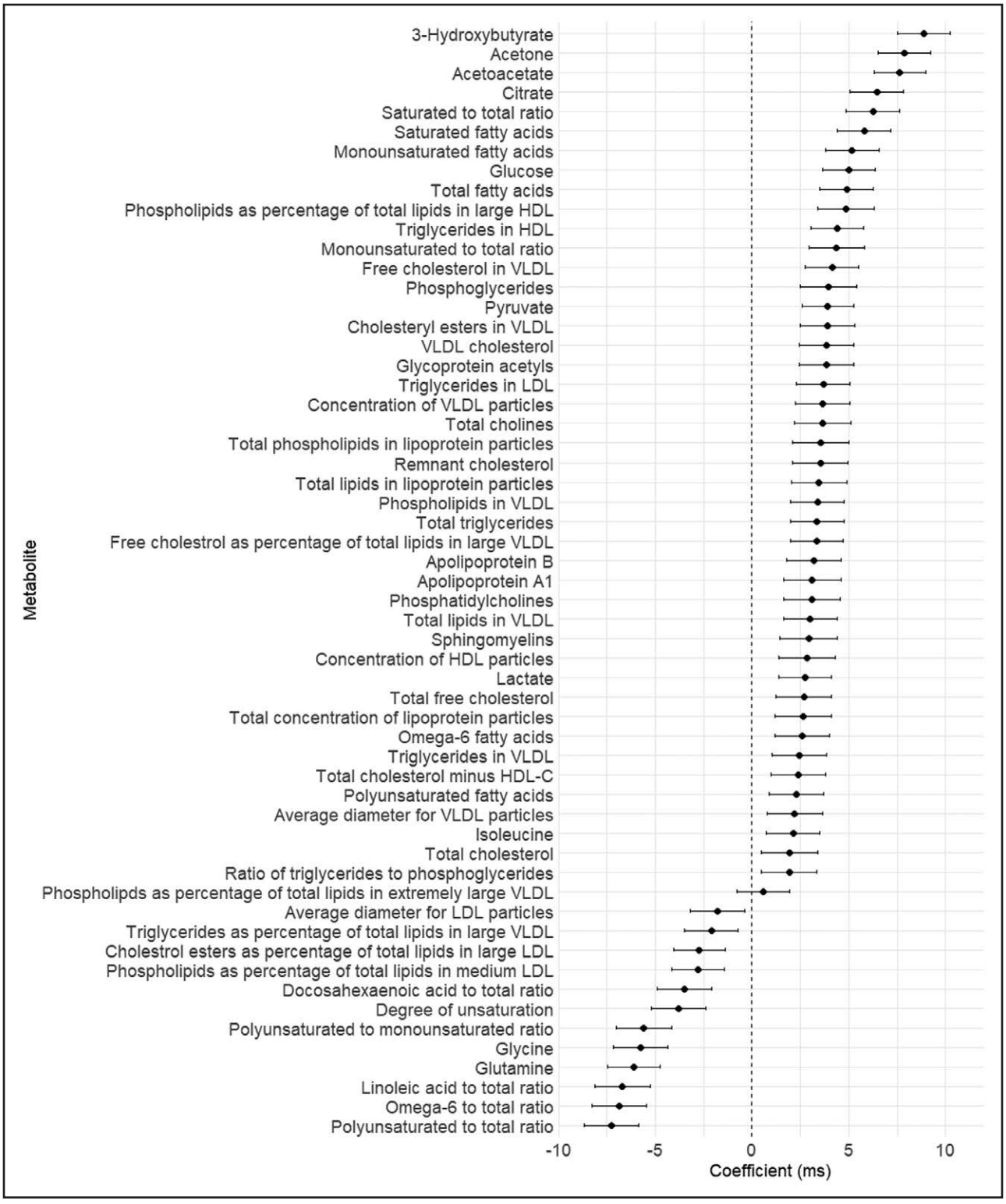

Figure 3. Heart rate corrected QT (QTc) interval effect sizes for metabolite concentrations in the top decile verses bottom decile of the population distribution in the training cohort (57 significant metabolites).
X axis; coefficient for each metabolite in milliseconds (ms). *Y* axis; metabolite. Significant associations were *P* values less than the Bonferroni-corrected threshold (0.05/100, $P<5\times10^{-4}$). Error bars indicate 95% CIs. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and VLDL, very low-density lipoprotein.

encode desaturase enzymes involved in the biosynthesis of highly unsaturated fatty acids from polyunsaturated fatty acids). Support for colocalization was also observed for citrate and glutamine at the *CASR* (calcium sensing receptor) locus, which has an important role in calcium homeostasis by regulation of parathyroid hormone. Colocalization was observed at 2 of the 7 QT loci for coronary artery disease (*CASR* locus) and metabolic syndrome (*FADS1/FADS2* locus; Table S13). No was no evidence of multitrait colocalization with diabetes or heart failure at any of the colocalizing regions.

