ANALYSIS OF SHORT-TERM TEMPORAL VARIABILITY OF CARDIAC VENTRICULAR REPOLARIZATION IN SUBJECTS WITH DIFFERENT CLINICAL CONDITIONS

Andrea Orosz MD

PhD Thesis

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Relevant Publications

I. **Orosz A**, Csajbók É, Czékus Cs, Gavallér H, Magony S, Valkusz Zs, Várkonyi TT, Nemes A, Baczkó I, Forster T, Wittmann T, Papp JGy, Varró A, Lengyel Cs: Increased Short-Term Beat-To-Beat Variability of QT Interval in Patients with Acromegaly. *PLoS One.* 2015;10: (4) p. e0125639.
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II. Lengyel Cs, **Orosz A**, Hegyi P, Komka Zs, Udvardy A, Bosnyák E, Trájer E, Pavlik G, Tóth M, Wittmann T, Papp JGy, Varró A, Baczkó I: Increased short-term variability of the QT interval in professional soccer players: possible implications for arrhythmia prediction. *PLoS One.* 2011;6: (4) Paper e18751. 10 p.
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III. **Orosz A**, Baczkó I, Nagy V, Gavallér H, Csanády M, Forster T, Papp JGy, Varró A, Lengyel Cs, Sepp R: Short-term beat-to-beat variability of the QT interval is increased and correlates with parameters of left ventricular hypertrophy in patients with hypertrophic cardiomyopathy. *Can J Physiol Pharmacol.* 2014; Accepted for publication, doi: 10.1139/cjpp-2014-0526.
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List of Other Publications

I. Nemes A, Piros GÁ, Domsik P, Kalapos A, Lengyel Cs, **Orosz A**, Forster T: Correlations between three-dimensional speckle-tracking echocardiography-derived left atrial functional parameters and aortic stiffness in healthy subjects - Results from the MAGYAR-Healthy Study. *Acta Physiol Hung.* 2015;102: (2) pp. 197-205.
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II. Piros GÁ, Domsik P, Kalapos A, Lengyel Cs, **Orosz A**, Forster T, Nemes A: A jobb pitvar és bal kamra méretének és funkciójának összefüggései egészségesekben. Eredmények a háromdimenziós speckle-tracking echokardiográfiás MAGYAR-Healthy Tanulmányból. *Orv Hetil.* 2015;156(24): 972-8.

III. Domsik P, Kalapos A, Lengyel Cs, **Orosz A**, Forster T, Nemes A: A mitralis anulus és a bal pitvar funkciója közötti összefüggések vizsgálata háromdimenziós speckle-tracking echokardiográfia segítségével egészséges önkéntesekben. Eredmények a MAGYAR-Healthy Tanulmányból. *Orv Hetil.* 2014;155: (38) pp. 1517-1523.

IV. Baczkó I, **Orosz A**, Lengyel Cs: A kamrai repolarizációs rezerv zavarai és hirtelen szívhalál élsportolókban. In: Lengyel Cs, Márton J, Török L (szerk.). Sportorvosi alapismeretek. Szeged: SZTE Általános Orvostudományi Kar, 2014. pp. 26-45. (ISBN:978-963-306-345-3).

V. Nemes A, Domsik P, Kalapos A, Lengyel Cs, **Orosz A**, Forster T: Comparison of Three-Dimensional Speckle Tracking Echocardiography and Two-Dimensional Echocardiography for Evaluation of Left Atrial Size and Function in Healthy Volunteers (Results from the MAGYAR-Healthy Study). *Echocardiography.* 2014;31(7): 865-71.
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VI. Nemes A, Kalapos A, Domsik P, Lengyel Cs, **Orosz A**, Forster T: Correlations between echocardiographic aortic elastic properties and left ventricular rotation and twist – Insights from the three-dimensional speckle-tracking echocardiographic MAGYAR-Healthy Study. *Clin Physiol Funct Imaging.* 2013;33(5): 381-5.
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VII. Baczkó I, **Orosz A**, Lengyel Cs, Varró A: Athletic Heart: The Possible Role of Impaired Repolarization Reserve in Development of Sudden Cardiac Death. In: Bastos JH, Silva AC (szerk.). Athlete Performance and Injuries. 197 p. Hauppauge: Nova Science Publishers, 2012. pp. 123-143. Sports and Athletics Preparation, Performance, and Psychology. (ISBN:9781619426580).

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Table of Contents

Abbreviations

APD: action potential duration

AN: autonomic neuropathy

AV: atrioventricular

BMI: body mass index

BP: blood pressure

cAMP: cyclic adenosine monophosphate

DAD: delayed afterdepolarization

EAD: early afterdepolarization

ECG: electrocardiogram

EF: ejection fraction

ERP: effective refractory period

ES: extrasystole

HbA1c: glycosylated hemoglobin

HCM: hypertrophic cardiomyopathy

hERG: human ether-à-go-go-related gene

hGH: human growth hormone

HR: heart rate

ICD: implantable cardioverter defibrillator

I_f: pacemaker current

IGF-1: insulin-like growth factor-1

I_{K1}: inward rectifier potassium current

I_{Kr}: rapid component of the delayed rectifier potassium current

I_{Ks}: slow component of the delayed rectifier potassium current

$I_{Na,late}$: late sodium current

I_{to}: transient outward potassium current

IVS: interventricular septum thickness

LA: left atrial diameter

LGE: late gadolinium enhancement

LV: left ventricule

LVEDD: left ventricular end-diastolic diameter

LVESD: left ventricular end-systolic diameter

LVM: left ventricular mass

LVmax: maximal left ventricular wall thickness

LVmax BSA: maximal left ventricular wall thickness normalized for body surface area

