Calcium, magnesium and potassium concentrations affect ventricular repolarization in a large-scale analysis of 150,000 individuals

Running title: Electrolytes and electrographic intervals in the population

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Conflict of Interest

M.J. Caulfield is Chief Scientist for Genomics England, a UK Government company. P. Sever has received research awards from Pfizer Inc. D.O. Mook-Kanamori works as a part-time clinical research consultant at Metabolon, Inc. All other authors have no conflict of interest to declare.

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Abstract
Background: Subclinical changes on the electrocardiogram are risk factors for cardiovascular mortality. Recognition and knowledge of electrolyte associations in cardiac electrophysiology are based on only *in-vitro* models and observations in patients with severe medical conditions.
Objectives: Investigate associations between serum electrolyte concentrations and changes in cardiac electrophysiology in the general population.
Methods: Summary results collected from 153,014 individuals (54.4% women; mean [SD] age: 55.1 [12.1] years) from 33 studies (of 5 ancestries) were meta-analysed. Linear regression analyses examining associations between electrolyte concentrations (mmol/L of calcium, potassium, sodium and magnesium), and electrocardiographic intervals (RR, QT, QRS, JT and PR) were performed. We adjusted for potential confounders and also stratified by ancestry, sex and use of antihypertensive drugs.
Results: Lower calcium was associated with longer QT (-11.5 ms, 99.75%CI: -13.7,-9.3) and JT, with sex-specific effects. In contrast, higher magnesium was associated with longer QT (7.2 ms, 99.75%CI: 1.3,13.1) and JT. Lower potassium was associated with longer QT (-2.8 ms, 99.75%CI: -3.5,-2.0), JT, QRS and PR durations, but all potassium associations were driven by use of antihypertensive drugs. No physiologically relevant associations were observed for sodium or RR intervals.
Conclusions: We identified physiologically relevant associations between electrolytes and electrocardiographic intervals in a large-scale analysis combining cohorts from different settings. The results provide insights for further cardiac electrophysiology research and could potentially influence clinical practice, especially the association between calcium and QT duration, by which calcium levels at the bottom 2% of the population distribution led to clinically relevant QT prolongation by >5 ms.
Condensed Abstract
Subclinical changes on the electrocardiogram are risk factors for cardiovascular mortality. Recognition and knowledge of electrolyte associations in cardiac electrophysiology are based on only *in-vitro* models and observations in patients with medical conditions. In our large-scale analysis (N=153,014), we identified physiologically relevant associations between electrolytes and electrocardiographic intervals. The results provide insights for further cardiac electrophysiology research and could potentially influence clinical practice, especially the association between calcium and QT duration, by which calcium levels at the bottom 2% of the population distribution led to clinically relevant QT prolongation by >5 ms.

Key words: Electrolytes, electrocardiographic intervals, epidemiology, meta-analysis, cohort studies

List of abbreviations:
BMI, body mass index
BP, blood pressure
CI, confidence interval
ECG, electrocardiogram
FDA, Food and Drug Administration
HTN, hypertension
QC, quality control
Q, quantiles
SD, standard deviation

Introduction

Disturbances in cardiac electrophysiology are well-recognized risk factors for cardiovascular morbidity and mortality. Prolonged QT intervals, as collective measures of ventricular depolarisation and repolarisation, and elevated resting heart rates have been consistently associated with adverse outcomes in epidemiological studies (1-4). Electrocardiogram (ECG) parameters correlate well with cardiac electrophysiology -- in particular cardiac action potential measurements made in single cells or tissue preparations. The duration of the action potential often mirrors the QT interval, whilst the maximum rate of depolarisation determines conduction velocity and influences PR and QRS durations (5,6).

Extreme serum electrolyte concentrations, particularly for potassium and calcium, are well-known risk factors for repolarisation disturbances, conduction abnormalities and cardiac arrhythmias (7,8). The influence of electrolytes on cardiac action potentials can be studied unambiguously (9). For example, increases in extracellular calcium concentration shortens the action potential duration (10). However, the mechanisms are counterintuitive and not clearly explained. In a modelling study, in addition to increases in repolarising potassium currents with increasing extracellular and intracellular calcium, it was also necessary to include increasing calcium dependent inactivation of the calcium current, to reproduce the observed relationship (11). A decrease in the sodium-calcium exchange may also contribute to the shortened action potential duration (12). In contrast, the effects of changes in action potential duration with extracellular magnesium are much smaller (13).

Importantly, variation in serum electrolyte concentrations within normal ranges is associated with occurrence of cardiovascular disease (14). For example, risks for myocardial infarction increased by 20% for every 0.1 mmol/L rise in calcium (15). Increased hazard ratios for mortality have been observed at the extreme limits of the normal range for potassium (16). Studies evaluating electrolyte effects, however, are often cellular experiments

or analyses in patient populations with multiple comorbidities. Such studies have difficulties in disentangling electrolyte versus direct disease effects. There is little information on associations between electrolytes and cardiac electrophysiology amongst relatively healthy individuals.

A direct link between serum electrolyte levels and cardiac physiology is indicated by genetic studies. Genome-wide association studies have identified multiple genes encoding electrolyte transporter and signalling proteins involved in cardiac physiology (17-19). For example, *KCNH2* encodes a subunit of the voltage-gated potassium channel hERG, and genetic variation in *KCNH2* can be associated with either a prolonged or shortened QT interval (17). Loss of function variants in *KCNH2* were identified in 30% of long QT syndrome cases, and gain of function mutations in *KCNH2* were identified in individuals with short QT syndrome (20,21).

Our hypothesis is that variation in serum electrolyte levels in the general population may alter cardiac electrophysiology. Identifying individuals at risk of electrophysiological disturbances may aid in prevention of cardiovascular disease and mortality. In this study, we performed a large-scale systematic analysis to investigate associations between serum electrolyte concentrations and electrocardiogram intervals within a healthy population free of severe cardiac abnormalities and including various ancestries.

Methods

Study setting

Studies were eligible to join the project, if participants had data on at least one electrolyte measured in serum and at least one ECG trait, both measured at a similar time point. All contributing studies are described in the **Online Appendix** and in **Online Table 1**.

All studies were approved by local Medical Ethical Committees in agreement with the declaration of Helsinki, and all participants provided written informed consent.

Participant exclusions

Individuals were excluded if they were <18 years old or pregnant. For quality control (QC), outliers were excluded from the final electrolyte and ECG dataset (>5 standard deviations from the mean). Extreme RR-intervals were also excluded (<500 or >1,500 ms). To minimise bias, and to exclude individuals with cardiovascular disease, we excluded individuals with atrial fibrillation, Wolff-Parkinson-White syndrome, 2nd or 3rd degree AV block, a history of myocardial infarction or heart failure, or a pacemaker, and anyone taking class I and/or class III blocking medication (ATC code: CO1B).