DISCUSSION

In this study we have performed the largest analysis to date establishing the relationships of serum metabolites concentrations and the QTc interval. We identified and replicated 22 metabolites associations, with the largest effects observed for ketone bodies (3-hydroxybutyrate, acetoacetate) and polyunsaturated fatty acids. Only 2 lipid measures were significant with the remaining replicated metabolites belonging to amino acid and glycolysis classes. Metabolites increase the QTc variance explained in a model including baseline clinical variables. We also identified support for a causal relationship with genetically determined linoleic acid to fatty acid ration, and shared causal variants were also observed at a locus level.

The QT interval is an important marker of ventricular repolarization time and prolongation is associated with an increased risk for ventricular arrhythmia and sudden cardiac death.[1] Therefore, there is significant public health interest in identifying biomarkers that may have relevance in acquired and congenital long-QT syndromes. Circulating electrolytes, inflammatory cytokines and antibodies have direct and indirect effects on cardiac ion channel expression and function.[38,39] Our study suggests serum metabolite concentrations may also influence the QTc interval with potential for adverse and beneficial effects. The significant associations observed in this study has generated several hypotheses that should lead to further investigation including experimental validation in cellular models.

Ketone bodies and polyunsaturated fatty acids consistently had the largest effect sizes, which were up to 9 ms when comparing top and bottom deciles. The US Food and Drug Administration threshold for regulatory concern in drug development Through QT/QTc studies is 5 ms[40] and it is estimated that for each 10ms increase in the QT interval above normal limits the incidence of ventricular arrhythmia increases by 5% to 7%[2] . Therefore, the effect sizes observed in this study may be of relevance when considering arrhythmic risk in acquired and congenital long-QT syndromes.

Ketone bodies are endogenously produced and most abundant during period of physiological and nutritional stress.[41] Diabetic ketoacidosis, which leads to elevated lactate and ketone bodies, is associated with QT prolongation.[42] The mechanism driving the association of ketone bodies with the QT interval is unknown. One possible explanation is ketone body driven increased energy metabolism that leads to an anion gap metabolic acidosis and a reduction in serum potassium and magnesium concentrations that have direct effects on repolarizing currents.[43] Elevated plasma β-hydroxybutyrate is also associated with arrhythmic events and disease progression in patients with arrhythmogenic cardiomyopathy.[44] A proteomics analysis in explanted arrhythmogenic cardiomyopathy hearts demonstrated accumulation of β-hydroxybutyrate in cardiomyocytes due to upregulation of ketone metabolic enzymes.[44] Further insight is available from the study of individuals undertaking ketogenic diets where longer QT intervals and higher β-hydroxybutyrate concentrations have been described along with reports of multiple sudden cardiac death.[45–47] Our study provides additional support for a link between ketone bodies and the QT interval. Validation in further data sets and experimental studies should be performed to provide biological insights to the mechanisms driving these relationships and inform recommendations for individuals with other risk factors that predispose to QT prolongation.

Our findings identify for the first time in humans, a potential protective effect of polyunsaturated fatty acids. Interestingly, the proportion of polyunsaturated fatty acids (including omega-6, linoleic acid and docosahexaenoic acid) to total fatty acids was consistently more significant than absolute levels. The potential QT shortening effects of polyunsaturated fatty acids has previously been reported in cellular and animal models where they have been observed to bind to voltage and pore sites of the cardiac *KCNQ1* channel resulting in modification of the inward potassium cardiac repolarizing current which will affect ventricular repolarization time.[13,14] These may be genotype-specific effects that could have therapeutic applications in some congenital LQTS subtypes.[48,49] An increase in linoleic acid as a proportion of polyunsaturated fatty acids is associated with reduced odds for ventricular fibrillation and fatal ischemic heart disease in case-control studies[50,51] and our study provides further support that this could be through protective effects on ventricular repolarization time.