LVM BSA: left ventricular mass normalized for body surface area

LVOT: resting left ventricular outflow tract

MRI: magnetic resonance imaging

NYHA: New York Heart Association

NSVT: non-sustained ventricular tachycardia

OGTT: oral glucose tolerance test

PW: posterior wall thickness

QTc: frequency corrected QT interval

QTd: QT dispersion

QTVI: normalized QT variability index

QTVN: normalized QT variability

SCD: sudden cardiac death

SD: standard deviation

SEM: standard error of mean

SR: sinus rhythm

STV_{QT}: short-term beat-to-beat temporal variability of the QT interval

STV_{RR}: short-term beat-to-beat temporal variability of the RR interval

TdP: Torsades de Pointes

Tpeak-Tend: duration of the T wave from the peak to the end

ULN: upper limit of normal value

1. Introduction

Myocardial hypertrophy has been shown to cause electrophysiological remodeling where the expression of different ion channels is altered. Myocardial hypertrophy in pathological settings in humans [Janse 2004; Li et al. 2004; Nattel et al. 2007] and in animal models, especially in the chronic atrioventricular (AV) block dog model [Vos et al. 1998] and heart failure models [Nuss et al. 1999; Li et al. 2002; Rose et al. 2005], has been shown to cause electrophysiological remodeling where the expression of different ion channels, including potassium channels critical for repolarization (i.e. I_{Ks}, I_{Kr} and I_{K1}), is downregulated. These changes have been associated with increased incidence of serious ventricular arrhythmias probably due to decreased repolarization reserve [Vos et al. 1998; Nuss et al. 1999; Volders et al. 1999; Li et al. 2002; Rose et al. 2005;]. It is conceivable that prolonged repolarization and a possibly impaired repolarization reserve might represent increased risk for the development of ventricular arrhythmias, including Torsades de Pointes ventricular tachycardia (TdP) that can degenerate into ventricular fibrillation and lead to sudden cardiac death [Biliczki et al. 2002; Lengyel et al. 2004].

The identification of patients at risk for serious ventricular arrhythmia and sudden cardiac death is critically important. Current techniques for the reliable prediction of TdP and other, potentially fatal ventricular arrhythmias remain unsatisfactory. Electrocardiographic (ECG) parameters have been studied for their utility as prognostic non-invasive markers in sudden cardiac death (SCD) risk assessment. The prolongation of the frequency corrected QT interval (QTc) and increased QTc dispersion (characterizing spatial repolarization heterogeneity) were observed in patients with hypertrophic cardiomyopathy (HCM) [Buja et al. 1993; Dritsas et al. 1992; Yi et al. 1998]. However, QTc prolongation and QTc dispersion have been found not to be predictive for SCD in HCM patients [Yi et al. 1998; Maron et al. 2001]. Moreover, QT interval prolongation alone cannot reliably predict the development of ventricular arrhythmias including the chaotic ventricular tachycardia, Torsades de Pointes (TdP), since cardiac repolarization reserve may be reduced even without significant changes in the duration of cardiac repolarization [Varró et al. 2011].

The Tpeak-Tend interval, another ECG parameter representing spatial (including transmural) dispersion of repolarization [Antzelevitch 2001], has been shown to more reliably predict the development of Torsades de Pointes (TdP) polymorphic ventricular tachycardia in congenital [Schwartz et al. 2001] and acquired long QT syndromes than QTc prolongation or increased QT dispersion [Yamaguchi et al. 2003].

Based on recent evidence, in addition to the prolonged QTc or Tpeak-Tend intervals, the short-term variability of the duration of repolarization (STV_{QT}) [Berger et al. 1997] might be a better parameter to predict serious ventricular arrhythmias and sudden cardiac death, as it has been suggested by both animal experimental work [Thomsen et al. 2004; Thomsen et al. 2005; Lengyel et al. 2007; Hanton et al. 2008] and recent clinical studies [Hinterseer et al. 2008; Hinterseer et al. 2009; Hinterseer et al. 2010; Oosterhof et al. 2011]. On the basis of these observations, Varkevisser et al. [Varkevisser et al. 2012] suggested that beat-to-beat STV_{QT} could be superior to QT interval prolongation in identifying patient populations at risk for ventricular arrhythmias and might be able to accurately predict individual risk.

Physical conditioning in *competitive athletes* induces cardiovascular adaptation including lower resting heart rate (increased vagal tone) and increased cardiac mass (hypertrophy) and volume as a consequence of increased demand on the cardiovascular system, called „athlete's heart" [Atchley et al. 2007]. Echocardiography studies show that myocardial hypertrophy develops following long-term sports activities [Scharhag et al. 2002; Atchley et al. 2007; Paolo et al. 2007]. Sudden death among young athletes is rare (1:50 000–1:100 000), however, it is still 2–4 times more frequent than in age-matched controls [Corrado et al. 2007]. Numerous congenital and acquired cardiac diseases have been identified as causes of SCD in athletes [Pigozzi and Rizzo 2008]; however, in 5-10 % of SCD cases no structural abnormalities are detected in the heart during autopsy [Maron et al. 1980; Maron et al. 1996], the exact mechanism of SCD in these cases is not established and is mostly attributed to ventricular fibrillation.

Hypertrophic cardiomyopathy (HCM) is a common inherited cardiac disease with a prevalence of one in 500 in the general population [Maron et al. 1995], characterized by marked but variable left ventricular hypertrophy and myocardial fibrosis [Gersh et al. 2011]. HCM is associated with lethal ventricular arrhythmias [Wigle et al. 1995; Maron 2002; Gersh et al. 2011], and it is the most common cause of SCD in young individuals [Decker et al. 2009; Maron 2010] and in competitive athletes younger than 35 years [Maron et al. 2009].

The reliable assessment of SCD risk in individual HCM patients and the identification of patients for implantable cardioverter defibrillator (ICD) implantation, the most effective intervention for SCD prevention, are critically important. Currently models for SCD risk stratification uses non-invasive conventional clinical markers and all of them exhibit a low positive predictive value and the current SCD risk assessment algorithm in HCM is still

considered incomplete and hampered by lack of sufficient evidence for all elements [McKeown and Muir 2013]. This notion is supported by SCD events in HCM patients who were not considered to be at high risk for SCD [Maron et al. 2008].

Acromegaly, caused by pituitary tumors, is well-known to be associated with cardiovascular complications, such as hypertension, left ventricular hypertrophy, asymmetric septal hypertrophy, cardiomyopathy, and congestive heart failure [Melmed 2006]. Excessive secretion of growth hormone and insulin-like growth factor 1 (IGF-1) can result in major structural and functional changes in cardiac system and arrhythmias [Melmed 2006], and a specific cardiomyopathy develops in acromegaly associated with life-threatening dysrhythmias [Clayton 2003]. Moreover, acromegaly can also be associated with cardiovascular diseases contributing to increased mortality among patients [Clayton 2003; Melmed 2006]. Dysrhythmias, atrioventricular conduction delay and sick sinus syndrome were reported in sudden death in acromegalic heart disease.

2. Aims

The aims of this study were:

2.1. to compare conventional ECG parameters as well as the short-term beat-to-beat temporal variability of the RR and QT intervals of professional soccer players to age-matched controls who do not participate in competitive sports;

2.2. to compare conventional ECG parameters of repolarization and STV_{QT} in HCM patients and age-matched healthy volunteers;

2.3. to determine beat-to-beat QT variability in patients with acromegaly.

