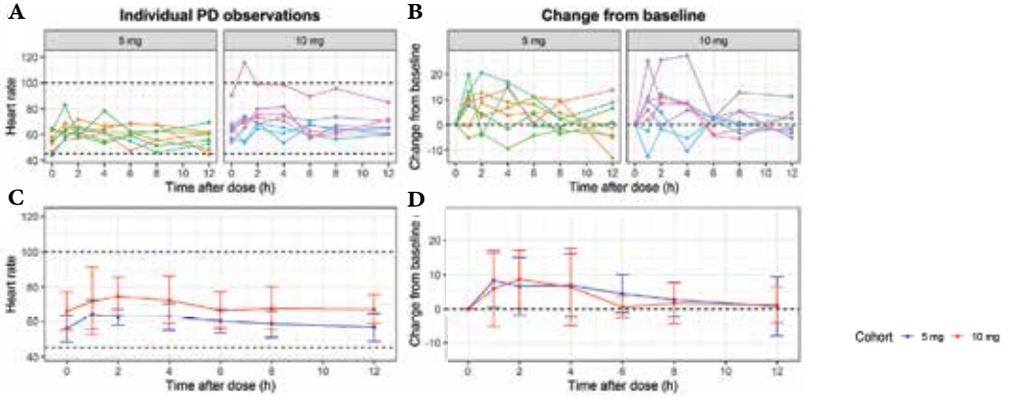
FEASIBILITY OF INTERPRETING BLINDED PHARMACODYNAMIC DATA – INVESTIGATING NORMAL VARIATIONS IN HEALTHY SUBJECTS

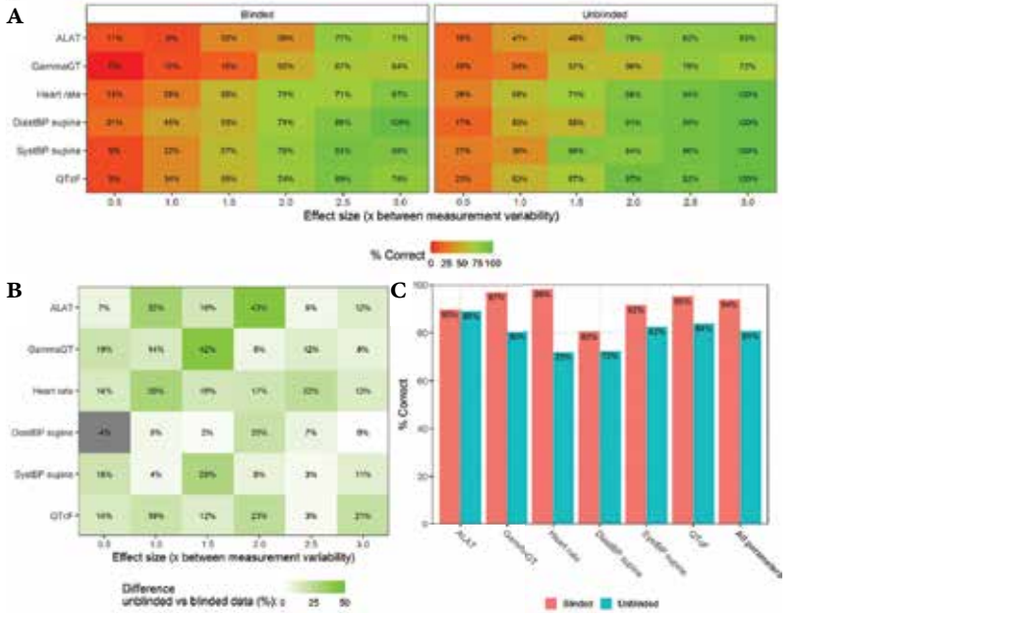
G.J.M. Hassing

PAGE 29 ➢ CHAPTER 2 FIGURE 1 **Stochastic simulation of blinded response data for two cohorts with 6 active and 2 placebo subjects, presented as individual measurements (A), change from baseline (B), and summarized as mean ± standard deviation (C, D) over time.** Slope of 2 × between measurement variability of the heart rate parameter was simulated in this scenario. Dashed horizontal lines in figure A and C present normal range based on literature. Dashed horizontal lines in figure B and D depict reference line for no change from baseline.



h = hours

PAGE 30 ➢ CHAPTER 2 FIGURE 4 **Heatmap with % correct decision of each parameter over effect size, based on blinded or unblinded data (A), the calculated delta (B), and all data combined (C).** Data is presented of two individuals who were shown both blinded or unblinded data. The % correct when no effect was simulated is presented in C. Parameters are sorted on the level of between measurement variability (high to low).



ALAT = alanine aminotransferase; DiastBP supine = diastolic blood pressure in supine position; SystBP supine = systolic blood pressure in supine position; QTcF = Fridericia corrected QT interval.

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CHAPTER I

INTRODUCTION

Compound development

A drug is defined as any compound that brings about a biological change in function through its chemical actions on a specific target.[1] The development of a new drug begins with a disease or clinical condition with an unmet clinical need which ideally is followed by extensive basic biomedical research to understand the pathophysiological processes behind a disease or clinical condition on a cellular and molecular level.[1,2] This basic biomedical research identifies potential drug targets through target validation and postulates hypotheses that inhibition or activation of the potential drug targets will result in a desired therapeutic effect.[1,2] Following identification of a potential drug target, the search for a suitable compound can be initiated either through screening of current chemical compound databases, development of newly synthesized compounds or through discovery of natural compounds.[1,2] Strategies for compound screening and design include structure-activity relationship, computer-aided design, combinational chemistry or synthetic human enzyme production.[1,3] After a new compound is deemed potentially eligible, extensive laboratory and preclinical testing follows prior to clinical development.[4] Once preclinical studies indicate that the compound is acceptably safe for administration in humans and shows pharmacodynamic effects, the owner of the development of the new compound will seek approval from health authorities such as the European Medicines Agency (EMA) or the United States Food and Drug Administration (FDA) and local ethics committees or investigational review boards to advance to the clinical phase.[4]

CLINICAL PHASE

The clinical phase is classically divided in four temporal phases (phase I-IV) and each clinical trial can be classified based their study type (human pharmacology, therapeutic exploratory, therapeutic confirmatory, therapeutic use).[5,6] Each phase provides a milestone in which the progress of development is reflected, while study type indicates the clinical trial's respective objectives. However, this concept is challenged and the current thinking is shifting to divide the clinical development period in early and late-stage development.

The present thesis will primarily apply to the interpretation of results from early stage development clinical trials (also called human pharmacology trials), although results from each phase allows implementation and modification of the development strategy of later phases and/or study types.[4-6] The initial human pharmacology clinical trials start with a first-in-human (FIH) administration of an investigational compound and aims to address one or more of the following objectives: assessment of tolerance, define/describe pharmacokinetics and pharmacodynamics, explore drug metabolism and drug interactions, and estimate activity.[5,6] Based on the risk profile and therapeutic potential of the compound it is decided if it would be appropriate to evaluate the compound first in healthy volunteers or whether it may only be administered to for example oncology patients where the risk/benefit profile of the drug may be more favourable.[4-6]

However, achieving these objectives is subject to limitations, especially when only one single objective is pursued. As an example, the tolerability of a single dose of a new compound, which is often one of the primary objectives of any given phase I clinical study, is not always as predictive of tolerability in the clinic.[7] Adverse reactions determining tolerability may occur at doses or plasma concentrations that are too high (direct toxic effects), or occur at doses or concentrations that are therapeutic, presumably through some other mechanism (collateral effects), or at relatively low concentrations in susceptible subjects (hyper susceptibility reactions).[7] While the former two are assumed to be more easily identifiable and quantifiable with direct and reversible dose-response (side-) effects, no data are available on the effect sizes that are required before a pharmacological (beneficial or adverse) effect can be observed in (blinded) evaluations in these early phase human pharmacological studies. Furthermore, patient specific characteristics, such as concomitant drug use, may further influence results and should be considered while evaluating the data even in healthy volunteers as illustrated in this manuscript. Hyper susceptibility reactions are less common and may lead to more serious adverse effects with consequentially irreversible damage.[7] Therefore, identification of such serious events are left to chance with for example a life-threatening event that occurs in 1:1000 subjects will only have a 1:100 chance of being detected in the typical early development group sizes of 20-200 subjects.[7]

Regarding the objective to evaluate a compound's pharmacokinetic and pharmacodynamic characteristics, starting dose and dosing regimen are paramount and are largely predicted using the preclinically pharmacokinetic and pharmacodynamic identified properties.[4,8] Predictions from the preclinical pharmacokinetic studies will drive how often the drug is administered, and

the nonclinical safety findings in animal models from the toxicology studies will inform the drug dose and how human subjects are monitored for potential adverse side effects.[6] Cautiously balanced contradictory requirements are weighted as dose selection for FIH studies should be sufficiently low to avoid toxicity, but not so low that any pharmacokinetic and pharmacodynamic effects cannot be observed.[8] Each end of the balance may add unnecessary testing in human trials, increase costs and potentially raise ethical reservations.[8] Data are continuously evaluated, usually blinded to the study treatment allocation, by scientists and physicians to judge the safety and futility of continuation of the trial. These blinded evaluations of data are formalized in the study protocol as dose escalation meetings and are used to project the safety and tolerability profile of the compound in a higher dose. This implies that reviewers must make a subjective interpretation of the presented, blinded data, on whether a pharmacologically induced (side) effect is present or not. A positive review from the dose escalation review committee is necessary to continue the study, with an increase in the dose of the investigated compound. These data presentation ordinarily do for obvious reasons not include statistical analyses and no differentiation which subjects were administered active or placebo treatment.

Next to evaluating the safety and tolerability of the increased dose level, intended or unintended pharmacology is evaluated. For example, in case intended pharmacology is observed at a level that is considered therapeutically relevant, additional cohorts with increased doses may not be ethical or rational. Furthermore, if unintended pharmacology is observed this may raise a safety concern, which may result in a premature termination of the study. Finally, it is not uncommon that follow-up studies are designed in parallel with an ongoing phase I study, using the preliminary blinded data. Thus, the subjective interpretation of blinded data plays a key role in the conduct of these studies and the design of subsequent studies.

However, no data are available on the effect sizes that are required before a pharmacological effect can be observed in these blinded evaluations of data. Therefore, **chapter** 2 of this thesis will describe the feasibility of interpreting blinded pharmacodynamic data of interim analyses in human pharmacology phase I studies. This chapter aims to describe the probability that effects are observed in these blinded evaluations of data in a simulated phase I human pharmacology study design. Moreover, this chapter will explore the effects of unblinding the data for reviewers to differentiate the effects of blinding on the interpretation.

Increasing the likelihood of detecting of a potential biomarkers linked to arrhythmia hazard has been a matter of great attention in early drug research.[9,10] An adverse event of considerable interest is the drug-induced ventricular arrhythmia, particularly when associated to prolongation of the QT(c)-interval. These drug-induced ventricular arrhythmia are linked to formation of polymorphic ventricular tachycardia called Torsades de Pointes and are potentially fatal.[9] However, because of their relatively low incidence, these potential biomarkers may only be identified after exposure of large numbers of subjects, and following investment of large quantities of resources.[10]

ELECTROCARDIOGRAM

The most used screening method to detect these drug-induced ventricular arrhythmia biomarkers is the electrocardiogram (ECG). First recorded with the use of a mercury capillary electrometer by Augustus Waller in 1887 (London, United Kingdom) and further improved with the introduction of string galvanometers by Willem Einthoven in 1901 (Leiden, The Netherlands), the use of the ECG has become an integral part of cardiac evaluation and management.[11,12] An ECG reflects the summation of the individual cardiac cell action potentials generated by the different transcellular ionic currents in the atrial and ventricular cardiac compartments and is measured at the body surface in a standardized method through 12 leads.[10,13]

The cardiac action potential occurs when a cardiomyocyte membrane potential depolarizes and then repolarizes back to the resting state and typically ranges from 200 to 400 milliseconds.[10,13] The plateau phase of the action potential is a time of elevated membrane resistance and/or low current flows and any disturbance of the balance between depolarizing and repolarizing currents can therefore drastically change the duration of the plateau phase and consequently the duration of the action potential.[10,14] Depolarization (upstroke in current) is primarily driven by the fast Na+ and slower Ca^2+ currents while the repolarization (plateau phase) is predominantly driven by the K+ and Ca^2+ currents.[10,13] Each electrolyte current is mediated by their respective ion-channel, as illustrated in figure 1. Of particular interest is the potassium ion channel, whose alpha subunit is highly sensitive and quickly blocked by specific drugs which results in slower potassium currents and thus repolarization prolongation.[15] This alpha subunit of the potassium channel is sometimes referred to simply as 'hERG' as the human Ether-à-go-go-Related Gene is the specific gene that codes for this channels' subunit.[16]

QT INTERVAL AND MEASUREMENT

Translated clinically, the QT interval represents the duration of ventricular depolarization and subsequent repolarization and is measured from the beginning of the QRS complex to the end of the T wave. Duration of the cardiac action potential mirrors the QT interval, while the maximum rate of depolarization determines conduction velocity and influences PR and QRS durations.[17,18] Ideally, QT interval measurements are performed on a 12 lead surface ECG to improve accuracy and correct for the different projections of the ventricular complex on the limb axis in different leads.[19] The QT interval is inversely proportionally subject to RR interval (time elapsed between two successive R-waves of the QRS signal on the ECG) with shorter QT intervals with increasing heart rates. In turn, RR interval is a function of intrinsic properties of the sinus node as well as autonomic influences and within normal healthy subjects, age and heart rate are the major determinants of heart rate variability.[19,20]

To accurately measure QT intervals, a stable RR is required and the Society for Cardiological Science & Technology therefore states that time should be taken for any given individual to be relaxed and comfortable prior to ECG measurements, although there are also groups advocating a 10 minute or longer supine period prior to ECG measurements.[1] However, in a clinical research unit volunteers are generally domiciled during phase I studies and may therefore require shorter resting periods prior to ECG measurements. Therefore, it is desirable to maintain a resting window that is on one hand long enough to allow stabilization of the QT interval, but that is short enough so that there is sufficient time for other measurements. Chapter 3 of this thesis will aim to address how much time is required to acquire a stabilized RR interval in a typical clinical setting and after a short exercise.

QT INTERVAL COMPONENTS

To address the relational problem of QT dependency on RR interval, many formulas have been proposed with the most commonly utilized are Bazett's and Fridericia's formula.[21,22] Both formulas have their respective limitations which ultimately results in a consistent overestimation of changes in QT interval corrections (QTc interval) at faster heart rates (e.g. above 120 beats-per-minute) or underestimation at lower heart rates (e.g. below 60 beats-per-minute). However, as stated in the E14 guideline of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), the Bazett's formula is deemed an inferior method of correcting for differences in heart rate among and within subjects.[23] Therefore, Friderica's formula is preferable as it is more reliable in assessing the effects of drugs on the duration of repolarization.[24,25]

Alternative analysis methods have been proposed to integrate the entire ECG, and not solely encompassing the QT interval, to identify compounds that may block specific electrolyte channels such as one by Johannesen et al.[15] Johannesen's aim is to pull apart the QT interval and analyse all the QT interval components separately. This method should help distinguish between drugs that prolong the QT interval through blockage of the so-called 'hERG' channels and drugs that block other ion-channels (such as calcium/late sodium channels) and are less likely to cause ventricular arrhythmias. The latter type of drugs may be considered "safe" despite their QT interval prolongation and therefore do not require expensive additional studies for the cardiac analysis, while the former does warrant more extensive cardiological analyses.[15]

The subintervals used by Johannesen et al are ventricular activation time, QRS duration, J-point – T-peak interval (corrected for RR interval by his own proposed formula) and T-peak-T-end interval.[15] As mentioned, this QTc subinterval differentiation allows for improved characterization of the drug effects on the QT interval and differentiation between early and late repolarization.[26] This subinterval analysis has been reported to be of use to differentiate channel interactions of novel compounds.[26] For example, hERG potassium channel blockages prolongs both early repolarization duration (J–T peak : end of QRS to global peak of T-wave) and late repolarization (Tpeak–Tend: global peak to end of T-wave).[27] However, adding late sodium channel blockers to a pure hERG blocker leads to a reduction in early repolarization duration.[27] Ultimately, J-Tpeak interval was identified as the best biomarker for differentiating QTc prolonging drugs with predominant hERG block from drugs with multichannel (hERG plus late sodium and/or calcium block).[28]

QTc INTERVAL AND PATIENT CHARACTERISTICS

Again translated clinically, surprisingly little is known to which extent the QTc subintervals are influenced by patient characteristics within a healthy population. This concerns for instance body temperature, body composition, blood pressure, and sex. As an illustration, sex appears to have no effect on drug-induced QT interval changes, though pure hERG potassium channel blockade does increase early repolarization duration, late repolarization and shortening of T-wave in women compared to men.[29] This appears at odds with the

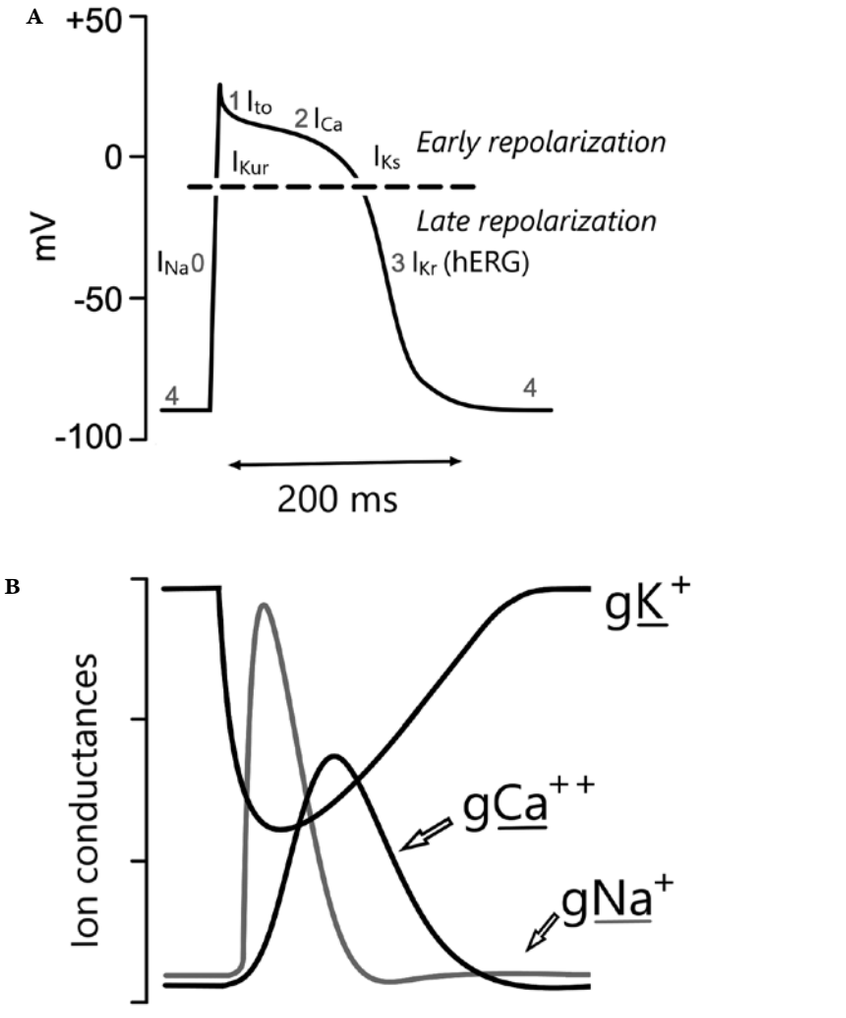
finding that women have an increased risk of Torsade de Pointes.[30] However, to which degree other patient characteristics may influence these QTc subintervals in healthy individuals remains unknown. Therefore, chapter 4 will cover the body temperature related ECG changes in normothermic healthy individuals. Chapter 5 will cover the blood pressure related ECG changes in healthy young individuals. In chapter 6, body mass index related ECG changes in healthy volunteers with a normal body mass index will be outlined. Chapter 7 will briefly discuss the relevance of total bilirubin levels on QT interval. Finally, chapter 8 will outline the effects of haemoglobin levels on ECG related parameters.

The outline of this thesis

Chapter 2 will describe the feasibility of interpreting blinded and unblinded pharmacodynamic data of interim analyses in human pharmacology studies. In chapter 3, heart rate variability and stabilization in a human pharmacology study setting will be discussed. Chapter 4 will cover the body temperature related ECG changes in normothermic healthy individuals. Chapter 5 will cover the blood pressure related ECG changes in healthy young individuals. In chapter 6, body mass index related ECG changes in healthy volunteers with a normal body mass index will be outlined. Chapter 7 will briefly discuss the relevance of total bilirubin levels on QT interval. Finally, chapter 8 will outline the effects of haemoglobin levels on ECG related parameters.

Jointly, these investigations described in this theses aim to help physicians to make informed decisions on the risk-benefit balance in early phase drug development trials.

FIGURE 1 Changes in a ventricular myocyte action potential (phases 0-4) with the linked ion-channels (part 1A) and the associated ion conductances changes (part 1B).



mV = millivolts, ms = milliseconds, K = potassium ion, Ca = calcium ion, Na = sodium ion, Ina = inward sodium ion channel, Ito = transient outward current, Ica = calcium current, IKur = potassium current, ultra rapid, Iks = potassium current, slow, IKr = potassium current, rapid. Phase 4 = resting, phase 0 = upstroke, phase 1 = early repolarization, phase 2 =plateau, phase 3 = late repolarization.

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CHAPTER II

DOSE ESCALATIONS IN PHASE I STUDIES: FEASIBILITY OF INTERPRETING BLINDED PHARMACODYNAMIC DATA: EFFECT IDENTIFICATION – TO BLIND OR TO UNBLIND

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What is already known about this subject

In early-phase clinical trials, conventionally reviewers must make subjective interpretations of generally blinded data on whether a pharmacologically induced (side) effect is present or not. However, no data are available on the effect sizes that are required before such a pharmacological effect can be observed in these blinded subjective evaluations of data.

WHAT THIS STUDY ADDS

Effect sizes smaller than 2 times the between-measurement standard deviation the investigated outcome frequently go unnoticed by blinded reviewers. Though unblinding reviewers resulted in about a 20% increase in correctly identifying a given effect, unblinding also resulted in more false positives when no effect was present.

Abstract

AIM During phase I study conduct, blinded data are reviewed to predict the safety of increasing the dose level. The aim of the present study was to describe the probability that effects are observed in blinded evaluations of data in a simulated phase I study design.

METHODS An application was created to simulate blinded pharmacological response curves over time for six common safety/efficacy measurements in phase I studies for 1 or 2 cohorts (6 active, 2 placebo per cohort). Effect sizes between 0 and 3 between-measurement standard deviations(SD) were simulated. Each set of simulated graphs contained the individual response and mean ± standard deviation over time. Reviewers (n=34) reviewed a median of 100 simulated datasets and indicated whether an effect was present.

RESULTS Increasing effect sizes resulted in a higher chance of the effect being identified by the blinded reviewer. On average, 6% of effect sizes of 0.5 between-measurement SD were correctly identified, increasing to 72% in 3.0 between-measurement SD effect sizes. In contrast, on average 92 to 95% of simulations with no effect were correctly identified, with little effect of between-measurement variability in single cohort simulations. Adding a dataset of a second cohort at half the simulated dose did not appear to improve the interpretation.

CONCLUSION Our analysis showed that effect sizes smaller than 2 times the between-measurement SD of the investigated outcome frequently go unnoticed by blinded reviewers, indicating that the weight given to these blinded analyses in current phase I practice is inappropriate and should be re-evaluated.

Introduction

During the conduct of clinical studies, scientists evaluate data to judge the safety and futility of continuation of the trial. Responsible scientists and physicians are typically blinded to the study treatment allocation during these early-phase evaluations. These blinded evaluations of data are formalized in the study protocol as dose escalation meetings and are used to project the safety and tolerability profile of the compound in a higher dose. This implies that reviewers must make a subjective interpretation of the presented, blinded data, on whether a pharmacologically induced (side) effect is present or not. A positive review from the dose escalation review committee is necessary to continue the study, with an increase in the dose of the investigated compound. Data presentation ordinarily does not include statistical analyses and no differentiation which subjects were administered active or placebo treatment.

Next to evaluating the safety and tolerability of the increased dose level, intended or unintended pharmacology is evaluated. For example, in case intended pharmacology is observed at a level that is considered therapeutically relevant, additional cohorts with increased doses may not be ethical or rational. Furthermore, if unintended pharmacology is observed this may raise a safety concern, which may result in a premature termination of the study. Finally, it is not uncommon that follow-up studies are designed in parallel with an ongoing phase I study, using the preliminary blinded data. Thus, the subjective interpretation of blinded data plays a key role in the conduct of these studies and the design of subsequent studies.

However, no data are available on the effect sizes that are required before a pharmacological effect can be observed in these blinded evaluations of data. Therefore, the aim of the present study is to describe the probability that effects are observed in these blinded evaluations of data in a simulated phase I study design and data evaluation.

Methods

OVERALL DESIGN

A custom browser-based application was developed that facilitated the review of simulated blinded evaluations of data of the datasets reviewed during routine phase I clinical studies conducted at the Centre for Human Drug Research in Leiden, The Netherlands. Various effect sizes, expressed as multiples of the between measurement standard deviation (SD) were simulated for 6 different parameters. Scientific staff members (n=34) each reviewed a median of 100 simulated datasets.

DATA SIMULATION

PHARMACOKINETICS

A one-compartment pharmacokinetic model simulated a pharmacokinetic profile of an orally administered fictional compound, with the following parameters: volume of distribution = 5 L/h, clearance = 2 L/h, absorption rate constant=0.8/h. Doses of 5 mg and/or 10 mg were simulated, resulting in a C_{max} of 0.5 and 1.0 ng/mL respectively, with a t_{max} of 1.7h. A variance of 0.01 was introduced on the volume of distribution and clearance parameter, resulting in a low level of inter-individual variability in the pharmacokinetic profile. This variance was not altered between simulations as the focus of this analysis was on the variability in the pharmacodynamic response.

SIMULATION OF PHARMACODYNAMIC RESPONSE

The response over time was simulated using a linear effect model based on the estimated parameters (see Parameter description section):

$$Slope = Between\ measurement\ variability\ (SD) \times Effect\ size$$

$$Response = (Baseline + Slope \times Concentration) \times (1 + N[0, \sigma^2])$$

Where Between measurement variability (SD) is the estimated standard deviation of the between-measurement variability for each parameter, multiplied by an Effect size, ranging between –3 and 3. The occurrence of a no effect scenario in the simulation (Effect size = 0) was higher to account for potential bias due to chance. This results in slopes being simulated that show no effect (effect size = 0) up to slopes that show an effect of three times the SD of the between-measurement variability. Baseline is the typical value of a parameter in a population, simulated with the estimated lognormal distribution of the inter-individual variability. Concentration is the simulated pharmacokinetic concentration (ng/mL) of an individual. Furthermore, a proportional residual error term was added on the response which was identical to the variance of the between-measurement variance, sampled from a normal distribution with mean 0 and variance σ^2.

For example. as the C_{max} of the 10 mg cohort was equal to 1 ng/mL, a Between measurement variability SD of 10% and an Effect size of 1 would result in a pharmacodynamic response being simulated that has 10% effect (equal to 1 SD) at the t_{max}, with an identical between-measurement variability (residual error) being implemented. Dependent on the simulated effect size, this would result in effect sizes of up to 3× (30%) the between-measurement variability being simulated at the t_{max}.

Sampling of pharmacokinetic and the measurements of the response were performed at timepoint oh (pre-dose), 1, 2, 4, 6, 8 and 12h post-dose. Parametrical diurnal effects were left out of the simulation to prevent clouding of the evaluation.

GRAPHICAL USER INTERFACE DESCRIPTION

An internal browser-based R Shiny application was created to simulate pharmacological dose-response curves that mimicked the typical phase I dose escalation profiles frequently encountered.[1] Each set of simulated graphs contained 5 graphs of a single parameter, in which the data of 8 subjects, 6 that received the active compound and 2 that received a placebo treatment, were simulated. The following graphs were generated and displayed:

- Individual datapoints simulated over time
- Individual change from baseline data over time
- Mean data over time, including whiskers displaying the standard deviation (SD), of each cohort
- Mean ± SD change from baseline data over time, of each cohort
- The mean ± SD of the pharmacokinetic profile, of each cohort

The model either simulated a single 10 mg dose of the fictional compound or simulated both a single 5 mg and 10 mg dose of the fictional compound. In the latter case, all graphs were generated for both the 5 and 10 mg data. An example of a simulated response profile over time for the heart rate parameter, based on data of 2 cohorts, is presented in Figure 1. The pharmacokinetic profile, which was also presented in the graphical user interface, is shown in figure S1.

PARAMETER DESCRIPTION

For 18 frequently recorded parameters, the baseline response, the between-measurement and the inter-individual variability was calculated based on previously collected data during mandatory medical screening in healthy volunteers. Out of these 18 parameters, six were identified that covered a 10-fold range in coefficients of variation for the between-measurement variability, ranging from 2.2% for the QTcF to 24.7% for Alanine aminotransferase. The selected variables are shown in table 1. Each variable was simulated with either a negative, positive or no effect following dosage except for Alanine aminotransferase (ALAT) and Gamma-GT, which were simulated as either a positive effect or no effect.

REVIEWERS

All reviewers (n=34) were part of the scientific staff of the Centre for Human Drug Research in Leiden, the Netherlands with ample experience at different research institutions or pharma companies. This included research physicians or clinical scientist (n= 20), post-docs (n=12) or professors (n= 2) with a mean of 1.7, 4.3, and 15.5 years of clinical experience, respectively. Reviewers were provided with standardized instructions, among which that they could only consider an effect positive or negative in case they would defend this effect against a study sponsor that is developing the fictional compound irrespective of whether the effect would be desirable or not.

UNBLINDED EVALUATION

To differentiate the effects of blinding on the interpretation of these data, a simulation was performed with the only difference that participants on active treatment were identifiable from participants treated with placebo. The cohort average plots also displayed the averages of the actively treated participants from the placebo-treated participants. The same parameters with the same settings were used. Two Centre for Human Drug Research staff members (GJH and PG) reviewed a total of 1423 blinded and 1230 unblinded datasets.

STATISTICAL ANALYSIS

Data are presented as % of correctly identified as an effect in a heatmap, specified for each effect size and parameter, or % of correctly identified as no effect for each parameter as a bar plot. A sigmoidal nonlinear least squares model was fitted in R on blinded data with a simulated effect for each parameter, and for comparison of the blinded and unblinded simulations.[1]

To achieve a resolution of maximum 5% for each parameter, effect size and number of cohorts (72 combinations) it was estimated that 1440 simulated datasets with an effect size higher or lower than 0 needed to be reviewed. A linear mixed effect model to identify a minimal detectable effect size with a statistical power of 80% was used based on 1 or 2 (4 placebo subjects, 6 active per cohort) cohorts for each parameter with a contrast up to 6h after dosing. The minimal detectable effect size was based on simulated effect sizes ranging

from 2 to 4.5 with 500 iterations for each effect size, parameter, and number of cohorts. The statistical power of each scenario was derived, and an overall mean power was calculated.

Results

In total, 34 scientific reviewers evaluated between 3 and 773 simulated datasets (median =100, mean = 139), a total of 3779 data simulations were evaluated, of which 1945 data simulation with an effect size higher or lower than 0.

EFFECT IDENTIFICATION

An increasing effect size resulted in a higher chance of the effect being identified by the reviewer, as can be observed in figure 2. On average, only 6% of effect sizes of 0.5 SD and 20% of effect sizes of 1 time the SD of the between-measurement variability were correctly identified by the reviewers. For example for systolic blood pressure effects, this resulted in a 5% chance to identify a 3.6 mmHg effect and 23% probability to identify a 7.2 mmHg effect. Also, a lower between-measurement variability resulted in a higher chance of the effect being identified by the reviewer, in particular for ALAT and Gamma-GT at the largest effect sizes, which have the highest between-measurement variability, as is best observed in figure S2. The effect of reviewing 2 dose level as opposed to 1 dose level resulted in a similar probability of the effect being identified by the reviewer and no clear improvement was identified, as shown in figure 2B and S3.