Exposure, outcome and covariate data

Four electrolytes were considered as exposures: calcium, sodium, potassium and magnesium, measured in serum in mmol/L. Five ECG measures were included in the analysis: QT, JT (as a measure of ventricular repolarization; where JT = QT – QRS), QRS, PR and RR intervals, measured in milliseconds (ms). Studies without ECG data contributed data only for RR interval, if heart rate (HR) was available from pulse measurements (by converting to RR using the formula: RR (ms) = 60,000 / HR (bpm)). Participant age, sex, body mass index (BMI), creatinine level, diabetes mellitus status and hypertension (HTN) status were included as covariates. Individuals were defined as being hypertensive if they met any one of the following criteria: (i) systolic blood pressure (BP) ≥ 140 mmHg; (ii) diastolic BP ≥ 90 mmHg; or (iii) taking BP-lowering medication (ATC codes: C02, C03, C04, C07, C08, C09). Sensitivity analyses were performed using new cut-offs for systolic and diastolic blood pressures from AHA/ACC (22). Diabetes was defined according to: (i) a doctor's diagnosis of diabetes mellitus; (ii) a fasting glucose concentration > 6.9 mmol/L; or (iii) taking any glucose-lowering medication (ATC code: A10), which is a generally accepted definition for harmonization across cohorts from different countries (23). Serum creatinine concentration levels were measured in µmol/L and were log-transformed within all models.

Study-level statistical analyses

A centrally-written script using R statistical software (24) was provided to each participating study, and the generated output files were submitted centrally (which included study characteristics, histograms of variable distributions for QC and summary statistics of regression analysis results), so that all studies ran identical analyses according to a harmonised protocol. Each study contributed to as many different models as possible, based on available data on exposures, outcomes and covariates. Analyses were performed for each ECG trait separately, to allow for differing sample sizes for each combination of ECG trait and electrolyte. For studies with individuals of different ancestries (notably CHS, HABC and MESA), analyses were stratified by each ancestry.

As our primary analyses, we performed linear regression analyses regressing each ECG trait on each electrolyte, adjusting for sex and age. Expect for analyses on RR interval itself, all analyses were statistically adjusted for RR interval which is generally a more suitable heart-rate correction method than other methods such as Bazett (25,26). Our cohorts essentially had unrelated individuals except for the CHRIS, MICROS, ORCADES and VIKING studies which used mixed models to correct for relatedness between study participants. By analysing all paired combinations of the five ECG traits and four electrolytes, there were 20 primary linear regression analyses in all participants. For each electrolyte-ECG trait association, we assessed the role of confounding by adding – one by one – the other covariates to the statistical models (BMI [kg/m^2, present in all subsequent adjusted models] diabetes mellitus status, HTN status, and creatinine). Our fully adjusted model using all covariates contained data from only the studies with all covariates available.

We also performed analyses stratified by sex, use of antihypertensive drugs (+ digoxin) and HTN status. Use of antihypertensive drugs (+ digoxin) was defined according to ATC codes (C01AA05 for digoxin; or C02, C03, C04, C07, C08, C09 for any BP-lowering

medication). Note that individuals would belong to the "drug-users" subgroup but not to the "HTN-only" subgroup, if they were taking only digoxin. Similarly, participants would belong to the "HTN-only" subgroup but not in the "drug-users" subgroup, if they were untreated hypertensives. Due to the overlap with HTN as a covariate in the adjusted sub-models, these subgroup analyses stratified by drug use and by HTN status were performed for only the two basic models adjusted for age, sex (and RR interval), and additionally for BMI.

Finally, to investigate the trend of associations across electrolyte levels and across the population distribution for the main electrolyte-ECG associations, we performed analyses stratified by quintiles of the electrolyte concentrations. The five quintiles were generated from the distribution of each electrolyte: Q1 to Q5. Pairwise comparison analyses were performed, using the minimally adjusted model (adjusted only for age and sex), with the middle quintile (Q3) as reference.

Meta-analyses

After QC of the received summary statistics data, fixed-effects inverse-variance-weighted meta-analyses were performed centrally using the "rmeta" CRAN package in R statistical software (24), pooling together the beta effect estimates and standard errors from all studies. As further QC, prior to the meta-analyses, we excluded any analysis model results from studies that were estimated in small sample sizes (<100 individuals). Two sets of meta-analyses were performed: an all-ancestry meta-analysis and five ancestry-stratified meta-analyses.

Due to the 20 different ECG-electrolyte associations, we corrected for multiple testing by the Bonferroni method and present results with 99.75% confidence intervals (CI). For any significant association from our primary analysis in the minimally adjusted model, we used the following sequential strategy for reporting results: (1) Significant associations from the minimally adjusted model were checked for robustness to covariate adjustment, by

comparing them to results from the fully adjusted model. (2) The effect sizes of the robust associations were evaluated for their physiological importance, and the plots from the quintile analyses were checked for clear linear trends supporting association results. (3) Associations meeting these requirements were reported and considered further within subgroup analyses by sex, drug use and HTN status.

Post-meta-analysis interaction analyses

Based on the coefficients from the meta-analyses from the models stratified by sex, drug use and HTN status, we additionally tested for evidence of effect modification on a multiplicative scale, using the methodology that has been previously described by Altman and Bland (27). Two-sided p-values for interaction <0.05 were considered significant.

Results

Characteristics of the study population

In the present study, we used data from a total of 38 study groups from 33 cohorts representing 5 different ancestries: European (N_{max} = 129,169 from 30 studies); African-American (N_{max} = 7,693 from 4 studies); "mixed" ancestry from Brazil (N_{max} = 14,612 from 2 studies: BAMBUI and ELSA); Asian (N_{max} = 555 from 1 study: MESA); and Hispanic/Latino (N_{max} = 985 from 1 study: MESA). Overall, we collected data from a total of 153,014 individuals (45.6% men) (**Table 1**). Mean (SD) age was 55.1 (12.1) years, and individuals were on average slightly overweight (mean [SD] BMI: 27.3 (4.8) kg/m^2). Serum electrolyte levels were distributed similarly among men and women and among drug-users and non-users. Study-specific characteristics are presented in **Online Tables 1-3**.

Association between serum electrolyte concentrations and ECG parameters

After observing no substantial heterogeneity between the different ancestry groups from inspection of forest plots (**Online Figure 1)**, the all-ancestry meta-analyses were used as the primary analysis results, to maximise sample size. Of the 20 main electrolyte-ECG trait

associations, we found evidence for 14 associations between a serum electrolyte concentration and an ECG trait (**Table 2, Figure 1**). There was no consistent heterogeneity observed between the participating studies from inspection of cohort-level forest plots (**Online Figure 2**).

High calcium was associated with shorter QT (-11.5 [99.75%CI: -13.7,-9.3] ms per mmol/L) and JT (-15.6 [99.75%CI: -18.3,-12.9] ms per mmol/L) intervals, and effect sizes increased in the fully adjusted model (**Table 2**), due to adjustment for HTN and diabetes status. In contrast, high magnesium was associated with longer QT (7.2 [99.75%CI: 1.3,13.1] ms per mmol/L) and JT intervals (9.9 [99.75%CI: 4.1,15.6] ms per mmol/L), with similar results observed in the fully adjusted model (**Table 2**). High potassium was associated with shorter QT (-2.8 [99.75%CI: -3.5,-2.0] ms per mmol/L), QRS (-1.6 [99.75%CI: -1.9,-1.3] ms per mmol/L), JT (-1.0 [99.75%CI: -1.8,-0.3] ms per mmol/L) and PR intervals (-1.7 [99.75%CI: -2.4,-1.1] ms per mmol/L), also with similar effect sizes in the fully adjusted model (**Table 2**). There were clear trends for the calcium, magnesium and potassium associations when study populations were stratified by quintiles, indicating support for the associations across the population distribution of electrolyte levels (**Online Figure 3**).