3. Methods

3.1. Patient population (general considerations)

The study population consisted of male professional soccer players from the Hungarian Premier League, patients with hypertrophic cardiomyopathy (HCM) and patients with acromegaly. Age- and sex-matched healthy volunteers, who did not participate in sports activities, were eligible for this study as control subjects. Professional soccer players, patients, or age-matched controls were excluded if they had excessive (>5%) ectopic atrial or ventricular beats, were in a rhythm other than normal sinus, had repolarization abnormalities (i.e. early repolarization pattern, T wave inversion and complete left bundle branch block or right bundle branch block), had a permanent pacemaker or any other disorders such as serious

retinopathy, symptomatic cardiac and/or pulmonary disease, acute metabolic disease, had excessive noise on the electrocardiographic signal that precluded analysis of the ECG waveform, were on any medication likely to affect the investigated ECG parameters or consumed significant amount of food within 3 hours or drank alcohol, coffee or smoked within 10 hours. All of the control individuals, soccer players and patients were of European descent.

3.2. Ethics Statement

The studies described here were carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and were approved by the Scientific and Research Ethical Committee of the Medical Scientific Board at the Hungarian Ministry of Health (ETT-TUKEB), under ethical approval No. 4987-0/2010-1018EKU (338/PI/010). All subjects have given written informed consent of the study.

3.3. Electrocardiography

Five-minute 12-lead electrocardiograms were recorded from patients with HCM or acromegaly and age-matched healthy human volunteers at rest, in the supine position to obtain signals with the least amount of motion artefact. In athletes, baseline ECG recordings were taken before a competitive soccer game (Hungarian Premier League) and also approximately 20 minutes after the end of the game in the dressing room. In all leads the ECG signals were digitized at 2000 Hz sampling rate with a multichannel data acquisition system (Cardiosys A01 software, Experimetria Ltd., Budapest, Hungary; MDE Heidelberg GMBH, Heidelberg, Germany) connected to a personal computer and stored for later off-line analysis.

The RR, QT intervals and duration of the T wave from the peak to the end (Tpeak-Tend) intervals were measured using automated algorithms as the average of consecutive 30 beats (minimum number of intervals needed for variability measurements), were checked by the investigator and manually corrected if needed and were calculated as the average of 30 beats. Out of the repolarization parameters we analyzed the QT dispersion (QTd), the frequency corrected QT intervals (QTc) performed by the Bazett's ($QTc = QT / \sqrt{RR}$), Fridericia ($QTc = QT / [RR/1000]1/3$), Framingham ($QTc = QT + [0.154 * \{1000-RR\}]$) and the Hodges formulas ($QTc = QT + 1.75 * [60\ 000 / RR-60]$). The QTc interval duration was defined as the mean duration of all QTc intervals measured. As no statistical difference was noted between different correction methods with regard to corrected QT interval, QTc corrected with the Bazett's formula was used for further comparisons. The PQ and QRS

intervals were measured as the average of 15 consecutive beats. All measurements were carried out using lead II and in case of excessive noise lead II, lead V5.

The calculation of the short-term beat-to-beat variability of repolarization was chosen since it is a relatively simple method that has been suggested as a future screening tool; moreover it has been shown in animal studies [Thomsen et al. 2004; Lengyel et al. 2007] and in certain patient populations [Hinterseer et al. 2009; Hinterseer et al. 2010] to reliably predict increased arrhythmia propensity.

Using 30 consecutive beats, RR and QT intervals were plotted against their respective previous interval and Poincaré plots were constructed as described previously [Volders et al. 1999]. The instability of beat-to-beat heart rate and repolarization were characterized by the short-term variability (STV) of the RR and QT intervals, and were calculated using the following formula: $STV = \sum|D_{n+1}-D_n| / (30 * \sqrt{2})$, where D is the duration of the RR or QT intervals. This calculation defines the STV as the mean distance of points perpendicular to the line of identity in the Poincaré plot and relies on previous mathematical analysis [Brennan et al. 2001].

3.4. Echocardiography

3.4.1. Professional soccer players

Echocardiographic measurements were performed at rest on 23 professional soccer players and 23 age-matched controls using a Dornier AI 4800 (Germany) echocardiograph with a 2.5 MHz transducer. Two-dimensionally guided M-mode recordings were obtained parasternally in accordance with the recommendations of the American Society of Echocardiography [Sahn et al. 1978]. Measurements were carried out as described previously [Pavlik et al. 1996]. For purely logistic reasons, not all control individuals and soccer players were subjected to echocardiography.

3.4.2. Patients with HCM and acromegalic patients

All HCM and acromegalic patients and all controls underwent transthoracic echocardiographic examination performed by the single observer blinded to subject data for all participants. Two-dimensional echocardiographic images were obtained by a commercially available Toshiba Powervision 8000 echocardiography equipment, in a number of cross-sectional planes using standard imaging positions to determine standard morphological and functional parameters [left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), ejection fraction (EF), left atrial diameter (LA), resting left

ventricular outflow tract (LVOT) peak gradient]. Maximal left ventricular wall thickness (LVmax) was defined as the largest wall thickness of the left ventricle at any left ventricular segment. LVmax was also normalized for body surface area (LVmax BSA).

3.5. Cardiac MRI in HCM patients

In all HCM patients, cardiac magnetic resonance imaging (MRI) was carried out to determine the left ventricular mass (LVM). MRI assessments were performed in supine position with the head first on a commercially available 1.5T scanner (Signa Excite HDxT, GE Medical Systems) using a phased-array body coil. Sequential gradient-echo short-axis cine images (base to apex; slice thickness: 8 mm; field of view: 43 mm; matrix: 224 * 224; repetition time: 100 milliseconds) covering the entire left ventricule (LV) were acquired during breath hold after normal expiration. Three long-axis images (2-, 3- and 4-chamber views) were also acquired. The acquisition was triggered by ECG. The gradient-echo short-axis images were used to measure LVM by planimetry of the manually defined endocardial and epicardial borders on each short-axis image covering the entire LV. The measurement was performed in both end diastole and end systole to enable calculation of LV ejection fraction. Papillary muscles were not included in the LVM. LVM was also normalized for body surface area (LVM BSA).

3.6. Autonomic function and laboratory assessment in patients with acromegaly

Autonomic function was assessed by means of five standard cardiovascular reflex tests: the heart rate (HR) responses to deep breathing and to standing up (30/15 ratio), the Valsalva maneuver, the systolic blood pressure response to standing up, and the diastolic pressure change during a sustained handgrip. A score was created to express the severity of autonomic neuropathy (AN), based on the results of the five tests (normal: 0, borderline: 1, abnormal: 2). The total score was in the interval of 0 to 10.