NO EFFECT IDENTIFICATION

On average, the reviewer correctly identified 92 to 95% of simulations with no effect, with negligible effect of intrasubject variability as illustrated in figure 3. Also, there appears to be no effect of reviewing data from 1 cohort as compared to 2 cohorts.

UNBLINDED ANALYSIS

A substantial improvement in the ability to identify effects was made by unblinding the reviewer to the treatment allocation. Unblinding the reviewer resulted in about a 20% increase in the ability of reviewers to identify the simulated effects correctly. However, this also resulted in an increased incorrect assessment when no effect was simulated, which was on average 13% less as shown in figure 3 and in figure 4.

MINIMAL DETECTABLE EFFECT SIZE

Correct identification of a minimal detectable effect size with an 80% statistical power was not reached with a linear mixed effects model for effect sizes of up to 4.5 SD for a single cohort data simulation, as illustrated in figure 5. However, for 2 cohort data simulations, the minimal detectable effect size with an 80% power was reduced to 3.5 SD because of the increased number of placebo subjects.

Discussion

This analysis found that effect sizes smaller than 2 times the SD of the between-measurement variability are frequently not observed during the blinded reviewers subjective interpretation. This probability does not seem to improve when multiple dose levels were modelled. In contrast, when no effect is present, this is usually correctly observed. Unblinding the data resulted in some improvement, but effect sizes smaller than 1 time the SD of between-measurement variability frequently remain unobserved and a false positive effect is more common in this scenario. Moreover, to detect a minimal effect with an 80% statistical power in linear mixed effect model required effect sizes of 3.5 or higher in simulations with 2 cohorts while single cohort simulations did not even reach those levels at effect sizes of 4.5. These results indicate that the weight given to these blinded analyses for predicting the safety and efficacy of an increasing dose level, as is common in current phase I practice, may be too big and should be re-evaluated.

Although the mainstay of phase I dose escalation studies is the ability to review safety, pharmacokinetic and intended or unintended pharmacology, there are surprisingly few data available to support the rationale for this approach. Most effect sizes in phase I studies, particularly in otherwise healthy volunteers, are relatively small in comparison to the variability. For example, the maximum pharmacodynamic effect of anti-hypertensive drugs is usually about 10 mmHg, which is about 1 SD between-measurement variability of systolic blood pressure.[2] The reviewers of our data detected this only 23% of the times. Another example is QTc prolongation, in which some non-cardiovascular drugs with a mean increase in the QT interval of 5 to 10 milliseconds have been withdrawn from the market because of an increased risk of torsade de pointes after coadministration with a strong CYP inhibitor.[3] Most notably is Terfenadine which averaged a QT interval prolongation of approximately 6 milliseconds in healthy individuals which was about 0.5 SD intrasubject vari-

ability in our data set.[3] The reviewers of our data detected this only 6% of the times. Unblinded review of the same data resulted in a higher possibility of correctly identifying smaller effect sizes for all parameters including systolic blood pressure and QTc. Therefore, our data support unblinded review of systolic blood pressure and QTc in phase I. This should result in a higher chance of detection of effect sizes smaller than 1 time the SD of intrasubject measurement variability at the cost of an increased probability of incorrect identification of an effect.

Conversely, incorrect identification of an effect may have unwanted consequences. First, overlooking a pharmacodynamic effect may lead to inaccurate prediction of the pharmacodynamic effects of the next dosing step. This leads to misinterpretation of safety risks. Also, our analysis shows that an investigator's capabilities to identify the maximum tolerable dose are limited. We have previously published that identifying a maximum tolerated dose should never be the purpose of phase I trials.[4] Moreover, when desired pharmacodynamic effects are overlooked this may lead to irrational continuation of the study to a dose level that is excessive. This may even lead to discontinuation of development when desired pharmacodynamic effects are not observed especially when further dose escalation is unsafe based on preclinical studies. Such a false-negative finding may also affect the design of follow-up studies.

Secondly, our results suggest that investigators should design their study keeping in mind the observable effect sizes with their respective statistical power in blinded interim analyses given the study population. For pharmacodynamic readouts that are implemented to evaluate desired pharmacodynamic effects, the effect size should exceed at least 1 SD, but preferably 2 times the SD of the intrasubject measurement variability. Obviously, increasing the number of study participants will lead to smaller effect size to be detectable and an adequate power analysis must be performed. However, from a safety perspective, increasing the entire study population is not considered feasible. Moreover, our data shows that having intermediate dosing levels up until the effect size that was simulated does not improve the interpretation of the data. Rather, it is recommended to include sensitive methodologies in phase I studies with a lower intrasubject variability to allow more accurate identification of pharmacologically induced effects.

A workable solution for key variables would be to conduct grouped or unblinded analyses to allow more accurate evaluation of pharmacodynamic and pharmacokinetic effects. Nevertheless, it must be noted that it may still not be possible to detect small effects considering the limited group size typically used in phase I studies. In addition, it is not unimaginable that the traditional phase 3 studies may be abolished and replaced with intensive measurements with sensitive methodologies in phase 1 and 2 studies in the future. This will allow a more accurate assessment of the chance that a compound will demonstrate favourable effects in clinical practice. Lastly, compound or even disease specific model-informed drug development as proposed by the Food and Drug Administration may ultimately improve decision-making through integration of data from each new (pre-) clinical study into biological and statistical models during development.[5]

LIMITATIONS

The initial training simulations with medical screening data provided a fit for purpose selection of parameters with a broad intrasubject distribution and inter-subject variability specific for the selected dataset. However, through implementation of additional data per subjects, or potential circadian rhythm in an outcome, may change the level of inter- and intra-subject variability in each parameter. Moreover, effect sizes are also variable between individuals in real-life and variability in the pharmacokinetics is more present, which may further affect the total level of variability in the data, though both effects were not accounted for in the present simulations. Finally, our results were solely based on the evaluation by reviewers of a single institute while perhaps variability exists between research centers. In order to minimize this potential bias, we included reviewers with a wide range of years of clinical experience of up to 15 years with work experience at various different institutes.

Conclusion

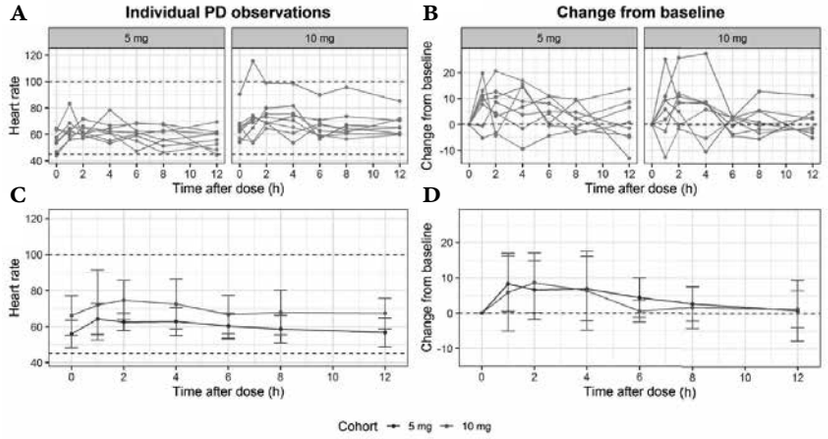
Our analysis showed that effect sizes smaller than 2 times the between-measurement SD of the investigated outcome frequently go unnoticed by blinded reviewers even when multiple dose levels were modelled. Unblinding the data resulted in some improvement, but effect sizes smaller than 1 time the between-measurement SD frequently remain unobserved and a false positive effect is more common in this scenario. These results indicate that the weight given to these blinded analyses for predicting the safety and efficacy of an increasing dose level, as is common in current phase I practice, may be too big and should be re-evaluated.

TABLE 1 Dataset information and model estimates for each parameter.

Parameter	Number of individuals with one/two measurements in dataset	Baseline parameter	IIV CV % (ω2)	BMV % (σ2)	BMV group	Simulated effect profiles
Alanine aminotransferase (U/L)	84/711	20.76	39% (0.14)	24.7% (0.06086)	High	None/positive effect
Gamma-GT (U/L)	154/625	19.95	52% (0.24)	17.6% (0.03081)	High	None/positive effect
Heart rate (BPM)	261/495	60.44	12% (0.015)	10.1% (0.01027)	Medium	None/negative/ positive effect
Diastolic blood pressure (mmHg)	256/495	71.99	10% (0.010)	7.9% (0.0062)	Medium	None/negative/ positive effect
Systolic blood pressure (mmHg)	153/636	123	8.9% (0.0079)	5.8% (0.003398)	Low	None/negative/ positive effect
QTCF (ms)	170/626	409.7	4.0% (0.0016)	2.2% (0.000503)	Low	None/negative/ positive effect

CV% = sqrt(exp(ω2)-1) Each parameter with corresponding levels of inter-individual (IIV) and between measurement (BMV) variability. CV% = Coefficient of variation, U/L = units/liter.

FIGURE 1 Stochastic simulation of blinded response data for two cohorts with 6 active and 2 placebo subjects, presented as individual measurements (A), change from baseline (B), and summarized as mean ± standard deviation (C, D) over time. Slope of 2 × between measurement variability of the heart rate parameter was simulated in this scenario. Dashed horizontal lines in figure A and C present normal range based on literature. Dashed horizontal lines in figure B and D depict reference line for no change from baseline.
➢ *See inside front cover for the coloured version of this image.*



H = hours

FIGURE 2 Heatmap with % correct decision of each parameter over effect size, based on blinded data of one or two cohorts (A), the calculated delta (B), and all data combined (C). The % correct and the simulated effect size of a parameter is reported in each cell (C). Parameters are sorted on the level of between measurement variability (high to low).
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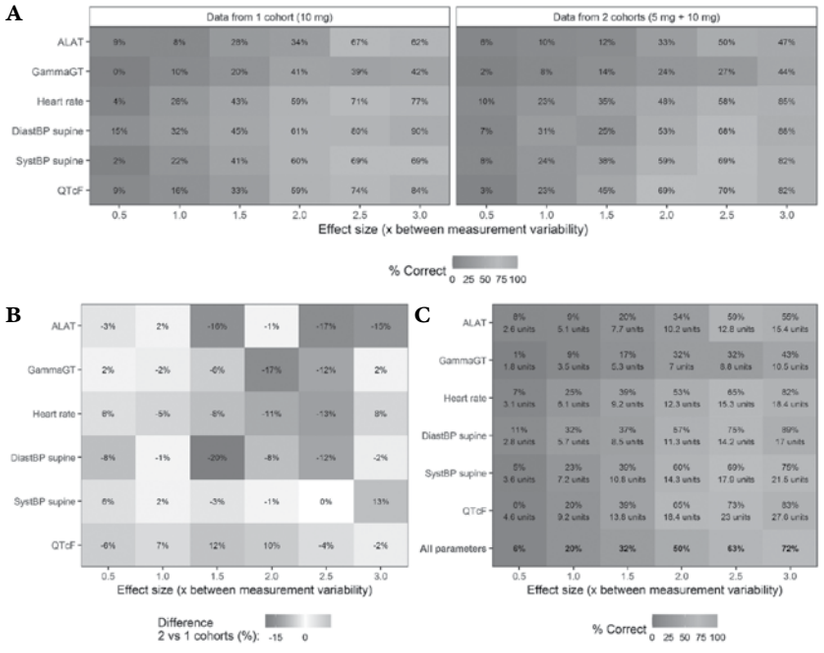
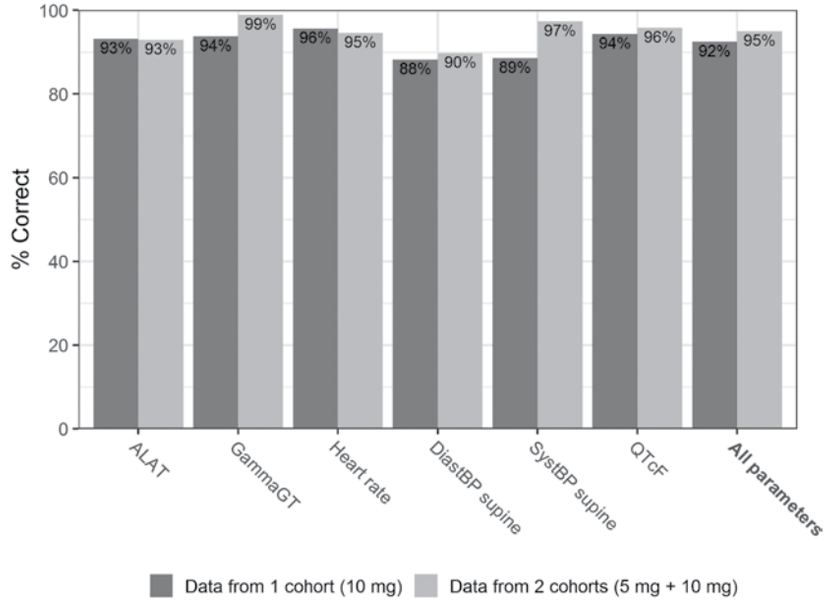


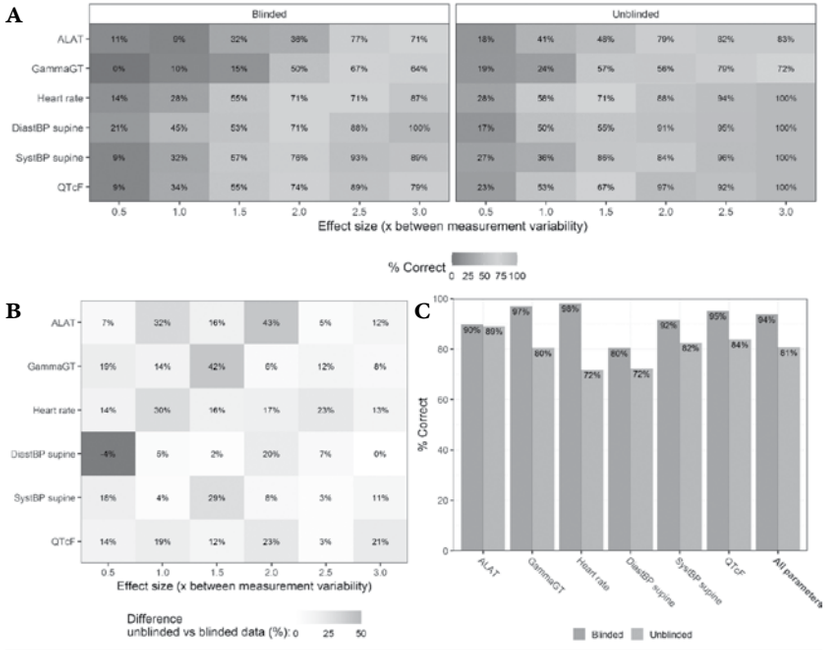
ALAT = alanine aminotransferase; DiastBP supine = diastolic blood pressure in supine position; SystBP supine = systolic blood pressure in supine position; QTcF = Fridericia corrected QT interval.

FIGURE 3 Barplot with % correct decision when no effect was present in different simulation scenarios.



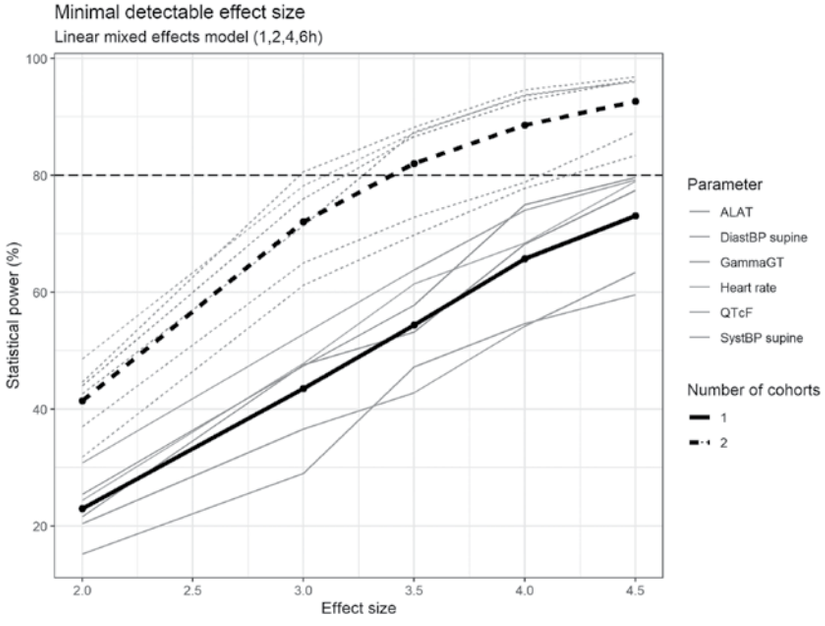
ALAT = alanine aminotransferase; DiastBP supine = diastolic blood pressure in supine position; SystBP supine = systolic blood pressure in supine position; QTcF = Fridericia corrected QT interval.

FIGURE 4 Heatmap with % correct decision of each parameter over effect size, based on blinded or unblinded data (A), the calculated delta (B), and all data combined (C). Data is presented of two individuals who were shown both blinded or unblinded data. The % correct when no effect was simulated is presented in C. Parameters are sorted on the level of between measurement variability (high to low).



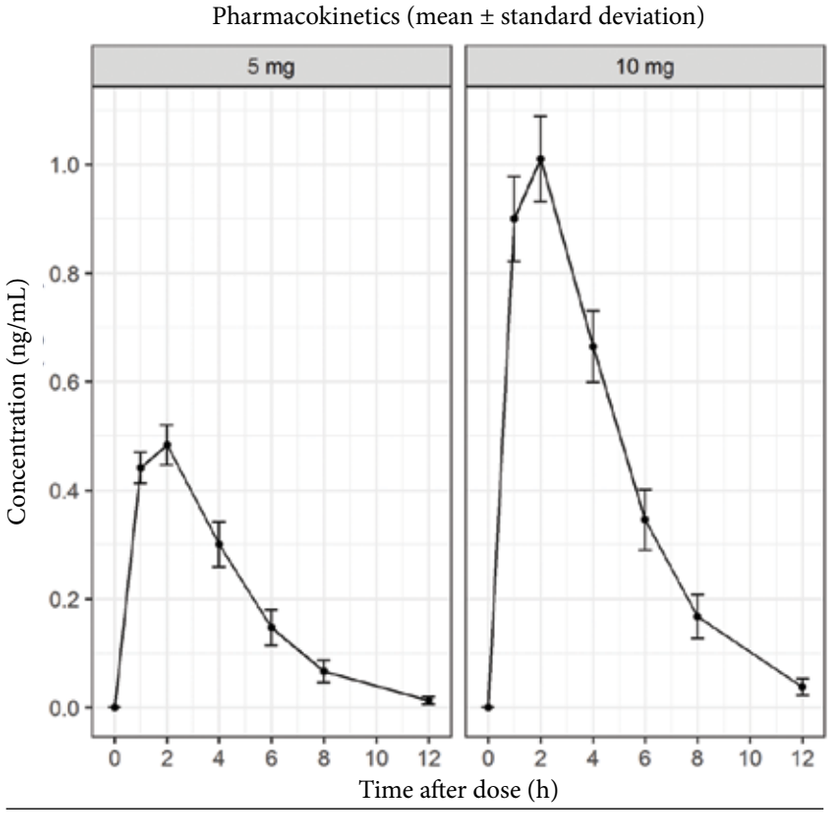
ALAT = alanine aminotransferase; DiastBP supine = diastolic blood pressure in supine position; SystBP supine = systolic blood pressure in supine position; QTcF = Fridericia corrected QT interval.

FIGURE 5 Statistical power for each parameter with an effect size between 2.0 and 4.5 standard deviation effects as calculated with a linear mixed effect model for both single or double drug cohort simulations. Dotted horizontal line highlights the statistical power to detect a minimal detectable effect size with 80% certainty.
➢ *See inside back cover for the coloured version of this image.*



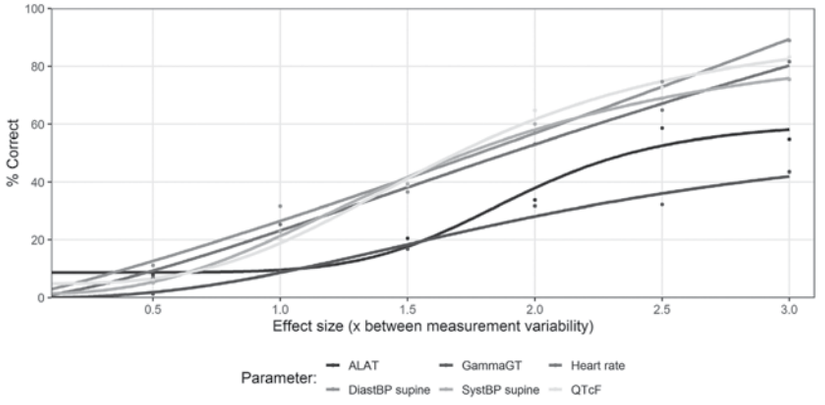
SUPPLEMENTAL FIGURES CHAPTER 2

FIGURE S1 Simulated mean ± standard deviation of the pharmacokinetic profiles of 6 subjects.



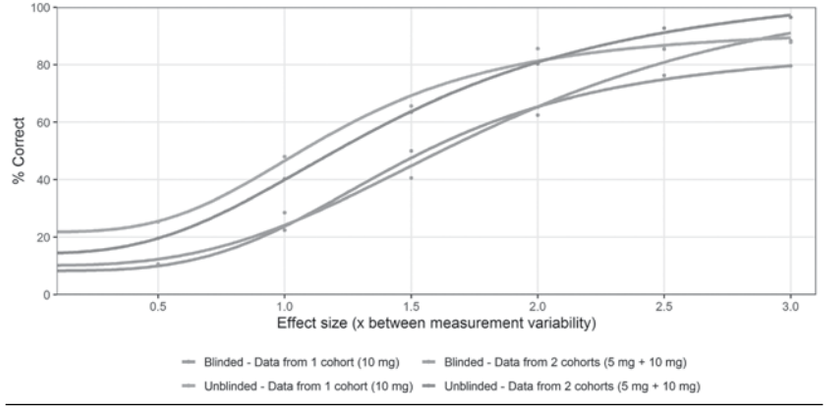
ng/mL = nanograms per millilitre, h = hours

FIGURE S2 Non-linear model fit of % correct for each parameter on the x-axis with effect size in standard deviations on the y-axis.



ALAT = alanine aminotransferase; DiastBP supine = diastolic blood pressure in supine position; SystBP supine = systolic blood pressure in supine position; QTcF = Fridericia corrected QT interval.

FIGURE S3 Non-linear model fit of % correct on the x-axis of the blinded versus unblinded simulations for 1 or 2 cohorts scenarios with effect size in standard deviations on the y-axis. Data from two reviewers being presented only.



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CHAPTER III

HEART RATE STABILITY IN A CLINICAL SETTING AND AFTER A SHORT EXERCISE IN HEALTHY MALE VOLUNTEERS

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Abstract

INTRODUCTION Limited data exists on heart rate stabilization in the domiciled nature of phase I clinical studies, particularly when frequent measurements of QT intervals are involved. The present analysis aimed to evaluate heart rate stability in the domiciled nature of, and stabilization after a short exercise.

METHODS 56 healthy male subjects were included in this analysis. Data during a domiciled clinical setting and after a short exercise were analysed. Mean values of 30 second intervals of collected electrocardiographical data (PR, RR, QT and QTcF intervals) during a 10-minute supine resting period in a domiciled nature or after walking up and down three stories (100 steps) were compared to baseline values using paired t-tests or compared to the intrasubject standard deviation.

RESULTS Stable heart rates and stable QTcF intervals observed immediately upon assuming a supine position in the domiciled clinical setting. After the short exercise, PR interval and RR interval were significantly ($p<0.05$) shorter for up to 120 seconds (mean value -9.8 ± 7.2 ms) and 30 seconds (-160 ± 165 ms, $p<0.05$), respectively. QT and QTcF intervals were significantly ($p<0.05$) shorter for up to 90- and 120-seconds post exercise, respectively. Both QT and QTcF intervals stabilized after 2 minutes, but QT interval remained prolonged while QTcF interval returned to baseline levels.

CONCLUSION In a clinical setting, male volunteers do not require a waiting period for electrocardiographic parameter normalization. However, accurate measurement of these parameters following a short exercise necessitates a minimum two-minute resting interval.

Introduction

Twelve-lead surface electrocardiograms (ECGs) are frequently performed in phase I human pharmacology trials to evaluate a novel compound's effect on cardiac conductivity and as a screening method to detect potentially fatal drug-induced ventricular electrical instability.[1] Of particular interest is the QT interval, which reflects the duration of ventricular depolarization and subsequent repolarization on an ECG, and QT interval prolongation which is linked to drug-induced ventricular arrhythmia. However, the QT interval varies over time and is inversely related to a subject's RR interval (time elapsed between two successive R-waves of the QRS signal on an ECG) with shorter QT intervals with increasing heart rates. And although the RR interval is in turn a function of intrinsic properties of the sinus node as well as autonomic influences, the subsequent QT interval changes encompasses both a swift and a gradual process.[2-5]

The process of how swiftly the QT interval adapts to RR interval fluctuations over time is referred to as QT/RR hysteresis.[6] Previous research has shown that where heart rhythm exhibits evident variations, it is necessary to take into account the hysteresis lag present in the QT interval adaptation to RR changes.[7] Ample research about heart rate recovery following exercises with different degrees of intensity report recovery times of RR interval stabilization to pre-exercise levels of up to 45 to 60 minutes.[8-12] Hence a stable heart rate is preferable for accurate QT interval measurements, and thus the Society for Cardiological Science & Technology states that time should be taken for any given individual to be relaxed and comfortable prior to ECG measurements without further detailing a certain time window.[13] Alternatively, other groups advocate a minimum period of 10 minutes or longer in a supine position prior to the QT measurement.[14]

However, in a clinical research unit, subjects are typically domiciled during phase I human pharmacology studies, making the previously cited studies not necessarily applicable. Hypothetically, the time period for the heart rate to return to baseline in domiciled healthy volunteers is shorter.[9,14,15] It is desirable to maintain a resting window that is on one hand long enough to allow stabilization of the RR interval, but as short as possible short to minimize interference in operational study conduct. Therefore, the aim of the present analysis was to evaluate heart rate stability in the domiciled nature of, and stabilization after a short exercise in male subjects.

Methods

In the present analysis, data of two clinical studies were included. The first clinical study covered the effects of supine resting in a clinical trial setting in male subjects, the second clinical study covered the effects of supine resting after a limited exercise bout in male subjects. All data were collected at the Centre for Human Drug Research in Leiden, the Netherlands, a clinical research organization specialized in early phase drug development studies. Prior to any study-related activities, an informed consent according to Declarations of Helsinki recommendations was signed by all subjects. Both clinical studies were performed in compliance with Good Clinical Practice (GCP). The clinical trial setting data were collected as part of a large study, registered under toetsingonline number NL68390.056.19 and approved by an independent ethics committee. The exercise bout setting data were collected as part of a study that was not submitted to the ethics committee as per Dutch legislation. A written informed consent was obtained prior to any study activities. All activities were performed in accordance with applicable standard operating procedures.

CLINICAL TRIAL SETTING STUDY

Included subjects were screened prior to study participation and were either young (18-25 years) or elderly (>60 years) males. Key exclusion criteria were evidence of any active or chronic disease or condition that could interfere with the study objectives. Furthermore, the use of any medication within less than 5 half-life times prior to study participation was prohibited. Subjects were instructed to roam about in the clinical research unit between 45 to 60 minutes prior to the first measurement. In accordance with the standard operating procedures use of staircases, any form of physical exercises or activities that may induce excessive heart rate fluctuations were prohibited. They were then instructed to assume a supine position during which a Holter-ECG was performed for at least ten minutes. This setting was chosen to mimic a typical clinical research setting.

EXERCISE BOUT STUDY

Recruited male subjects were included in case they claimed to be otherwise healthy and were not taking any cardiac related medication within less than 5 half-life times prior to study participation. After subjects were fitted with a HOLTER-ECG, they were instructed to remain in a resting supine position for five minutes, after which they had to walk up and down three stories (100 steps). Immediately upon completion, they were instructed to assume a supine resting position for 15 minutes. A HOLTER-ECG measurement was performed continuously.

HOLTER ECG

Data were acquired with Mortara H12+ ™monitors (Mortara Instrument, MKE, WI), with 10 disposable electrodes placed in the standard anatomical position. Data from the holter monitors were automatically analysed with validated SuperECG Software (Mortara Instrument, MKE, WI). Validation consisted of automated and manual calculations of various ECG measurements in 30 patients in normal sinus rhythm with continuous 12-lead Holter recordings for a 24-hour period, which illustrated a mean difference of 5 milliseconds in QT interval analyses.[16] Also, this software has previously been used in other research to analyse ECG measurements.[17]

The ECG parameters that were included in the present analysis were PR, RR, QT, and corrected QT (QTcF) interval. The QTcF interval values were calculated with the Fridericia formula:

$$QTcFridericia = \frac{QT\ interval}{\sqrt[3]{RR}}$$

Intervals of 30 seconds were used to calculate means of each parameter. In the clinical setting study, the final 120 seconds of recording were considered a stable heart rate and were used as baseline for analysis. In the exercise bout study, the final 300 seconds of the first 10 minutes in supine position were used to create a baseline.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM SPSS version 20 (IBM corporation, Armonk, NY, USA). Data are reported as mean ± standard deviation (SD), or with a percentage where appropriate. The collected data was visually assessed for normal distribution, indicating that it followed a normal distribution pattern. Paired t-tests were used to compare the means of the 30 second intervals with baseline means. Two criteria were set to determine a significant difference with the baseline. The first criterion was a statistically significant p-value between the evaluated epoch and the baseline measurement. The second criterion was a change from baseline greater than 25% for the PR interval or above the threshold of >240 ms, 20 ms for the RR interval, 1.35 ms for the QT interval and 1.9 ms for the QTcF interval.[16-18] These values were based on reported literature for PR interval, calculated using half the intrasubject SD reported in literature for RR and QT interval or based the data set from our own exercise bout study for QTcF, using the last 5 minutes from the baseline holter recordings.

Results

Data of two studies, one in a typical domiciled clinical setting and one with a limited exercise bout, were retrospectively analysed. Baseline characteristics of each group are shown in table 1.

CLINICAL SETTING ECG PARAMETER MEASUREMENTS

The clinical study was performed in young (n=29) and elderly (n=17) male subjects with a mean age of respectively 21.1 ± 2.0 years and 71.6 ± 6.2 years. In the younger domiciled clinical setting subject group, PR interval was 0.69 ± 4.83 ms (p=0.47) longer while RR interval was 2.65 ± 58.90 ms (p=0.82) longer at the 30-second timepoint compared to the first baseline measurement immediately after assuming a supine position following the roaming period (figure 1A and 1B). QT interval was 1.03 ± 5.34 ms (p=0.31) longer, while QTcF interval was 0.88 ± 4.42 ms (p=0.31) longer at the 30-second timepoint compared to the baseline measurement (figures 1C and 1D).

In the elderly male domiciled clinical setting subject group, PR interval was -1.20 ± 5.69 ms (p=0.38) shorter while RR interval was 7.45 ± 24.21 ms (p=0.22) longer at the 30-second timepoint compared to the first measurement immediately after assuming a supine position following the roaming period (figure 2A and 2B). QT interval was 1.14 ± 3.51 ms (p=0.20) longer, while QTcF interval was 0.21 ± 3.15 ms (p=0.79) longer at the 30-second timepoint compared to the baseline measurement (figures 2C and 2D).

Each of the subsequently measured ECG parameter values over time (mean per 30 seconds) of both the younger and older male clinical setting subject groups showed no significant difference from the first baseline measurement immediately after assuming a supine position following the roaming period. Both the p-value of 0.05 from the Paired samples t-test and the critical values were not exceeded as shown in figures 1 and 2.