High sodium was associated with longer QRS (0.1 [99.75%CI: 0.0, 0.1] ms per mmol/L) and JT intervals (0.2 [99.75%CI: 0.1, 0.3] ms per mmol/L). But sodium was associated with only QRS interval in the fully adjusted model, suggesting an influence of confounding, which was found to be from HTN and diabetes (**Table 2, Figure 1**). Moreover, the small effect sizes for sodium would not be viewed as physiologically relevant (0.1-0.2 ms increase in QRS per 1 mmol/L increase in sodium), and there was no meaningful trend in the quintile analyses to support an association (**Online Figure 3**).

In general, all electrolytes examined were associated with RR interval, and results were similar in the fully adjusted model (**Table 2**). Although associations with RR intervals

reached statistical significance, effect sizes were very small (e.g., 64.1 ms change in RR per 1 mmol/L increase in magnesium). Such changes would not be viewed as physiologically important, considering the population distributions of electrolyte levels and RR durations. Furthermore, the electrolyte-RR associations did not show clear trends when electrolyte levels were stratified by quintiles (**Online Figure 3**).

Hence, none of the associations for RR interval or sodium were considered further. Therefore the eight key associations of interest are: calcium and magnesium with QT and JT intervals; and potassium with four ECG traits (QT, QRS, JT and PR).

Subgroup analyses

Based on the eight electrolyte-ECG trait associations observed in the main analysis, we additionally stratified by sex, drug use or HTN status. With the minimally adjusted model, we found evidence of sex-specific associations (p-value interaction < 0.05) for only calcium and QT ($p_{interaction}$ = 0.008) and JT ($p_{interaction}$ = 0.008) intervals (**Online Table 4, Figure 2**), with stronger associations in women than in men. Specifically, per mmol/L increase, calcium was associated with 8.8 (99.75%CI: -12.1,-8.0) ms shorter QT intervals and 12.1 (99.75%CI: -16.3,-8.0) ms shorter JT intervals in men, compared to 12.6 (99.75%CI: -15.5,-9.8) and 16.9 (99.75%CI: -20.4,-13.4) ms, respectively, for women.

When stratified according to drug use, non-drug-users had attenuated associations between potassium and QT, QRS, JT, and PR intervals (**Online Table 5, Figure 3**). For each mmol/L increase in potassium, QT intervals were -5.5 (99.75%CI: -6.9,-4.2) ms shorter in drug-users, but only -0.9 (99.75%CI: -1.8, 0.0) ms shorter in non-users, in the minimally adjusted model ($p_{interaction}$ <0.001). Similar results were observed for JT intervals, and to a lesser extent for PR and QRS intervals. Attenuation also occurred for calcium, but to a much lesser extent, and associations were still observed in non-users. An increase of 1 mmol/L of calcium was associated with a -16.1 (99.75%CI: -21.1,-10.9) ms shorter QT interval in drug-

users, but with a -11.1 (99.75%CI: -13.4, -8.7) ms shorter QT interval in non-users (p-value for interaction = 0.007). Results were similar when we stratified by HTN status (drug use/140/90 mmHg), but the differences in associations were usually less pronounced than when we stratified by drug use (**Online Table 6**), particularly with lower hypertension cut-offs (drug use/130/90 mmHg or drug use/120/80 mmHg) in the analyses on potassium (**Online Table 7**). All subgroup results remained comparable in the adjusted model.

Discussion

We investigated associations between serum electrolyte levels and measures of cardiac electrophysiology in a large-scale population-based meta-analysis. We observed eight associations that had cardiac electrophysiological relevance. After full adjustment for considered confounding factors, we found that higher calcium levels were associated with shorter QT and JT intervals, and magnesium with longer QT and JT intervals, reflecting shortened and prolonged ventricular repolarisation, respectively. Interestingly, the relationship between shortened ventricular repolarisation and calcium was stronger in women. Higher potassium levels were associated with shorter QT, QRS, JT and PR intervals. However, associations with potassium were observed specifically in drug users (mainly antihypertensive drugs) and hypertensive individuals. The associations with potassium are therefore assumed to be related to antihypertension treatment. No physiologically relevant associations were observed for sodium. Although all four electrolytes were significantly associated with RR interval, none of the associations was viewed as clinically or electrophysiologically important. Collectively, our findings from a collection of (population-based) cohort studies of different settings contribute to understanding the role of electrolytes in cardiac electrophysiology in the general population.

Lower calcium levels were robustly associated with longer QT and JT intervals -- but not QRS duration -- across different subgroups, reflecting ventricular repolarisation

primarily. Biologically, calcium is stored in large amounts in the sarcoplasm reticulum, ready to be released for cardiac muscle contraction initiated by an inward L-type calcium current.

Based on the effect sizes in the fully adjusted model, we estimated the proportion of the general population that has clinically significant changes in the QT duration (which has clinical cut-off values; **Figure 4A**). According to our data, 2% of the general population (irrespective of sex) have a calcium concentration that prolongs the QT interval by 5 ms or more. The U.S. Food and Drug Administration (FDA) uses 5 ms as the threshold level for regulatory concern following a "thorough QT/QTc study" in healthy volunteers -- a required part of the evaluation of new treatment compounds before market launch (28). The FDA practices potentially highlight the clinical importance of our findings and suggest the possible usefulness of ECG assessment in patients with low calcium levels, to prevent arrhythmic events, particularly in the presence of other interacting risk factors for ventricular repolarisation prolongation.

Our findings may be more clinically relevant to women, due to the larger observed effects, although we are unable to explain the sex-specific differences. To the best of our knowledge, there are no reports on sex-specific expression profiles of calcium channels or receptors in cardiac myocytes. Interestingly, 17β-estradiol -- an estrogen hormone -- inhibits calcium channels (29-31). However, considering the mean age (55 years), women in our study population are likely to be mostly postmenopausal, with significantly lower estradiol levels. More research is therefore required to elucidate the cause of the sex-specific observations.

A higher magnesium concentration was associated with longer QT and JT intervals. However, magnesium effect sizes were fairly small (**Figure 4B**). Nevertheless, our results suggest a biological role for magnesium in ventricular repolarisation. In animal tissue samples, the effect of magnesium on transmembrane potentials of cardiac myocytes is also

less substantial, in contrast to other electrolytes [13]. Clinically, previous research suggested a linear relationship between magnesium and coronary heart disease mortality, where a 0.1 mmol/L increase in serum magnesium -- even within normal ranges -- was associated with decreased risk (32). Our associations for magnesium in a large-scale study represent novel contributions, considering the fewer published reports on magnesium, compared to other electrolytes.

We observed shorter PR, QRS, QT and JT intervals with increasing potassium. The effects of increasing potassium concentrations are recognised to be biphasic. Within the physiological range, increasing extracellular potassium causes a paradoxical increase in outward current mediated by hERG channels which initially shortens the action potential and stabilises the resting membrane potential (33). Combined with an increase in the velocity of phase 3 of the action potential, this manifests as shortening of the QT interval and peaking of the T wave (34,35). When potassium concentrations reach those associated with clinically defined hyperkalaemia, the resting membrane potential decreases, reducing the upstroke velocity of the action potential thus delaying interventricular conduction (34). This results in the classical ECG characteristics of hyperkalaemia such as a prolonged QRS duration.