Fasting venous blood samples were obtained from each patient and controls for the determination of serum glucose, blood urea nitrogen, creatinine, sodium and potassium levels. Human growth hormone (hGH) and IGF-1 were measured by chemiluminescent immunoassay (IMMULITE 1000 Immunoassay System, Siemens. hGH measurement comparator: Recombinant 98/574; detection limit: 0.01 ng/ml; intra-assay coefficients of variation: 6.0 %; interassay coefficients of variation: 6.2 %. IGF-1 measurement comparator: WHO IRP 87/517; detection limit: 20.0 ng/ml; intra-assay coefficients of variation: 5.0 %; interassay coefficients of variation: 9.0 %).

3.7. Statistics

3.7.1. Professional soccer players

Body weight, body mass index (BMI), age and ECG interval data are expressed as means ± standard error of the mean (SEM). Comparisons between controls and soccer players were made using the unpaired Student's *t*-test. ECG parameters of athletes before and after the game were compared by one-way analysis of variance (ANOVA) followed by a paired *t*-test. A p value of < 0.05 was considered significantly different. Statistical analyses were performed using Statistica for Windows (version 9).

3.7.2. Patients with HCM

All data are expressed as mean ± standard deviation (SD). Comparisons between HCM patients and controls for the study variables were done using the independent samples Student's *t*-test for normally distributed parameters. Normal distribution was verified by the Kolmogorov-Smirnov test. Degree of association between two variables was expressed by the Pearson correlation coefficient (r). The statistical analyses were performed using the MedCalc software package (ver. 14.12.0). Statistical significance was accepted at the $p < 0.05$ level.

3.7.3. Acromegalic patients

All data are expressed as mean ± SD. Comparisons between acromegalic patients and controls for the study variables were done using the unpaired Student's *t*-test for normally distributed parameters, nonparametric Mann-Whitney U test for non-normal distributions, and linear regression for revealing correlations. The statistical analyses were performed using the SPSS 16.0 software package. Statistical significance was accepted at the $p < 0.05$ level.

4. Results

4.1. Short-term variability of the QT interval in professional soccer players

4.1.1. Study population

The study population consisted of 76 male professional soccer players from the Hungarian Premier League (age 16 to 39, mean 22.0 ± 0.61 years; weight 76.2 ± 0.95 kg, BMI 23.2 ± 0.18 kg/m^2), and 76 male, age-matched healthy control sedentary subjects who did not participate in sports activities (age 15 to 39, mean 22.0 ± 0.54 years; weight 77 ± 1.7 kg, BMI 23.3 ± 0.48 kg/m^2).

4.1.2. Echocardiographic measurements in study subjects

Professional soccer players exhibited significantly higher values in interventricular septum, left ventricular posterior wall thickness and in left ventricular end-diastolic diameter compared to age-matched controls (Table 1). These results were not unexpected and were supportive of the presence of athlete's heart in these professional soccer players.

Table 1. Echocardiographic parameters in professional soccer players and age-matched controls

	Controls	Soccer players
IVS (mm)	**9.0 ± 0.31**	**10.2 ± 0.20****
PW (mm)	**9.1 ± 0.9**	**9.9 ± 0.14****
LVEDD (mm)	**48.1 ± 0.95**	**50.6 ± 0.80***
LVESD (mm)	**31.9 ± 0.96**	**33.3 ± 0.66**

Values are presented as mean ± SEM.

Abbreviations: IVS: interventricular septum thickness; PW: posterior wall thickness; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter.

*p < 0.05, **p < 0.01 *vs* control. n = 23 in each group.

4.1.3. Heart rate, QT and QTc intervals in study subjects

The development of athlete's heart in response to long-term physical conditioning is characterized by increased vagal tone. As expected, the RR intervals in soccer players were significantly longer before the game compared to age-matched volunteers (Table 2 and Figure 1A). Consequently, the heart rate of professional soccer players was lower compared to the control group before the game (Figure 1B). However, after the soccer game the heart rates of athletes were higher than in controls (Figure 1B). The duration of cardiac repolarization is cycle length dependent where slower heart rates lead to prolonged repolarization. Accordingly, significantly longer QT intervals were measured in soccer players before the game (Table 2 and Figure 2). However, after the game these differences in QT intervals were not observed, since heart rates of athletes were close to controls, while the QT intervals in soccer players were significantly shorter than before the game (Table 2 and Figure 2).

Table 2. Heart rate, QT and QTc intervals in professional soccer players and age-matched controls

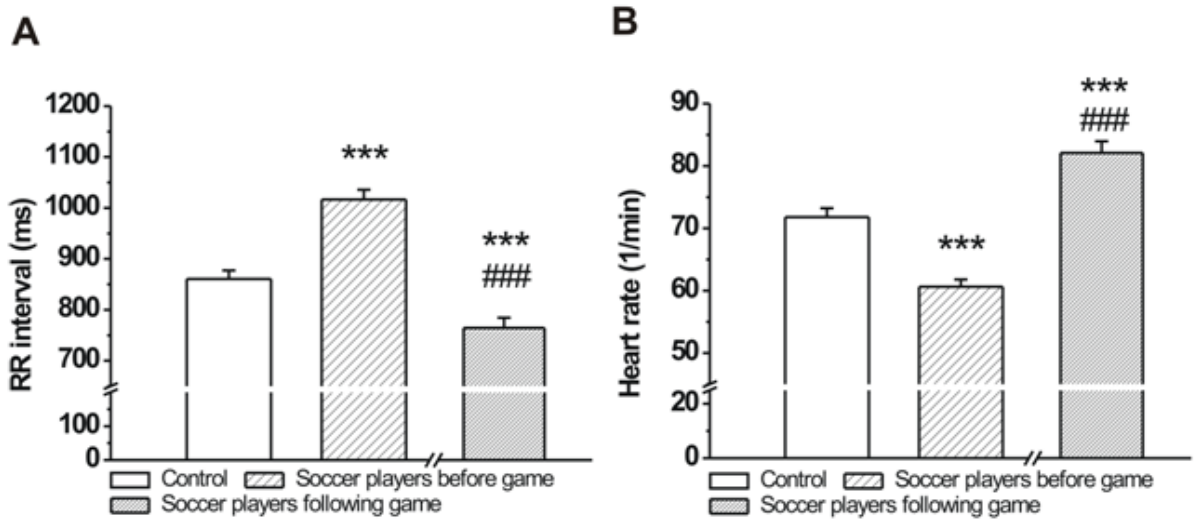
	Control	Soccer players before game	Soccer players following game
RR (ms)	**861 ± 17**	**1017 ± 19*** **	**765 ± 21*** ###**
QT (ms)	**390 ± 4**	**419 ± 3*** **	**383 ± 3*** ###**
QTc (ms) Bazett	**423 ± 3**	**418 ± 2**	**443 ± 3*** ###**
QTc (ms) Fridericia	**411 ± 2**	**418 ± 2***	**421 ± 2*** **
QTc (ms) Framingham	**411 ± 2**	**417 ± 2**	**420 ± 2****
QTc (ms) Hodges	**411 ± 2**	**420 ± 2*** **	**422 ± 2*** **

Values are presented as mean ± SEM.