EXERCISE STUDY ECG PARAMETER MEASUREMENTS

The male subjects of the exercise challenge (n=10) had a mean age of 26.9 ± 4.7 years which differed significantly from both clinical trial study groups. Other than mean age, no relevant differences between the groups were observed. Different mean post-exercise ECG parameters to baseline measurements were found in the exercise group as illustrated in figure 3. True values for each measurement are shown in supplementary table S1. Baseline heart rate was 74.0 ± 9.6 beats per minute. Mean duration of the short exercise was 121 seconds with a maximum mean heart rate of 107.3 ± 15.5 beats per minute. PR interval was significantly ($p<0.05$) shorter for up to 120 seconds after assuming a supine position following the exercise with a mean value of -9.8 ± 7.2 ms, but never crossed the threshold of 25% change from baseline. RR interval was significantly ($p<0.05$) shorter for up to 30 seconds after assuming a supine position following the exercise, while the final change from baseline larger than 20 ms is after 60 seconds with a mean value of -56.5 ± 73.5 ms. Finally, for QT and QTcF interval, the last statistically significant shortening compared to the baseline values were at the 90 ($p=0.04$) and 120 ($p<0.1$) second time points, respectively. Based on the critical clinical values, both QT interval and QTcF interval duration were initially shorter but stabilized after 2 minutes with between measurement variability less than the critical value (1.35 ms and 1.9 ms respectively). However, QT interval duration remained prolonged compared to baseline values with a mean prolongation of +5.8 ± 6.3 ms while QTcF interval duration did normalize and returned to baseline values after the initial 2 minutes.

Discussion

In the present analysis, we observed a stable heart rate in male subjects that were in a domiciled clinical setting after they assumed a supine position, whereas heart rate stabilization took up to two minutes after attaining a supine position following a short exercise. In line with this, PR and QTcF intervals remained stable in the clinical setting, whereas this took up to two minutes after the short exercise. These data suggest that the current practice of an extended duration of supine positioning to normalize heart rate and QTcF interval could be reduced without affecting the ECG data quality.

Heart rate is influenced by several mechanisms including autonomic function.[5,13] However, given that in virtually all patients and healthy subjects the RR interval and in turn QT interval varies on a beat to beat basis even during resting, controlled conditions, there is no clear consensus on the resting period prior to an ECG measurement.[14,19] Previous studies focused on QT/RR hysteresis, the process of how swiftly the QT interval adapts to RR interval fluctuations over time, reported stabilization recovery times up to 45 to 60 minutes varying based on the level of intensity following exercises.[8-12] Therefore, at minimum a resting period of up to five to ten minutes is used in a clinical research setting. However, our data suggest that these heart rate stabilization studies are

not applicable and do not translate well to clinical trials. In our analysis, both RR and PR interval recovered to baseline levels after a short exercise up to two minutes after assuming a supine position and reflects the normal parasympathetic reactivation following an exercise.[8] And although a relatively low level of intensity exercise was chosen with a mean heart rate elevation of less than 35 beats per minute, QTcF interval still took longer to recover to baseline levels. This is in line with previous studies where QT adaptation in response to RR changes were reported with an initially rapid QT interval reaction during the first 50 seconds followed by a slower adjustment that takes up to 2 minutes to complete.[20] Therefore, the time for the ECG parameters to normalize in heart rate stabilization studies and our clinical study differ a lot from each other. These differences make it inappropriate to compare these two types of studies and applying these recovery times in a clinical setting.

However, in contrast to these exercise focused heart rate recovery studies, in clinical trials these markedly increased heart rates will not be reached because subjects are prohibited to any form of physical exercise or activity that may induce excessive heart rate fluctuations. And although the Society for Cardiological Science & Technology states that time should be taken for any given individual to be relaxed and comfortable prior to ECG measurements a specific time window is not provided.[13] In this study, the exercise test was intentionally not designed to be maximal. Instead, we aimed to create a more natural and representative daily setting during a clinical trial where individuals may roam freely within a facility. Our intention was to simulate a moderate level of physical activity that individuals might typically engage in during their daily routines. The present analysis illustrates that in such a typically domiciled clinical setting any waiting period for ECG parameters normalization is not needed and thus guidelines of resting time (five to ten minutes) may be eliminated. An interval of only five minutes may translate into operational/logistical benefits for clinical study conduct. In the vast majority of clinical trials there is a tight schedule for measurements. Shortening the resting time in supine position of subjects can leave time for extra non-ECG measurements or it can lead to a better performance of the existing schedule.

Another point of concern that may also induce autonomic imbalance and thus lead to irregular RR intervals and inaccurate QT interval analyses are positional change.[21] Previous studies mainly focused on changing body positions from a supine to standing position, which resulted in short term (<1 minute) increased heart rates and elevated blood pressures to compensate for the initial fall in cardiac output.[21-24] However, in our study subjects were instructed to assume a supine position from a standing position after which we observed no significant ECG measurement changes. These findings suggest a limited roll of autonomic imbalances caused by assuming a supine position on cardiac conductivity within a clinical setting.

After this study, the question still arises if the ECG parameters have the same values after three minutes of rest and after 10 minutes of rest. To further investigate this, the ECG parameters should be measured again in a clinical trial and the values of the parameters after three minutes should be compared with timepoints 10 and 15 minutes to see if it is similar or that there are any changes from the first timepoint.

LIMITATIONS

In this analysis, automated ECG analyses were used. These analyses are reliable, but analyses done manually by medical experts can be even more accurate.[25] In automated analyses outliers in the data can be overlooked, and even cause an excessive value of a parameter that can influence the data. In this study this potential impact was limited by evaluating the automatically generated data through visual inspection of histograms and QQ plots of the data. Though automated assessments have been validated, it is nevertheless not considered the gold standard.[26] Moreover, it is important to note that the exercise group was primarily used to illustrate the relatively quick stabilization of RR intervals following a short exercise bout. This specific finding may not directly extrapolate to elderly volunteers. Exercise-induced changes in RR intervals can vary among different age groups, and caution should be exercised when generalizing these specific findings to elderly populations. Finally, a limitation of this study is that it only included male subjects, therefore restricting the generalizability of the findings to female individuals.

Conclusion

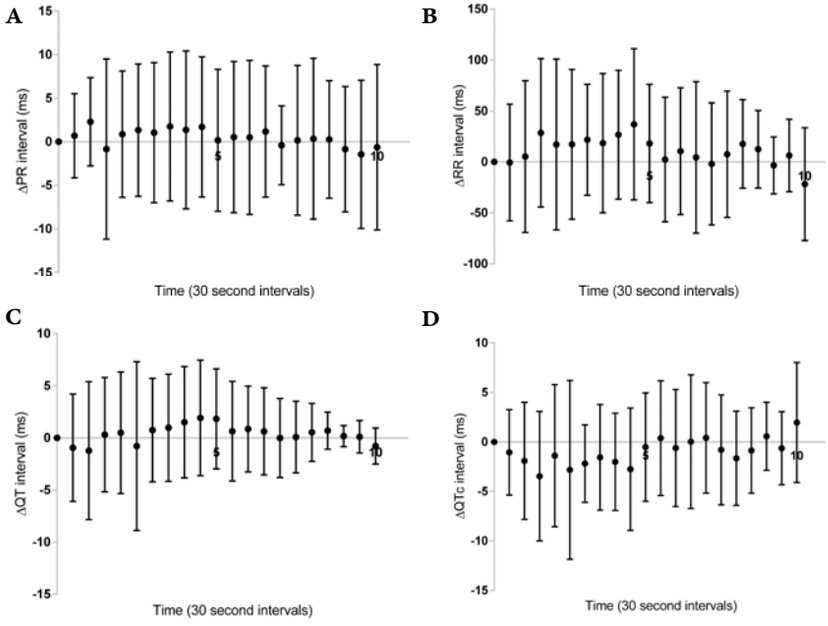
These results indicate that in a clinical setting with male volunteers any waiting period for ECG parameters normalization is not needed, because in our clinical study the first minute of recording the resting time was already in line with baseline measurement. Furthermore, after an exercise challenge, in which male subjects performed an exercise of walking 100 steps, a maximum of up to three minutes is needed to normalize the heart rate parameters.

TABLE 1 **Baseline characteristics of young and elderly male subjects in the domiciled clinical setting and the exercise bout group were compared.**

	Young (n=29)	Elderly (n=17)	Exercise (n=10)
Age (years)	21.1 ± 2.0	71.6 ± 6.2	26.9 ± 4.7
Range	18-25	>60	20-36
Body Mass Index (kg/m²)	22.1 ± 1.7	24.0 ± 1.8	26.3 ± 1.7
Heart rate frequency (beats/min)	57.2 ± 8.5	58.4 ± 6.5	68.8 ± 8.0
ECG intervals (ms)			
PR interval	149.2 ± 34.9	175.1 ± 22.5	156.5 ± 10.7
QRS duration	99.6 ± 10.5	97.6 ± 9.5	101.1 ± 9.6
QT interval	412.8 ± 29.5	418.8 ± 22.5	381.5 ± 18.0

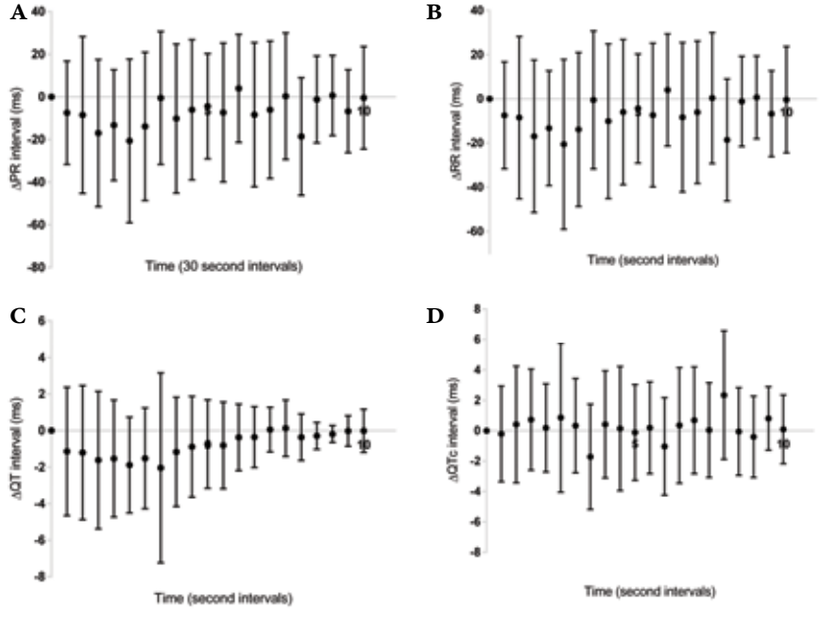
ms = milliseconds.

FIGURE 1 **Electrocardiographic parameters (PR interval in A, RR interval in B, QT interval in C and QTcF interval in D) over time as measured in the younger (18-25 years) male domiciled clinical setting subject group, where each timepoint indicates the change from baseline (time point 0) means with standard deviations over intervals of 30 seconds.** Each of the electrocardiographic parameters showed no significant difference from baseline.



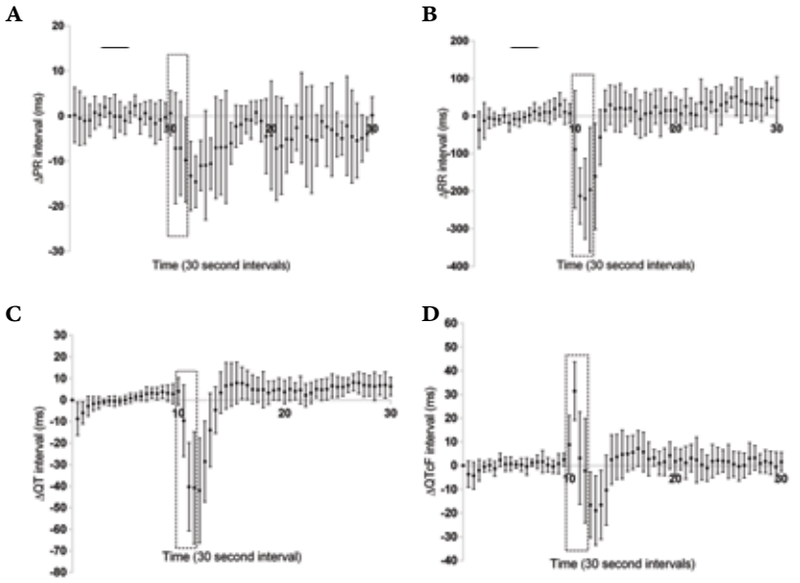
ms = milliseconds

FIGURE 2 **Electrocardiographic parameters (PR interval in A, RR interval in B, QT interval in C and QTcF interval in D) over time as measured in the elderly (>60 years) male domiciled clinical setting subject group where each timepoint indicates the change from baseline (time point 0) means with standard deviation over intervals of 30 seconds.** Each of the electrocardiographic parameters showed no significant difference from baseline.



ms = milliseconds

FIGURE 3 **Electrocardiographic parameters (PR interval in A, RR interval in B, QT interval in C and QTcF interval in D) as measured in the male exercise bout group where each timepoint indicates the change from baseline (first 10 minutes) means with standard deviation over intervals of 30 seconds.** Dotted square indicates the exercise period. A. PR interval significantly ($p<0.05$) shorter for up to 120 seconds but never crossed the threshold of 25% change from baseline. B) RR interval significantly ($p<0.05$) shorter for up to 30 seconds and change from baseline larger than 20 ms for up to 60 seconds. C) QT interval significantly ($p<0.05$) shorter for up to 90 seconds and stabilized after 120 seconds but remained prolonged compared to baseline. D) QTcF interval significantly ($p<0.05$) shorter for up to 120 seconds and remained stable afterwards within baseline values.
➢ *See inside back cover for the coloured version of this image.*



SUPPLEMENTARY TABLE

TABLE S1 The ECG parameter values of the 30 seconds intervals of the exercise bout study. Values are shown as parameter value in milliseconds ± standard deviation. Mean duration of the exercise was 120 seconds.

	PR interval	RR interval	QT interval	QTcF interval
Baseline	158.7 ± 8.1	824 ± 25.2	366.8 ± 3.1	391.8 ± 3.2
0 30 min	158.9 ± 7.0	787.2 ± 48.0	358.1 ± 7.6	388.2 ± 5.6
1 00 min	158.1 ± 6.0	811.7 ± 26.6	360.9 ± 5.1	387.6 ± 5.7
1 30 min	157.6 ± 5.1	818.4 ± 17.5	364.0 ± 4.6	389.8 ± 4.5
2 00 min	157.7 ± 3.5	816.3 ± 12.5	365.2 ± 3.2	391.3 ± 2.1
2 30 min	159.4 ± 3.6	814.1 ± 18.9	365.6 ± 2.8	392.0 ± 2.4
3 00 min	159.0 ± 2.3	825.0 ± 22.9	365.9 ± 1.2	390.5 ± 3.1
3 30 min	160.7 ± 2.3	805.7 ± 16.0	366.4 ± 2.2	394.1 ± 2.8
4 00 min	159.4 ± 3.2	816.0 ± 20.3	366.3 ± 2.4	392.4 ± 3.0
4 30 min	158.6 ± 4.8	815.4 ± 12.8	366.3 ± 1.7	392.5 ± 2.9
5 00 min	158.6 ± 3.3	820.8 ± 19.3	366.9 ± 2.0	392.3 ± 1.6
5 30 min	157.6 ± 3.0	826.8 ± 22.8	367.8 ± 2.2	392.2 ± 2.7
6 00 min	159.5 ± 2.2	836.5 ± 18.6	368.3 ± 1.6	391.4 ± 3.5
6 30 min	161.0 ± 2.3	829.4 ± 24.1	368.7 ± 2.1	392.9 ± 1.5
7 00 min	158.0 ± 3.3	834.3 ± 31.0	369.7 ± 3.3	393.4 ± 2.9
7 30 min	159.0 ± 2.5	834.4 ± 28.6	370.1 ± 2.4	393.5 ± 4.7
8 00 min	158.3 ± 4.0	843.0 ± 31.7	369.8 ± 2.8	392.1 ± 3.0
8 30 min	157 ± 4.5	854.6 ± 33.1	370.6 ± 2.9	391.4 ± 2.7
9 00 min	157.8 ± 4.4	834.9 ± 26.1	370.1 ± 3.7	392.6 ± 4.2
9 30 min	158.4 ± 3.2	831.1 ± 156	369.7 ± 4.5	394.3 ± 2.7
10 00 min	159.3 ± 4.9	735.2 ± 75.0	370.9 ± 6.2	400.6 ± 12.3
10 30 min	151.5 ± 12.2	611.3 ± 107	357.2 ± 16.6	423.2 ± 12.3
11 00 min	151.5 ± 10.4	604.0 ± 166	326.5 ± 20.4	395.0 ± 19.5
11 30 min	148.9 ± 9.3	627.6 ± 141	326.1 ± 25.9	389.6 ± 22.0
12 00 min	145.4 ± 7.6	663.3 ± 73.5	324.9 ± 24.3	375.2 ± 13.8
12 30 min	144.1 ± 5.7	767.5 ± 54.0	338.3 ± 18.8	372.9 ± 14.6
13 00 min	147.7 ± 5.5	839.3 ± 52.4	352.9 ± 17.1	375.2 ± 14.5
13 30 min	147.7 ± 12.0	854.0 ± 60.5	362.2 ± 10.7	381.4 ± 14.7
14 00 min	148.1 ± 5.6	843.4 ± 64.7	370.2 ± 9.6	394.3 ± 10.4
14 30 min	151.6 ± 11.2	845.6 ± 45.5	373.5 ± 10.7	395.5 ± 9.5
15 00 min	151.7 ± 10.5	843.3 ± 35.7	374.0 ± 10.0	396.5 ± 10.3
15 30 min	151.8 ± 12.5	845.2 ± 60.8	374.8 ± 9.6	396.8 ± 7.4
16 00 min	152.6 ± 4.6	836.6 ± 50.1	374.6 ± 7.4	397.4 ± 7.1
16 30 min	156.4 ± 4.7	815.8 ± 57.4	373.8 ± 6.7	399.0 ± 7.6
17 00 min	156.8 ± 4.2	830.6 ± 50.7	371.7 ± 6.7	397.6 ± 8.3
17 30 min	157.9 ± 3.0	832.7 ± 44.9	371.5 ± 5.8	394.6 ± 7.1

CONTINUATION SUPPLEMENTARY TABLE 1

	PR interval	RR interval	QT interval	QTcF interval
18 00 min	157.8 ± 4.2	848.7 ± 62.5	371.7 ± 8.4	395.1 ± 3.5
18 30 min	159.5 ± 2.5	832.5 ± 34.0	370.2 ± 5.3	392.8 ± 5.0
19 00 min	157.6 ± 4.6	838.7 ± 48.6	371.3 ± 4.5	393.4 ± 5.8
19 30 min	154.2 ± 8.3	838.8 ± 33.6	371.8 ± 5.1	396.1 ± 5.7
20 00 min	154.4 ± 12.0	840.4 ± 37.5	370.5 ± 5.3	393.6 ± 5.7
20 30 min	151.5 ± 11.5	830.3 ± 46.4	371.9 ± 3.9	394.5 ± 4.4
21 00 min	152 ± 10.7	850.1 ± 41.6	370.9 ± 6.4	393.0 ± 6.8
21 30 min	153.6 ± 7.4	830.6 ± 31.0	371.4 ± 5.2	394.9 ± 5.7
22 00 min	153.5 ± 5.1	827.0 ± 62.8	369.1 ± 4.8	394.2 ± 9.5
22 30 min	156.1 ± 5.0	859.9 ± 54.2	370.1 ± 4.0	392.1 ± 6.4
23 00 min	158.2 ± 10.0	844.0 ± 28.0	371.9 ±4.9	390.8 ± 5.6
23 30 min	154.1 ± 11.1	861.7 ± 62.8	371.6 ± 5.0	393.7 ± 6.9
24 00 min	153.4 ± 11.9	839.0 ± 53.8	371.9 ± 5.4	394.0 ± 5.5
24 30 min	153.3 ± 5.7	853.9 ± 24.9	373.4 ± 4.8	393.8 ± 4.7
25 00 min	157.5 ± 3.5	860.2 ± 26.2	372.9 ± 4.3	393.5 ± 7.1
25 30 min	156.3 ± 8.8	873.6 ± 51.0	372.9 ± 3.3	392.4 ± 5.3
26 00 min	155.7 ± 10.3	876.0 ± 58.3	373.7 ± 3.9	391.5 ± 6.1
26 30 min	154.6 ± 4.9	864.7 ± 32.0	375.0 ± 4.9	391.9 ± 5.2
27 00 min	153.7 ± 3.7	858.6 ± 44.8	374.8 ± 5.3	394.8 ± 6.5
27 30 min	156.5 ± 11.0	855.7 ± 39.7	373.8 ± 4.8	395.0 ± 5.2
28 00 min	154.1 ± 10.2	857.4 ± 43.2	373.5 ± 6.5	392.8 ± 3.9
28 30 min	153.2 ± 8.3	852.9 ± 43.4	373.2 ± 4.5	393.6 ± 4.8
29 00 min	153.7 ± 5.2	871.5 ± 26.6	373.6 ± 6.1	393.0 ± 4.9
29 30 min	154.7 ± 3.6	872.4 ± 61.9	373.7 ± 4.3	391.2 ± 6.3
30 00 min	158.9 ± 4.0	866.7 ± 55.0	373.2 ± 5.3	393.4 ± 4.0
30 30 min	157.6 ± 4.6	852.6 ± 62.0	375.0 ± 6.3	391.2 ± 5.5

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CHAPTER IV

THE ASSOCIATION BETWEEN BODY TEMPERATURE AND ELECTROCARDIOGRAPHIC PARAMETERS IN NORMOTHERMIC HEALTHY VOLUNTEERS

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Abstract

BACKGROUND Previous studies reported that hypo- and hyperthermia are associated with several atrial and ventricular electrocardiographical parameters, including corrected QT (QTc) interval. Enhanced characterization of variations in QTc interval and normothermic body temperature aids in better understanding the underlying mechanism behind drug induced QTc interval effects. The analysis' objective was to investigate associations between body temperature and electrocardiographical parameters in normothermic healthy volunteers.

METHODS Data from 3023 volunteers collected at our center were retrospectively analysed. Subjects were considered healthy after review of collected data by a physician, including a normal tympanic body temperature (35.5-37.5°C) and in sinus rhythm. A linear multivariate model with body temperature as a continuous was performed. Another multivariate analysis was performed with only the QT subintervals as independent variables and body temperature as dependent variable.

RESULTS Mean age was 33.8±17.5 years, mean body temperature was 36.6±0.4°C. Body temperature was independently associated with age (standardized coefficient (SC)=-0.255, P<0.001), female gender (SC=+0.209, P<0.001), heart rate (SC=+0.231, P<0.001), P-wave axis (SC=-0.051, P<0.001), J-point elevation in lead V4 (SC=-0.121, P<0.001) and QTcF duration (SC=-0.061, P=0.002). In contrast, other atrial and AV nodal parameters were not independently associated with body temperature. QT subinterval analysis revealed that only QRS duration (SC=-0.121, P<0.001) was independently associated with body temperature.

CONCLUSION Body temperature in normothermic healthy volunteers was associated with heart rate, P-wave axis, J-point amplitude in lead V4 and ventricular conductivity, the latter primarily through prolongation of the QRS duration.

Introduction

Normal body temperature is defined as 35.5 to 37.5°C in healthy volunteers, although different ranges have been described depending on the site where the body temperature is measured.[1] Body temperature values outside the normal range have been reported to result in substantial changes to the surface electrocardiographs (ECG), such as heart rate,[2-5] QRS duration,[4-7] J-wave prevalence,[4,8-12] and QT interval duration.[4,6,13-15] Even within the normal range, body temperature has been reported to have an effect on the corrected QT interval (QTc INTERVAL).[4-6,15] Preclinical studies reported atrioventricular conduction and QTc interval duration changes for each increase or decrease of a degree in body temperature of 5.3 to 14 ms and 6.1 to 14 ms, respectively.[2,16,17] These findings are further supported by in vitro human tissue studies.[18-20]

However, the effect of body temperature on the surface ECG in healthy volunteers with a normal body temperature has been underreported. An increased characterization of the association between the QTc interval, including QT subinterval analysis, [21] and body temperature aids a better differentiation between the direct effects of pharmacological agents on the ECG or indirect effects on the ECG through body temperature modulating pharmacological agents such as opioids or 5-HT_{1A} serotonin receptor modulators.[22,23]

Therefore, the aim of the present analysis was to investigate the association between body temperature and selected surface ECG parameters in healthy volunteers with a normal body temperature.

Methods

Data from 3023 male and female volunteers, aged 18 years or older with body temperatures between 35.5°C and 37.5°C were included in the retrospective analysis. All data were collected at the Centre for Human Drug Research in Leiden, the Netherlands, a clinical research organization specialized in early phase drug development studies. Data collected during the mandatory medical screening to verify study eligibility for enrolment in the early phase drug development studies as a volunteer between 2010 and 2016 were included in the present analysis. Ethical approvals from the Medical Ethical Review Committee for the intended studies were acquired and informed consent documents were signed by the volunteers prior to any data collection. The present study was performed in accordance with local regulations. All activities were performed in accordance with applicable standard operating procedures.

MEDICAL SCREENING

In this analysis, only subjects considered healthy were eligible for inclusion. Healthy was defined as the absence of clinically significant abnormalities on a large selection of tests with no evident history of diseases. This battery of tests consisted of a single visit to the clinical unit during office hours (between 09:00-17:00 o'clock) where a detailed medical history, a physical examination, vital signs including blood pressure, body temperature, weight and height measurement, body mass index (BMI) calculation, and a twelve-lead ECG were recorded. Subjects were instructed not to eat or drink at least three hours prior to the medical screening. Additionally, hematologic and chemistry blood panel, urine dipstick, and a urine drug test were recorded. Only subjects with a normal body temperature (35.5°C-37.5°C) and considered healthy after review of collected data by a physician were included in the present analysis.

TEMPERATURE MEASUREMENT

Body temperature was measured tympanically using a BRAUN® ThermoScan ear thermometer (Kronberg im Taunus, Germany) by a trained medical assistant following the standardized operating procedure. The subject was placed in a supine position with his/her head stabilized on a pillow. A disposable Braun Thermoscan lens filter (Kronberg im Taunus, Germany) was placed prior to the measurement. The ear canal was straightened by gently pulling the outer ear, with the thermometer probe pointing towards the eardrum. The measured body temperature was entered immediately into the database system (Promasys, Fort Lauderdale, FL, USA).

ECG MEASUREMENTS

The twelve-lead ECGs were recorded with the volunteer in a resting supine position and after a five-minute resting period. The twelve-lead ECGs were recorded using an electrocardiograph (Marquette 800/5500/2000; General Electric Healthcare, MKE, USA) and ten disposable electrodes placed in the standard anatomical position. The ECG data were then uploaded into the ECG warehouse (Muse Cardiology Data Management System v7, General Electric Healthcare, Chicago, IL, USA). The Marquette Cubic Spline and Finite Residual Filter filters were used for artefact and noise management. In addition, a physician manually reviewed all ECGs for quality, legibility, and abnormalities. The ECG warehouse automatically assesses interval and amplitude data from the digital ECGs with the Marquette 12SL algorithm, which provides a variety of validated ECG measurements on median beats and have been used in previous studies.[24,25] The ECG measurements were performed only by the Marquette 12SL algorithm, there were no manual adjustments or manual measurements. Independent evaluation showed that the Marquette 12SL algorithm passed all of the amplitude measurement requirements (maximum of 10 ms deviation) as defined in International Electrotechnical Commission, as described in the General Electric Physician's Guide (version 2036070-006). Description, methods of determination and calculation, and units of the ECG parameters are described in table 1.

STATISTICAL ANALYSIS

Only subjects who were considered healthy by a physician after review of all the collected data, including an ECG with sinus rhythm were included in the current analysis. Male and female subjects were analysed collectively as a group. A separate analysis where male and female subjects were analysed separately is provided as supplemental data. Data are reported as mean ± standard deviation (SD), or median with interquartile range or percentage where appropriate. Subjects were divided into quartiles based on their body temperature (35.5-36.3°C, 36.4-36.6°C, 36.7-36.9°C and 37.0-37.4°C). Because of the large number of data samples, normality was tested through visual confirmation of graphical plots. Variances were compared using the Analysis of Variance (ANOVA) test with a post-hoc Tukey analysis. A linear univariate and a backward linear multivariate regression model analysis were performed with body temperature as the dependent variable. Probabilities of less than 0.10 in the linear univariate regression model were added to the backward linear multivariate regression model. In the case that variables reversed in association between the univariate and multivariate analysis, they were excluded from the multivariate analysis. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value. Statistical analyses were performed using IBM SPSS version 20 (IBM corporation, Armonk, NY, USA).

Results

In total, 3023 subjects were included in the present analysis. Average age was 33.8 ±17.5 years, 74.4% were male and the mean body temperature was 36.63 ±0.38°C. Subject characteristics are shown in table 2. Incrementally with body temperature, the percentage of male gender, systolic and diastolic blood pressure, serum

sodium and serum potassium decreased between the groups as can be observed in table 2. Additionally, heart rate increased incrementally between the groups as can also be observed in table 2, figure 1 and figure 2. Other baseline characteristics were not significantly different among body temperature groups.

BODY TEMPERATURE AND ECG PARAMETERS

Table 2 displays the association between the body temperature groups and the evaluated ECG parameters. Maximum P-wave duration, PR-interval, QRS duration, R-amplitude in lead aVL, heart axis, J-point elevation in lead V4, and the JpTpc interval were significantly different between the body temperature groups, as also displayed in figure 1. Differences in these electrocardiographical parameters in lead V4 between body temperature groups are depicted figure 2.

LINEAR REGRESSION ANALYSIS BODY TEMPERATURE

In the univariate analysis, body temperature was associated with age (SC=-0.198, P<0.001), female gender (SC=+0.222, P<0.001), systolic blood pressure (SC=-0.096, P<0.001), diastolic blood pressure (SC=-0.059, P=0.003), heart rate (SC=+0.277, P<0.001), serum sodium concentration (SC=-0.065, P<0.001) and serum potassium concentration (SC=-0.080, P<0.001). Electrocardiographically, maximum P-wave duration (SC=-0.058, P=0.002), P-wave dispersion (SC=-0.060, P=0.002), PR-interval duration (SC=-0.130, P<0.001), QRS duration (SC=-0.125, P<0.001), R-wave amplitude in lead I (SC=-0.032, P=0.082), R-wave amplitude in lead aVL (SC=-0.061, P=0.001), R-wave amplitude in lead V1 (SC=-0.034, P=0.064), R-wave amplitude in lead V3 (SC=-0.041, P=0.023), R-wave amplitude in lead V4 (SC=-0.061, P=0.001), R-wave amplitude in lead V5 (SC=-0.070, P<0.001), R-wave amplitude in lead V6 (SC=-0.053, P=0.004), heart axis (SC=+0.070, P<0.001), J-point elevation in lead V4 (SC=-0.110, P<0.001) and QTcF duration (SC=-0.036, P=0.050) were associated with body temperature, as shown in table 3. Diastolic blood pressure, maximum P-wave duration, R-amplitude in lead V4, R-amplitude in lead V5, JpTp, and TpTe were excluded from the multivariate analysis, because these variables reversed in association between the univariate and multivariate analysis. No collinearity was found in the final multivariate analysis model with no parameter displaying a Variance Inflation Factor of >3. In the multivariate analysis, age (SC=-0.255, P<0.001), female gender (SC=+0.209, P<0.001), heart rate (SC=+0.231, P<0.001), P-wave axis (SC=-0.051, P<0.001), J-point elevation in lead V4 (SC=-0.121, P<0.001) and QTcF duration (SC=-0.061, P=0.002) were significantly associated with body temperature, as shown in table 4. The R-squared value of the multivariate analysis was 0.182. Gender-specific regression models were also made and are presented in the supplemental material.