Interestingly, potassium effects were significantly greater in individuals on antihypertensive medication, with prolongation of the QT interval of 5 ms or more in ~4% of participants (**Figure 4D**). The greater effect of potassium on ECG intervals in individuals taking antihypertensive medication may be explained by direct and indirect drug effects. *In-vitro* studies have demonstrated inhibitory effects of beta-blockers on HERG channels, through direct blocking of the channel. Angiotensin receptor blockers (ARBs) impede currents carried by hKv1.5, KvLQT1, KCNQ1 and HERG(Ikr) subunits (36,37). Overexpression of angiotensin II type 1 receptors in mouse ventricular myocytes decreases

myocyte potassium currents, lengthen action potential duration and significantly prolong QT intervals (even after adjustment for QRS duration) (38).

There are few reports on long-term effects of antihypertensive medication on ventricular repolarisation in humans. Three small studies of individuals with left ventricular hypertrophy related to hypertension showed improvement in echocardiographic and ECG findings of hypertrophy -- with shortening of the QT interval -- following use of an ACE inhibitor, ARB or beta-blocker (atenolol) (39-41). These ECG changes may be due to ventricular remodelling, or also to changes in autonomic tone. For example, the QT/RR slope relationship can be influenced by autonomic tone, which could augment effects of serum potassium on ECG intervals (42), as suggested by our study. Our study did not have complete information on the exact medications used (antihypertensive agents or other drugs that may affect ECG intervals). However, individuals taking class I or III anti-arrhythmics were excluded meaning that the number of individuals taking digoxin is expected to be very low and unlikely to impact results. Furthermore, indications for taking these drugs may differ among individuals, and the various underlying aetiologies may influence ECG characteristics.

Historically, an influence of circulating electrolytes on the ECG has been known for ~100 years. For example, 20 years after Einthoven reported his string galvanometer in 1903 (43), Carter and Andrus observed long QT durations in infants with tetany from hypocalcemia (44). The QT duration decreased when the tetanic infants were given oral calcium. Prolonged QT intervals were seen with low potassium levels as early as 1950 (45). Several other reports on electrolytes and ECGs followed shortly thereafter (35,46-50). However, electrolyte effects have not been well described apart from patient populations. For example, electrolyte effects were not analysed in a study of 32,949 normal ECGs at Vanderbilt University (in subjects without heart disease, medications that affect ECGs, or

abnormal electrolytes (51)). Our report represents the first large-scale ECG analysis in relation to electrolyte levels in the general population.

Study strengths and limitations

A major strength is that our study is sufficiently powered to investigate associations between serum electrolyte levels and cardiac electrophysiology measures. The large collection of (population-based) cohorts in this study minimizes the risk of reporting cohort-specific (false-positive) results. Also, all data were analysed by use of a standardised protocol, to minimise differences in analyses among the individual studies. This would be a useful strategy to adopt for future analyses incorporating data from multiple different cohorts, although meta-analyses techniques should always be performed and the assessment of nonlinearity remains difficult. Our analyses of different ancestries did not show major heterogeneity in our findings, and confounders were taken into consideration (where possible). However, the list of confounders considered was limited by access to individual level data available among the participating studies. The limitations in our study were that we were not able to study dynamic interrelations among all serum electrolytes jointly in relation to ECG intervals, because only a few cohorts had data on all four electrolytes. This would be an interesting area for follow-up in a subset of the cohorts. Calcium is usually bound to albumin, and low calcium can be caused by low albumin levels. However, we believe albumin plays a negligible role in the present study, because low albumin levels are rare in the general population. Although the observational nature of our study limits causal inferences, biological evidence supporting our results favours an interpretation the electrolyte-ECG interval associations are causal. Finally, we stratified according to use of any antihypertensive treatment overall, rather than to the use of specific antihypertensive drug classes as this information was not available. Possible alterations in potassium effects due to different antihypertensive drugs is an area to be explored in future studies.

Conclusions

In summary, within our large-scale study, we identified multiple electrolyte-ECG associations relevant to ventricular repolarisation, involving calcium, magnesium and potassium, although causality has yet to be determined. Regarding calcium and ventricular repolarisation, a subgroup of the general population has an increase in QT-interval that may be medically relevant, based on the effect sizes observed. Further research is necessary to improve our understanding of the underlying (causal) mechanisms involved.

Perspectives

Competency in patient care and procedural skills: Low calcium levels in the general population can result in a clinically significant prolongation of ventricular repolarization, reflected by prolonged QT and JT interval durations.

Translational outlook: Future studies should examine causality of the observed associations, investigate the underlying biology, and examine whether frequent calcium measurements in individuals at risk can prevent or delay cardiovascular events.

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Figure Legends

Figure 1: Associations between serum electrolyte concentrations and measures on the electrocardiogram. Beta effect results can be interpreted as the difference in milliseconds of the ECG (electrocardiogram) measure per one standard deviation increase in serum electrolyte concentration. Red bars reflect positive associations, blue bars reflect negative associations. The intensity of the colour refers to the precision of the association. Analyses in model 1 were adjusted for age, sex, and RR interval ("minimally adjusted model"). Analyses in model 4 were adjusted for age, sex, RR interval, body mass index, hypertension status, diabetes mellitus status and natural log of serum creatinine concentration ("fully adjusted model"). A two-sided p-value < 0.0025 according to Bonferroni correction was considered statistically significant (Note: RR-interval associations not shown here).

Figure 2: Sex-specific association between serum calcium and QT and JT intervals. Results are presented as the beta effect sizes with 99.75% confidence intervals (in milliseconds per mmol/L increase in calcium concentration), for men (shown as circles) and women (shown as squares) separately. The minimally adjusted model included covariate adjustment for age, sex, RR-interval and cohort-specific covariates. The fully adjusted model additionally included body mass index, diabetes mellitus status, hypertension status and natural log of serum creatinine concentration. "P-int" is the p-value from the interaction analysis.

Figure 3: Associations for potassium and calcium stratified by drug use. Results are presented as the beta effect sizes with 99.75% confidence intervals (in milliseconds per mmol/L increase in calcium or potassium concentration), for drug use (shown as circles) and non-drug use (shown as squares) separately. The minimally adjusted model included covariate adjustment for age, sex, RR-interval and cohort-specific covariates. The fully adjusted model additionally included body mass index, diabetes mellitus status, hypertension

status and natural log of serum creatinine concentration. “P-int” is the p-value from the interaction analysis.

Figure 4: Differences in effects of electrolytes on QT durations, for different percentiles of electrolyte levels. For each percentile point, the graphs indicate the difference between the beta estimate at that percentile and the beta estimate at the 50^{th} percentile (in milliseconds of QT per mmol/L increase in calcium). For plots A-C, the estimated effects are calculated according to the fully adjusted model. For plots D and E from subgroup analyses, the estimated effects are calculated according to the model adjusted for age, sex, RR-interval and body mass index. The shading represents the 95% confidence interval.