Abbreviations: QTc: frequency corrected QT interval (calculated by the Bazett's, Fridericia, Framingham and Hodges formulas).

*p < 0.05; **p < 0.01; ***p < 0.001 *vs* age-matched control; ###p < 0.001 *vs* before game values. n = 76 in each group.

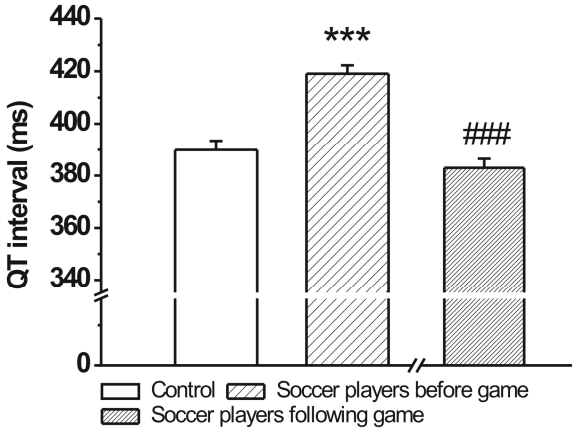
Figure 1. The RR interval (A) and heart rate (B) of age-matched controls and professional soccer players before and following a competitive game



Values are presented as mean ± SEM.

***p < 0.001 *vs* age-matched control; ###p < 0.001 *vs* before game values. n = 76 in each group.

Figure 2. The QT intervals of age-matched controls and professional soccer players before and following a competitive game

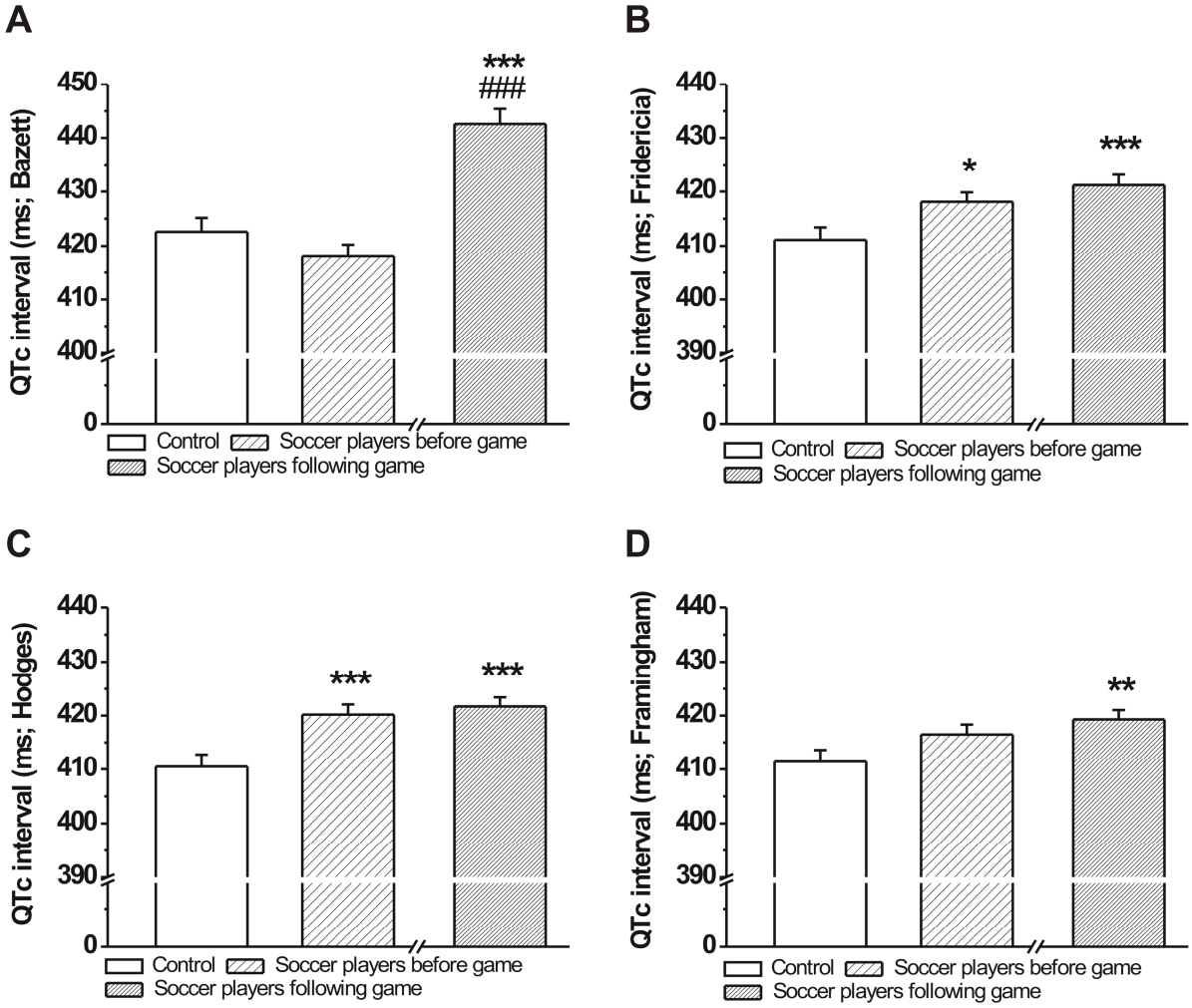


Values are presented as mean ± SEM.

***p < 0.001 *vs* age-matched control; $^{###}$p < 0.001 *vs* before game values. n = 76 in each group.

In order to reliably assess the duration of ventricular repolarization and to minimize the influence of changing heart rate on the QT interval, it is necessary to carry out frequency correction of the QT interval. However, it has been shown that Bazett's and Fridericia correction formulas described over 90 years ago [Bazett 1920; Fridericia 1920] overestimate changes in QT interval [Indik et al. 2006]. Therefore, in this study, to calculate the frequency corrected QT interval (QTc) we also used Framingham and Hodges correction formulas shown to alter the accuracy of QT interval changes due to heart rate in a smaller degree [Indik et al. 2006]. In this regard, QTc calculated using the Bazett's and Framingham formulas were not different in athletes before the game compared to controls (Table 2; Figures 3A and D), while QTc values calculated with the other two formulas were significantly longer in players before the game (Table 2; Figures 3B and D). In addition, QTc was significantly prolonged in soccer players following the game compared to control values calculated with all correction formulas in the present study (Table 2 and Figures 3A–D). Only QTc calculated with the Bazett's formula showed a large and significant prolongation in soccer players after the game compared to pre-game values (Table 2 and Figures 3A–D).