QT SUBINTERVALS

In the multivariate analysis of the QT subintervals, JpTpc was excluded from the analysis because of reversing in association from positive in the univariate analysis to negative in the multivariate analysis. Body temperature showed the strongest association with the QRS interval duration (SC=-0.121, P<0.001), with the TpTe interval nearly reaching statistical significance (SC=-0.035, P=0.054), as can be observed from table 5. The R-square of the second backward multivariate model was 0.017.

Discussion

In the present analysis of 3032 normothermic healthy volunteers, body temperature was found to be independently associated with heart rate, P-wave axis, J-point amplitude in lead V4 and ventricular conductivity. QT subinterval analysis revealed that the association was primarily mediated through prolongation of the QRS duration and not through repolarization prolongation. In contrast, other atrial and atrioventricular nodal ECG parameters were not independently associated with body temperature. These findings suggest that body temperature induces ventricular electrocardiographical changes that can be observed in a healthy population with a normal body temperature.

Previous studies already evaluated the cellular mechanism of the association between body temperature and the cardiac electrophysiological characteristics.[6,19,20,26-29] These studies showed that electrophysiological characteristics were predominantly caused by the effects of temperature on late current Na+ influx channels.[6,19,20,26-29] Animal and in vitro studies showed Na+ channels are temperature sensitive, with increased rates at higher temperatures of Na+ channel activation, deactivation, fast and slow inactivation.[13,18,30-32] These Na+ channel kinetics were also apparent in physiological body temperature conditions,[19] and appear to be reflected in our electrocardiographic findings in normothermic healthy individuals.

Furthermore, an association between body temperature and heart rate has previously been reported. Hyperthermia in humans increases heart rate independently from age and gender and is associated with increased incidences

of ventricular arrhythmias in Brugada syndrome and long QT-syndrome patients.[3,13,27,29] Conversely, hypothermia is associated with sinus bradycardia though not always observed in moderately hypothermic patients, [3-5] possibly because of a stress induced sympathetically mediated increased heart rate secondary to initial cooling.[33] In our analysis, heart was independently associated with body temperature in normothermic heathy adults. Heart rate increased incrementally with body temperature, which is in line with previous findings in both hypo- and hyperthermia studies.

In the present analysis, we also observed an independent association between the J-point elevation in lead V4 and body temperature, although this was only observed in males in the multivariate analysis and not in females, presumable due to the increased amount of tissue between the heart and the electrodes in female subjects. Osborn waves, characterized by J-point elevation and being most apparent in precordial lead V4 are a well-recognized effect of a hypothermia.[9,11,12] Hypothermia-induced accelerated Na+ channel inactivation leads a reduced amplitude of the action potential duration primarily in the epicardium but not in the endocardium.[27] This difference leads to a transmural voltage gradient, materializing as J-point elevation on the ECG.[12] The independent association found between body temperature and J-point elevation in lead V4 in the present analysis suggests that the gradient between epicardium and endocardium exists even in healthy volunteers with a normal body temperature and is modulated by body temperature within the normal range.

There are ample human and animal studies on the effects of hypo- and hyperthermia on the ventricular action potential.[2,16,17,33] Preclinically, for each degree centigrade that the body temperature was reduced below 38°C, the ventricular action potential was prolonged by 6.1 to 14 milliseconds, and for each degree of body temperature increase above 38°C the ventricular action potential was shortened by 5.3 to 14 milliseconds.[2,16,17 33] A prolongation of the QTc interval occurred in about 73% of hypothermia patients [34] and in 100% of patients treated with targeted temperature management after cardiac arrest, which normalized after the body temperature was restored to the normal range.[2,4,34] In the current analysis in normothermic healthy volunteers, we also observed the inverse association between body temperature and the QTcF duration. These findings provide confirmation that the effects observed in hypo- and hyperthermia also occur within the clinically more common body temperature range.

However, QT subinterval analysis revealed that the main driver for QTc prolongation was prolongation of the QRS duration and not the duration of repolarization (JpTpc and TpTe). The effect of body temperature on the QTc interval is thereby substantially different from QTc interval prolongation induced by drug effects on the human ether-a-go-go-related gene (hERG / Kv11.1), which is a K+channel, where no effect on the QRS interval was observed, but rather a comparable prolongation of the JpTpc and TpTe intervals.[21] These data also appear to support the observation that hypothermia was not found to be associated with increased ventricular arrhythmias or mortality.[4,6,7,14,35] In summary, body temperature related QTc interval prolongation is presumably mediated through reduced Na+channel activation materializing as QRS duration prolongation on the surface ECG and thereby distinguishable from hERG channel mediated QTc interval prolongation. Our analysis supports this notion and may contribute an additional method to differentiate between direct pharmacological effects on hERG channels or through indirect effects through fluctuations in body temperature.

Finally, previous studies also reported on an association between body temperature and atrial and AV conduction time, such as P-wave amplitude and width and the PR-interval.[7,33,36] Although these associations were also observed in our univariate analysis, only P-wave axis remained independently associated with body temperature. Compared with the ventricular action potential, the atrial action potential has a less negative resting potential, an abbreviated plateau phase and slower terminal repolarisation, which are predominantly induced by altered potassium currents.[20,37] Preclinically, significant decreases in body temperature of 18 to 27°C in rats were required to induce AV pathology.[33,38] However, in humans severe AV-pathology, for example a total AV-block, was only observed in severe hypothermia cases (< 28˚C) and may explain the loss of significance in our multivariate analysis in normothermic healthy adults.[5,7,34]

LIMITATIONS

In the present analysis, body temperature was measured peripherally with a tympanic thermometer, while the golden standard for the core body temperature measurement is pulmonary artery temperature.[39] Each peripheral body temperature measurement method has their own estimated error but can be reduced to a minimum when considering method specific factors prior to the measurements.[39,40] Comparative studies found that tympanic body temperature

measurements had the highest accuracy compared to other peripheral temperature measurements and had a mean difference between the gold standard and tympanic measurements of 0.02°C with a 0.99 correlation.[39-41] Moreover, the present retrospective study design with the lack of paired data limits the sensitivity of our analysis to find true associations between body temperature and the measured ECG parameters. Finally, additional factors which may potentially influence both body temperature and ECG parameters through autonomic nerves system separately, such as menstrual cycle[42] or time of day[43], were not included in this analysis. However, we believe that both menstrual cycle and the body temperature fluctuations during office hours and their potential influences are rather limited within the timespan of a screening and are partially corrected through the large sample size. Moreover, we provided a separate univariate and multivariate analysis for both genders as a supplement of this manuscript.

Conclusion

Body temperature in normothermic healthy volunteers was associated with heart rate, P-wave axis, J-point amplitude in lead V4 and ventricular conductivity, primarily through prolongation of the QRS duration. In contrast, other atrial and AV nodal ECG parameters were not independently associated with body temperature.

TABLE 1 Methods of determination or calculation of the electrocardiographic variables used in healthy volunteers aged 18 years of older with a tympanically measured body temperature between 35.5°C and 37.5°C.

Variable	Description
Maximum P-wave duration (ms)	Longest P-wave duration sampled from all leads.
P-wave dispersion (ms)	Difference between the longest minus the shortest P-wave duration from all leads
Total P-wave area in lead V1 (mm×ms)	Sum of the total area under and above the isoelectric line from onset to termination of the P-wave.44
P-wave axis (degrees)	Net vector of the P-wave axis based on the extremity leads.
PR-interval (ms)	Beginning of the P-wave until the beginning of the QRS complex.
QRS duration (ms)	Mean first deflection from the isoelectric line following the P-wave until the J-point.
R-amplitude in lead I (μV)	Amplitude of the first upward deflection of the QRS complex (R-wave) in lead I.
R-amplitude in lead aVL (μV)	Amplitude of the first upward deflection of the QRS complex (R-wave) in lead aVL
R-Amplitude in lead V1-V6 (μV)	Amplitude of the first upward deflection of the QRS complex (R-wave) in lead V1 through V6
Heart axis (degrees)	Net vector of the R-wave axis based on the extremity leads.
Cornell product (ms×mm)	Product of the QRS duration and the Cornell voltage.45 Cornell voltage is the sum of the amplitude of the R-wave in lead aVL and the amplitude of the S-wave in lead V3.45
J-point amplitude lead V4 (mm)	Deflection of the downward deflection of the QRS complex at the R-ST junction measured in lead V4.9
J-point - T-peak interval correct for heart rate (ms)	Duration of QRS complex offset to peak of the T-wave / RR-interval as measured in lead II to the power of 0.58 as proposed by Johannesen.21 RR-interval is the interval between the onset of one QRS complex to the onset of the next QRS complex, measured in seconds, derived from the heart rate (HR) as 60/HR.
T-peak – T-wave interval (ms)	Duration of peak of the T-wave to end of the T-wave as measured in lead II.21
Maximum T-wave duration (ms)	Longest T-wave duration sampled from all leads
Minimum T-wave duration (ms)	Shortest T-wave duration sampled from all leads
T-wave dispersion (ms)	Difference between the longest and shortest T-wave duration selected from all leads
T-wave axis (degrees)	Net vector of the T-wave axis based on the extremity leads.
QTcF duration (ms)	QTcF duration is calculated using the Fridericia formula, which divides the QT-interval by the cube-root of RR-interval. QT-interval is the interval between the start of the Q-wave and the end of the T-wave.

TABLE 2 Relation between patient characteristics and electrocardiographic parameters to body temperature in normothermic healthy volunteers aged 18 years or older (n=3023) with a tympanically measured body temperature between 35.5°C and 37.5°C. Categorical variables were compared using Chi-square test, variances were compared using the Analysis of Variance test with a post hoc Tukey analysis. Results are reported as mean ±standard deviation or as percentage. The symbols α, ß, γ, and δ represent a significant difference (P<0.05) compared to that group. If no symbols are present, no significance was found between the groups.

	Body temperature (°C)			
	35.5-36.3 (n=742)	36.4-36.6 (n=783)	36.7-36.9 (n=837)	37.0-37.5 (n=661)
Corresponding groups	**α**	**ß**	**γ**	**δ**
Age (years)	38.5± 19.7 ^{ßγδ}	35.2 ± 18.3 ^{αγδ}	32.3 ± 16.2 ^{αßδ}	28.8 ± 13.7 ^{αßγ}
Gender male (% male)	85.8	78.6	74.1	57.6
Body mass index (kg/m²)	23.7	23.7	23.7	23.7
Systolic blood pressure (mmHg)	125.0 ± 14.1 ^{ßγδ}	123.6 ± 12.9 ^{α}	122.6 ± 12.6 ^{α}	121.9 ± 12.8 ^{α}
Diastolic blood pressure (mmHg)	73.0 ± 9.6 ^{γδ}	72.2 ± 9.6	71.5 ± 9.5 ^{α}	71.3 ± 9.0 ^{α}
Heart rate (beats/min)	59.5 ± 9.3 ^{ßγδ}	61.9 ± 9.9 ^{αγδ}	64.1 ± 9.9 ^{αßδ}	67.1 ± 10.5 ^{αßγ}
Serum Sodium (mmol/L)	141.7 ± 1.9 ^{δ}	141.6 ± 2.0 ^{δ}	141.6 ± 1.9 ^{δ}	141.4 ± 1.8 ^{αßγ}
Serum Potassium (mmol/L)	4.37 ± 0.31 ^{δ}	4.36 ± 0.31 ^{δ}	4.34 ± 0.34 ^{δ}	4.29 ± 0.29 ^{αßγ}
Serum Calcium (mmol/L)	2.40 ± 0.09	2.41 ± 0.09	2.42 ± 0.09	2.41 ± 0.10
Maximum P-wave Duration (ms)	104.4 ± 12.8 ^{δ}	103.1 ± 12.5	103.6 ± 11.6 ^{δ}	101.8 ± 12.2 ^{αγ}
P-wave dispersion (ms)	55.4 ± 22.0	54.9 ± 20.7	53.9 ± 20.9	52.9 ± 21.0
Total P-wave area in lead V1 (mm×ms)	49.31 ± 81.39	49.32 ± 80.90	52.64 ± 81.64	50.55 ± 84.28
PR-interval (ms)	160.6 ± 23.8 ^{ßγδ}	155.6 ± 23.6 ^{αδ}	153.4 ± 22.0 ^{α}	152.0 ± 22.6 ^{αß}
QRS duration (ms)	97.85 ± 10.87 ^{δ}	96.83 ± 11.21 ^{δ}	96.69 ± 10.88 ^{δ}	93.58 ± 10.68 ^{αßγ}
R-amplitude lead I (µV)	700.3 ± 309.2	674.1 ± 283.2	682.4 ± 296.3	671.5 ± 296.6
R-amplitude lead aVL (µV)	365.4 ± 293.0 ^{γδ}	332.1 ± 275.0	329.4 ± 268.3 ^{α}	318.6 ± 256.3 ^{α}
Heart axis (degrees)	45.7 ± 34.2 ^{δ}	48.7 ± 33.2	49.8 ± 33.5	52.7 ± 32.8 ^{α}
J-point amplitude lead V4 (mm)	55.0 ± 54.5 ^{ßγδ}	47.4 ± 49.6 ^{αδ}	48.5 ± 47.9 ^{αδ}	37.9 ± 47.1 ^{αßγ}
J-point - T-peak duration (corrected for heart rate) (ms)	217.9 ± 22.8	217.0 ± 22.9 ^{δ}	217.9 ± 23.6	220.3 ± 24.0 ^{ß}
T-peak – T-end duration (ms)	95.7 ± 12.0	95.1 ± 12.4	94.7 ± 11.4	94.1 ± 12.0
Maximum T-wave duration (ms)	188.7 ± 23.1	187.1 ± 21.0	187.8 ± 21.3	189.2 ± 21.3
Minimum T-wave duration (ms)	111.0 ± 55.3	109.8 ± 57.8	113.0 ± 56.0	113.7 ± 53.3
T-wave dispersion (µV)	77.7 ± 56.8	77.3 ± 58.9	74.8 ± 57.5	75.5 ± 55.5
QTcF duration (ms)	411.3 ± 20.2	409.3 ± 19.1	409.2 ± 18.5	409.2 ± 18.5

mV = millivolt, µV = microvolt, ms = milliseconds, mm = millimeters.

TABLE 3 Linear univariate regression model analysis in normothermic healthy volunteers aged 18 years or older (n=3023) with a tympanically measured body temperature between 35.5°C and 37.5°C. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value and the R-square value in the linear univariate regression model.

	Univariate analysis			
Variable	**USC**	**SC**	***R*-square**	***P*-value**
Age (years)	-0.004	-0.198	0.039	<0.001
Female gender	0.192	0.222	0.049	<0.001
Body mass index (kg/m²)	0.002	0.017	<0.001	0.337
Systolic blood pressure (mmHg)	-0.003	-0.096	0.009	<0.001
Diastolic blood pressure (mmHg)	-0.002	-0.059	0.003	0.003
Heart rate (beats/min)	0.010	0.277	0.076	<0.001
Serum Sodium (mmol/L)	-0.013	-0.065	0.004	<0.001
Serum Potassium (mmol/L)	-0.095	-0.080	0.006	<0.001
Serum Calcium (mmol/L)	0.092	0.023	<0.001	0.222
Maximum P-wave Duration (ms)	-0.002	-0.058	0.003	0.002
P-wave dispersion (ms)	-0.001	-0.046	0.002	0.011
Total P-wave area in lead V1 (mm×ms)	4.77×10^{-5}	0.010	<0.001	0.571
PR-interval (ms)	-0.002	-0.130	0.017	<0.001
QRS duration (ms)	-0.004	-0.125	0.015	<0.001
R-amplitude lead I (µV)	-4.04×10^{-5}	-0.032	0.001	0.082
R-amplitude lead aVL (µV)	-8.39×10^{-5}	-0.061	0.003	0.001
Heart axis (degrees)	0.001	0.070	0.005	<0.001
J-point amplitude lead V4 (mm)	-0.001	-0.110	0.012	<0.001
J-point - T-peak duration (corrected for heart rate) (ms)	0.001	0.034	0.001	0.064
T-peak – T-end duration (ms)	-0.002	-0.049	0.002	0.007
Maximum T-wave duration (ms)	9.52×10^{-5}	0.005	<0.001	0.765
Minimum T-wave duration (ms)	0.000	0.016	<0.001	0.365
T-wave dispersion (µV)	-9.25×10^{-5}	-0.014	<0.001	0.443
QTcF duration (ms)	-0.001	-0.036	0.001	0.050

mV = millivolt, µV = microvolt, ms = milliseconds, mm = millimeters, QTcF=corrected QT interval with Fridericia's method.

TABLE 4 Backward linear multivariate regression model analysis in normothermic healthy volunteers aged 18 years or older (n=3023) with a tympanically measured body temperature between 35.5°C and 37.5°C. Probabilities of less than 0.10 in the linear univariate regression model were added to the backward linear multivariate regression model. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value. The R-square of the backward linear multivariate regression model was 0.178.

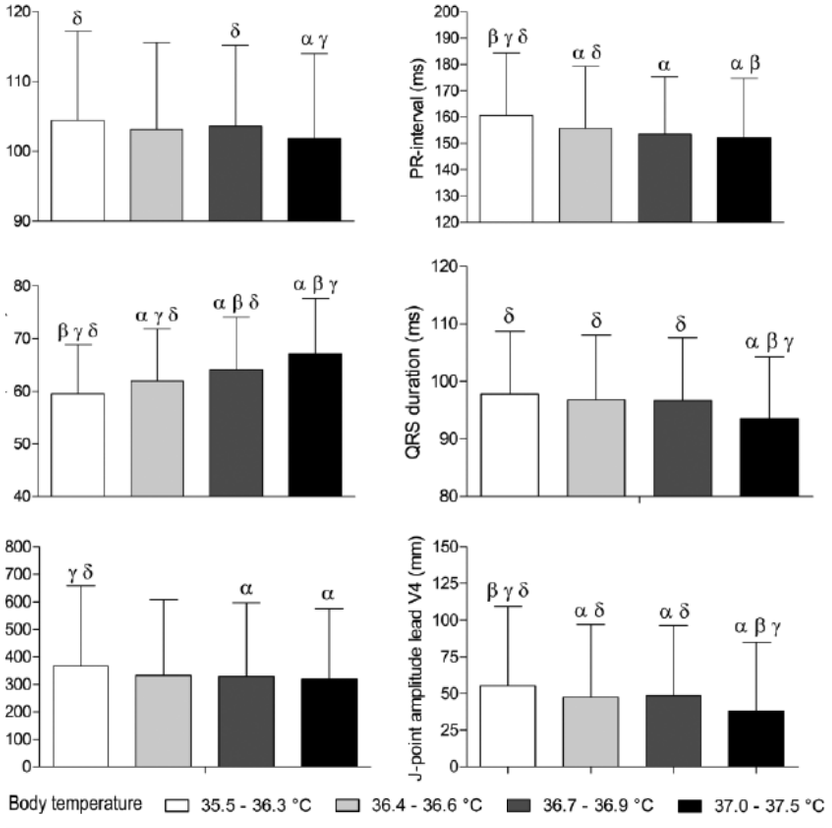
	Multivariate analysis		
Variable	**USC**	**SC**	***P*-value**
Age (years)	-0.005	-0.252	<0.001
Female gender	0.183	0.208	<0.001
Systolic blood pressure (mmHg)	Dropped		
Diastolic blood pressure (mmHg)	Dropped		
Heart rate (beats/min)	0.008	0.230	<0.001
Serum Sodium (mmol/L)	Dropped		
Serum Potassium (mmol/L)	Dropped		
Maximum P-wave Duration (ms)	Excluded		
P-wave dispersion (ms)	Dropped		
PR-interval (ms)	Dropped		
QRS duration (ms)	Dropped		
R-amplitude lead aVL (μV)	Dropped		
Heart axis (degrees)	Dropped		
J-point amplitude lead V4 (mm)	-0.001	-0.118	<0.001
QTcF (ms)	-0.001	-0.061	0.002

mV = millivolt, μV = microvolt, ms = milliseconds, mm = millimeters, QTcF=corrected QT interval with Fridericia's method.

TABLE 5 Backward linear multivariate regression model analysis with body temperature as dependent variable to identify which subintervals of the QT-interval were dependent on body temperature in normothermic healthy volunteers aged 18 years or older (n=3023) with a tympanically measured body temperature between 35.5°C and 37.5°C. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value. The R-square of the backward linear multivariate regression model was 0.017., ms = milliseconds.

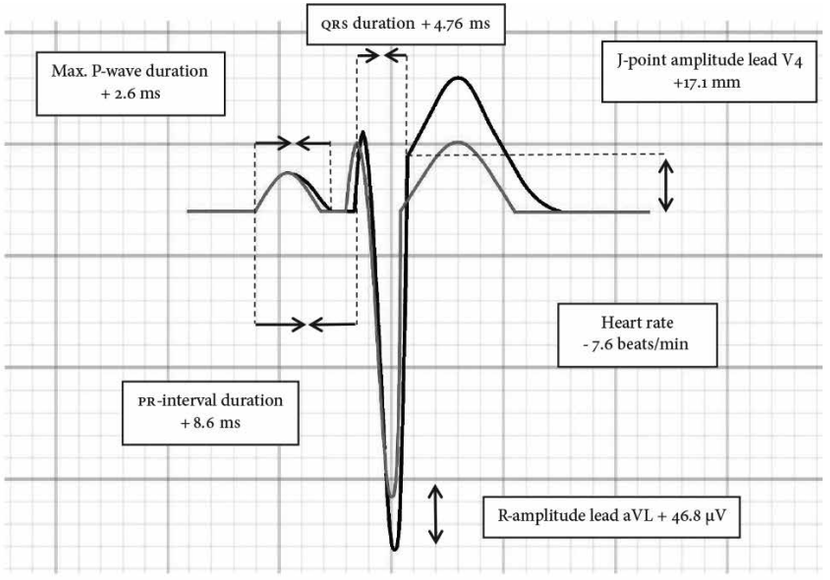
	Multivariate analysis		
Variable	**USC**	**SC**	***P*-value**
QRS duration (ms)	-0.004	-0.121	<0.001
J-point - T-peak duration (corrected for heart rate) (ms)	Excluded		
T-peak – T-end duration (ms)	-0.001	-0.035	0.054

FIGURE 1 Overview of significant changes of electrographic parameters to body temperature in normothermic healthy volunteers aged 18 years or older (n=3023) with a tympanically measured body temperature between 35.5°C and 37.5°C. Results were based on Analysis of Variance (ANOVA) test between body temperature groups, and expressed as difference in the electrocardiographic parameter (with 95% confidence interval) per body temperature groups using a post hoc Tukey analysis.



µV = microvolt, ms = milliseconds, mm = millimeters, min = minutes.

FIGURE 2 Overview of significant changes of electrographic parameters to body temperature in normothermic healthy volunteers aged 18 years or older (n=3023) with a tympanically measured body temperature between 35.5 - 36.3°C (grey line) and 37.0-37.5°C (black line). Results were based on Analysis of Variance (ANOVA) test between body temperature groups and expressed as difference in the electrocardiographic parameter per body temperature groups using a post hoc Tukey analysis



µV = microvolt, ms = milliseconds, mm = millimeters

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CHAPTER V

BLOOD PRESSURE-RELATED ELECTROCARDIOGRAPHIC FINDINGS IN HEALTHY YOUNG INDIVIDUALS

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Abstract

PURPOSE Elevated blood pressure induces electrocardiographic changes and is associated with an increase in cardiovascular disease later in life compared to normal blood pressure levels. The purpose of this study was to evaluate the association between normal to high normal blood pressure values (90-139/50-89 mmHg) and electrocardiographic parameters related to cardiac changes in hypertension in healthy young adults.

METHODS Data from 1449 volunteers aged 18 to 30 years collected at our center were analysed. Only subjects considered healthy by a physician after review of collected data with systolic blood pressure values between 90 to 139 mmHg and diastolic blood pressure values between 50 to 89 mmHg were included. Subjects were divided into groups with 10 mmHg systolic blood pressure increment between groups for analysis of electrocardiographic differences. Backward multivariate regression analysis with systolic and diastolic blood pressure as a continuous variable were performed.

RESULTS Mean age was 22.7 ±3.0 years, 73.7% were male. P-wave area, ventricular activation time, QRS-duration, Sokolow-Lyon voltages, Cornell Product, J-point – T-peak duration corrected for heart rate and maximum T-wave duration were significantly different between systolic blood pressure groups. In the multivariate model with gender, body mass index and cholesterol, ventricular rate (standardized coefficient (SC): +0.182, P<0.001), ventricular activation time in lead V6 (SC=+0.065, P=0.048), Sokolow-Lyon voltage (SC=+0.135, P<0.001), and Cornell product (SC=+0.137, P<0.001) were independently associated with systolic blood pressure, while ventricular rate (SC=+0.179, P<0.001), P-wave area in lead V1 (SC=+0.079, P=0.020), and Cornell product (SC=+0.091, P=0.006) were independently associated with diastolic blood pressure.

CONCLUSION Blood pressure related electrocardiographic changes were observed incrementally in a healthy young population with blood pressure in the normal range. These changes were an increased ventricular rate, increased atrial surface area, ventricular activation time and increased ventricular hypertrophy indices on a standard twelve lead electrocardiogram.

Introduction

Hypertension induces structural and functional adaptations to the heart such as myocardial fibrosis formation and microvascular endothelial dysfunction which results clinically in left ventricular hypertrophy (LVH), diastolic dysfunction, and ultimately heart failure.[1,2] Electrocardiographically, in hypertensive patients, the abovementioned changes result in an increased P-wave duration, P-wave dispersion, PR-interval,[3-8] increased QRS duration,[9-11] increased LVH criteria such as Sokolow-Lyon and Cornell product,[12-15] and an increased QT interval and QT dispersion.[14-18] These electrocardiographical (ECG) findings are independently associated with an increased prevalence of atrial fibrillation, stroke, and coronary artery disease.[6-13,19-22]

The ECG changes have already been reported to be present in high normal blood pressure (130-139/80-89 mmHg).[3,4,15,16] However, to which extent these ECG changes can be observed in normotensive individuals is unknown. Furthermore, systolic blood pressure induced ECG changes, such as ventricular activation time, QT-interval and T-wave changes independently from anatomical adaptations in animal experiments.[23-25] To which extent these findings can be translated to humans is currently unknown. The improved characterization of blood pressure related ECG changes in humans is of particular interest in drug research, where knowledge of normal physiological changes help differentiate between physiological and potentially harmful or unknown pharmacodynamic effects such as prolongation of the QRS duration or QT-interval through QT-subinterval analysis.[26] The purpose of the present study was to characterize the association between blood pressure and selected ECG parameters recognized to be associated with hypertension in a healthy, young, normotensive, and elevated blood pressure population.

Methods

Data from 1449 male and female volunteers aged 18-30 years with a systolic blood pressure between 90 and 139 mmHg and diastolic blood pressure between 50 and 89 mmHg were included in the present study. All data were collected at the Centre for Human Drug Research in Leiden, the Netherlands, a clinical research organization specialized in early phase drug development studies. Data collected during the mandatory medical screening to verify study eligibility

between 2010 and 2016 were included in the present study. Ethical approvals from the Medical Ethical Review Committee for the intended studies were acquired and informed consent documents were signed by the volunteers prior to any data collection. The present study was performed in accordance with local regulations. All activities were performed in accordance with applicable standard operating procedures.

MEDICAL SCREENING

The medical screening consisted of a single visit to the clinical unit where a detailed anamnesis, a physical examination, vital signs including blood pressure, temperature, weight and height measurement, body mass index calculation, and a twelve-lead electrocardiogram (ECG) were recorded. Additionally, haematology and chemistry blood panels, urine dipstick, and a urine drug test were analysed.

BLOOD PRESSURE MEASUREMENT

Subjects were placed in a supine, resting position for five minutes preceding the blood pressure measurement. The blood pressure cuff was placed on the upper arm just above the elbow crease. Brachial blood pressure was measured using a calibrated and yearly maintained Dash 3000/4000 or a Carescape V100 Dinamap device (General Electric Healthcare, Chicago, Illinois, USA). The blood pressure measurement was immediately entered electronically into a validated database system (Promasys, OmniComm, Fort Lauderdale, FL, USA).

ECG MEASUREMENT

The twelve-lead ECGs were recorded with the volunteer in supine position and after a five-minute resting period. The twelve-lead ECGs were recorded using an electrocardiograph (Marquette 800/5500/2000 or Dash 3000; General Electric Healthcare, MKE, USA) and twelve disposable electrodes placed in the standard anatomical position. The ECG data were then uploaded into the ECG warehouse (Muse Cardiology Data Management System v7, General Electric Healthcare, Chicago, IL, USA). The Marquette Cubic Spline filter and Finite Residual Filter were used for artefact and noise management. The ECG warehouse automatically assesses interval and amplitude data from the digital ECGs with the Marquette 12SL algorithm, which provides a variety of ECG measurements which have been used in previous studies.[27,28] Independent evaluation showed that the Marquette 12SL algorithm passed all of the amplitude measurement requirements (maximum of 10 milliseconds deviation) as defined in International Electrotechnical Commission, as described in the GE Physician's Guide (version 2036070-006). In addition to the algorithm, a physician reviewed all ECGs for quality, legibility, and abnormalities. Description, methods of determination and calculation, and units of the ECG parameters are described in table 1.

STATISTICAL ANALYSIS

Only subjects who were considered healthy by a physician after review of all the collected data, including an ECG with sinus rhythm were included in the current study. Data are reported as mean ± standard deviation (SD), median with interquartile range or percentage where appropriate. Subjects were divided in groups based on systolic blood pressure with 10 mmHg increments (90-99 mmHg, 100-109 mmHg, 110-129 mmHg, 120-129 mmHg, 130-139 mmHg) and based on diastolic blood pressure with 10 mmHg increments (50-59 mmHg, 60-69 mmHg, 70-79 mmHg, 80-89 mmHg) for analysis. Possible gender-related differences were investigated using a Chi-square test. Variances were compared using the Analysis of Variance (ANOVA) test with a post-hoc Tukey analysis. A linear univariate and a backward linear multivariate regression model analysis were performed with systolic blood pressure and diastolic blood pressure as the dependent continuous variable, respectively. Due to collinearity, selected variables were dropped from the backward linear multivariate regression model analysis based on a Variance Inflation Factor higher than 3.0. This concerned QRS duration, JpTpc interval, and QTcF duration. The decision which parameter to exclude was based on R squared values; the combination of variables that yielded the largest R squared value without any parameter displaying a VIF of >3 was reported in the multivariate analysis. Additionally, because we found an association between systolic blood pressure and QTcF, a separate backward multivariate analysis to identify which subintervals of the QT-interval with systolic blood pressure as dependent variable was performed. These subintervals were: ventricular activation time, QRS duration, J-point – T-peak interval, corrected for heart rate (JpTpc) and T-peak-T-end interval (TpTe). The correction of the JpTpc interval was performed using the formula proposed by Johannesen:[26]

$$\mathrm{JpTpc} = \frac{Jpoint - Tpeak\ interval}{RR\ Interval^{0.58}}$$

Probabilities of less than 0.10 in the linear univariate regression model were added to the backward linear multivariate regression model. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value. Statistical analyses were performed using IBM SPSS version 25 (IBM corporation, Armonk, NY, USA).

Results

In total, 1449 subjects were included in the present study. Mean age was 22.7 ±3.0 years, 73.7% were male. Subject characteristics for the systolic and diastolic blood pressure groups are shown in table 2 and 3, respectively. Incrementally with systolic blood pressure, the percentage of male gender increased significantly between the systolic groups. Furthermore, body mass index, diastolic blood pressure, ventricular rate, and serum calcium were significantly different between the systolic blood pressure groups as can be observed in table 2. Between diastolic blood pressure groups, age, temperature, systolic blood pressure, ventricular rate, serum cholesterol and Cornell Product differed significantly between the groups as can be observed in table 3. Other baseline characteristics were not significantly different among systolic or diastolic blood pressure groups.