Table 1: Pooled characteristics of the study populations

	Total		By sex: mean (SD)		By drug use: mean (SD)	
	N	Mean (SD)	Men	Women	Users	Non-Users
Age in years	153,014	55.1 (12.1)	55.5 (11.9)	54.7 (12.2)	59.0 (9.9)	51.5 (11.6)
Body mass index in kg/m^2	152,481	27.3 (4.8)	27.5 (4.2)	27.1 (5.3)	27.8 (5.0)	26.2 (4.6)
Creatinine in µmol/L	151,691	81.3 (22.4)	91.1 (22.9)	73.2 (18.6)	-	-
Electrolytes[1]						
Calcium in mmol/L	90,575	2.33 (0.11)	2.33 (0.11)	2.33 (0.11)	2.36 (0.11)	2.32 (0.11)
Potassium in mmol/L	129,464	4.23 (0.37)	4.27 (0.37)	4.19 (0.36)	4.20 (0.43)	4.23 (0.35)
Sodium in mmol/L	125,760	141 (2.7)	141 (2.6)	141 (2.7)	141 (2.8)	141 (2.6)
Magnesium in mmol/L	42,720	0.83 (0.08)	0.83 (0.08)	0.82 (0.08)	0.83 (0.08)	0.83 (0.07)

ECG measures						
RR interval in ms	153,014	917 (148)	935 (155)	903 (139)	872 (148)	903 (144)
QT interval in ms	125,104	399 (28.7)	403 (29.3)	400 (28.1)	388 (30.0)	395 (27.3)
QRS interval in ms	123,695	92.7 (12.9)	97.8 (12.9)	90.3 (11.6)	90.3 (13.9)	91.8 (12.1)
JT interval in ms	121,355	311 (28.4)	304 (28.3)	316 (27.4)	297 (29.4)	303 (27.2)
PR interval in ms	124,078	159 (24.3)	164 (24.4)	158 (23.5)	156 (25.3)	156 (22.9)

Study-level characteristics were collected from each study, with summary descriptive statistics for all continuous variables used within the analysis models: covariates; electrolytes and ECG (electrocardiogram) measures. These characteristics were then pooled together centrally across all studies. The "Total" columns are for all individuals, with "N" showing the number of individuals; the "By sex" columns correspond to the subgroup analyses stratified by sex, showing separate summaries for Men and Women; the "By drug-use" columns correspond to the subgroup analyses stratified by drug-use, showing separate summaries for Users and Non-Users (where drug-use is defined as the use of antihypertensive drugs + digoxin). Within each set of columns are the Mean and SD (Standard Deviation) values. [1]Due to the alternative analysis pipeline used in the ORCADES and VIKING studies, these studies were not able to provide means and standard deviations for the electrolytes.

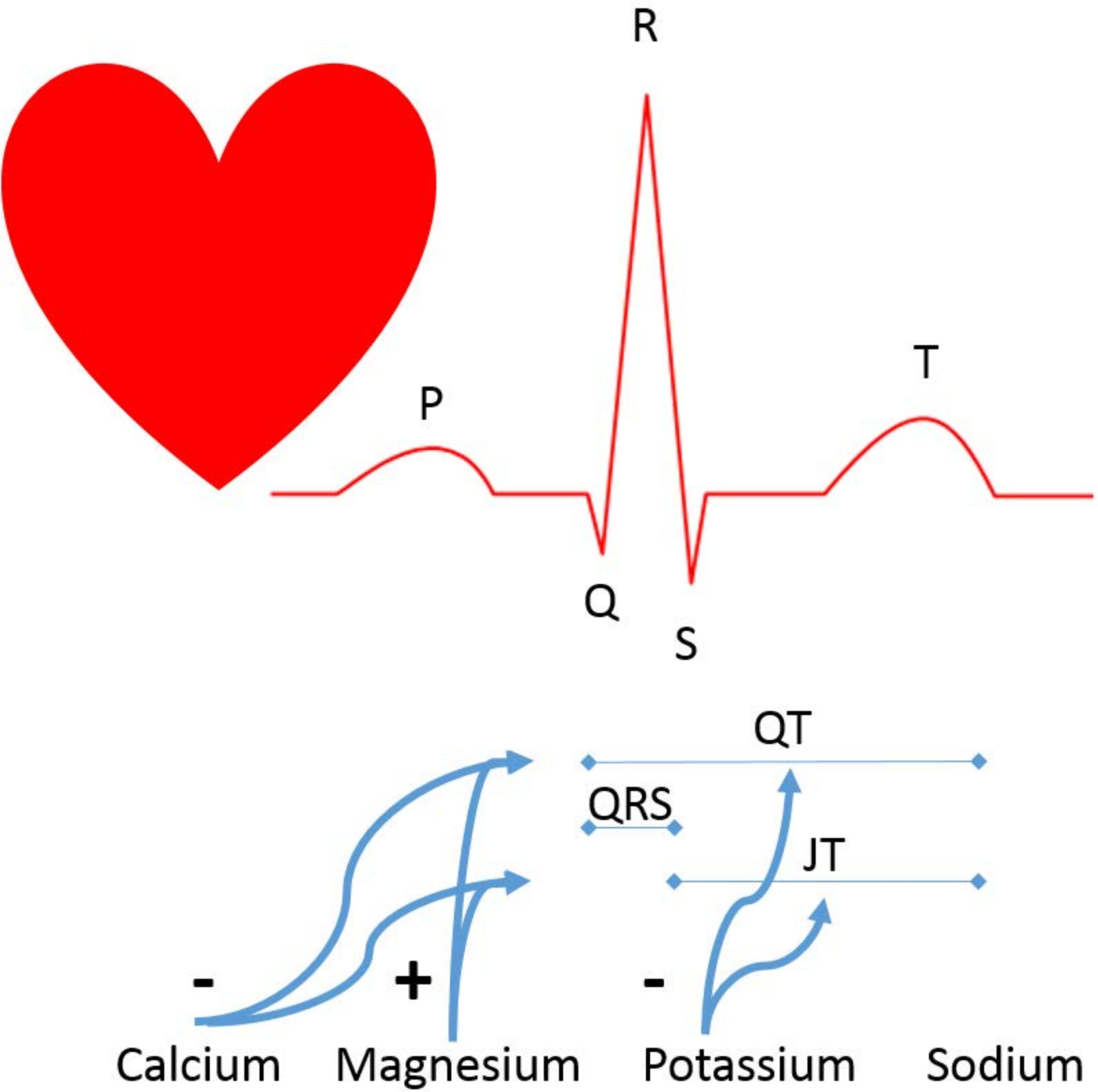
Table 2: Association between serum electrolyte concentrations and ECG measures in the general population