Figure 3. Frequency corrected QT interval of age-matched controls and professional soccer players before and following a competitive game



Values are presented as mean ± SEM.

Abbreviation: QTc: frequency corrected QT interval (calculated by the Bazett's, Fridericia, Framingham and Hodges formulas).

*p < 0.05; **p < 0.01; ***p < 0.001 *vs* age-matched control; $^{###}$p < 0.001 *vs* before game values. n = 76 in each group.

4.1.4. Short-term beat-to-beat temporal variability of the RR and QT intervals

To characterize the instability of cardiac ventricular repolarization, the short-term beat-to-beat variability of the QT interval was calculated in professional soccer players and age-matched controls. Since it is reasonable to assume that STV_{QT} can be, at least in part, influenced by the short-term beat-to-beat temporal variability of the RR interval (STV_{RR}), the STV_{RR} was also calculated in both groups. Soccer players before the competitive game exhibited a

significantly larger STV_{RR} compared to controls; however, this difference disappeared after the game, when their heart rates were close to controls (Table 3 and Figure 4A). As individual representative examples (Poincaré plots) and grouped average data show, the short-term beat-to-beat variability of the QT interval was significantly higher in soccer players compared to controls (Table 3; Figures 4B and C). Importantly, and unlike the STV_{RR}, the STV_{QT} was still significantly higher in soccer players compared to controls but was also reduced after the game compared to pre-game values (Table 3; Figure 4C).

Table 3. Short-term beat-to-beat temporal variability of the RR and QT intervals in professional soccer players and age-matched controls

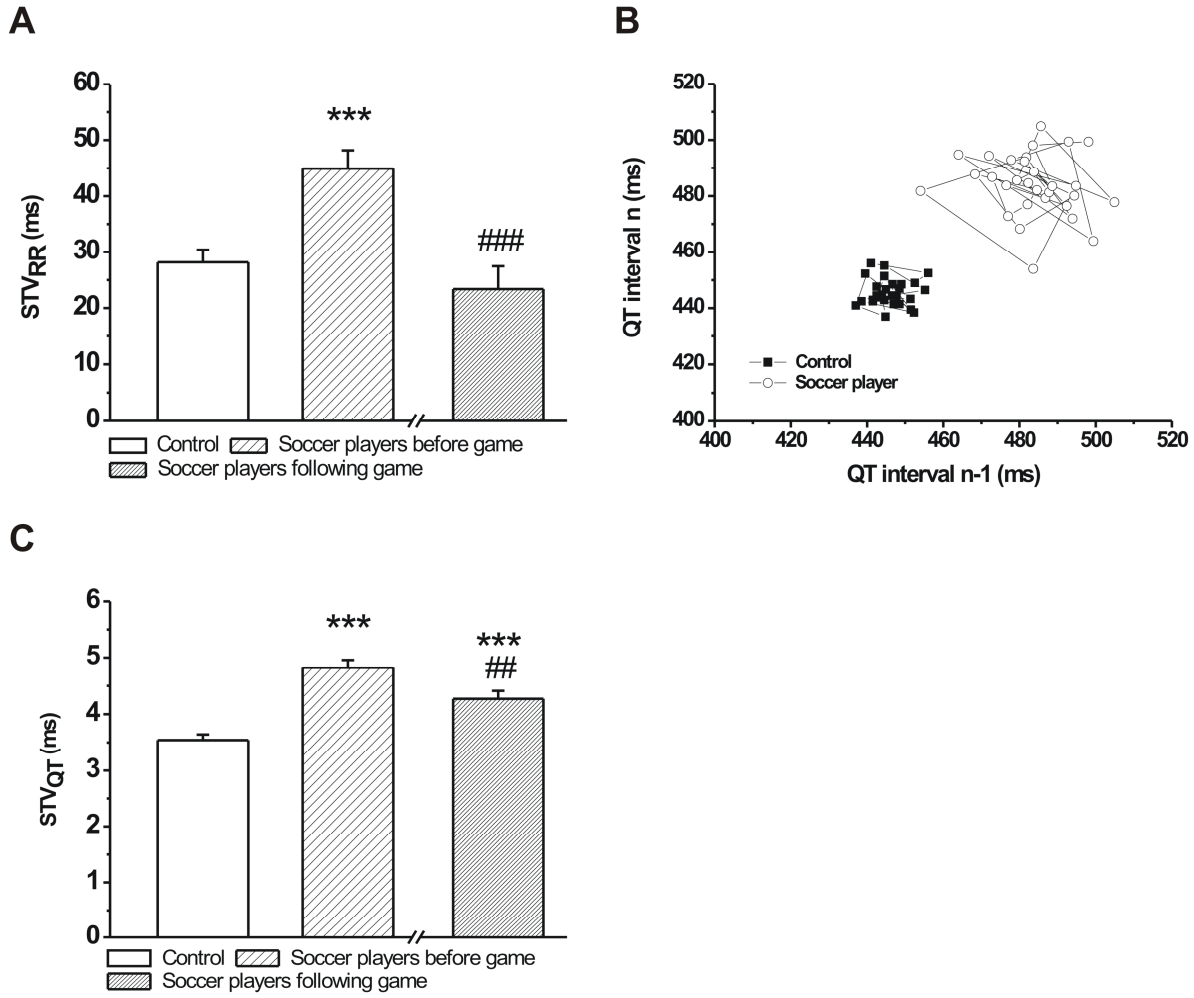
	Control	Soccer players before game	Soccer players following game
STV_{RR} (ms)	**28.2 ± 2.2**	**44.9 ± 3.3*****	**23.4 ± 4.1**$^{\#\#\#}$
STV_{QT} (ms)	**3.5 ± 0.1**	**4.8 ± 0.1*****	**4.3 ± 0.1***** $^{\#\#}$

Values are presented as mean ± SEM.

Abbreviations: STV_{RR}: short-term beat-to-beat temporal variability of the RR interval; STV_{QT}: short-term beat-to-beat temporal variability of the QT interval.

***$p < 0.001$ *vs* age-matched control; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ *vs* before game values. n = 76 in each group.

Figure 4. Short-term beat-to-beat temporal variability of the RR (STV_{RR}) and QT (STV_{QT}) intervals in age-matched controls and professional soccer players before and following a competitive game



Values are presented as mean ± SEM.