SYSTOLIC BLOOD PRESSURE AND ECG PARAMETERS

Table 2 displays the association between the systolic blood pressure groups and the evaluated ECG parameters, respectively. P-wave area in lead V1, ventricular activation time in lead V6, QRS-duration, Sokolow-Lyon voltages, Cornell Product, JpTpc interval, and maximum T-wave duration were significantly different between systolic blood pressure groups, as displayed in figure 1.

LINEAR REGRESSION ANALYSIS SYSTOLIC BLOOD PRESSURE

In the univariate analysis of the data, systolic blood pressure was significantly associated with female gender (SC=-0.349, P<0.001), body mass index (SC=+0.216, P<0.001), ventricular rate (SC=+0.138, P<0.001), serum cholesterol (SC=+0.095, P=0.003), serum sodium (SC=+0.082, P=0.002), serum calcium (SC=+0.180, P<0.001), P-wave area in lead V1 (SC=+0.136, P<0.001), ventricular activation time in lead V6 (SC=+0.156, P<0.001), QRS duration (SC=+0.171, P<0.001), Sokolow-Lyon voltage (SC=+0.221, P<0.001), Cornell product (SC=+0.235, P<0.001), JpTpc interval (SC=-0.175, P<0.001), maximum T-wave duration (SC=-0.164, P<0.001), and QTcF duration (SC=-0.073, P=0.005), as can also be observed from table 4.

Due to collinearity between JpTpc interval and maximum T-wave duration, maximum T-wave duration was dropped from the multivariate analysis. In addition, ventricular activation time in lead V6 was collinear with QTcF duration, therefore QTcF duration was dropped from the multivariate analysis. This resulted in a drop of the R-square value from 0.244 to 0.237 of the overall multivariate model.

In the backward multivariate analysis, female gender (SC=-0.232, P<0.001), body mass index (SC=+0.198, P<0.001), ventricular rate (SC=+0.189, P<0.001), serum cholesterol (SC=+0.082, P=0.004), ventricular activation time in lead V6 (SC=+0.065, P=0.049), Sokolow-Lyon voltage (SC=+0.136, P<0.001), and Cornell product (SC=+0.137, P<0.001) were independently associated with systolic blood pressure, as can also be observed from table 5. The R-square of the multivariate model was 0.237.

QT SUBINTERVALS

In the multivariate analysis with the QT subintervals, systolic blood pressure was associated with QRS duration (SC=+0.109, P<0.001), and JpTpc interval (SC-0.117, P<0.001), but not to TpTe interval (SC=-0.032, P=0.250), as can be observed from table 6. The R-square of the second backward multivariate model was 0.039.

DIASTOLIC BLOOD PRESSURE AND ECG PARAMETERS

Table 3 displays the association between the diastolic blood pressure groups or diastolic blood pressure groups and the evaluated ECG parameters. Only Cornell Product was significantly different between diastolic blood pressure groups. Other ECG parameters were not significantly different among diastolic blood pressure groups.

LINEAR REGRESSION ANALYSIS DIASTOLIC BLOOD PRESSURE

In the univariate analysis of the data, diastolic blood pressure was significantly associated with age (SC=+0.132, P<0.001), female gender (SC=-0.063, P=0.024), temperature (SC=+0.074, P=0.010), ventricular rate (SC=+0.151, P<0.001), serum cholesterol (SC=+0.161, P<0.001), serum calcium (SC=+0.075, P=0.008), P-wave area in lead V1 (SC=+0.054, P=0.055), Sokolow-Lyon voltage

(SC=+0.048, P=0.088), Cornell product (SC=+0.068, P=0.015), and minimum T-wave duration (SC=-0.051, P=0.069) as can also be observed from table 7.

In the backward multivariate analysis, age (SC=+0.129, P<0.001), ventricular rate (SC=+0.179, P<0.001), serum cholesterol (SC=+0.136, P<0.001), P-wave area in lead V1 (SC=+0.079, P=0.020), and Cornell product (SC=+0.091, P=0.006) were independently associated with systolic blood pressure, as can also be observed from table 8. No variables were dropped from the multivariate analysis because of collinearity. The R-square of the multivariate model was 0.090.

Discussion

This study found an association between hypertension associated ECG parameters and blood pressure in healthy, young (18-30 years) adults with a systolic blood pressure between 90 and 139 mmHg and a diastolic blood pressure between 50 and 89 mmHg. A higher systolic blood pressure was independently associated with an increased ventricular rate, prolongation of ventricular activation time in lead V6, increased Sokolow-Lyon voltages, Cornell product, and an increased maximum T-wave duration. Furthermore, a higher diastolic blood pressure was independently associated with an increased P-wave area in lead V1 and Cornell Product, though comparatively less than systolic blood pressure. These results indicate that blood pressure-related cardiac changes can be observed in a surface ECG in a healthy, young population.

Hypertension induced atrial remodelling consists of cardiomyocyte hypertrophy and differentiation of fibroblasts into myofibroblasts as well as atrial dilation.[1,2] These ECG changes can be observed as prolongation of the P-wave and PR-interval on surface ECGs.[3-8] In the present study, we also observed these changes although these were not independent from other parameters. We hypothesize that blood pressure-related atrial structural changes may occur but are preceded by ventricular changes, which is supported by previously reported findings in animal studies.[29,30] Nevertheless, the P-wave area in lead V1 was independently associated with diastolic blood pressure and showed an association with systolic blood pressure. A previous study reported that P-wave area in lead V1 was associated with atrial dilation on cardiac MRI, thus, hypothetically, the present study suggests that blood pressure induces atrial geometrical changes in subjects with a normal blood pressure.[31]

Hypertension induced ventricular structural changes mainly constitute an increased left ventricular mass (LVM), thickness, and stiffness,[1,2,9,32] which ultimately results in restricted left ventricular filling and function.[1,9-11] These adaptations induce conduction changes, such as prolongation of the ventricular activation time (46 ± 0.4 ms) or prolongation of QRS duration (100-109 ± 5 ms), [10,11,25,32] and increased LVH markers such as increased Sokolow-Lyon (2.4-3.8 ± 1.0 mV) and Cornell (148-282 ± 110 mm×ms) on a surface ECG.[12-15] All of these were also observed in the present study involving healthy young volunteers, although in general these were more subtle in the present study as compared to studies in elderly hypertensive patients.

Previous studies indicated that the QTc interval duration was increased in high normal and stage 1 hypertensive patients compared to normotensive patients and was correlated to increases in LVM and LVH criteria on an ECG.[15-18,33] However, systolic blood pressure may also induce a prolonged ventricular activation time and duration of the QT-interval and T-wave changes independently from the LVM index.[23-25] Moreover, regional differences in static QT interval measurement on an ECG may provide an indication of an underlying non-homogeneity of ventricular repolarization caused by LVH and left ventricular size in hypertensive patients.[17,34,35]

In the present study, we also observed an association between the QTc interval and systolic blood pressure. QTc subinterval analysis was performed, which allows for improved characterization of the effect on the QT interval and differentiation between early and late repolarization.[26] The subinterval analysis has been reported to be of use to differentiate channel interactions of novel compounds.[26] Our subinterval analysis found that blood pressure increases the QTc interval mainly through prolongation of the QRS interval. This is substantially different from I_{kr} channel induced QTc interval prolongation, which is typically of interest in drug induced QT prolongation. The results in the present study can be used in drugs that have an effect on blood pressure. When evaluating a compound that induces an increase in blood pressure, this is likely to be associated with an increase in QT interval. QT subinterval analysis can be used to discriminate direct blood pressure induced effects (QRS prolongation) from I_{kr} channel induced effects (prolongation of repolarization duration). Future research should be aimed at describing the interaction between blood pressure and the ECG over time. The present study does not allow for differentiation between acute effects and chronic effects of blood pressure on the surface ECG. The present study also does not allow to assess the time course over which these ECG changes are induced, although hypothetically, these effects will be induced over multiple years.

LIMITATIONS

The limitations of this study are the retrospective, cross-sectional design, and the usage of automatically calculated ECG data. The automated assessment has been validated but is nevertheless not considered the gold standard.[36]

Conclusion

In conclusion, we found that hypertension related electrocardiographic changes can be observed incrementally in healthy young individuals with a normal to high normal elevated blood pressure. These changes were related to an increased ventricular rate, increased atrial surface area, increased ventricular activation time, increased ventricular hypertrophy indices and an altered ventricular repolarization on a standard twelve lead electrocardiogram.

TABLE 1 Methods of determination or calculation of the electrocardiographic (ECG) variables used in the analysis of the 1554 healthy volunteers aged 18 to 30 years with a body mass index (BMI) between 18.5 and 30.0 kg/m^2.

Variable	Description
Maximum P-wave duration (ms)	Longest P-wave duration sampled from all leads
P-wave balance in lead V1 (μV)	Difference between the upward and downward deflection of the P-wave
P-wave dispersion (ms)	Difference between the longest minus the shortest P-wave duration from all leads
Total P-wave area in lead V1 (mm×ms)	Sum of the total area under and above the isoelectric line from onset to termination of the P-wave[33]
PR-interval (ms)	Beginning of the P-wave until the beginning of the QRS complex
QRS duration (ms)	First deflection from the isoelectric line following the P-wave until the J-point.
Heart axis (degrees)	Net vector of the R-wave axis based on the extremity leads.
Sokolow-Lyon voltage (mm)	Sum amplitude of the S-wave in lead V1 and the amplitude of the R-wave in lead V5 or V6 (whichever is larger)[34]
Cornell product (ms×mm)	Product of the QRS duration and the Cornell voltage[35] Cornell voltage is the sum of the amplitude of the R-wave in lead aVL and the amplitude of the S-wave in lead V3[35]
Maximum T-wave duration (ms)	Longest T-wave duration sampled from all leads
Minimum T-wave duration (ms)	Shortest T-wave duration sampled from all leads
T-wave dispersion (ms)	Difference between the longest and shortest T-wave duration selected from all leads
QTcF duration (ms)	QTcF duration is calculated using the Fridericia formula, which divides the QT-interval by the cube-root of RR-interval.[36] QT-interval is the interval between the start of the Q-wave and the end of the T-wave. RR-interval is the interval between the onset of one QRS complex to the onset of the next QRS complex, measured in seconds, derived from the heart rate (HR) as 60/HR.

mV = millivolt, μV = microvolt, ms = milliseconds, mm = millimeters.

TABLE 2 Relation between patient characteristics and electrocardiographic parameters to BMI of included healthy volunteers. Categorical variables were compared using Chi-square test, variances were compared using the Analysis of Variance test with a post hoc Tukey analysis. Results are reported as mean ± SD or as percentage. The symbols α, ß, γ, δ, and ε represent a significant difference (P<0.05) compared to that group. If no symbols are present, no significance was found between the groups.

	Body mass index (kg/m²)				
	18.5-20.7 (n= 323)	20.8-22.0 (n= 334)	22.1-23.4 (n= 324)	23.5-25.0 (n= 309)	25.1-30.0 (n=255)
Corresponding group	**α**	**ß**	**γ**	**δ**	**ε**
Age (years)	22.2 ±2.93 $^{\gamma\delta\varepsilon}$	22.6 ±3.02	23.0 ±2.93 $^{\alpha}$	23.1 ±3.05 $^{\alpha}$	23.1 ±2.89$^{\alpha}$
Gender (% male)	70.0	72.8	76.5	75.4	77.5
Temperature (°C)	36.7 ±0.35	36.7 ±0.40	36.7 ±0.40	36.7 ±0.39	36.8 ±0.39
Systolic blood pressure (mmHg)	117 ±10.2 $^{\gamma\delta\varepsilon}$	119 ±10.5 $^{\varepsilon}$	120 ±9.63 $^{\alpha\varepsilon}$	121 ±10.0 $^{\alpha}$	123 ±10.0$^{\alpha\beta\gamma}$
Diastolic blood pressure (mmHg)	67.9 ±7.77	68.3 7.74	68.6 ±7.68	68.4 ±7.50	69.1 ± 8.34
Ventricular rate (beats/min)	64.4 ±9.99	62.7 ±9.69	62.5 ±10.6	62.6 ±9.83	64.7 ±10.6
Serum Sodium (mmol/L)	141 ±1.82	141 ±1.90	141 ±1.92	141 ±1.85	141 ±1.91
Serum Potassium (mmol/L)	4.31 ±0.33	4.33 ±0.31	4.33 ±0.29	4.36 ±0.30	4.33 ±0.30
Serum Calcium (mmol/L)	2.42 ±0.09	2.42 ±0.09	2.41 ±0.09	2.42 ±0.09	2.42 ±0.10
Maximum P-wave duration (ms)	99.09 ± 10.4 $^{\delta\varepsilon}$	100.5 ± 11.5 $^{\delta\varepsilon}$	101.2 ± 10.7$^{\varepsilon}$	102.9 ± 10.6 $^{\alpha\beta}$	103.8 ± 11.1$^{\alpha\beta\gamma}$
P-wave balance in lead V1 (µV)	0.31 ± 0.45 $^{\delta\varepsilon}$	0.32 ± 0.42 $^{\delta\varepsilon}$	0.33 ± 0.43	0.41 ± 0.41 $^{\alpha\beta}$	0.42 ± 0.35$^{\alpha\beta}$
P-wave dispersion (ms)	51.5 ± 20.8	53.6 ± 19.9	52.0 ± 21.0	51.7± 21.3	50.9 ± 20.9
Total P-wave area in lead V1 (mm×ms)	47.53 ± 82.6 $^{\delta\varepsilon}$	47.15 ± 76.3 $^{\delta\varepsilon}$	50.50 ± 79.5 $^{\delta\varepsilon}$	67.91 ± 80.0 $^{\alpha\beta\gamma}$	70.96 ± 74.0$^{\alpha\beta\gamma}$
PR-interval (ms)	146.9 ± 18.1 $^{\gamma\delta\varepsilon}$	149.9 ± 21.9	152.2 ± 21.0 $^{\alpha}$	151.5 ± 20.7 $^{\alpha}$	153.1 ± 19.0$^{\alpha}$
QRS duration (ms)	96.0 ± 10	96.4 ± 10	97.7 ± 10	97.0 ± 10	96.9 ± 10
Heart axis (degrees)	68.65 ± 25.8 $^{\gamma\delta\varepsilon}$	63.90 ± 26.0 $^{\delta\varepsilon}$	59.77 ± 27.0 $^{\alpha\varepsilon}$	54.57 ± 28.3 $^{\alpha\beta}$	51.91 ± 28.2$^{\alpha\beta\gamma}$
Sokolow-Lyon voltage (mm)	28.02 ± 8.28	27.30 ± 7.78	27.83 ± 8.27	26.80 ± 7.69	26.77 ± 7.66
Cornell product (mm×µV)	11.45 ± 6.29	12.29 ± 6.52	12.47 ± 6.21	11.74 ± 5.78	12.07 ± 6.09
Maximum T-wave duration (ms)	183 ± 20	184 ± 20	182 ± 20	183 ± 20	181 ± 21
Minimum T-wave duration (ms)	107 ± 54	115 ± 52	110 ± 49	114 ± 51	109 ± 50
T-wave dispersion (µV)	75.9 ± 54.7	69.4 ± 52.9	72.5 ± 54.7	68.3 ± 51.8	72.4 ± 50.5
QTcF duration (ms)	404.8 ± 17	406.7 ± 18	405.4 ± 18	405.1 ±19	404.0 ± 19

mV = millivolt, µV = microvolt, ms = milliseconds, mm = millimeters.

TABLE 3 Relation between patient characteristics and electrocardiographic parameters to diastolic blood pressure of included healthy volunteers (n=1285). Categorical variables were compared using Chi-square test, variances were compared using the Analysis of Variance test with a post hoc Tukey analysis. Results are reported as mean ± standard deviation or as percentage. The symbols α, ß, γ, and δ represent a significant difference (P<0.05) compared to that group. If no symbols are present, no significance was found between the groups.

	Diastolic blood pressure (mmHg)			
	50-59 (n=158)	60-69 (n=582)	70-79 (n=440)	80-89 (n=105)
Corresponding group	**α**	**ß**	**γ**	**δ**
Age (years)	22.1 ± 2.69$^{\gamma\delta}$	22.5 ± 2.87$^{\gamma}$	23.0 ± 3.10$^{\alpha\beta}$	23.3 ± 2.94$^{\alpha}$
Gender (% male)	75.1	69.2	75.9	76.9
Body mass index	22.5 ± 2.2	22.7 ± 2.5	22.6 ± 2.6	22.9 ± 2.66
Temperature (°C)	36.6 ± 0.37$^{\delta}$	36.7 ± 0.38	36.7 ±0.39	36.8 ± 0.36$^{\alpha}$
Systolic blood pressure (mmHg)	113.40 ± 8.86$^{\beta\gamma\delta}$	117.11 ± 9.05$^{\alpha\gamma\delta}$	122.2 ± 8.96$^{\alpha\beta\delta}$	128.4 ± 7.46$^{\alpha\beta\gamma}$
Ventricular rate (beats/min)	63.84 ± 10.5$^{\beta\gamma\delta}$	63.07 ± 9.64$^{\alpha\delta}$	63.70 ± 10.3$^{\alpha\delta}$	67.08 ± 10.4$^{\alpha\beta\gamma}$
Serum Cholesterol (mmol/L)	4.09 ± 0.73$^{\gamma\delta}$	4.19 ± 0.79$^{\gamma\delta}$	4.34 ± 0.81$^{\gamma\delta}$	4.45 ± 0.78$^{\gamma\delta}$
Serum Sodium (mmol/L)	141 ± 1.81	141 ± 1.93	141 ± 1.86	141 ±1.9
Serum Potassium (mmol/L)	4.34 ± 0.35	4.33 ±0.30	4.34 ± 0.28	4.32 ± 0.36
Serum Calcium (mmol/L)	2.41 ± 0.09	2.41 ± 0.08	2.43 ± 0.09	2.42 ± 0.10
Maximum P-wave duration (ms)	101.1 ± 11.6	101.1 ± 10.8	100.9 ± 10.7	101.2 ± 41.4
P-wave dispersion (ms)	50.2 ± 22.9	51.9 ± 21.2	51.6 ± 20.3	52.0 ± 20.1
P-wave area in lead V1 (mm×ms)	55.4 ± 72.5	48.78 ± 78.9	59.96 ± 84.5	66.3 ± 83.6
PR-interval (ms)	151.2 ± 22.4	150.2 ± 19.9	149.5 ± 19.4	151.9 ± 19.3
VAT in lead V6 (ms)	43.04 ± 5.31	42.40 ± 4.95	42.92 ± 5.14	42.57 ± 4.98
QRS duration (ms)	96.7 ± 9.98	95.7 ± 10.0	96.9 ± 10.2	96.6 ± 9.80
Heart axis (degrees)	60.3 ± 28.0	61.1 ± 25.2	61.8 ± 28.4	59.1 ± 34.4
Sokolow-Lyon voltage (mm)	2.64 ± 0.72	2.71 ± 0.83	2.78 ± 0.80	2.78 ± 0.75
Cornell product (ms×mm)	118.3 ± 60.0	113.6 ± 59.9$^{\delta}$	121.0 ± 65.6	132.0 ± 63.3 $^{\beta}$
J-point - T-peak duration (corrected for heart rate) (ms)	212.9 ± 20.3	214.6 ± 21.3	214.1 ± 20.7	210.8 ± 23.7
T-peak – T-end duration (ms)	94.57 ± 8.41	94.53 ± 8.99	93.45 ± 9.62	95.3 ± 13.3
Maximum T-wave duration (ms)	182.5 ± 20	184.2 ± 21	182.5 ± 20	181.4 ± 21
Minimum T-wave duration (ms)	111.1 ± 51	114.1 ± 52	107.0 ± 52	101.4 ± 48
T-wave dispersion (µV)	71.32 ± 52	70.1 ± 53	75.5 ± 52	79.9 ± 51
QTcF duration (ms)	404.1 ± 18	405.3 ± 18	405.1 ± 18	404.2 ± 18

mV = millivolt, µV = microvolt, ms = milliseconds, mm = millimeters, VAT = Ventricular activation time, QTcF=corrected QT interval with Fridericia's method.

TABLE 4 Univariate regression model analysis with systolic blood pressure as dependent variable. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value and the R-square value in the linear univariate regression model.

	Univariate analysis			
Variable	**USC**	**SC**	**R-square**	**P-value**
Age (per year)	-0.129	-0.040	0.002	0.130
Female gender	-7.922	-0.349	0.122	<0.001
Body mass index	0.838	0.216	0.047	<0.001
Temperature (°C)	-0.192	-0.008	<0.001	0.777
Ventricular rate (beats/min)	0.133	0.138	0.019	<0.001
Serum Cholesterol (mmol/L)	1.138	0.095	0.008	0.003
Serum Sodium (mmol/L)	0.423	0.082	0.007	0.002
Serum Potassium (mmol/L)	-0.455	-0.014	<0.001	0.588
Serum Calcium (mmol/L)	19.483	0.180	0.032	<0.001
Maximum P-wave duration (ms)	0.011	0.013	<0.001	0.627
P-wave dispersion (ms)	0.002	0.005	<0.001	0.856
P-wave area in lead V1 (mm×ms)	0.017	0.136	0.019	<0.001
PR-interval (ms)	0.012	0.026	0.001	0.325
VAT in lead V6 (ms)	0.298	0.156	0.024	<0.001
QRS duration (ms)	0.166	0.171	0.029	<0.001
Heart axis (degrees)	-0.018	-0.052	0.003	0.050
Sokolow-Lyon voltage (mm)	0.3×10^{-5}	0.221	0.049	<0.001
Cornell product (ms×mm)	3.70×10^{-5}	0.235	0.055	<0.001
J-point - T-peak duration (corrected for heart rate) (ms)	-0.081	-0.175	0.031	<0.001
T-peak – T-end duration (ms)	0.021	0.020	<0.001	0.438
Maximum T-wave duration (ms)	-0.079	-0.164	0.027	<0.001
Minimum T-wave duration (ms)	-0.007	-0.038	0.001	0.148
T-wave dispersion (ms)	-0.005	-0.027	0.001	0.310
QTcF (ms)	-0.040	-0.073	0.005	0.005

mV = millivolt, μV = microvolt, ms = milliseconds, mm = millimeters, VAT = Ventricular activation time, QTcF=corrected QT interval with Fridericia's method.

TABLE 5 Backward linear multivariate regression model analysis with systolic blood pressure as dependent variable. Probabilities of less than 0.10 in the linear univariate regression model and Variance Inflation Factor lower than 3.0 were added to the backward linear multivariate regression model. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value and the R-square value in the linear univariate regression model. The R-square of the backward linear multivariate regression model was 0.237.

	Multivariate analysis		
Variable	**USC**	**SC**	**P-value**
Female gender	-5.046	-0.231	<0.001
Body mass index	0.753	0.198	<0.001
Ventricular rate (beats/min)	0.182	0.187	<0.001
Serum Cholesterol (mmol/L)	1.005	0.084	0.004
Serum Sodium (mmol/L)	Excluded		
Serum Calcium (mmol/L)	Excluded		
P-wave area in lead V1 (mm×ms)	0.006	0.056	0.057
VAT in lead V6 (ms)	0.125	0.065	0.048
QRS duration (ms)	Excluded		
Heart axis (degrees)	Excluded		
Sokolow-Lyon voltage (mm)	0.20×10^{-5}	0.135	<0.001
Cornell product (ms×mm)	2.09×10^{-5}	0.137	<0.001
J-point - T-peak duration (corrected for heart rate) (ms)	Excluded		
Maximum T-wave duration (ms)	Dropped		
QTcF (ms)	Dropped		

mV = millivolt, μV = microvolt, ms = milliseconds, mm = millimeters, VAT = Ventricular activation time, QTcF=corrected QT interval with Fridericia's method.

TABLE 6 Backward linear multivariate regression model analysis with systolic blood pressure as dependent variable to identify which subintervals of the QT-interval with systolic blood pressure as dependent variable. Probabilities of less than 0.10 in the linear univariate regression. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value and the R-square value in the linear univariate regression model. The R-square of the backward linear multivariate regression model was 0.039.

	Multivariate analysis		
Variable	**USC**	**SC**	**P-value**
QRS duration (ms)	0.106	0.109	<0.001
J-point - T-peak duration (corrected for heart rate) (ms)	-0.054	-0.117	<0.001
T-peak – T-end duration (ms)	-0.032	-0.031	0.250

ms = milliseconds, mm = millimeters.

TABLE 7 Univariate regression model analysis with diastolic blood pressure as dependent variable. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value and the R-square value in the linear univariate regression model.

	Univariate analysis			
Variable	**USC**	**SC**	**R-square**	**P-value**
Age (per year)	0.343	0.132	0.017	<0.001
Female gender	-1.093	-0.063	0.004	0.024
Body mass index	0.123	0.040	0.002	0.151
Temperature (°C)	1.482	0.074	0.006	0.010
Ventricular rate (beats/min)	0.116	0.151	0.023	<0.001
Serum Cholesterol (mmol/L)	1.521	0.161	0.026	<0.001
Serum Sodium (mmol/L)	0.024	0.006	0.000	0.836
Serum Potassium (mmol/L)	-0.460	-0.018	0.000	0.517
Serum Calcium (mmol/L)	6.370	0.075	0.006	0.008
Maximum P-wave duration (ms)	0.005	0.007	0.000	0.800
P-wave dispersion (ms)	0.002	0.006	0.000	0.822
P-wave area in lead V1 (mm×ms)	0.005	0.054	0.003	0.055
PR-interval (ms)	0.005	0.012	0.000	0.664
VAT in lead V6 (ms)	0.006	0.004	0.000	0.890
QRS duration (ms)	0.031	0.041	0.002	0.143
Heart axis (degrees)	-0.004	-0.014	0.000	0.607
Sokolow-Lyon voltage (mm)	0.000	0.048	0.002	0.088
Cornell product (ms×mm)	8.33×10^{-6}	0.068	0.005	0.015
J-point - T-peak duration (corrected for heart rate) (ms)	-0.011	-0.029	0.001	0.295
T-peak – T-end duration (ms)	-0.010	-0.012	0.000	0.660
Maximum T-wave duration (ms)	-0.013	-0.035	0.001	0.212
Minimum T-wave duration (ms)	-0.008	-0.051	0.003	0.069
T-wave dispersion (ms)	0.005	0.037	0.001	0.190
QTCF (ms)	0.001	0.002	0.000	0.947

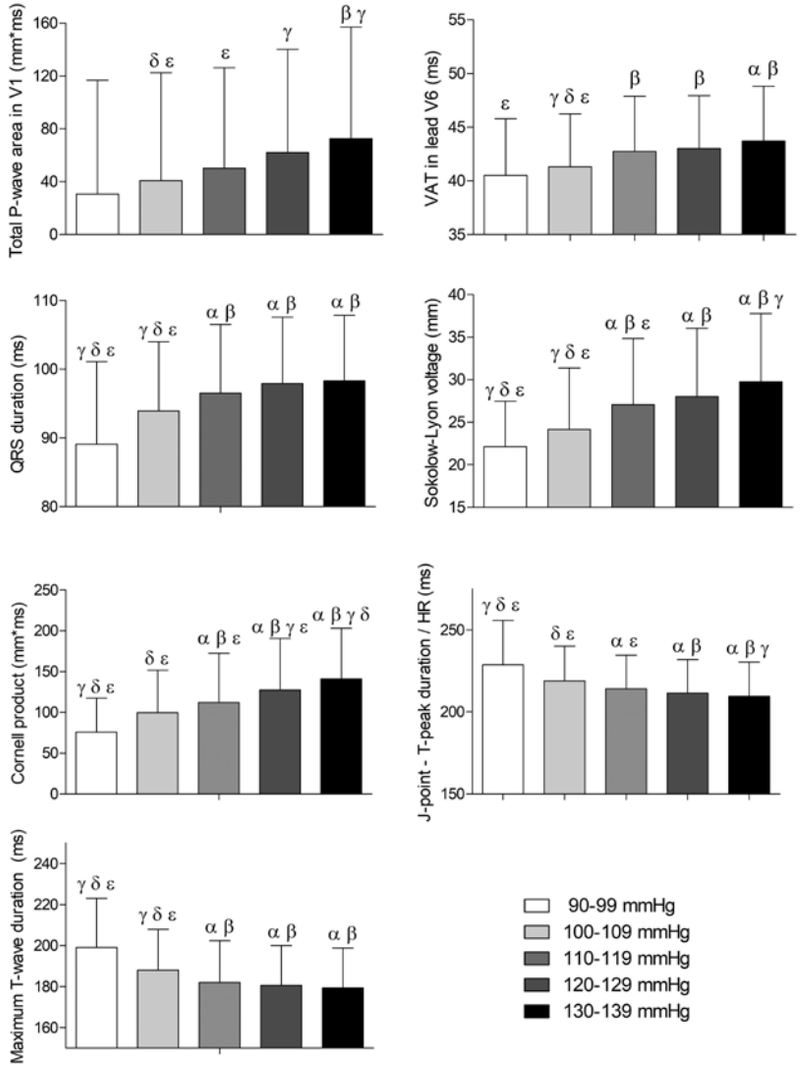
mV = millivolt, μV = microvolt, ms = milliseconds, mm = millimeters, VAT = Ventricular activation time, QTCF=corrected QT interval with Fridericia's method.

TABLE 8 Backward linear multivariate regression model analysis with diastolic blood pressure as dependent variable. Probabilities of less than 0.10 in the linear univariate regression model and Variance Inflation Factor lower than 3.0 were added to the backward linear multivariate regression model. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value and the R-square value in the linear univariate regression model. The R-square of the backward linear multivariate regression model was 0.090.

	Multivariate analysis		
Variable	**USC**	**SC**	**P-value**
Age (per year)	0.329	0.129	<0.001
Female gender	-1.184	-0.070	0.057
Temperature (°C)	1.301	0.067	0.068
Ventricular rate (beats/min)	0.136	0.179	<0.001
Serum Cholesterol (mmol/L)	1.290	0.136	<0.001
Serum Calcium (mmol/L)	Excluded		
P-wave area in lead V1 (mm×ms)	0.008	0.079	0.020
Sokolow-Lyon voltage (mm)	Excluded		
Cornell product (ms×mm)	1.22×10^{-5}	0.091	0.006
Minimum T-wave duration (ms)	Excluded		

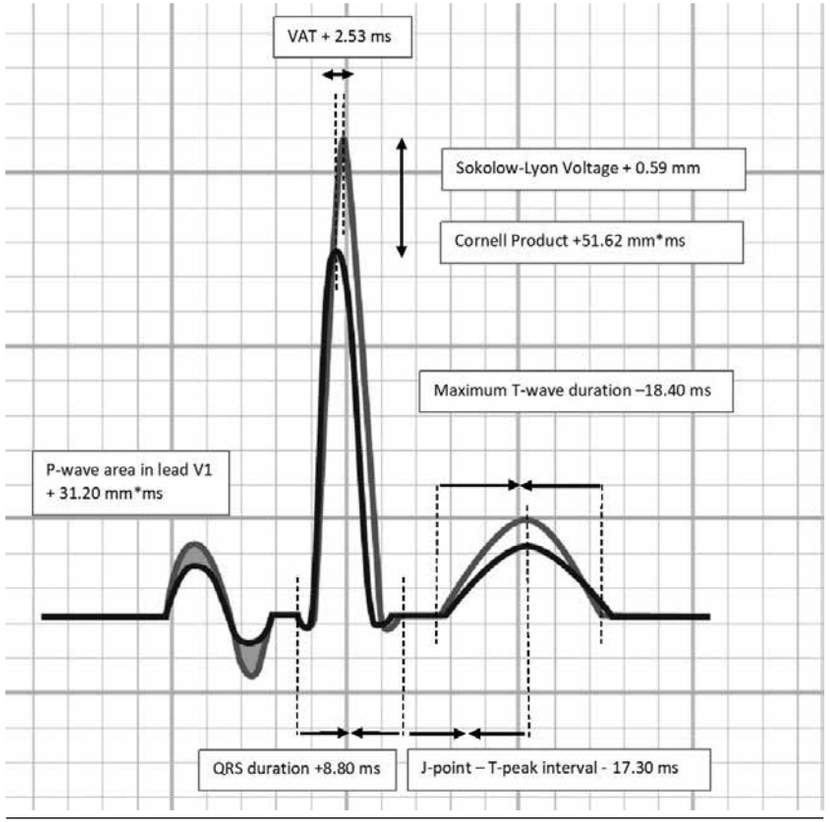
mV = millivolt, μV = microvolt, ms = milliseconds, mm = millimeters.