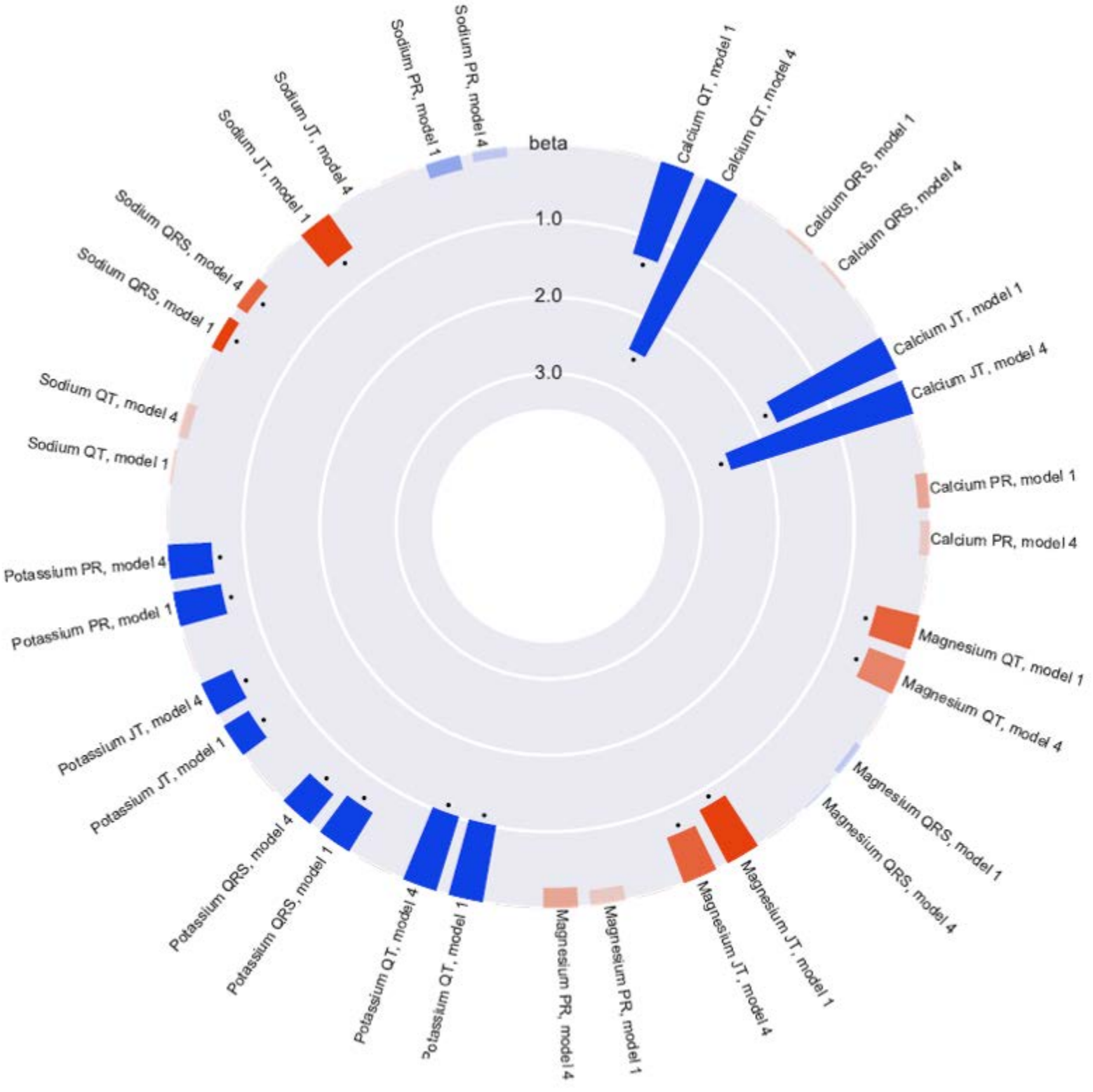
	Minimally adjusted model			Fully adjusted model for all potential confounders		
	N (studies)	Beta	99.75% CI	N (studies)	Beta	99.75% CI
Calcium						
RR interval	94,264 (33)	-21.1	-33.6; -8.7	77,520 (26)	-32.1	-46.6; -17.5
QT interval	77,479 (31)	-11.5	-13.7; -9.3	62,874 (25)	-22.3	-25.7; -18.9
QRS interval	77,471 (31)	0.45	-0.6; 1.5	62,869 (25)	0.4	-1.0; 1.8
JT interval	75,222 (29)	-15.6	-18.3; -12.9	62,342 (24)	-22.7	-26.0; -19.4
PR interval	76,834 (30)	1.4	-0.7; 3.6	62,267 (24)	1.2	-1.5; 3.9
Magnesium						
RR interval	44,682 (16)	64.1	37.5; 90.7	36,940 (13)	39.8	10.6; 69.0
QT interval	36,165 (14)	7.2	1.3; 13.1	30,509 (12)	6.4	0.0; 12.8
QRS interval	36,138 (14)	-1.0	-3.6; 1.5	30,509 (12)	-0.3	-3.0; 2.5
JT interval	36,165 (14)	9.9	4.1; 15.6	30,529 (12)	7.9	1.6; 14.2
PR interval	35,956 (14)	2.5	-2.2; 7.2	30,355 (12)	3.3	-1.9; 8.5

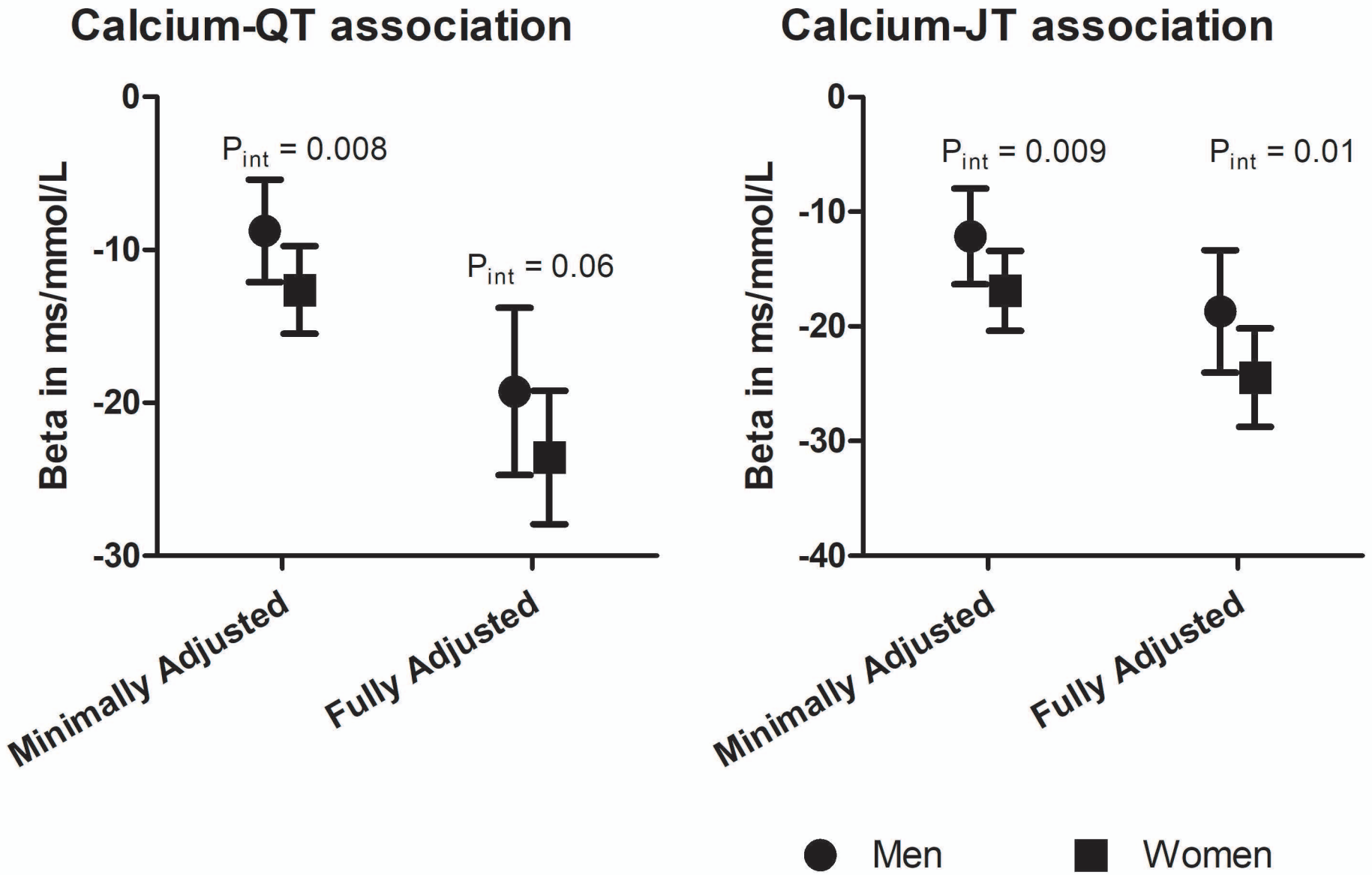
Potassium						
RR interval	126,528 (29)	13.9	10.6; 17.3	87,875 (23)	12.4	8.5; 16.3
QT interval	98,669 (26)	-2.8	-3.5; -2.0	66,941 (22)	-2.8	-3.6; -1.9
QRS interval	97,283 (26)	-1.6	-1.9; -1.3	65,576 (22)	-1.3	-1.7; -1.0
JT interval	96,656 (25)	-1.0	-1.8; -0.3	64,995 (21)	-1.2	-2.1; -0.3
PR interval	97,725 (25)	-1.7	-2.4; -1.1	66,312 (21)	-1.6	-2.3; -0.8
Sodium						
RR interval	122,732 (28)	2.4	1.9; 2.9	84,116 (22)	1.3	0.8; 1.8
QT interval	94,787 (25)	0.0	-0.1; 0.1	63,182 (21)	0.1	-0.1; 0.2
QRS interval	93,483 (25)	0.1	0.0; 0.1	61,815 (21)	0.1	0.0; 0.1
JT interval	92,857 (24)	0.2	0.1; 0.3	61,236 (20)	0.0	-0.1; 0.1
PR interval	93,914 (24)	-0.1	-0.2; 0.0	62,541 (20)	0.0	-0.2; 0.1