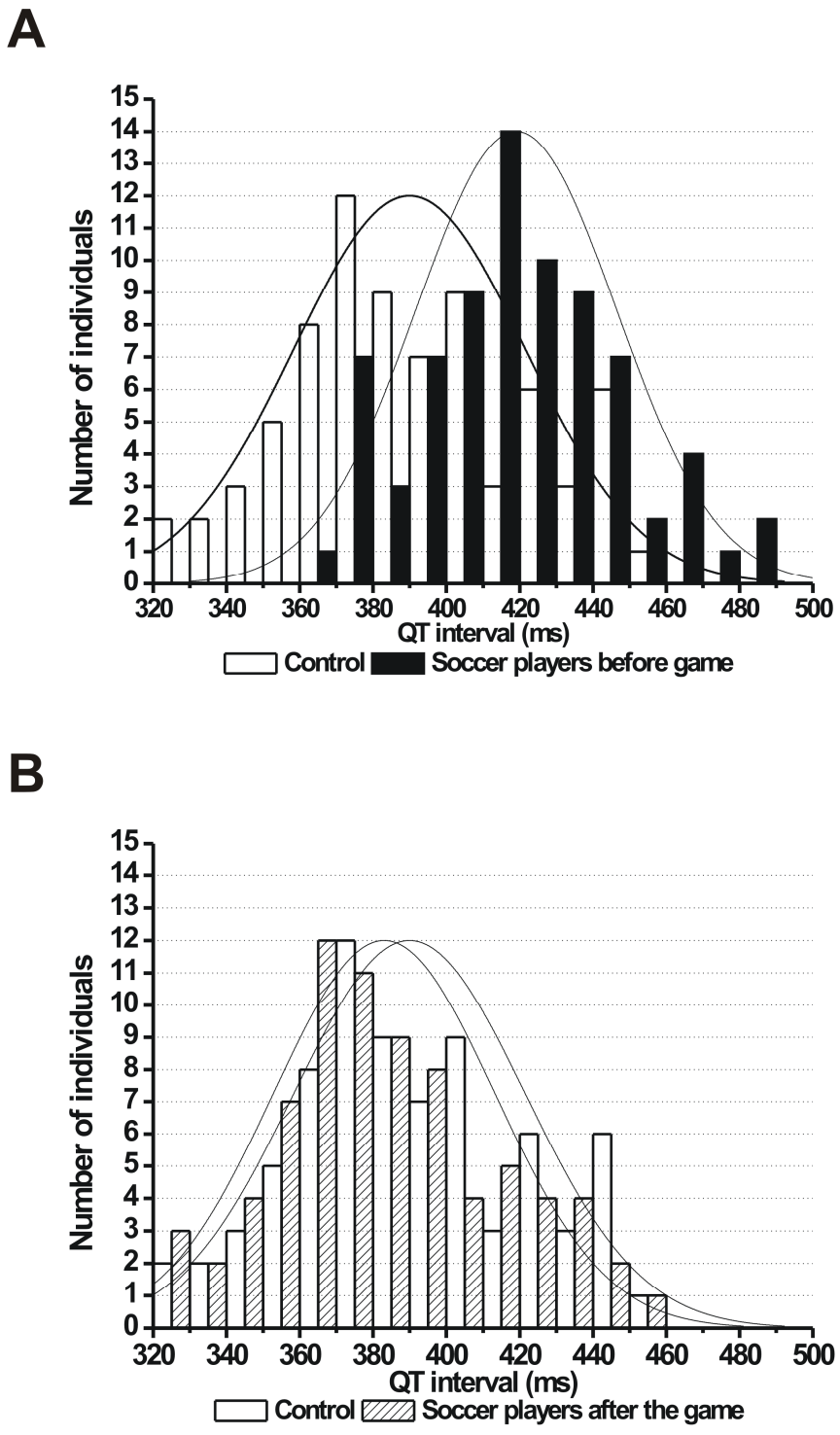
Abbreviations: STV_{RR}: short-term beat-to-beat temporal variability of the RR interval; STV_{QT}: short-term beat-to-beat temporal variability of the QT interval.

***$p < 0.001$ *vs* age-matched control; $^{##}p < 0.01$; $^{###}p < 0.001$ *vs* before game values. n = 76 in each group.

Histograms on Figures 5 and 6 show the distribution of QT interval and STV_{QT} values within the control and soccer player groups, respectively. The histograms clearly exhibit a shift to the right in the distribution of both QT intervals and STV_{QT} in soccer players before the competitive game compared to controls (Figures 5A and 6A). However, while the distribution of QT intervals shows a similar pattern in soccer players to controls following the