FIGURE 1 Associations of systolic blood pressure with electrocardiographic parameters. Results were based on Analysis of Variance (ANOVA) test between systolic blood pressure groups, and expressed as difference in the electrocardiographic parameter (with 95% confidence interval) per systolic blood pressure groups using a post hoc Tukey analysis. The symbols α, ß, γ, δ, and ε represent a significant difference (P<0.05) compared to that group. If no symbols are present, no significance was found between the groups.



*HR = heart rate, VAT = Ventricular activation time, ms = milliseconds, mm*ms = millimeter times milliseconds.*

FIGURE 2 Overview of significant changes of electrographic parameters in normotensive volunteers between 90-99 mmHg and 120-129 mmHg systolic blood pressure groups. Results were based on Analysis of Variance (ANOVA) test between systolic blood pressure groups, and expressed as difference in the electrocardiographic parameter (with 95% confidence interval) per systolic blood pressure groups using a post hoc Tukey analysis.



*VAT = Ventricular activation time, ms = milliseconds, mm*ms = millimeter times milliseconds.*

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CHAPTER VI

BODY MASS INDEX RELATED ELECTROCARDIOGRAPHIC FINDINGS IN HEALTHY YOUNG INDIVIDUALS WITH A NORMAL BODY MASS INDEX

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What's new

Obesity associated electrocardiographic changes are also found in healthy young individuals with a normal body mass index (BMI) (18.5-25 kg/m^2).

- Maximum P-wave duration, P-wave balance, total P-wave area in lead V1, PR-interval duration, and heart axis were significantly different between the different BMI quartiles.
- Within the normal range, an increased BMI was independently associated with an increased P-wave duration, increased P-wave balance, a leftward shift of the heart axis, and decreased Sokolow-Lyon voltage.

Abstract

INTRODUCTION An increased body mass index (BMI) (>25 kg/m^2) is associated with a wide range of electrocardiographic (ECG) changes. However, the association between ECG changes and BMI in healthy young individuals with a normal BMI (18.5-25 kg/m^2) is unknown. The aim of this study was to evaluate the association between BMI and ECG parameters.

METHODS Data from 1290 volunteers aged 18 to 30 years collected at our centre were analysed. Only subjects considered healthy by a physician after review of collected data with a normal BMI and in sinus rhythm were included in the analysis. Subjects with a normal BMI (18.5-25 kg/m^2) were divided into BMI quartiles analysis and a backward multivariate regression analysis with a normal BMI as a continuous variable was performed.

RESULTS Mean age was 22.7 ±3.0 years, mean BMI was 22.0, and 73.4% were male. Maximum P-wave duration, P-wave balance, total P-wave area in lead V1, PR-interval duration, and heart axis were significantly different between the BMI quartiles. In the multivariate model maximum P-wave duration (SC=+0.112, P<0.001), P-wave balance in lead V1 (SC=+0.072, P<0.001), heart axis (SC=-0.164, P<0.001), and Sokolow-Lyon voltage (SC=-0.097, P<0.001) were independently associated with BMI.

CONCLUSION Increased BMI was related with discrete ECG alterations including an increased P-wave duration, increased P-wave balance, a leftward shift of the heart axis, and decreased Sokolow-Lyon voltage on a standard twelve lead ECG in healthy young individuals with a normal BMI.

Introduction

Obesity causes several hemodynamic changes such as increased blood and stroke volume, and an increase in pulmonary and left atrial pressure.[1,2] These changes cause structurally altered cardiac tissue such as left atrial enlargement and remodelling, and ventricular hypertrophy.[1,2] These may ultimately result in obesity-induced left ventricular diastolic and systolic dysfunction and right and left ventricular heart failure.[1,2]

Some obesity-induced adverse effects on cardiac function can be identified on a 12-lead electrocardiogram (ECG). This includes an increased P-wave duration and dispersion, [3-6] prolongation of the PR-interval, [3-7] low QRS voltage in the limb leads, [7-9] leftward shift of the heart axis, [7-11] various markers of left ventricular hypertrophy [12-14] and prolongation of the corrected QT interval and prolonged QT interval duration.[8] Many of these ECG abnormalities have been reported to be reversible with substantial weight loss thereby reinforcing the association between BMI and ECG changes.[2,8]

These ECG changes are well-documented in obese individuals. However, to which extend these ECG changes are associated with BMI in healthy young individuals with a normal BMI (18.5-25 kg/m^2) is largely unknown. In addition, subtle physiological changes in these individuals are of particular interest in early phase pharmaceutical research because they help differentiate between normal physiological changes or potentially harmful or unknown pharmacodynamic effects. The aim of the present analysis was to evaluate the association between BMI and selected ECG parameters related to cardiac alterations in obesity in a healthy young population with a normal BMI.

Methods

Data from 1290 male and female volunteers with a normal BMI (18.5-25.0 kg/m^2) aged 18-30 years used in the present analysis were collected at the Centre for Human Drug Research in Leiden, the Netherlands, a clinical research organization specialized in early phase drug development studies. Data from studies that were performed in healthy volunteers between 2010 and 2016 were included in the present analysis. For all studies, healthy volunteers underwent a mandatory medical screening to verify eligibility for the study. The present analysis was performed in accordance with local regulations. All activities were performed in accordance with applicable standard operating procedures.

MEDICAL SCREENING

The medical screening consisted of a single visit to the clinical unit where a detailed anamnesis, a physical examination, vital signs including blood pressure, temperature, weight and height measurement, BMI calculation, and a twelve-lead electrocardiogram (ECG) were recorded. Additionally, haematology and chemistry blood panel, urine dipstick, and a urine drug test were recorded.

WEIGHT AND HEIGHT MEASUREMENT

For the weight and height measurement, the subject was asked to undress except for underwear and asked to stand on the platform with the back against the measuring rod, heels against the heel board and back and neck straight. A BMI was calculated automatically by the digital scale using the formula:

$$\text{BMI} = \frac{\text{Weight (kilogams)}}{\text{Height (meters)}^2}$$

Body weight and height measurements were recorded with a calibrated digital measuring rod (SECA 285; RevaMed BV, Kampen, the Netherlands) and immediately entered into a validated database system (Promasys, OmniComm, Fort Lauderdale, FL, USA).

ECG MEASUREMENTS

The twelve-lead ECGs were recorded with the volunteer in a resting supine position and after a five-minute resting period. The twelve-lead ECGs were recorded using an electrocardiograph (Marquette 800/5500/2000 or Dash 3000; General Electric Healthcare, MKE, USA) and twelve disposable electrodes placed in the standard anatomical position. The ECG data were then uploaded into the ECG warehouse (Muse Cardiology Data Management System v7, General Electric Healthcare, Chicago, IL, USA). The Marquette Cubic Spline and Finite Residual Filter filters were used for artefact and noise management. The ECG warehouse automatically assesses interval and amplitude data from the digital ECGs with the Marquette 12SL algorithm, which provides a variety of ECG measurements which have been used in previous studies.[15,16] In addition, a physician reviewed all ECGs for quality, legibility, and abnormalities. Independent evaluation showed that the Marquette 12SL algorithm passed all of the amplitude measurement requirements (maximum of 10 ms deviation) as defined in International Electrotechnical Commission, as described in the General Electric Physician's

Guide (version 2036070-006). Description, methods of determination and calculation, and units of the ECG parameters are described in table 1.

VALIDATION COHORT

Additionally, data from 255 male and female volunteers with an overweight BMI (25.1-30.0 kg/m^2) aged 18-30 years similarly collected as the volunteers with a normal BMI (18.5-25.0 kg/m^2) group were added to the analysis as a validation cohort. These data were used to test the persistence of any significant variances of ECG findings in the healthy subjects with a normal BMI (18.5-25.0 kg/m^2) in that of healthy subjects with an overweight BMI (25.1-30.0 kg/m^2). This data was not included in the univariate and multivariate analysis.

STATISTICAL ANALYSIS

Data are reported as mean ± standard deviation (SD), median with interquartile range or percentage where appropriate. Categorical variables were compared using Chi-square test. Variances were compared using the Analysis of Variance (ANOVA) test with a post-hoc Tukey analysis. A linear univariate and a backward linear multivariate regression model analysis were performed solely with the data of the subjects with a normal BMI (18.5-25.0 kg/m^2). Probabilities of less than 0.10 in the linear univariate regression model were added to the backward linear multivariate regression model. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value. Statistical analyses were performed using IBM SPSS version 25 (IBM corporation, Armonk, NY, USA).

Results

In total, 1290 subjects were included in the present analysis. Mean age was 22.6 ±3.0 years, 73.9% were male. Subjects with a normal BMI (n=1290) were divided based on BMI quartiles (18.5-20.7; 20.7-22.0; 22.0-23.4; 23.4-25.0 kg/m^2). Overweight subjects (n=255) were allocated into the overweight group. Subject characteristics are shown in table 2. Subjects in the lowest BMI quartile were significantly younger and had a significantly lower systolic blood pressure compared to subjects in the third (P=0.003 and P<0.001, respectively)) and fourth BMI quartile (P<0.001 and P<0.001, respectively). In addition, overweight subjects were significantly younger than the first quartile (P=0.003) and had a significantly higher systolic blood pressure compared to subjects in the first (P<0.001), second (P<0.001), and third BMI quartile (P=0.006). Other baseline characteristics were not significantly different among BMI groups.

BMI AND ECG PARAMETERS

Table 2 displays the association between the BMI quartiles and the evaluated ECG parameters. Maximum P-wave duration, P-wave balance, total P-wave area in lead V1, PR-interval, and heart axis were significantly different between the normal BMI quartiles and between the overweight BMI group, as displayed in figure 1.

LINEAR REGRESSION ANALYSIS

In the univariate analysis of the data of the subjects with a normal BMI (18.5-25.0 kg/m^2), BMI was significantly associated (P<0.05) with age (SC=+0.139, P<0.001), systolic blood pressure (SC+0.146, P<0.001), ventricular rate (SC=-0.076, P=0.006), serum creatinine (SC=+0.152, P<0.001), serum potassium (SC=+0.054, P=0.054), maximum P-wave duration (SC=+0.130, P<0.001), P-wave balance in lead V1(SC=+0.077, P=0.006), total P-wave area in lead V1 (SC=+0.084, P=0.003), PR-interval (SC=+0.090, P=0.001), and heart axis (SC=-0.191, P<0.001). In the backward multivariate model, age (SC=+0.108, P<0.001), systolic blood pressure (SC=+0.173, P<0.001), ventricular rate (SC=-0.081, P=0.005), maximum P-wave duration (SC=+0.112, P<0.001), P-wave balance in lead V1 (SC=+0.072, P<0.001), heart axis (SC=-0.164, P<0.001), and Sokolow-Lyon voltage (SC=-0.097, P<0.001) were independently associated with BMI, as can also be observed from table 3. The R-square of the multivariate model was 0.100.

Discussion

This analysis found an association between ECG parameters and BMI in healthy young (≤ 30 years) adults with a normal BMI (18.5-25.0 kg/m^2). A higher BMI was independently associated with an increased P-wave duration, an increased P-wave, a leftward shift of the heart axis, and a decreased Sokolow-Lyon voltage.

Left atrial enlargement (LAE) is associated with an increased prevalence of atrial fibrillation, cardiovascular events and death.[13] Obesity is found to be the most important risk factor for LAE development in the general population, [17] and is dependent on the extent of obesity.[2-6] Furthermore, LAE is also independently related with age, hypertension, BMI, waist circumference, and

metabolic syndrome.[18] Additionally, obesity is the strongest predictor of LAE in hypertensive patients, and is under the influence of race and gender.[19] These structural changes can be observed on the twelve-lead surface ECG through increased P-wave duration, P-wave area, and P-wave dispersion.[2-6] Obesity associated ECG changes such as an increased P-wave duration (5-22 ms) and P-wave dispersion (14-25 ms),[3-6] increased PR-interval (5-13 ms)[3-7] and a leftward shift of the heart axis (11-37 degrees) compared to adults with a normal BMI were reported.[7-11] In the present analysis, we found a relation between BMI and these indices of atrial size. Although no left atrial measurement was performed these results suggest that atrial size may also be related to BMI in healthy individuals with a normal BMI (18.5-25.0 kg/m^2).

Presumably, increased epicardial and pericardial fat, which are increased in obesity, further induce these changes.[2,20-25] Cardiac fat depositions were found to have metabolic and inflammatory functions which can contribute to the fibrotic remodelling of the atrial tissue.[1,2,20-25] These fat depositions are significantly increased in obesity and are believed to induce the abovementioned ECG changes.[1,2,20-25] Hypothetically, the volume of epicardial and pericardial fat is also dependent on BMI in young, non-obese individuals. This may be an additional explanation for the association that was observed in the present analysis between BMI and the abovementioned ECG changes.

Leftward shifts of the P-wave, QRS and T-wave axes (11-37 degrees) are reported in obese patients compared to healthy controls.[7-11] The cause of these shifts is uncertain but may be related to a leftward and more horizontal orientation of the heart attributed to the diaphragmatic pressure from central obesity, independent from left ventricular hypertrophy.[7-11] This explains the association between lower BMI and rightward P-wave and QRS-wave axes and independently from left ventricular mass.[11] This is in line with our findings and presumably, the leftward change in heart axis that was observed in the present analysis is caused by an increase in diaphragmatic pressure which is dependent on BMI.

Previous reports already advocated caution when using ECG markers for left ventricular hypertrophy (LVH) in obese patients.[14] The commonly used Sokolow-Lyon voltage criteria underestimate the prevalence of anatomic LVH in the presence of obesity, whereas Cornell product criteria for ECG LVH appear to provide a more accurate measure of LVH in obese and overweight patients.[12] Obesity results in three distinct processes that affect the surface ECG—lateral displacement of the anatomical left ventricular (LV) axis, increased chest wall fat and increased pericardial fat mass, all of which decrease voltage amplitude on the ECG.[26] We found decreased Sokolow-Lyon voltages in the multivariate analysis, but no associations with the Cornell product calculations. These findings illustrate the challenge of their use as marker for LVH even in healthy individuals with a normal BMI (18.5-25.0 kg/m^2).

LIMITATIONS

The limitations of this study are the retrospective, cross-sectional design, and the usage of automatically calculated ECG data. The reported associations were found to be significant in the multivariate model, however the R-squared of the multivariate model was only modest, suggesting that other co-existing factors play a role in atrial and ventricular structural and functional remodelling. Larger prospective cohort studies are needed to explore the prognostic value of these ECG findings. Additional information such as waist circumference, cardiac dimensions, and more detailed information about the body composition such as fat and muscle percentages may further differentiate between groups and provide new insights about the cardiac changes.

Conclusion

In conclusion, we found that BMI related discrete electrocardiographic changes can be observed in healthy young individuals with a normal BMI (18.5-25.0 kg/m^2). These were related to an altered atrial conduction, leftward shift of the heart axis, and decreased Sokolow-Lyon voltage.

TABLE 1 Methods of determination or calculation of the electrocardiographic (ECG) variables used in the analysis of the 1554 healthy volunteers aged 18 to 30 years with a body mass index (BMI) between 18.5 and 30.0 kg/m².

Variable	Description
Maximum P-wave duration (ms)	Longest P-wave duration sampled from all leads
P-wave balance in lead V1 (μV)	Difference between the upward and downward deflection of the P-wave
P-wave dispersion (ms)	Difference between the longest minus the shortest P-wave duration from all leads
Total P-wave area in lead V1 (mm×ms)	Sum of the total area under and above the isoelectric line from onset to termination of the P-wave [33]
PR-interval (ms)	Beginning of the P-wave until the beginning of the QRS complex
QRS duration (ms)	First deflection from the isoelectric line following the P-wave until the J-point.
Heart axis (degrees)	Net vector of the R-wave axis based on the extremity leads.
Sokolow-Lyon voltage (mm)	Sum amplitude of the S-wave in lead V1 and the amplitude of the R-wave in lead V5 or V6 (whichever is larger) [34]
Cornell product (ms×mm)	Product of the QRS duration and the Cornell voltage [35] Cornell voltage is the sum of the amplitude of the R-wave in lead aVL and the amplitude of the S-wave in lead V3 [35]
Maximum T-wave duration (ms)	Longest T-wave duration sampled from all leads
Minimum T-wave duration (ms)	Shortest T-wave duration sampled from all leads
T-wave dispersion (ms)	Difference between the longest and shortest T-wave duration selected from all leads
QTcF duration (ms)	QTcF duration is calculated using the Fridericia formula, which divides the QT-interval by the cube-root of RR-interval. [36] QT-interval is the interval between the start of the Q-wave and the end of the T-wave. RR-interval is the interval between the onset of one QRS complex to the onset of the next QRS complex, measured in seconds, derived from the heart rate (HR) as 60/HR.

mV = millivolt, μV = microvolt, ms = milliseconds, mm = millimeters.

TABLE 2 Relation between patient characteristics and electrocardiographic parameters to BMI of included healthy volunteers. Categorical variables were compared using Chi-square test, variances were compared using the Analysis of Variance test with a post hoc Tukey analysis. Results are reported as mean ± SD or as percentage. The symbols α, ß, γ, δ, and ε represent a significant difference (P<0.05) compared to that group. If no symbols are present, no significance was found between the groups.

	Body mass index (kg/m²)				
	18.5-20.7 (n= 323)	**20.8-22.0 (n= 334)**	**22.1-23.4 (n= 324)**	**23.5-25.0 (n= 309)**	**25.1-30.0 (n=255)**
Corresponding group	**α**	**ß**	**γ**	**δ**	**ε**
Age (years)	22.2 ±2.93 γδε	22.6 ±3.02	23.0 ±2.93 α	23.1 ±3.05 α	23.1 ±2.89α
Gender (% male)	70.0	72.8	76.5	75.4	77.5
Temperature (°C)	36.7 ±0.35	36.7 ±0.40	36.7 ±0.40	36.7 ±0.39	36.8 ±0.39
Systolic blood pressure (mmHg)	117 ±10.2 γδε	119 ±10.5 ε	120 ±9.63 αε	121 ±10.0 α	123 ±10.0αßγ
Diastolic blood pressure (mmHg)	67.9 ±7.77	68.3 7.74	68.6 ±7.68	68.4 ±7.50	69.1 ± 8.34
Ventricular rate (beats/min)	64.4 ±9.99	62.7 ±9.69	62.5 ±10.6	62.6 ±9.83	64.7 ±10.6
Serum Sodium (mmol/L)	141 ±1.82	141 ±1.90	141 ±1.92	141 ±1.85	141 ±1.91
Serum Potassium (mmol/L)	4.31 ±0.33	4.33 ±0.31	4.33 ±0.29	4.36 ±0.30	4.33 ±0.30
Serum Calcium (mmol/L)	2.42 ±0.09	2.42 ±0.09	2.41 ±0.09	2.42 ±0.09	2.42 ±0.10
Maximum P-wave duration (ms)	99.09 ± 10.4 δε	100.5 ± 11.5 δε	101.2 ± 10.7ε	102.9 ± 10.6 αß	103.8 ± 11.1αßγ
P-wave balance in lead V1 (μV)	0.31 ± 0.45 δε	0.32 ± 0.42 δε	0.33 ± 0.43	0.41 ± 0.41 αß	0.42 ± 0.35αß
P-wave dispersion (ms)	51.5 ± 20.8	53.6 ± 19.9	52.0 ± 21.0	51.7± 21.3	50.9 ± 20.9
Total P-wave area in lead V1 (mm×ms)	47.53 ± 82.6 δε	47.15 ± 76.3 δε	50.50 ± 79.5 δε	67.91 ± 80.0 αßγ	70.96 ± 74.0αßγ
PR-interval (ms)	146.9 ± 18.1 γδε	149.9 ± 21.9	152.2 ± 21.0 α	151.5 ± 20.7 α	153.1 ± 19.0α
QRS duration (ms)	96.0 ± 10	96.4 ± 10	97.7 ± 10	97.0 ± 10	96.9 ± 10
Heart axis (degrees)	68.65 ± 25.8 γ δε	63.90 ± 26.0 δε	59.77 ± 27.0 αε	54.57 ± 28.3 αß	51.91 ± 28.2αßγ
Sokolow-Lyon voltage (mm)	28.02 ± 8.28	27.30 ± 7.78	27.83 ± 8.27	26.80 ± 7.69	26.77 ± 7.66
Cornell product (mm×μV)	11.45 ± 6.29	12.29 ± 6.52	12.47 ± 6.21	11.74 ± 5.78	12.07 ± 6.09
Maximum T-wave duration (ms)	183 ± 20	184 ± 20	182 ± 20	183 ± 20	181 ± 21
Minimum T-wave duration (ms)	107 ± 54	115 ± 52	110 ± 49	114 ± 51	109 ± 50
T-wave dispersion (μV)	75.9 ± 54.7	69.4 ± 52.9	72.5 ± 54.7	68.3 ± 51.8	72.4 ± 50.5
QTcF duration (ms)	404.8 ± 17	406.7 ± 18	405.4 ± 18	405.1 ±19	404.0 ± 19

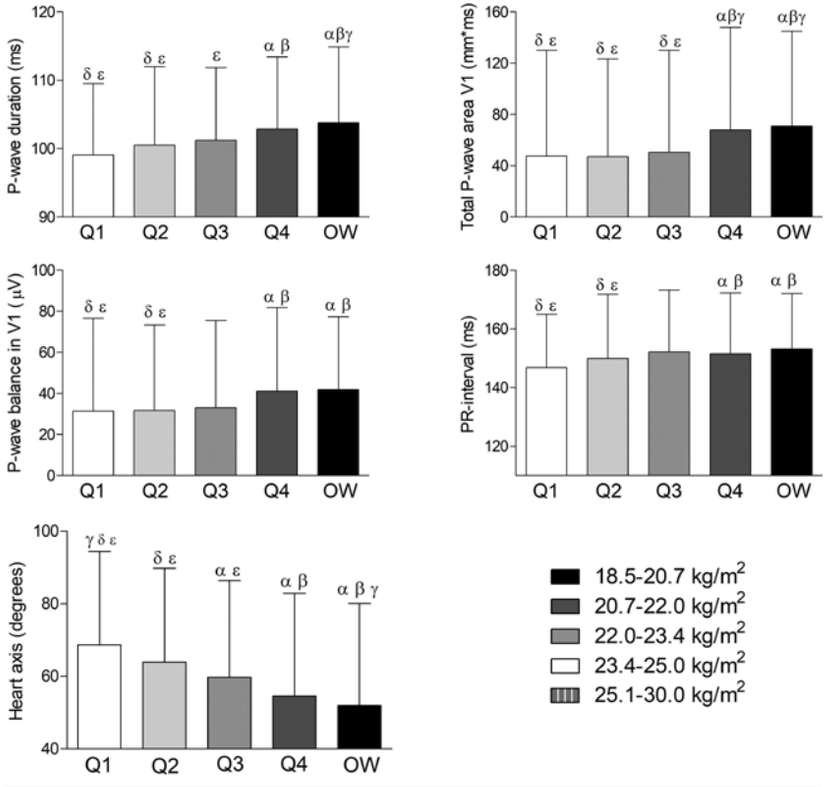
mV = millivolt, μV = microvolt, ms = milliseconds, mm = millimeters.

TABLE 3 Univariate and backward linear multivariate regression model analysis. Probabilities of less than 0.10 in the linear univariate regression model were added to the backward linear multivariate regression model. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value and the R-square value in the linear univariate regression model. The R-square of the backward linear multivariate regression model was 0.100.

	Univariate analysis				Multivariate analysis		
Variable	USC	SC	R-square	P-value	USC	SC	P-value
Age (per year)	0.079	0.139	0.019	<0.001	0.061	0.108	<0.001
Female gender	-0.211	-0.054	0.003	0.056	Excluded		
Temperature (°C)	0.038	0.009	0.000	0.764			
Systolic blood pressure (mmHg)	0.024	0.146	0.021	<0.001	0.029	0.173	<0.001
Diastolic blood pressure (mmHg)	0.005	0.022	0.000	0.479			
Ventricular rate (beats/min)	-0.013	-0.076	0.006	0.006	-0.014	-0.081	0.005
Serum Sodium (mmol/L)	-0.022	-0.024	0.001	0.399			
Serum Potassium (mmol/L)	0.299	0.054	0.003	0.054	0.253	0.046	0.098
Serum Calcium (mmol/L)	-0.735	-0.038	0.001	0.178			
Maximum P-wave duration (ms)	0.020	0.130	0.017	<0.001	0.018	0.112	<0.001
P-wave balance in lead V1 (μV)	0.003	0.077	0.006	0.006	0.003	0.072	0.010
P-wave dispersion (ms)	0.001	0.009	0.000	0.740			
Total P-wave area in lead V1 (mm×ms)	0.002	0.084	0.007	0.003	Excluded		
PR-interval (ms)	0.007	0.090	0.008	0.001	Excluded		
QRS duration (ms)	0.009	0.053	0.003	0.059	Excluded		
Heart axis (degrees)	-0.012	-0.191	0.036	<0.001	-0.010	-0.164	<0.001
Sokolow-Lyon voltage (mm)	0.000	-0.048	0.002	0.084	0.000	-0.097	<0.001
Cornell product (mm)	5.67×10^{-7}	0.021	0.000	0.458			
Maximum T-wave duration (ms)	-0.002	-0.019	0.000	0.503			
Minimum T-wave duration (ms)	0.001	0.038	0.001	0.171			
T-wave dispersion (ms)	-0.001	-0.045	0.002	0.107			
QTcF (ms)	5.38×10^{-5}	0.001	0.000	0.982			

mV = millivolt, μV = microvolt, ms = milliseconds, mm = millimeters, QTcF=corrected QT interval with Fridericia's method.

FIGURE 1 – Associations of body mass index (BMI) with electrocardiographic parameters. Results were based on Analysis of Variance test between BMI distribution, and expressed as difference in the electrocardiographic parameter (with 95% confidence interval) per quartile of BMI using a post hoc Tukey analysis. The symbols α, ß, γ, δ, and ε represent a significant difference (P<0.05) compared to that group. If no symbols are present, no significance was found between the groups.



*OW = overweight BMI (25.1-30.0 kg/m²) group. μV = microvolt, ms = milliseconds, mm*ms = millimeter times milliseconds.*

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CHAPTER VII

LETTER TO THE EDITOR

RESPONSE TO ARTICLE BY MAJEED ET AL. 'RELATION OF TOTAL BILIRUBIN AND QT INTERVAL PROLONGATION (FROM THE THIRD NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY)'

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Letter to the editor

As a center specialized in early drug development, we have read with great interest the article recently published in ANE by Majeed et al. (2019).[1] The authors evaluated the association of total bilirubin (TB) levels with QT interval in an elderly, multiracial cohort.

The authors found a clear association between higher levels of TB and the prevalence of a prolonged QT interval. Such findings are of particular interest in early drug development, where these results may help differentiate between physiological and potentially harmful or unknown pharmacodynamic effects on the QT interval. However, there are several aspects that should have been taken into account that may have influenced the results of this study.

Firstly, physiologically the QT interval is dependent on the heart rate. Subsequently, appropriate interpretation of QT interval data requires a correction for the heart rate as calculated through the RR interval. In the scope of total bilirubin levels, Zambruni et al. studied varies QT interval correction formulas in patients with liver cirrhosis and concluded that the corrected QT interval was no longer influenced by the RR interval with the application of the Fridericia's formula (shown below).[2,3] Unfortunately, no corrected QT interval or RR interval are reported throughout the article which impairs appropriate interpretation of the findings.

$$QTcFridericia = \frac{QT\ interval}{\sqrt[3]{RR}}$$

Secondly, associations between serum electrolyte concentrations and changes in cardiac electrophysiology must be taken into account when analysing ventricular repolarization as reported by Noordam et al.[4] Noordam et al. reported significant associations between QT interval prolongation and lower levels of calcium, and higher levels of magnesium, respectively.

Lastly, valuable clinical data can be revealed through QT subinterval analyses by dividing the QT interval into its components of depolarization (QRS), early repolarization (J-Tpeak), and late repolarization (Tpeak-Tend) as proposed by Johannesen et al.[5] Especially late repolarization prolongation is associated with mortality related ventricular arrhythmias and is of particular interest in drug development.[5]

With these remarks in consideration, we adapted Majeed et al.[1] their analysis and applied it to our own dataset. Data from 1290 male and female volunteers aged 18-30 years (mean age 22.7 ± 3.0) collected at our center were analysed. Only subjects considered healthy by a physician and free of medication use were included. Backward multivariate regression analysis with total bilirubin level as a continuous variable was performed. In the univariate analysis of the data, total bilirubin level was significantly associated with BMI (SC=-0.061, P=0.030), cholesterol (SC=-0.102, P=0.003), potassium (SC=-0.065, P=0.022), calcium (SC=+0.129, P<0.001), systolic blood pressure (SC=+0.124, P<0.001), ventricular rate (SC=-0.058, P=0.040), QRS duration (SC=+0.135, P<0.001), early repolarization (SC=-0.139, P<0.001) and QTcF interval (SC=-0.071, P=0.005), but not with QT interval (SC=0.004, P=0.879) or late repolarization (SC=+0.035, P=0.172).

In our backward multivariate analysis, cholesterol (SC=-0.108, P=0.001), potassium (SC=-0.087, P=0.010), calcium (SC=+0.107, P=0.002), systolic blood pressure (SC=+0.101, P=0.004), ventricular rate (SC=-0.069, P=0.048) and early repolarization (SC=-0.147, P<0.001) remained significantly associated with total bilirubin level. QTcF interval was no longer significantly associated with total bilirubin level. In conclusion, our results illustrate the value of the addition of QT interval correction, serum electrolytes, and QT subintervals to our analysis. These adaptations may explain the differences in our findings compared to Majeed et al. (2019).[1] Additionally, differences may also be explained because of the significantly different characteristics between the two groups. Our group consisted solely of healthy young individuals, while Majeed et al. (2019)[1] their group consisted of elderly individuals with comorbidities such as high normal elevated systolic blood pressure and diabetes mellitus, both of which are linked with QT-interval modulation in liver cirrhosis patients independently from Child Pugh score.[6] Therefor, we conclude that for our daily practices, total bilirubin levels appear to be of relatively minor influence to QT interval. However, further research is needed to confirm and our findings.

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CHAPTER VIII

HAEMOGLOBIN ASSOCIATED ELECTROCARDIOGRAPHIC PARAMETERS FINDINGS IN HEALTHY YOUNG INDIVIDUALS

Submitted

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Abstract

BACKGROUND Anaemia induces a physiological cardiac compensation through increased cardiac output and is associated with ventricular electrocardiographical parameters, including prolongation of the corrected QT (QTc) interval. Enhanced characterization of variations in QTc interval and serum haemoglobin level aids in better understanding the underlying mechanism behind drug induced QTc interval effects. This analysis' objective was to investigate associations between haemoglobin and electrocardiographical parameters in healthy volunteers.

METHODS Data from 1118 healthy male volunteers aged 18-30 years collected at our center were analysed. Subjects were considered healthy after review of collected data by a physician, including a serum haemoglobin level of 8.5-11.0 mmol/L and in sinus rhythm. A linear multivariate analysis with haemoglobin as a continuous variable was performed. Another multivariate analysis was performed with only the QT subintervals as independent variables and haemoglobin as dependent variable.