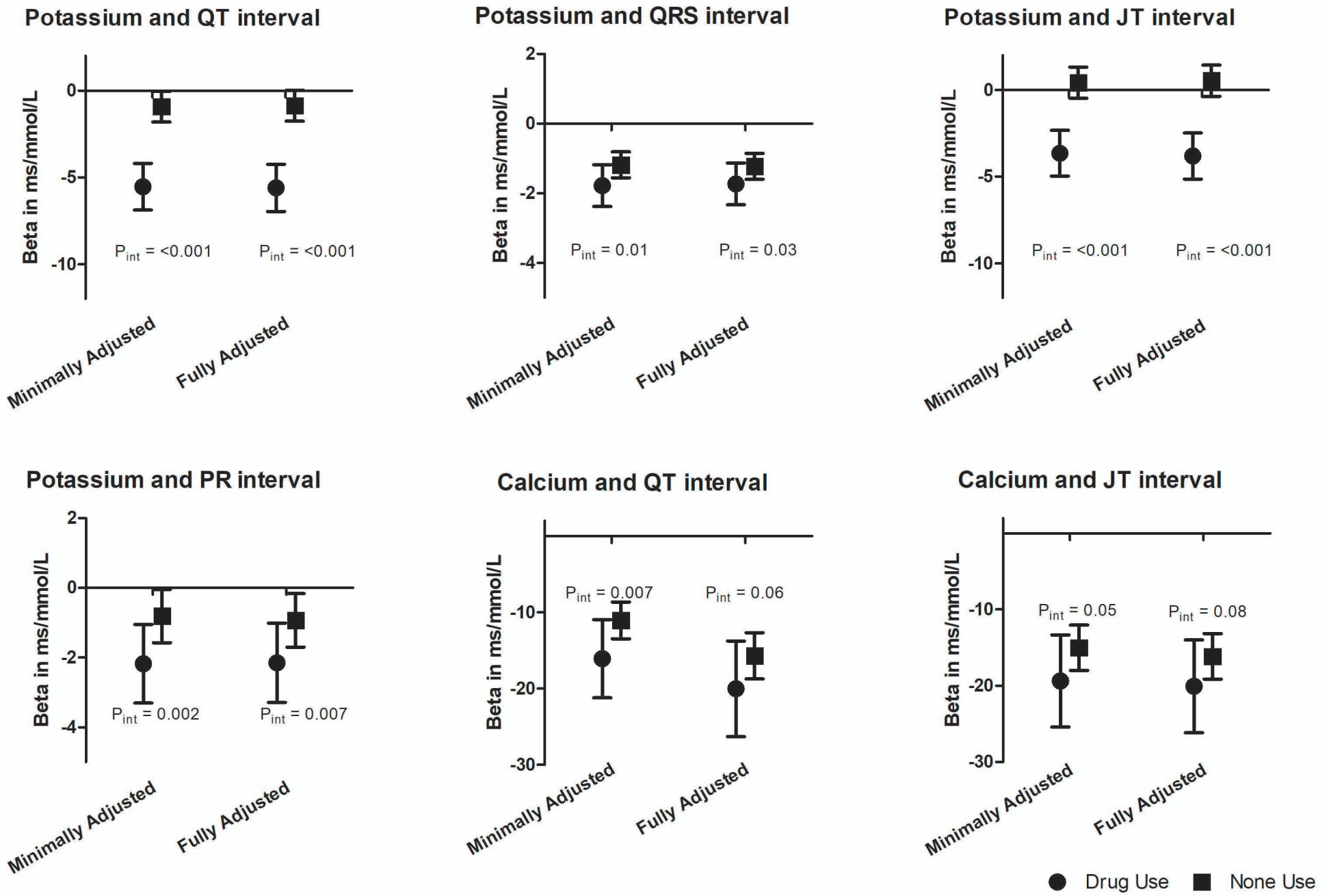
Abbreviations: N, number of individuals included in the analyses; “studies”, the number of studies contributing to the analysis; 95% CI, 95% confidence interval; Beta, the effect estimate from the linear regression model. The “Minimally adjusted model” included adjustment for age,

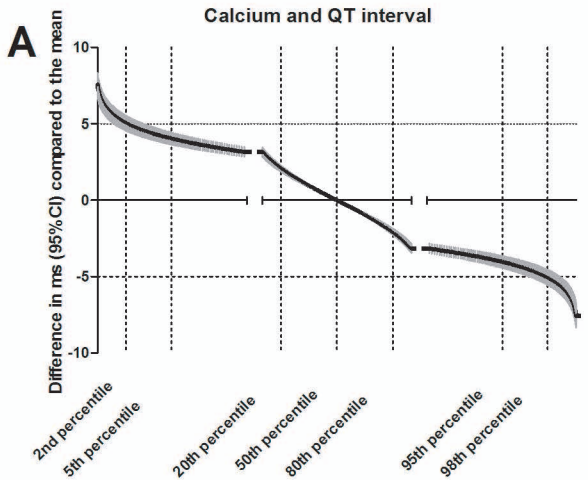
sex, RR-interval and cohort-specific covariates. The “Fully adjusted model,” in the cohorts with data on all covariates available, was additionally adjusted for body mass index, diabetes mellitus status, hypertension status and natural log of serum creatinine concentration. The Beta effect results presented are the changes in ECG (electrocardiogram) measure in milliseconds per 1 mmol/L increase in electrolyte concentration. A two-sided p-value was considered statistically significant.