We did not identify support for the presence of a linear or nonlinear relationship between HDL and the QTc interval despite also performing sex-stratified analyses, in contrast to a population-based study by Del Giorno et al.[17] This could be due to sample size differences (21 056 verses 1202) or because the previous study did not adjust for dietary factors and alcohol consumption that are potential confounding factors. In fact, in our study, out of 63 different lipoprotein measures, only 2 had a significant association with the QTc interval that replicated. This suggests lipoprotein levels may not have clinically meaningful effects on ventricular repolarization.

Despite experimental studies supporting a direct effect of some metabolites on cardiac ion channel function, only linoleic acid had support for a causal relationship in the MR study. The absence of an association for 21 of the 22 metabolites tested may indicate the presence of confounding factors. However, for some metabolites, the heritability estimates were low and the percentage variance explained by lead variants was small (eg, 6.1% and 0.51% for 3-hydroxybutyrate). Therefore, other possible explanations are the reliance of the MR study on genetic instruments with low explanatory power or that the biological relationships captured by the summation of included genotypes do not lie on the causal pathway. We did identify support for colocalization with 15 metabolites at 7 loci. These included the *FADS1/FADS2* locus which is a major determinant of fatty acid levels and for the *CASR* locus with citrate and glutamine levels. Citrate is derived from glutamine via reductive carboxylation that supports fatty acid synthesis under certain conditions including hypoxia, which is an important process in cellular metabolism.[52] It is unknown whether citrate may influence the QT interval through fatty acid or calcium regulation pathways. Glutamine is recognized to be involved in maladaptive cardiac remodeling, ischemia and reperfusion injury and its metabolism has been considered a potential target for pharmaceutical intervention.[53,54] It has previously been shown the L-glutamine supplementation in diabetic rates shortens the QT interval, which is the same direction of effect as this study.[55] Further GWAS for metabolites and the QT interval at larger sample sizes may identify additional colocalizing regions.

The QTc variance explained by the combined clinical and metabolite LASSO model was 11.2% and therefore other contributing factors are present and warrant further study. This may include common and rare genetic variation,[11] other-omics data including proteomics, other comorbidities (eg, thyroid dysfunction[56]) and serum electrolyte levels.[57] Future work should aim to further develop the model to improve QTc prediction and test utility in arrhythmic risk prediction.

In this study, we have used serum metabolite concentrations from samples taken on the same day as an ECG recording, however the time difference between the 2 were not available. As the direct effects of metabolites on cardiac electrophysiology are likely to be acute, and the concentrations of some serum metabolites are affected by diurnal variation[58] and activity levels,[59] it is possible that our results could be affected if there was a substantial time difference between measurements. However, by performing internal replication, we have identified consistent associations that likely reflect the most important metabolites. Replication in an external cohort is required when a data set of a suitable sample size becomes available. We have been conservative in our approach by using a Bonferroni correction to declare significant findings in discovery and replication analyses as they informed subsequent MR and colocalization analyses and we wished to robustly control for false positives. Using a false discovery rate correction would likely result in additional significant findings. Outlier metabolite concentration measurements were excluded as they may represent measurement error and increase the risk of false positive associations. Within sample repeat measurements of these outliers would help confirm if they are accurate, however this data is not available. We wished to determine metabolite-QT relationships in individuals without preexisting ischemic heart disease or heart failure as these are potential confounding factors. It is possible that metabolite associations with the QT interval differ in the presence of these comorbidities and warrants additional study. This study predominantly included individuals of European ancestry as sample sizes for non-European ancestry participants were too small for inclusion. The study should be repeated in the future when this data becomes available.

CONCLUSIONS

In summary, we have identified associations for 22 metabolites with the QTc interval and demonstrated genetic support for linoleic acid and shared causal variants at genomic regions including fatty acids, citrate, and glutamine. These metabolites may be risk factors for additive effects on the QT interval in acquired and congenital LQTS. Further research is warranted to validate these findings in an external cohort and establish metabolite relationships in diverse populations when these data become available. Future work should also consider developing models that incorporate additional-omics data for QTc and arrhythmic risk prediction in LQTS cohorts and provide experimental evidence that may support their potential as therapeutic targets to shorten QT intervals in high-risk patient groups.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S13
Figures S1–S2

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