RESULTS Mean age was 22.8 ±3.0 years, mean haemoglobin 9.46 ± 0.50 mmol/L. Electrocardiographically, haemoglobin was independently associated with heart rate (SC=+0.153, P<0.001), and Fridericia corrected QT interval (SC=-0.146, P<0.001). Atrial parameters were not independently associated with haemoglobin. QT subinterval analysis revealed that haemoglobin level had the highest impact on J-point – T-peak corrected for heart rate interval duration (SC=-0.155, P<0.001), followed by QRS duration (SC=-0.142, P<0.001) and T-peak T-end interval duration (SC=-0.061, P=0.051).

CONCLUSION A lower haemoglobin level in non-anaemic healthy male volunteers was independently associated with decreased heart rate and an altered ventricular conductivity, predominantly mediated through a prolongation of the early repolarization phase.

Introduction

Anaemia, defined as serum haemoglobin levels below 7.5 mmol/L (females, 12.0 g/dL) or 8.0 mmol/L (males, 12.9 g/dL) induces a physiological cardiac compensation through increased cardiac output in order to maintain adequate oxygen delivery.[1-3] In acute onset non-hypotensive hypovolemia, as observed in mild haemorrhages or during blood donation, physiological increases in blood pressure but not of heart rate are observed [4,5] and also in a chronic anaemic setting, higher cardiac output is mainly achieved through increased stroke volume with minor contributions of increased heart rate.[6-8] Echocardiographic imaging of healthy adults with chronic anaemia showed increased left ventricular diameters, with greater left-ventricular mass.[6-9] Following correction of anaemia, significant decreases in left ventricular end-diastolic and end-systolic diameters and left ventricular mass index compared to baseline levels were found.[6-8,10]

On a 12-lead surface electrocardiogram (ECG), the anaemic echocardiographic changes are predominantly reflected in ventricular indices, with increased Sokolow-Lyon voltages,[9] prolonged QTc-interval,[11-14] reduced T-wave amplitudes[14-16] and an increased interval between the peak and the end of the T wave (TpTe).[12,13] However, the effect of normal haemoglobin levels on the surface ECG in healthy volunteers has been underreported. An increased characterization of the association between the QTc interval, including QT subinterval analysis,[17] and haemoglobin levels aids a better differentiation between the direct effects of pharmacological agents on the ECG. Furthermore, in early phase drug research relatively large amounts of up to 500 mL of blood are drawn within a short time period. Frequently, this causes a substantial decrease in haemoglobin level of which there is a limited data on the effects on the surface ECG.

The purpose of the present analysis was to characterize the association between haemoglobin levels and selected ECG parameters recognized to be associated with anaemia in a healthy and young male population with normal haemoglobin levels.

Methods

Data from 1118 healthy young male volunteers aged 18-30 years with a normal haemoglobin level between 8.5 and 11.0 mmol/L (13.7 and 17.7 g/dL) were included in the present analysis.[3] All data were collected at the Centre for Human Drug Research in Leiden, the Netherlands, a clinical research organization spe-

cialized in early phase drug development studies. Data collected during the mandatory medical screening to verify study eligibility between 2010 and 2016 were included in the present analysis. Ethical approvals from the Medical Ethical Review Committee for the intended studies were acquired and informed consent documents were signed by the volunteers prior to any data collection. The present analysis was performed in accordance with local regulations. All activities were performed in accordance with applicable standard operating procedures.

MEDICAL SCREENING

In this analysis, only subjects considered healthy were eligible for inclusion. Healthy was defined as the absence of clinically significant abnormalities on a wide selection of tests with no evident history of diseases. This battery of tests consisted of a single visit to the clinical unit during office hours (between 09:00-17:00 o'clock) where a detailed medical history, a physical examination, vital signs including blood pressure, body temperature, weight and height measurement, body mass index (BMI) calculation, and a 12-lead ECG were recorded. Additionally, haematology and chemistry blood panels, urine dipstick, and a urine drug test were analysed.

BLOOD SAMPLING

Subjects were required to be fasting for a minimal duration of four hours prior to blood sampling. Blood sampling was performed in accordance with applicable standard operating procedure and in accordance to the World Health Organization guidelines on drawing blood.[18] Venous blood was sampled from a vein located at the antecubital fossa or forearm and after removal of the tourniquet. Haematology and chemistry blood panels included, but was not limited to, haemoglobin level, and sodium, potassium, calcium, thyroid hormone levels, creatinine, respectively. Haemoglobin levels were measured by a haematology automated analyser (Sysmex XE 2100, Sysmex Corporation, Kobe, Hyogo, Japan).

ECG MEASUREMENT

The 12-lead ECGs were recorded with the volunteer in supine position and after a five-minute resting period prior to the blood sampling. The 12-lead ECGs were recorded using an electrocardiograph (Marquette 800/5500/2000 or Dash 3000; General Electric Healthcare, MKE, USA) and ten disposable electrodes placed in the standard anatomical position. The ECG data were then uploaded into the ECG warehouse (Muse Cardiology Data Management System V7, General Electric Healthcare, Chicago, IL, USA). The Marquette Cubic Spline filter and Finite Residual Filter were used for artefact and noise management. In addition, a physician manually reviewed all ECGs for quality, legibility, and abnormalities. The ECG warehouse automatically assesses interval and amplitude data from the digital ECGs with the Marquette 12SL algorithm, which provides a variety of validated ECG measurements on median beats and have been used in previous studies.[19,20] The ECG measurements were performed only by the Marquette 12SL algorithm, there were no manual adjustments or manual measurements. Independent evaluation showed that the Marquette 12SL algorithm passed all amplitude measurement requirements (maximum of 10 ms deviation) as defined in International Electrotechnical Commission, as described in the General Electric Physician's Guide (version 2036070-006). In addition to the algorithm, a physician reviewed all ECGs for quality, legibility, and abnormalities. Description, methods of determination and calculation, and units of the ECG parameters are described in table 1.

STATISTICAL ANALYSIS

Only subjects who were considered healthy by a physician after review of all the collected data, including an ECG with sinus rhythm were included in the current analysis. Data are reported as mean ± standard deviation (SD), median with interquartile range or percentage where appropriate. Subjects were divided in quartile groups based on haemoglobin levels (8.4-9.0; 9.1-9.3; 9.4-9.8;9.9-11.1 mmol/L) for analysis. Because of the substantial number of data samples, normality was assessed through visual confirmation of graphical plots. Variances were compared using the Analysis of Variance (ANOVA) test with a post-hoc Tukey analysis. A linear univariate and a backward linear multivariate regression model analysis were performed with haemoglobin level as the dependent continuous variable.

Probabilities of less than 0.10 in the linear univariate regression model were added to the backward linear multivariate regression model. In the case that variables reversed in association between the univariate and multivariate analysis, they were excluded from the multivariate analysis. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value. Statistical analyses were performed using IBM SPSS version 25 (IBM corporation, Armonk, NY, USA).

Results

In total, 1118 male subjects were included in the present analysis. Mean age was 22.8 ± 3.0 years, mean haemoglobin level was 9.46 ± 0.50 mmol/L (15.2 ± 0.8 g/dL). Subject characteristics are shown in table 2. Incrementally with haemoglobin level groups systolic and diastolic blood pressure increased between the groups as can be observed in table 2. Other baseline characteristics were not significantly different among haemoglobin level groups.

HAEMOGLOBIN AND ECG PARAMETERS

Table 2 displays the association between the haemoglobin level groups and the evaluated ECG parameters. Heart rate, QRS duration, JpTpc interval, maximum T-wave duration, and QTcF duration were significantly different between the haemoglobin level groups. Differences in these electrocardiographical parameters between haemoglobin level groups are depicted in an illustrative ECG tracing of lead V5 in figure 1.

LINEAR REGRESSION ANALYSIS SERUM HAEMOGLOBIN LEVEL

In the univariate analysis, haemoglobin level was associated with body mass index (SC=+0.079, P=0.008), body temperature (SC=+0.097, P=0.002), systolic blood pressure (SC=+0.167, P<0.001) and diastolic blood pressure (SC=+0.162, P<0.001). Electrocardiographically, heart rate (SC=+0.175, P<0.001), PR-interval duration (SC=-0.060, P=0.045), QRS duration (SC=-0.095, P=0.002), JpTpc interval (SC=-0.083, P=0.005), maximum T-wave duration (SC=-0.094, P=0.002), and QTcF duration (SC=-0.131, P<0.001) were associated with haemoglobin level, as shown in table 3. Maximum T-wave duration was excluded from the multivariate analysis because of reversing in association with the univariate analysis to the multivariate analysis. Due to collinearity, selected variables were dropped from the backward linear multivariate regression model analysis based on a Variance Inflation Factor (VIF) higher than 3.0. The decision which parameter to exclude was based on R squared values; the combination of variables that yielded the largest R squared value without any parameter displaying a VIF of >3 was reported in the multivariate analysis. This concerned QRS duration, JpTpc interval, and QTcF duration. In the multivariate analysis, systolic blood pressure (SC=+0.084, P=0.026), diastolic blood pressure (SC=+0.100, P=0.007), heart rate (SC=+0.153, P<0.001), and QTcF duration (SC=-0.146, P<0.001) were significantly associated with haemoglobin level, as shown in table 3. The R-squared value of the multivariate analysis was 0.086.

QT SUBINTERVALS

Because we found an association between haemoglobin level and QTcF, a separate backward multivariate analysis to identify which subintervals of the QT-interval with haemoglobin level as dependent variable was performed. These subintervals were: QRS duration, J-point – T-peak interval, corrected for heart rate (JpTpc) and T-peak-T-end interval (TpTe). The correction of the JpTpc interval was performed using the formula proposed by Johannesen:[17]

$$\mathrm{JpTpc} = \frac{Jpoint - Tpeak\ interval}{RR\ Interval^{0.58}}$$

In the multivariate analysis of the QT subintervals, haemoglobin level showed the highest impact on the JpTpc interval duration (SC=-0.155, P<0.001), followed by the QRS duration (SC=-0.142, P<0.001) and the TpTe interval duration (SC=-0.061, P=0.051). The R-square of the second multivariate model was 0.029.

Discussion

In the present analysis of 1118 healthy young volunteers, haemoglobin level was found to be independently associated with incremental increases in heart rate and altered ventricular conductivity. These results indicate that haemoglobin-related cardiac changes can be observed in a surface ECG in a non-anaemic healthy, young male population.

Anaemia induces physiological cardiovascular compensations in both chronic and acute settings in order to maintain adequate oxygen delivery.[1,2,4,5] An acute decrease of haemoglobin in hypovolemic conditions, such as during blood donation, or in isovolumic haemodilution instances results in sympathetic activation.[4,5,21] This sympathetic activation is reflected in an increased blood pressure and increased heart rate variability and an increased in sympathovagal balance, but not with absolute increases in mean heart rate.[4,5,22] In chronic anaemic settings where haemoglobin levels decrease below 6.4 mmol/L (10.3 g/dL), higher cardiac output is predominantly achieved through increased stroke volume, and the increased heart rate contributes minimally.[6-8] However, in the present analysis heart rate was significantly lower in the lowest haemoglobin quartiles compared to the higher quartiles, and heart rate was positively associated with increased haemoglobin levels. These findings are in contrast with reported findings in an anaemic population, where acute and chronic low levels of haemoglobin marginally increase heart rates and suggest other factors may be at play in a non-anaemic population.

One explanation may be found in physiological cardiac remodelling in response to anaemia resulting in an increased stroke volume. For example, three studies with echocardiography in healthy adults and elderly with anaemia showed increased end-systolic and end-diastolic left ventricular diameters, with greater left ventricular mass.[6-8] Such anaemic echocardiographic adaptations may be reflected on ECG with changes in ventricular indices, such as an increased Sokolow-Lyon voltage[9] reflecting left ventricular hypertrophy which has been observed to occur in 25-66% of anaemic children and adults.[15,23] Conversely, collective decreases in QRS complex voltages[14,16,24] with no changes in heart axis[25] have been reported in both animal studies and in anaemic patients. Nonetheless, the present analysis did not find such an association between normal haemoglobin levels and ventricular depolarization indices such as the left ventricular Sokolow-Lyon voltages or changes in R-wave amplitude values in non-anaemic, healthy male volunteers.

An association between ventricular repolarization, reflected in the QTc interval, and haemoglobin level was also observed in the present analysis. This corroborates previous findings that showed that morphological changes in cardiac repolarization associated with haemoglobin are reflected on ECG in a prolonged QTc-interval,[11-14] reduced T-wave amplitudes[14-16] and an increased interval between the peak and the end of the T wave (TpTe).[12,13] Furthermore, in anaemic patients, both severe iron deficiency anaemia (haemoglobin <5.0 mmol/L/8.1 g/dL) and sickle cell disease, prolonged QTc duration was found in around 35-38% of patients.[13,23,26-29] These reports are in line with the present analysis, where a haemoglobin level within a normal range had an independent inverse relationship with QTcF interval duration.

To further improve characterization of the QTc interval, a QTc subinterval analysis was performed which allows a differentiation between early and late repolarization.[17] Especially since regional differences in static QT interval measurement on an ECG, caused by left ventricular hypertrophy and increased left ventricular size in hypertensive patients[30-32] may provide an indication of an underlying non-homogeneity of ventricular repolarization as anaemia is reported to predict prolonged QTc intervals in hypertensive patients[28] anaemic patients (78.97 ± 8.63 ms) compared to a control group (84.57 ± 10.13 ms).[13] Unfortunately, other subintervals such as QRS duration or JPTP-interval were not reported in their report. However, in the present analysis, no significant differences were found between the different non-anaemic haemoglobin level groups and the TpTe interval. Moreover, our QTc subinterval analysis suggest that the QTcF relationship with lower haemoglobin levels is mainly caused by QRS interval and JpTpc interval prolongation, and to a lesser extent TpTe interval. This is significantly different from pharmaceutical related blockage of the human ether-a-go-go related gene (hERG) potassium channel associated with the risk for torsade de pointes. Pharmaceutical hERG blockage has no effect on QRS duration but increases JpTpc interval and TpTe interval similarly.[17] Our findings might hypothetically be a result of the increased ventricular dimensional changes associated with anaemia, as previously mentioned.[6-8] A finding, which is of particular interest in early drug research, where pharmaceutical compounds predominantly impact late repolarization through hERG blockage.[17]

LIMITATIONS

The limitations of this study are the retrospective, cross-sectional design, and the usage of automatically calculated ECG data. The automated assessment has been validated but is nevertheless not considered the gold standard.[33] Moreover, the present retrospective study design with the lack of paired data limits the sensitivity of our analysis to find true associations between haemoglobin levels and the measured ECG parameters.

Conclusion

A lower haemoglobin level in non-anaemic, healthy male volunteers was independently associated with decreased heart rate and an altered ventricular conductivity, predominantly mediated through a prolongation of the early repolarization phase. These results indicate that haemoglobin-related cardiac changes can be observed in a surface ECG in a healthy, young population.

TABLE 1 Methods of determination or calculation of the electrocardiographic variables.

Variable	Description
Maximum P-wave duration (ms)	Longest P-wave duration sampled from all leads
P-wave balance in lead V1 (μV)	Difference between the upward and downward deflection of the P-wave
P-wave dispersion (ms)	Difference between the longest minus the shortest P-wave duration from all leads
Total P-wave area in lead V1 (mm×ms)	Sum of the total area under and above the isoelectric line from onset to termination of the P-wave
PR-interval (ms)	Beginning of the P-wave until the beginning of the QRS complex
QRS duration (ms)	Mean first deflection from the isoelectric line following the P-wave until the J-point
R-amplitude in lead I (μV)	Amplitude of the first upward deflection of the QRS complex (R-wave) in lead I
R-amplitude in lead aVL (μV)	Amplitude of the first upward deflection of the QRS complex (R-wave) in lead aVL
Heart axis (degrees)	Net vector of the R-wave axis based on the extremity leads.
Sokolow-Lyon voltage (mm)	Sum amplitude of the S-wave in lead V1 and the amplitude of the R-wave in lead V5 or V6 (whichever is larger)[29]
Cornell product (ms×mm)	Product of the QRS duration and the Cornell voltage[30] Cornell voltage is the sum of the amplitude of the R-wave in lead aVL and the amplitude of the S-wave in lead V3[30]
J-point - T-peak interval correct for heart rate (ms)	Duration of QRS complex offset to peak of the T-wave / RR-interval as measured in lead II to the power of 0.58 as proposed by Johannesen.[17] RR-interval is the interval between the onset of one QRS complex to the onset of the next QRS complex, measured in seconds, derived from the heart rate (HR) as 60/HR.
T-peak – T-wave interval (ms)	Duration of peak of the T-wave to end of the T-wave as measured in lead II.[17]
Maximum T-wave duration (ms)	Longest T-wave duration sampled from all leads
Minimum T-wave duration (ms)	Shortest T-wave duration sampled from all leads
T-wave dispersion (ms)	Difference between the longest and shortest T-wave duration selected from all leads
QTcF duration (ms)	QTcF duration is calculated using the Fridericia formula, which divides the QT-interval by the cube-root of RR-interval. QT-interval is the interval between the start of the Q-wave and the end of the T-wave.

mm = millimeters, ms = milliseconds, mV = millivolt, μV = microvolt.

TABLE 2 Relation between patient characteristics and electrocardiographic parameters to normal hemoglobin levels of included healthy male volunteers aged 18-30 years (n=1118). Categorical variables were compared using Chi-square test, variances were compared using the Analysis of Variance test with a post hoc Tukey analysis. Results are reported as as median (25%-75%), mean ± SD or as percentage. The symbols α, ß, γ, and δ represent a significant difference (P<0.05) compared to that group. If no symbols are present, no significance was found between the groups.

	Hemoglobin levels (mmol/L)			
	8.5 – 9.0 (n=246)	9.1 – 9.3 (n=241)	9.4 – 9.8 (n=391)	9.9-11.0 (n=240)
Corresponding groups	**α**	**ß**	**γ**	**δ**
Age (years)	23 (21 – 25)	22 (20 – 25)	22 (20 – 25)	22 (20 – 25)
Body mass index (kg/m²)	22.7	22.6	22.9	23.1
Temperature (°C)	36.6 ±0.37	36.6 ±0.37	36.6 ±0.35	36.7 ± 0.36
Systolic blood pressure (mmHg)	119.8 ± 9.7 $^{\beta\gamma\delta}$	121.5 ± 10.2 $^{\alpha\delta}$	122.3 ± 9.2 $^{\alpha}$	124.0 ± 9.7 $^{\alpha\beta}$
Diastolic blood pressure (mmHg)	67.3 ± 7.6 $^{\delta}$	68.4 ± 8.0 $^{\delta}$	68.8 ± 7.8 $^{\delta}$	71.1 ± 7.7 $^{\alpha\beta\gamma}$
Heart rate (beats/min)	60.5 ± 9.7 $^{\gamma\delta}$	62.1 ± 9.7 $^{\delta}$	63.0 ± 9.9 $^{\alpha\delta}$	65.4 ± 10.1 $^{\alpha\beta\gamma}$
Serum Sodium (mmol/L)	141.6 ± 1.7	141.9 ± 1.91	141.8 ± 1.8	141.8 ± 1.7
Serum Potassium (mmol/L)	4.36 ± 0.30	4.35 ± 0.29	4.34 ± 0.31	4.37 ± 0.33
Maximum P-wave Duration (ms)	102.1 ± 11.4	103.3 ± 10.6	102.0 ± 10.6	102.3 ± 11.0
P-wave balance in lead V1 (μV)	42.9 ± 38.2	41.6 ± 37.9	41.2 ± 43.4	41.0 ± 40.1
P-wave dispersion (ms)	56.5 ± 16.3	56.6 ± 12.3	56.4 ± 14.5	56.5 ± 14.7
Total P-wave area in lead V1 (mm×ms)	72.0 ± 74.7	65.90 ± 75.70	65.4 ± 82.7	67.2 ± 77.4
PR-interval (ms)	155.4 ± 23.5	151.1 ± 19.0	151.1 ± 19.8	151.5 ± 20.3
QRS duration (ms)	100.5 ± 8.9 $^{\delta}$	100.6 ± 7.7 $^{\delta}$	99.3 ± 8.6	98.0 ± 9.5 $^{\alpha\beta}$
R-amplitude lead I (μV)	659.0 ± 285.1	635.9 ± 276.4	640.0 ± 270.4	638.6 ± 278.0
R-amplitude lead aVL (μV)	269.7 ± 224.6	256.2 ± 227.0	256.1 ± 205.7	265.9 ± 205.4
Heart axis (degrees)	59.5 ± 27.0	59.5 ± 27.5	61.9 ± 24.7	59.7 ± 32.5
Sokolow-Lyon voltage (mm)	29.3 ± 7.9	29.0 ± 7.9	28.5 ± 7.7	28.7± 7..5
Cornell product (mm×μV)	13.4 ± 5.7	13.9 ± 6.5	13.5 ± 6.2	13.6 ± 5.9
J-point - T-peak duration (corrected for heart rate) (ms)	208.2 ± 17.7	210.3 ± 18.7 $^{\gamma}$	205.5 ± 17.2 $^{\beta}$	206.6 ± 17.8
T-peak – T-end duration (ms)	94.9 ± 8.4	94.0 ± 10.4	94.4 ± 10.2	93.7 ± 8.5
Maximum T-wave duration (ms)	178.0 ± 18.6	179.4 ± 17.0 $^{\gamma}$	175.0 ± 17.1 $^{\beta}$	175.8 ± 16.8
Minimum T-wave duration (ms)	107.2 ± 49.3	116.5 ± 51.8	106.6 ± 53.0	109.5 ± 48.5
T-wave dispersion (ms)	82.8 ± 32.7	76.5 ± 31.2	82.4 ± 33.3	82.3 ± 32.9
QTcF duration (ms)	403.5 ± 17.3 $^{\gamma\delta}$	405.4 ± 16.8 $^{\gamma\delta}$	399.8 ± 17.2 $^{\alpha\beta}$	399.5 ± 16.7 $^{\alpha\beta}$

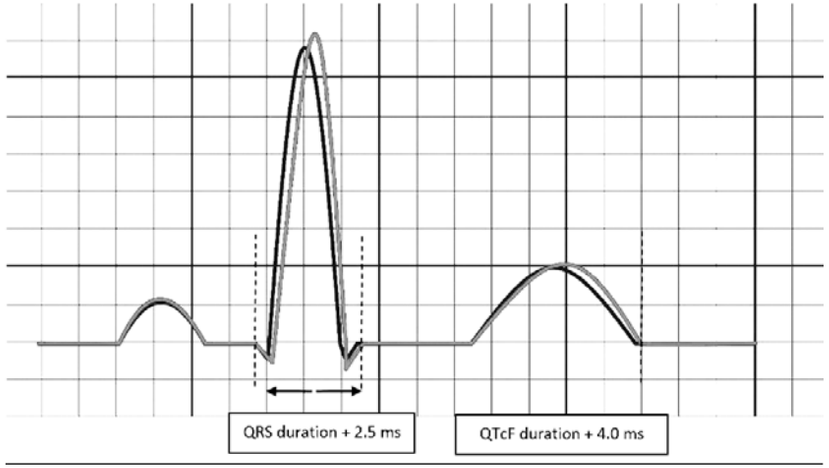
1 mmol/l = 1.6 g/dL, mm = millimeters, ms = milliseconds, μV = microvolt, mV = millivolt, QTcF=corrected QT interval with Fridericia's method.

TABLE 3 Univariate and backward linear multivariate regression model analysis. Probabilities of less than 0.10 in the linear univariate regression model were added to the backward linear multivariate regression model. Results are reported as unstandardized coefficient (USC) and standardized coefficient (SC) with the corresponding P-value and the R-square value in the linear univariate regression model. The R-square of the backward linear multivariate regression model was 0.086.

	Univariate analysis				Multivariate analysis		
Variable	**USC**	**SC**	***R*-square**	***P*-value**	**USC**	**SC**	***P*-value**
Age (per year)	0.007	-0.040	0.002	0.181			
Body mass index (kg/m²)	0.016	0.079	0.006	0.008	0.013	0.064	0.057
Temperature (°C)	0.134	0.097	0.009	0.002	Dropped		
Systolic blood pressure (mmHg)	0.009	0.167	0.028	<0.001	0.004	0.084	0.026
Diastolic blood pressure (mmHg)	0.011	0.162	0.026	<0.001	0.006	0.100	0.007
Heart rate (beats/min)	0.009	0.175	0.030	<0.001	0.008	0.153	<0.001
Serum Sodium (mmol/L)	0.005	0.017	<0.001	0.572			
Serum Potassium (mmol/L)	0.002	0.001	<0.001	0.964			
Maximum P-wave duration (ms)	0.000	0.006	<0.001	0.834			
P-wave balance in lead V1 (μV)	0.000	0.009	<0.001	0.754			
P-wave dispersion (ms)	0.000	0.012	0.001	0.692			
Total P-wave area in lead V1 (mm×ms)	1.88×10^{-5}	0.003	<0.001	0.922			
PR-interval (ms)	-0.001	-0.060	0.004	0.045	-0.002	-0.063	0.058
QRS duration (ms)	-0.005	-0.095	0.009	0.002	Dropped		
R-amplitude lead I (μV)	-3.51×10^{-5}	-0.019	<0.001	0.521			
R-amplitude lead aVL (μV)	2.56×10^{-6}	0.001	<0.001	0.971			
Heart axis (degrees)	0.000	-0.014	0.000	0.638			
Sokolow-Lyon voltage (mm)	-2.83×10^{-5}	-0.044	0.002	0.145			
Cornell product (mm)	1.12×10^{-8}	-0.001	<0.001	0.964			
J-point - T-peak duration (corrected for heart rate) (ms)	-0.002	-0.083	0.007	0.005	Excluded		
T-peak – T-end duration (ms)	-0.002	-0.039	0.002	0.188			
Maximum T-wave duration (ms)	-0.003	-0.094	0.009	0.002	Excluded		
Minimum T-wave duration (ms)	0.000	-0.023	0.001	0.434			
T-wave dispersion (ms)	0.000	0.011	<0.001	0.757			
QTcF (ms)	-0.004	-0.131	0.017	<0.001	-0.004	-0.146	<0.001

mm = millimeters, ms = milliseconds, μV = microvolt, mV = millivolt, QTcF=corrected QT interval with Fridericia's method.

FIGURE 1 Overview of significant (*) electrocardiographic changes between different hemoglobin levels in healthy male volunteers aged 18-30 years (n=1118) with a comparison between serum hemoglobin levels 8.5-9.0 mmol/L (grey line, 13.7-14.5 g/dL) and 9.9-11.0 mmol/L (black line, 16.0-17.7 g/dL). Results were based on Analysis of Variance (ANOVA) test between serum hemoglobin level groups and expressed as difference in the electrocardiographic parameter per serum hemoglobin level groups using a post hoc Tukey analysis.



mm = millimeters, ms = milliseconds, μV = microvolt, mV = millivolt, QTcF=corrected QT interval with Fridericia's method.

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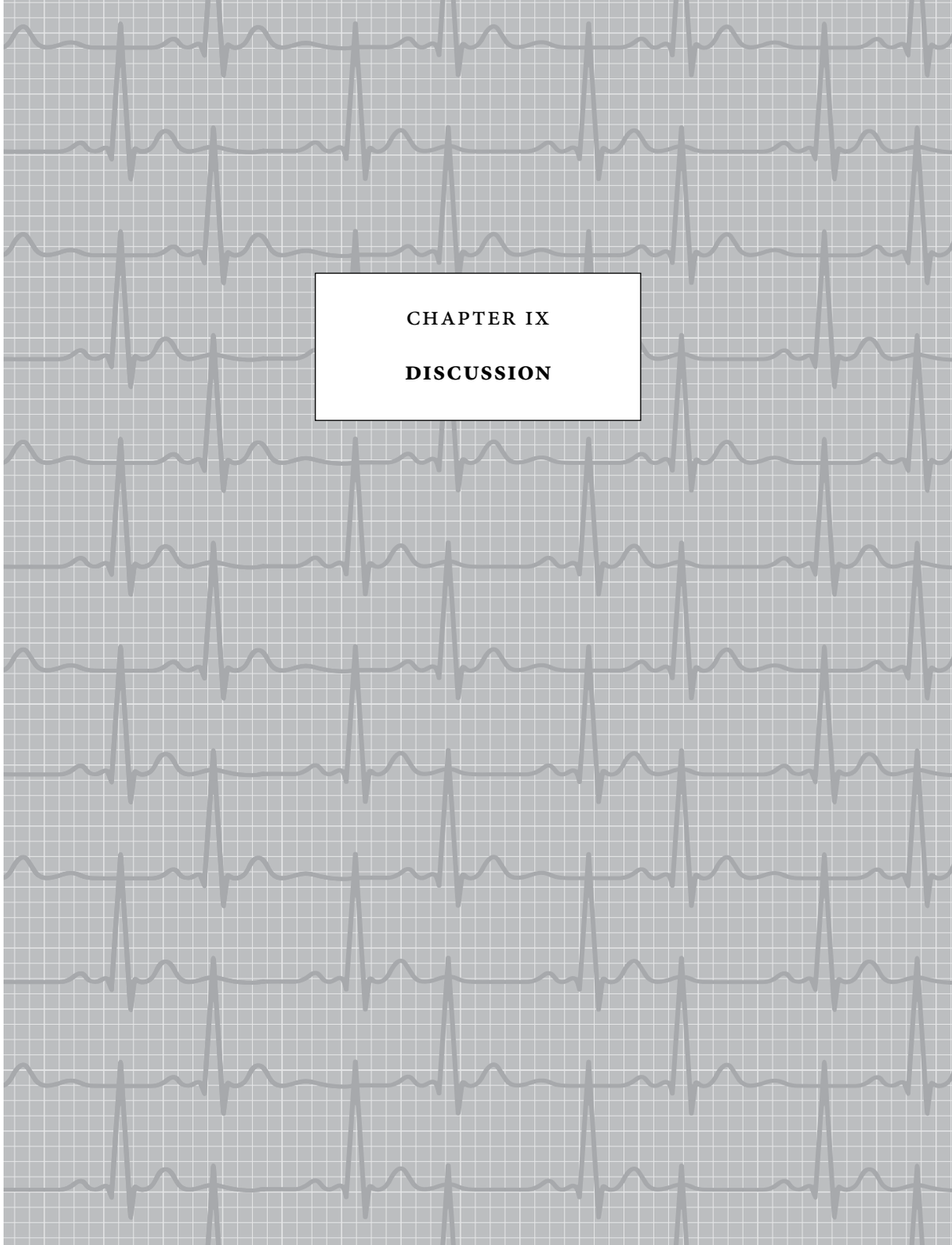

CHAPTER IX

DISCUSSION

Summary, general discussion and future perspectives

Early phase drug development trials aim to evaluate drug tolerance, pharmacokinetics and pharmacodynamics and explore drug metabolism and drug interactions.[1,2] However, each of these aims come with their respective limitations as addressed in the introduction of this dissertation. Therefore, the aim of this dissertation was to investigate tools and approaches that may help physicians to make informed decisions on the risk-benefit balance in these early phase drug development trials.

Foremost, **chapter 2** described our exploration of the feasibility of interpreting in a blinded and unblinded situation pharmacodynamic data in a simulated early phase trial. Overall, our results show that reviewers are capable to reliably detect effects exceeding twice the standard deviation, when reviewing data in a blinded manner, complicated by the normal variations within healthy individuals as described in later chapters. Unblinding data did offer some improvement, but effects smaller than 1 time the SD of between-measurement variability frequently remained unobserved and false positives became more common as a result of unblinding the reviewer. These findings suggest that reviewers should refrain from making firm statements about safety and efficacy of an increasing dose level.

Moreover, to be able to make better risk-benefit decision, knowledge about the general variations of commonly measured parameters in healthy individuals is essential. Electrocardiographic (ECG) data, and in particular QT interval evaluations, serve as a proxy for pharmacodynamic effects during early phase drug development studies for safety and/or desired pharmacodynamic effects. Yet, ECG data is highly heterogeneous and influenced by patient characteristics that may alter over time. Unfortunately, little is known on the 'normal' physiological variability. This makes ECG-derived parameters ideal for the subject of this thesis. Hence, in chapters 3-8 we highlight the various ECG changes associated with different patient characteristics, and if applicable to QT interval.