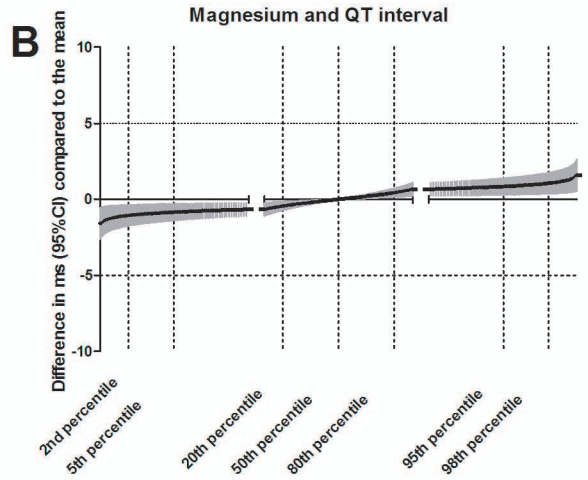

R
P
T
Q
S
QT
QRS
JT
-
+
-
Calcium
Magnesium
Potassium
Sodium

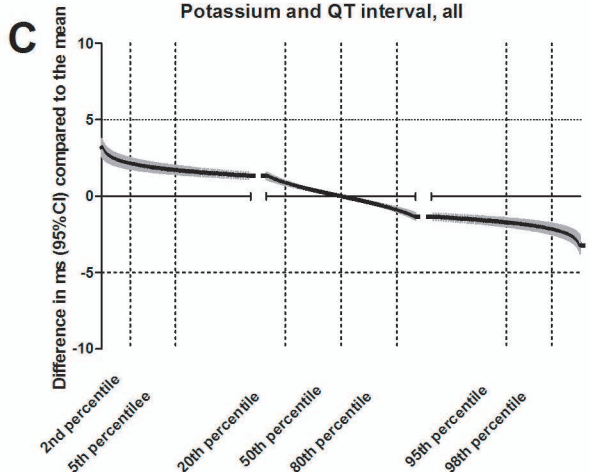

beta
1.0
2.0
3.0
Calcium QT, model 1
Calcium QT, model 4
Calcium QRS, model 1
Calcium QRS, model 4
Calcium JT, model 1
Calcium JT, model 4
Calcium PR, model 1
Calcium PR, model 4
Magnesium QT, model 1
Magnesium QT, model 4
Magnesium QRS, model 1
Magnesium QRS, model 4
Magnesium JT, model 1
Magnesium JT, model 4
Magnesium PR, model 1
Magnesium PR, model 4
Potassium QT, model 1
Potassium QT, model 4
Potassium QRS, model 1
Potassium QRS, model 4
Potassium JT, model 1
Potassium JT, model 4
Potassium PR, model 1
Potassium PR, model 4
Sodium QT, model 1
Sodium QT, model 4
Sodium QRS, model 1
Sodium QRS, model 4
Sodium JT, model 1
Sodium JT, model 4
Sodium PR, model 1
Sodium PR, model 4

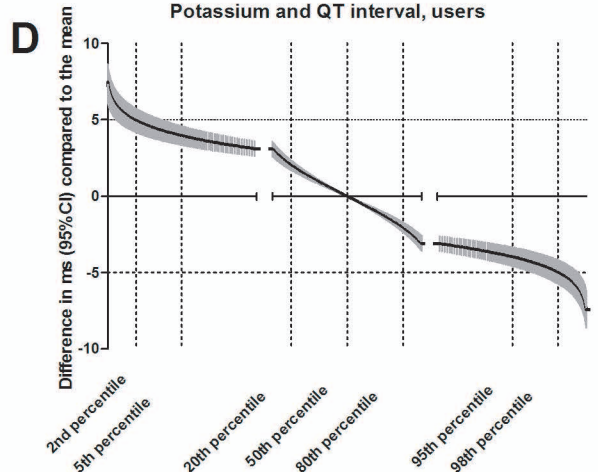

Calcium-QT association
Beta in ms/mmol/L
0
-10
-20
-30
P_int = 0.008
P_int = 0.06
Minimally Adjusted
Fully Adjusted
Calcium-JT association
Beta in ms/mmol/L
0
-10
-20
-30
-40
P_int = 0.009
P_int = 0.01
Minimally Adjusted
Fully Adjusted
Men
Women

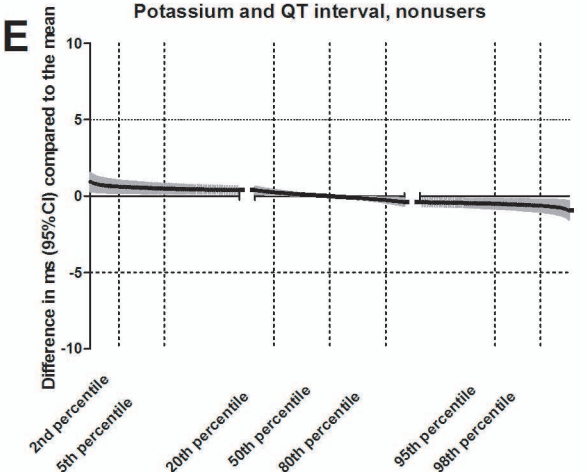

Potassium and QT interval
Beta in ms/mmol/L
0
-5
-10
P int = <0.001
P int = <0.001
Minimally Adjusted
Fully Adjusted
Potassium and QRS interval
Beta in ms/mmol/L
2
0
-2
-4
P int = 0.01
P int = 0.03
Minimally Adjusted
Fully Adjusted
Potassium and JT interval
Beta in ms/mmol/L
0
-5
-10
P int = <0.001
P int = <0.001
Minimally Adjusted
Fully Adjusted
Potassium and PR interval
Beta in ms/mmol/L
2
0
-2
-4
P int = 0.002
P int = 0.007
Minimally Adjusted
Fully Adjusted
Calcium and QT interval
Beta in ms/mmol/L
-10
-20
-30
P int = 0.007
P int = 0.06
Minimally Adjusted
Fully Adjusted
Calcium and JT interval
Beta in ms/mmol/L
-10
-20
-30
P int = 0.05
P int = 0.08
Minimally Adjusted
Fully Adjusted
Drug Use
None Use


A
Calcium and QT interval
Difference in ms (95%CI) compared to the mean
10
5
0
-5
-10
2nd percentile
5th percentile
20th percentile
50th percentile
80th percentile
95th percentile
98th percentile


B
Magnesium and QT interval
Difference in ms (95%CI) compared to the mean
10
5
0
-5
-10
2nd percentile
5th percentile
20th percentile
50th percentile
80th percentile
95th percentile
98th percentile


C
Potassium and QT interval, all
Difference in ms (95%CI) compared to the mean
10
5
0
-5
-10
2nd percentile
5th percentilee
20th percentile
50th percentile
80th percentile
95th percentile
98th percentile


D
Potassium and QT interval, users
Difference in ms (95%CI) compared to the mean
10
5
0
-5
-10
2nd percentile
5th percentile
20th percentile
50th percentile
80th percentile
95th percentile
98th percentile


E
Potassium and QT interval, nonusers
Difference in ms (95%CI) compared to the mean
10
5
0
-5
-10
2nd percentile
5th percentile
20th percentile
50th percentile
80th percentile
95th percentile
98th percentile