For a start, measurement of the QT interval and the QT dependency on RR interval is preferably corrected for with Friderica's formula and measured during a stable RR interval period for optimal accuracy. **Chapter 3** of this dissertation illustrated the limited impact of RR interval variations on heart rate-corrected QT-intervals in an early phase drug development trial. This may reflect that these volunteers were studies while hospitalized with relatively little physical activity. We found that (maximally) a two-minute resting interval following a short exercise is required to allow for accurate electrocardiographic parameter measurements. Meanwhile, heart rate normalization after assuming a supine position in a typical domiciled phase I clinical setting occurred instantaneously. This finding suggests that the current practice of an extended duration of supine positioning to normalize heart rate and QTcF interval could be reduced and open the opportunity to perform additional measurements without affecting the ECG data quality. Additionally, we concluded that RR interval correction of the QT-interval using Friderica's formula allowed reliable assessment of the drug effect on the duration of repolarization.

In line with these findings, we think it is conceivable that different evaluation of the QT interval could provide more information on this drug effect. One such alternative method is QT subinterval analysis, as proposed by Johannesen et al. This may allow for improved risk assessment and identify desired pharmacodynamic effects through characterization of drugs that may block specific electrolyte channels differently.[3] However, again surprisingly little is known to which extent the QTc intervals and its subintervals are influenced by patient characteristics. Therefore, this dissertation analysed various patient characteristics to which degree they influenced these QTc (sub)intervals.

Firstly, **chapter 4** aimed to investigate associations between body temperature and electrocardiographical parameters in normothermic healthy volunteers. Our analysis found that body temperature within normal ranges was independently associated with heart rate, P-wave axis, J-point amplitude in lead V4 and ventricular conductivity. Further, the QT subinterval analysis showed that the QT-interval dependence on temperature was primarily associated with prolongation of the ventricular polarization (QRS duration) and not by modulation of ventricular repolarization. Thus, the effects of body temperature on QTc interval are thereby substantially different from QTc interval prolongation induced by drug effects of hERG blockage. We suggest that body temperature related QTc interval prolongation is presumably mediated through reduced Na+ channel activation materializing as QRS duration prolongation on the surface ECG as has been suggested in existing literature. Importantly, this provides a tool to distinguish this QTc prolongation from hERG channel mediated QTc interval prolongation, and we propose to include QT subinterval analysis.

Another commonly investigated parameter for both pharmacodynamic safety and desired effect is blood pressure. In hypertensive patients, an abundance of ECG changes are reported. This includes evidence that QTc duration is

longer in hypertensive individuals compared to non-hypertensive individuals.[4] However, to which extent these ECG changes can be observed in normotensive individuals was unknown. In **chapter 5**, we aimed to characterize the association between blood pressure and selected ECG parameters associated in patients with hypertension in a healthy, young, normotensive, and elevated blood pressure population. We observed shorter QTc intervals with 10 mmHg incremental steps of systolic blood pressure, though no significant difference was found between the different groups. However, the QTc subinterval analysis showed that an increase in systolic blood pressures resulted in a significant prolongation of the QRS interval while shortening the repolarization intervals. This is again substantially different from hERG drugs blockage induced QTc interval prolongation. Accordingly, QTc subinterval analysis should be used to discriminate between direct blood pressure induced QT prolongation drug effects through QRS prolongation and hERG channel drug blockage effects with may result in repolarization prolongation.

Similarly to elevated blood pressure, obesity is known to cause several hemodynamic changes which may ultimately alter cardiac tissues that are identifiable on a 12-lead ECG. We investigated in **chapter 6** if these well-documented obesity-related ECG changes are also associated in subjects with body mass index (BMI) within a normal (18.5-25.0 kg/m^2) range. Altered atrial conduction, a leftward shift of the heart axis, and decreased Sokolow-Lyon voltage, though more discretely compared to obese individuals, were observed within this population. And in contrast to reported literature which reported increased QTc intervals in obese individuals, chapter 6 illustrated a lack of significant QTc interval changes in healthy adults with a normal BMI.[5-7] We hypothesize that obesity-related QTc interval changes may be a result of the accompanied metabolic syndrome and cardiac fat deposition than to hemodynamic changes associated with obesity.

Chapter 7 highlights the importance of QT interval correction with the help of Fridericia's formula in relation to possible QT interval prolongation associated with total bilirubin serum levels. Though QT prolongation has been reported to occur in 37-84% of cirrhotic individuals with either alcoholic or non-alcoholic liver disease, syndromically related increased bilirubin (Gilbert's syndrome) is associated with altered heart rates.[8,9] Chronotropic incompetence is a consistent finding in alcoholic as well as non-alcoholic cirrhosis, and refers to inability of the sinus node to increase heart rate or contractility after appropriate exercise or pharmacological stimulation.[9] Ultimately, altered activation of Ca^2+ homeostasis in cirrhotic liver patients results in disturbed several transmembrane plasma membrane ion channels such as potassium and Ca^2+ and have been shown to be dysfunctional both in cirrhotic subjects and cirrhotic animals.[9] Therefore, the uncorrected QT interval prolongation associated with increased total bilirubin levels as reported by Majeed et al. may have been caused solely by an association between the heart rate and total bilirubin levels.[10] Our own analysis in a pool of healthy individuals aged 18 to 30 years found a negative association between total bilirubin levels and QTcF interval, in contrast to the positive association found by Majeed et al.[10] Thus, within an early phase trial with health individuals, bilirubin serum levels may subtly mask QTcF prolongation and warrant caution.

Finally, **chapter 8** explores the relevance of serum haemoglobin levels in relation to ECG measurements as it is not uncommon in early phase research to draw relatively large amounts of up to 500 mL of blood within a short time frame.

Anaemia induced cardiac changes are reflected on a standard 12-lead ECG in ventricular indices, with increased Sokolow-Lyon voltages, [11] prolonged QTc-interval,[12-15] reduced T-wave amplitudes [15-17] and an increased interval between the peak and the end of the T wave (TpTe).[13,14] However, the effect of normal haemoglobin levels on the surface ECG in healthy volunteers participating in a clinical trial with blood draws accumulating to a relatively large amounts have been underreported. Our analysis showed that a lower haemoglobin level in non-anaemic, healthy male volunteers was independently associated with lower heart rate and an altered ventricular conductivity, predominantly mediated through a prolongation of the early repolarization phase. This is again significantly different from drug related blockage of the hERG channel blockage and thus underpins the importance of the QTc subinterval method.

In conclusion, this thesis described that careful and thus a more detailed QT analysis should be done to adequately interpret ventricular repolarization in small, early phase drug development studies. The presented findings suggest that the QT interval should be cautiously interpreted in these studies despite the strategies to improve the interpretation of the QT effect.

We suggest that our findings using the QT interval as a model readout, may also be applicable to other frequently used read-outs in early phase drug development studies.

FUTURE PERSPECTIVE

This thesis utilized QT interval measurements as a proxy for clinical biomarkers used in early phase drug development. As with these biomarkers, the ubiquitously used QTc interval analysis has its limitations in drug development and is further limited because of the subtle influence of patient characteristics. For several of these biomarkers, calculations can be performed to improve their accuracy. This is also the case with the QT interval: the QT subintervals analysis as proposed by Johannesen et al allows differentiation between early and late repolarization changes and may be helpful to unravel the contribution of the various ion channels which contribute to the action potential of the cardiomyocyte. The present dissertation illustrated the added value of these QT subinterval analysis by relating certain patient characteristics, some that may even change over time, to a subinterval segment in the QT interval that is not related to the I_{kr} channel induced QTc interval prolongation.

An important next step to take is to have innovative methods that incorporate patient characteristics and thereby have the potential to support the development of compound specific profiles which outline compounds' effect on the various cardiomyocytes' ion channels. For the QT interval, one method as proposed by the ICH E14 guideline is concentration QTc modelling, which incorporates all collected data across each dose level to characterize a compound's given influence on the QTc interval.[18] Such characterization provides an alternative to the conventional by-timepoint analysis or intersection–union test to classify the risk of QTc prolongation of a compound.[18]

Alternatively, one highly popularised novel method is the use of advanced algorithms or deep learning algorithms. Although not widely used in practice, for QT interval analysis, it is postulated that this allows more accurate identification of drug-induced QT-interval prolongation compared to conventional QTc measurement methods.[19] Interestingly, ECG subinterval analysis identified the J-Tpeak interval as the best discriminator of sotalol intake, a drug extensively used as a positive control in an assay-sensitivity study.[19] Evidently, additional research which further validates these findings with ideally an open-source publicly available algorithm perhaps even from the monitoring authorities such as the European Medicines Agency is warranted prior to any form of standardization. Perhaps even, in an ideal world, the stream of (anonymized) data collected at the later stages of drug development or even post-development marketed drugs could further refine the profiles through centralized deep learning algorithms.

In conclusion, this dissertation showed that physicians must be aware of the constraints that allow them to identify or obviate (un)desirable effects most notably if they evaluate these effects in a blinded matter. Unblinding might partially mitigate the limitation, but current measurement methods have gaps that we should remain aware of. Detailed measurements of subintervals with characterization of ion channel profiles, concentration QTc modelling, or machine learning might help physicians in their decision making in the future.

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CHAPTER X

NEDERLANDSE SAMENVATTING EN DISCUSSIE

Onderzoek naar geneesmiddelen in een vroege fase, de zogenoemde 'early phase drug development trials', hebben tot doel de tolerantie, farmacokinetiek en farmacodynamiek van geneesmiddelen te evalueren en het metabolisme en de interacties tussen geneesmiddelen te onderzoeken.[1,2] Maar elk van deze doelstellingen heeft zijn eigen beperkingen, zoals besproken in de inleiding van dit proefschrift. Daarom was het doel van dit proefschrift om instrumenten en benaderingen te onderzoeken die artsen kunnen helpen geïnformeerde beslissingen te nemen over de risico-batenbalans in deze vroege fase van geneesmiddelenontwikkeling.

Allereerst werd in **hoofdstuk 2** ons onderzoek naar de haalbaarheid van de interpretatie van geblindeerde en ongeblindeerde farmacodynamische gegevens in een gesimuleerde early phase drug development beschreven. Onze resultaten laten zien dat geblindeerde beoordelaars farmacologische effecten van meer dan tweemaal de standaarddeviatie (een maat voor de spreiding van een variabele) betrouwbaar te kunnen detecteren, maar worden bemoeilijkt door de normale variaties binnen gezonde individuen zoals beschreven in latere hoofdstukken. Het ontblinden van de data bood enige verbetering, maar effecten kleiner dan 1 maal de standaarddeviatie van de variabiliteit tussen metingen bleven vaak onopgemerkt en fout-positieven kwamen vaker voor als gevolg van het deblokkeren van de beoordelaar. Deze bevindingen suggereren dat beoordelaars geen harde uitspraken moeten doen over veiligheid en werkzaamheid van een nieuw medicijnen ten behoeve van het verhogen van de dosering.

Kennis over de algemene variaties van frequent gemeten parameters bij gezonde personen essentieel om betere risico-batenbeslissingen te kunnen nemen. Elektrocardiografische (ECG) gegevens, en in het bijzonder QT-interval evaluaties, dienen als een proxy voor farmacodynamische effecten tijdens early phase drug development voor veiligheid en/of gewenste farmacodynamische effecten. ECG-gegevens zijn echter zeer heterogeen en worden beïnvloed door patiëntkenmerken die in de loop van de tijd kunnen veranderen. Helaas is er weinig bekend over de 'normale' fysiologische variabiliteit. Dit maakt ECG-afgeleide parameters ideaal voor het onderwerp van dit proefschrift. Daarom belichten we in de hoofdstukken 3-8 de verschillende ECG-veranderingen die samenhangen met verschillende patiëntkenmerken, en indien van toepassing met het QT-interval.

Om te beginnen wordt de meting van het QT-interval en de QT-afhankelijkheid van het RR-interval in farmacologisch onderzoek bij voorkeur gecorrigeerd

met de formule van Friderica en gemeten tijdens een stabiele RR-intervalperiode voor optimale nauwkeurigheid. **Hoofdstuk 3** van dit proefschrift illustreerde de beperkte invloed van RR-interval schommelingen op de voor hartslag gecorrigeerde QT-intervallen in een early phase drug development omgeving. Dit kan een weerspiegeling zijn van het feit dat deze vrijwilligers werden onderzocht terwijl ze in het onderzoeksinstituut verbleven met relatief weinig fysieke activiteiten. Wij vonden dat (maximaal) een rustperiode van twee minuten na een korte inspanning nodig is voor nauwkeurige metingen van ECG-parameters. Bovendien normaliseerde de hartslag zich onmiddellijk na het aannemen van een liggende positie in deze typische onderzoeksetting. Deze bevinding suggereert dat de huidige praktijk van het aannemen van een liggende positie voor tot wel 10 minuten met als doel de hartslag en het QTcF-interval te normaliseren, kan worden verminderd en hierdoor de mogelijkheid ontstaat om aanvullende metingen te uit te voeren zonder de kwaliteit van de ECG-gegevens aan te tasten. Bovendien concludeerden wij dat een correctie van het QT-interval met behulp van de formule van Friderica een betrouwbare beoordeling mogelijk maakte van het effect van het geneesmiddel op de duur van de repolarisatie.

In het verlengde van deze bevindingen achten wij het denkbaar dat een andere evaluatie van het QT-interval meer informatie zou kunnen opleveren over geneesmiddeleffecten. Een dergelijke alternatieve methode is QT subinterval analyse, zoals voorgesteld door Johannesen et al. Deze methode kan een betere risicobeoordeling mogelijk maken en gewenste farmacodynamische effecten identificeren door karakterisering van geneesmiddelen die specifieke elektrolytische kanalen verschillend kunnen blokkeren.[3] Echter, opnieuw is verrassend weinig bekend in hoeverre de QTc intervallen en zijn subintervallen worden beïnvloed door patiëntkarakteristieken. Daarom werden in dit proefschrift verschillende patiëntkarakteristieken geanalyseerd en in welke mate zij deze QTc (sub)intervallen beïnvloedden.

Ten eerste werd in **hoofdstuk 4** het verband onderzocht tussen lichaamstemperatuur en elektrocardiografische parameters bij normotherme gezonde vrijwilligers. Uit onze analyse bleek dat normotherme lichaamstemperaturen onafhankelijk samenhingen met de hartslag, de as van de P-golf, de amplitude van het J-punt in afleiding V4 en de ventriculaire geleiding. Verder bleek uit de QT-subinterval analyse dat de QT-intervalafhankelijkheid van de temperatuur voornamelijk samenhing met verlenging van de ventriculaire polarisatie (QRS-duur) en niet met modulatie van de ventriculaire repolarisatie.

De effecten van lichaamstemperatuur op het QTc-interval zijn dus wezenlijk anders dan QTc-intervalverlenging geïnduceerd door medicamenteuze effecten door hERG-blokkade. Wij opperen dat de verlenging van het QTc-interval bij lichaamstemperatuur vermoedelijk gemedieerd wordt door verminderde activering van het Na+ kanaal, hetgeen zich manifesteert als verlenging van de QRS-duur op een ECG, zoals ook in de bestaande literatuur wordt gesuggereerd. Belangrijk is dat deze kennis een hulpmiddel biedt om deze QT-verlenging te onderscheiden van door het hERG-kanaal gemedieerde QTc-intervalverlenging, en wij stellen voor de QT-subinterval analyse op te nemen.

Een andere vaak onderzochte parameter voor zowel de farmacodynamische veiligheid als het gewenste effect is de bloeddruk. Bij hypertensieve patiënten wordt een overvloed aan ECG-veranderingen gemeld. Zo zijn er aanwijzingen dat de QTc-duur langer is bij hypertensieve personen in vergelijking met niet-hypertensieve personen.[4] In hoeverre deze ECG-veranderingen kunnen worden waargenomen bij normotensieve personen was echter onbekend. In **hoofdstuk 5** wilden wij de associatie tussen bloeddruk en geselecteerde ECG-parameters bij patiënten met hypertensie karakteriseren in een gezonde, jonge, normotensieve en verhoogde bloeddrukpopulatie. Wij observeerden kortere QTc-intervallen bij elke 10 mmHg oplopende stap van de systolische bloeddruk, hoewel er geen significant verschil werd gevonden tussen de verschillende groepen. Uit de QTc-subinterval analyse bleek echter dat een verhoging van de systolische bloeddruk resulteerde in een significante verlenging van het QRS-interval, terwijl de repolarisatie-intervallen werden verkort. Dit verschilt wederom wezenlijk van de door hERG-medicijnen geblokkeerde QTc-intervalverlenging. Derhalve moet QTc-subinterval analyse worden gebruikt om onderscheid te maken tussen directe door bloeddruk geïnduceerde QT-verlengingseffecten door QRS-verlenging en effecten van hERG-kanaalblokkade die kunnen leiden tot repolarisatieverlenging.

Net als verhoogde bloeddruk is van obesitas bekend dat het verschillende hemodynamische veranderingen veroorzaakt die uiteindelijk het hartweefsel kunnen veranderen dat herkenbaar is op een ECG. In **hoofdstuk 6** onderzochten wij of deze goed gedocumenteerde obesitasgerelateerde ECG-veranderingen ook voorkomen bij personen met een body mass index (BMI) binnen de normale grenzen (18,5-25,0 kg/m^2). Een veranderde atriale geleiding, een verschuiving van de hartas naar links, en een verminderde Sokolow-Lyon spanning werden in deze populatie waargenomen, zij het meer discreet in vergelijking met zwaarlijvige personen. En in tegenstelling tot literatuur die verhoogde QTc intervallen rapporteerde bij zwaarlijvige personen, toonde hoofdstuk 6 een gebrek aan significante QTc interval veranderingen bij gezonde volwassenen met een normale BMI.[5-7]

Wij veronderstellen dat de aan obesitas gerelateerde QTc interval veranderingen eerder het gevolg zijn van het aan obesitas geassocieerde metabool syndroom en cardiale vetafzetting dan aan de hemodynamische veranderingen geassocieerd met obesitas.

Hoofdstuk 7 belicht het belang van QT-intervalcorrectie met behulp van de Fridericia formule in het kader van mogelijke QT-intervalverlenging geassocieerd met totale bilirubineserumspiegels. Hoewel QT-verlenging voorkomt bij 37-84% van de cirrhotische personen met alcoholische of niet-alcoholische leverziekte, wordt syndromaal gerelateerde verhoogde bilirubine (syndroom van Gilbert) in verband gebracht met veranderde hartfrequenties.[8,9] Chronotropische incompetentie is een consistente bevinding bij zowel alcoholische als niet-alcoholische cirrose, en verwijst naar het onvermogen van de sinusknoop om de hartfrequentie of contractiliteit te verhogen na passende inspanning of farmacologische stimulatie.[9] Uiteindelijk resulteert een veranderde activering van de Ca^2+-homeostase bij cirrotische leverpatiënten in een verstoring van verschillende transmembrane plasmamembraan-ionenkanalen zoals kalium en Ca^2+ en is aangetoond dat deze zowel bij cirrotische personen als bij cirrotische dieren disfunctioneel zijn.[9] Daarom kan de ongecorrigeerde QT-intervalverlenging die gepaard gaat met een verhoogd totaal bilirubinegehalte, zoals gemeld door Majeed e.a., mogelijk enkel veroorzaakt zijn door een relatie tussen de hartslag en het totale bilirubinegehalte.[10] Onze eigen analyse in een pool van gezonde personen van 18 tot 30 jaar vond juist een negatief verband tussen het totale bilirubinegehalte en het QTcF-interval, in tegenstelling tot het positieve verband dat door Majeed et al.[10] werd gevonden. Derhalve kan bilirubine in een early phase drugonderzoek met gezonde personen de QTcF-verlenging op subtiele wijze maskeren en is voorzichtigheid geboden.

Ten slotte wordt in **hoofdstuk 8** de relevantie van het serum hemoglobinegehalte in relatie tot ECG-metingen onderzocht, aangezien het niet ongewoon is om in een early phase drugonderzoek relatief grote hoeveelheden tot wel 500 mL bloed af te nemen in een kort tijdsbestek.

Door bloedarmoede veroorzaakte cardiale veranderingen worden op een ECG weerspiegeld in ventriculaire indices, met verhoogde Sokolow-Lyon spanningen,[11] verlengd QTc-interval,[12-15] verlaagde T-golf amplitudes[15-17] en een verhoogd interval tussen de piek en het einde van de T-golf (TpTe).[13,14] Het

effect van een normaal hemoglobinegehalte op een ECG bij gezonde vrijwilligers die deelnemen aan een klinisch onderzoek met relatief grote bloedafnames is echter onderbelicht gebleven. Uit onze analyse bleek dat een lager hemoglobinegehalte bij niet-anemische, gezonde mannelijke vrijwilligers onafhankelijk geassocieerd was met een lagere hartslag en een gewijzigde ventriculaire geleidbaarheid, voornamelijk gemedieerd door een verlenging van de vroege repolarisatiefase. Echter, deze verandering in de vroege repolarisatiefase is opnieuw verschillend van een geneesmiddel gerelateerde blokkering van het hERG-kanaal en onderstreept dus het belang van de QTc-subinterval methode.

Concluderend wordt in dit proefschrift beschreven dat een nauwkeurige en derhalve gedetailleerdere QT-analyses moeten worden uitgevoerd om ventriculaire repolarisatie in early phase drug onderzoeken adequaat te interpreteren. De huidige bevindingen duiden erop dat het QT-interval in deze early phase drugstudies behoedzaam moet worden geïnterpreteerd, ondanks strategieën om de interpretatie van het QT-effect te verbeteren.

Wij stellen voor dat onze bevindingen, waarbij het QT-interval als model wordt gebruikt, ook van toepassing kunnen zijn op andere vaak gebruikte meetwaarden in early phase drug development studies.

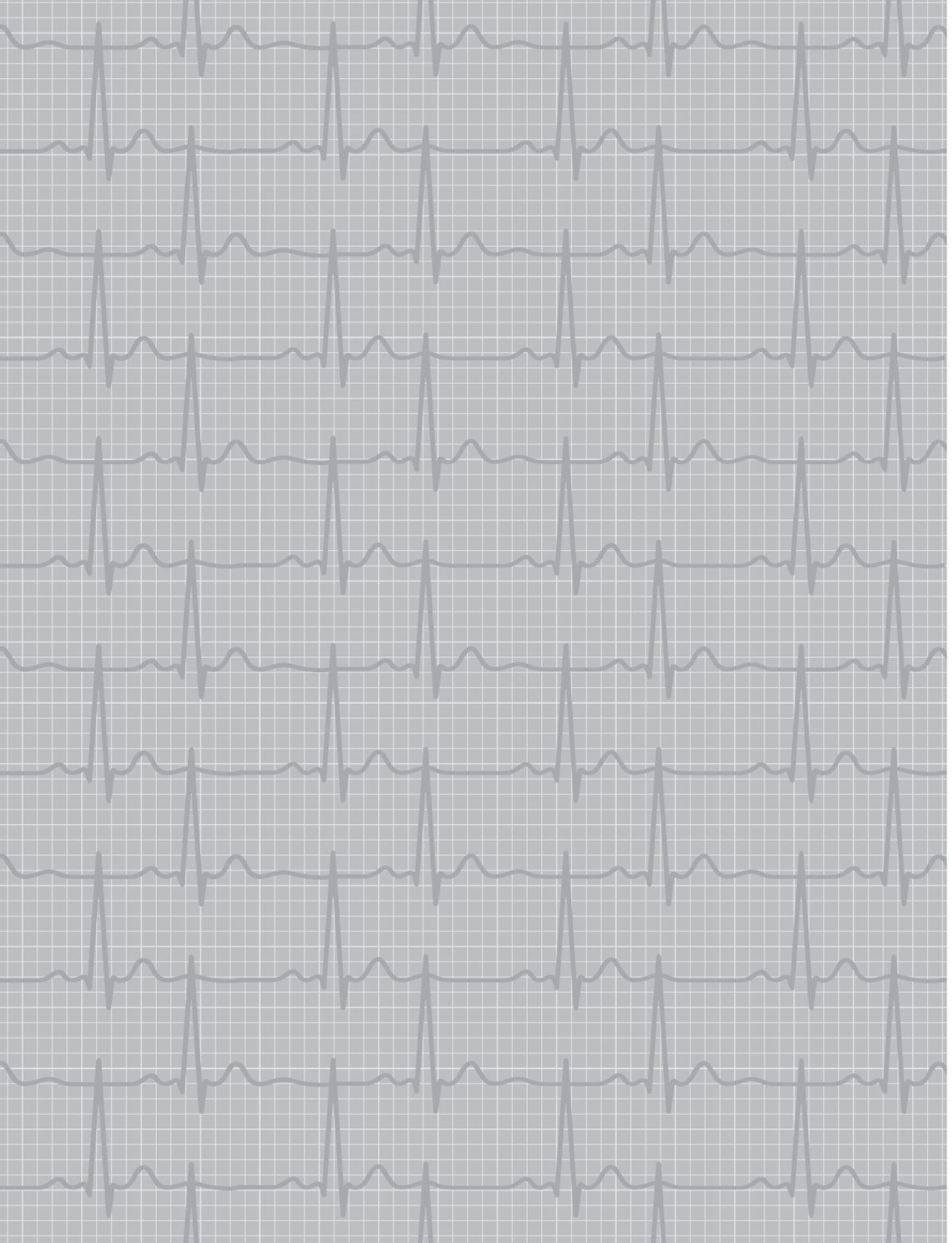
TOEKOMSTPERSPECTIEVEN

Dit proefschrift gebruikte QT-interval metingen als een proxy voor klinische biomarkers gebruikt in early phase drug development trials. Net als bij deze biomarkers heeft de QTc interval analyse haar beperkingen in de ontwikkeling van geneesmiddelen en zijn deze biomarkers daarnaast gehinderd door de subtiele invloed van patiëntkenmerken. Voor deze verscheidene biomarkers kunnen berekeningen worden uitgevoerd om de nauwkeurigheid ervan te verbeteren. Dit is ook het geval met het QT-interval: de QT-subinterval analyse zoals voorgesteld door Johannesen et al., maakt het mogelijk onderscheid te maken tussen vroege en late repolarisatie veranderingen. Deze methode zou dan ook kunnen worden gebruikt om de verschillende ionenkanalen die bijdragen tot de actiepotentiaal van de cardiomyocyt en de invloed van nieuwe medicijnen op deze ion-kanalen te ontrafelen. Het huidige proefschrift illustreerde de toegevoegde waarde van deze QT subinterval analyse door bepaalde patiëntkenmerken, waarvan sommige die in de loop van de tijd kunnen veranderen, aan een subinterval segment in het QT-interval dat niet verband houdt met de door het I_{kr}-kanaal geïnduceerde QTc-intervalverlenging te koppelen.

Een belangrijke volgende stap is te beschikken over innovatieve methoden die rekening houden met deze patiëntkenmerken in de QTc analyses. Deze methoden hebben de potentie om gedetailleerde profielen van nieuwe medicijnen te ontwikkelen waarin de effecten van het medicijn op de verschillende ionenkanalen van cardiomyocyten te verkennen. Voor het QT-interval is één methode voorgesteld in de ICH E14-richtlijn genaamd de concentratie QTc-modellering, die alle verzamelde gegevens over elk dosisniveau omvat om de invloed van een medicijn op het QTc-interval te karakteriseren.

Een andere zeer populaire nieuwe methode is het gebruik van geavanceerde algoritmen of deep learning-algoritmen. Hoewel dit in de praktijk niet veel wordt gebruikt, wordt verondersteld dat dit voor QT-intervalanalyse een nauwkeurigere identificatie van door geneesmiddelen veroorzaakte QT-intervalverlenging mogelijk maakt dan conventionele QTc-meetmethoden.[19] Het is duidelijk dat aanvullend onderzoek met idealiter een open-source publiek beschikbaar algoritme, misschien zelfs van de controlerende instanties zoals het Europees Geneesmiddelenbureau, gerechtvaardigd is voordat enige vorm van standaardisatie plaatsvindt. In een ideale wereld zou de stroom van (geanonimiseerde) gegevens die in latere stadia van de geneesmiddelenontwikkeling of zelfs voor op de markt gebrachte geneesmiddelen worden verzameld, zodat de profielen verder kunnen worden verfijnd met behulp van de gecentraliseerde deep learning-algoritmen.

Concluderend toonde dit proefschrift aan dat artsen zich bewust moeten zijn van de beperkingen waarmee zij (on)gewenste effecten kunnen identificeren of voorkomen, vooral als zij deze effecten geblindeerd evalueren. Ontblinden zou de beperking gedeeltelijk kunnen verzachten, maar de huidige meetmethoden vertonen lacunes waarvan we ons bewust moeten blijven. Gedetailleerde metingen van subintervallen met karakterisering van ion-kanaal profielen, concentratie QTc-modellering of machine learning zouden artsen in de toekomst kunnen helpen bij hun besluitvorming.



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CURRICULUM VITAE

Gert-Jan Hassing was born July 25th, 1991 in Amsterdam, the Netherlands. After completing school (atheneum level) in 2010, he attended medical school at Leiden University Medical Center in Leiden, The Netherlands. From 2011 he was employed as a research assistant at the Centre for Human Drug Research, Leiden, The Netherlands, where he assisted in the clinical research unit. After completion of medical school in November 2016, he enrolled in the Dentistry program at the Catholic University of Leuven in September 2017, with the aim to become a maxillofacial surgeon. In the beginning of 2017 he was employed as a screening physician at the Centre for Human Drug Research, during which period he started his PhD project.

Following his Dentistry graduation on 30th of June 2021, Gert-Jan started with his maxillofacial residency on the 1st of July at the University Medical Center of Utrecht under supervision of Professor Rosenberg.

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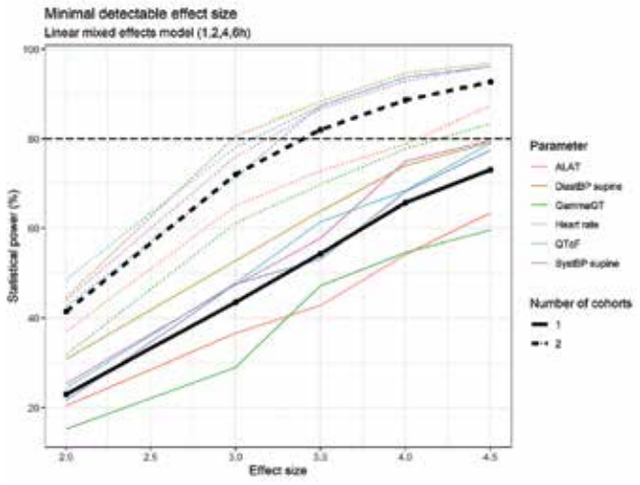
PAGE 33 ➢ CHAPTER 2 FIGURE 5 **Statistical power for each parameter with an effect size between 2.0 and 4.5 standard deviation effects as calculated with a linear mixed effect model for both single or double drug cohort simulations.** Dotted horizontal line highlights the statistical power to detect a minimal detectable effect size with 80% certainty.



PAGE 49 ➢ CHAPTER 3 FIGURE 3 **Electrocardiographic parameters (PR interval in A, RR interval in B, QT interval in C and QTcF interval in D) as measured in the male exercise bout group where each timepoint indicates the change from baseline (first 10 minutes) means with standard deviation over intervals of 30 seconds.** Dotted square indicates the exercise period. A. PR interval significantly (p<0.05) shorter for up to 120 seconds but never crossed the threshold of 25% change from baseline. B) RR interval significantly (p<0.05) shorter for up to 30 seconds and change from baseline larger than 20 ms for up to 60 seconds. C) QT interval significantly (p<0.05) shorter for up to 90 seconds and stabilized after 120 seconds but remained prolonged compared to baseline. D) QTcF interval significantly (p<0.05) shorter for up to 120 seconds and remained stable afterwards within baseline values.