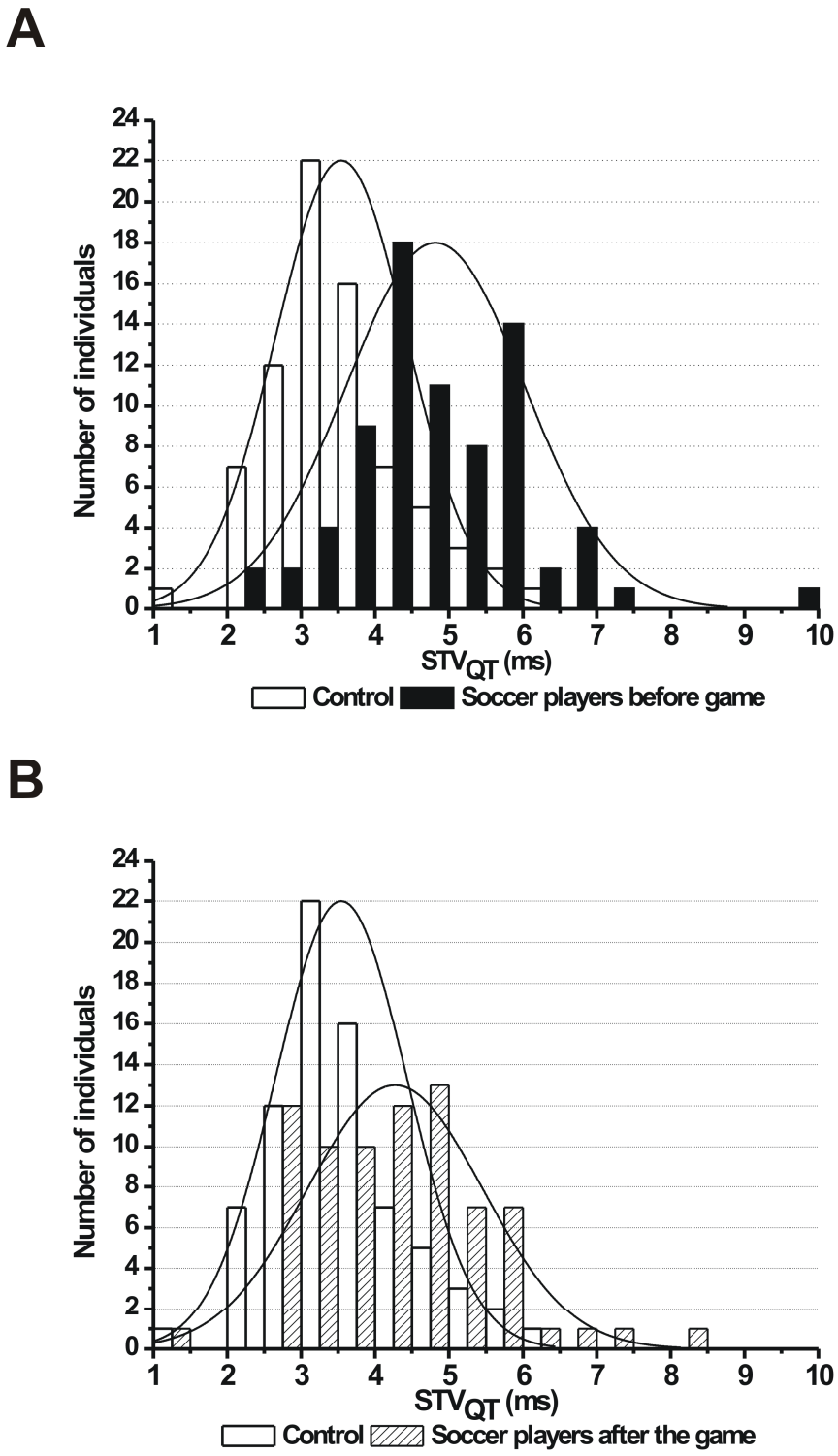
game (Figure 5B); the distribution pattern of STV_{QT} largely remained unchanged after the game (Figure 6B). These results suggest that the increased STV_{QT} in soccer players is very unlikely caused by the prolonged QT interval itself in these athletes.

Figure 5. Histograms showing the distribution of the QT interval



(**A**) Controls (empty bars) and soccer players before game (full bars) and (**B**) controls (empty bars) and soccer players after the game (hashed bars). Bin size is 10 ms. n = 76 in each group.

Figure 6. Histograms showing the distribution of short-term beat-to-beat temporal variability of the QT interval (STV_{QT})



(**A**) Controls (empty bars) and soccer players before game (full bars) and (**B**) controls (empty bars) and soccer players after the game (hashed bars). Bin size is 0.5 ms.

Abbreviation: STV_{QT}: short-term beat-to-beat temporal variability of the QT interval.

n = 76 in each group.

In six players before the game the STV_{QT} was markedly larger than the average STV_{QT} in the soccer player group (9.7, 7.2, 7.0, 7.0, 6.7, 6.7 and group average was 4.8 ± 0.14 ms; Figure 6). Since increased STV_{QT} has been associated with increased proarrhythmic risk in certain patient populations, the player who had 9.7 ms STV_{QT} was contacted and the measurement was repeated to yield a heart rate of 47/min and an STV_{QT} of 5.0 ms, however, before the repeated measurement he had been injured for 2 months. Whether this smaller (but still higher and close to the group average) STV_{QT} on repeated measurement was due to the well-known de-training phenomenon in an athlete who had been out of training due to injury was unclear.

4.2. Short-term variability of the QT interval and correlation with parameters of left ventricular hypertrophy in patients with hypertrophic cardiomyopathy

4.2.1. Study population

Thirty-seven consecutive patients with hypertrophic cardiomyopathy (HCM) were enrolled into the study. The diagnosis of HCM was based on established diagnostic criteria [Gersh et al. 2011]. Among the 37 HCM patients, 24 patients were taking beta blockers and 8 patients were taking verapamil as first line therapy. Three patients were taking cardiac medications known to prolong QT interval (two were taking amiodarone and one was taking propafenone). None of the patients were on any other drug therapy with known QT interval prolonging effect.

A total of 37 age- and gender-matched healthy volunteers (mean age 43 ± 12 years, males/females 21/16) without a history or evidence of heart disease was enrolled in the study as controls. Body mass index was significantly lower in the control group (25 ± 4 *vs* 28 ± 6 kg/m^2, $p = 0.007$, see Table 4).

Table 4. The main demographical, clinical and echocardiographic parameters of the patients with HCM and controls

	Control	HCM
n	**37**	**37**
Sex (male/female)	**21/16**	**21/16**
Age (year)	**43 ± 12**	**48 ± 15**
BMI (kg/m^2)	**25 ± 4**	**28 ± 6***
BMI ≥30/≥25/<25 kg/m^2 (n)	**5/13/19**	**11/16/10**
NYHA class 0/1/2/3 (n)	**19/18/0/0**	**0/5/26/6****
EF (%)	**68 ± 6**	**69 ± 9**
LVEDD (mm)	**48 ± 4**	**46 ± 7**
LVESD (mm)	**30 ± 4**	**27 ± 7***
IVS (mm)	**9 ± 1**	**20 ± 6*****
PW (mm)	**9 ± 1**	**11 ± 2*****

Values are represented as mean ± SD.

Abbreviations: HCM: hypertrophic cardiomyopathy; BMI: body mass index; NYHA: New York Heart Association; EF: ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; IVS: interventricular septum thickness; PW: posterior wall thickness.

*p < 0.05, ***p < 0.001, ****p < 0.0001 *vs* control.

4.2.2. Electrocardiographic parameters in HCM patients and controls

Comparison of ECG parameters between HCM patients and controls are presented in Table 5. Patients with HCM exhibited significantly increased RR, PQ and QRS intervals. QTc was significantly prolonged in HCM patients (Figure 7A), regardless of the method used for QTc correction (using Bazett's, Fridericia, Framingham or the Hodges formulas). There was no difference between QTc intervals in the HCM group obtained by the different correction methods. The terminal part of the T wave, the Tpeak-Tend interval was also markedly longer in HCM patients (Figure 7B). QT dispersion (Figure 7C) and short-term QT variability was also markedly increased in patients with HCM (Figure 7D). The largest relative increase among the different parameters was seen with regard to short-term QT variability with a

relative increase of 41%. Differences between the HCM and control groups remained highly significant when we excluded patients taking QT prolonging drugs (amiodarone or propafenone, n = 3) from the comparisons. BMI or obesity status did not correlate with either of the repolarization parameters.

Table 5. Electrocardiographic parameters in HCM patients and controls

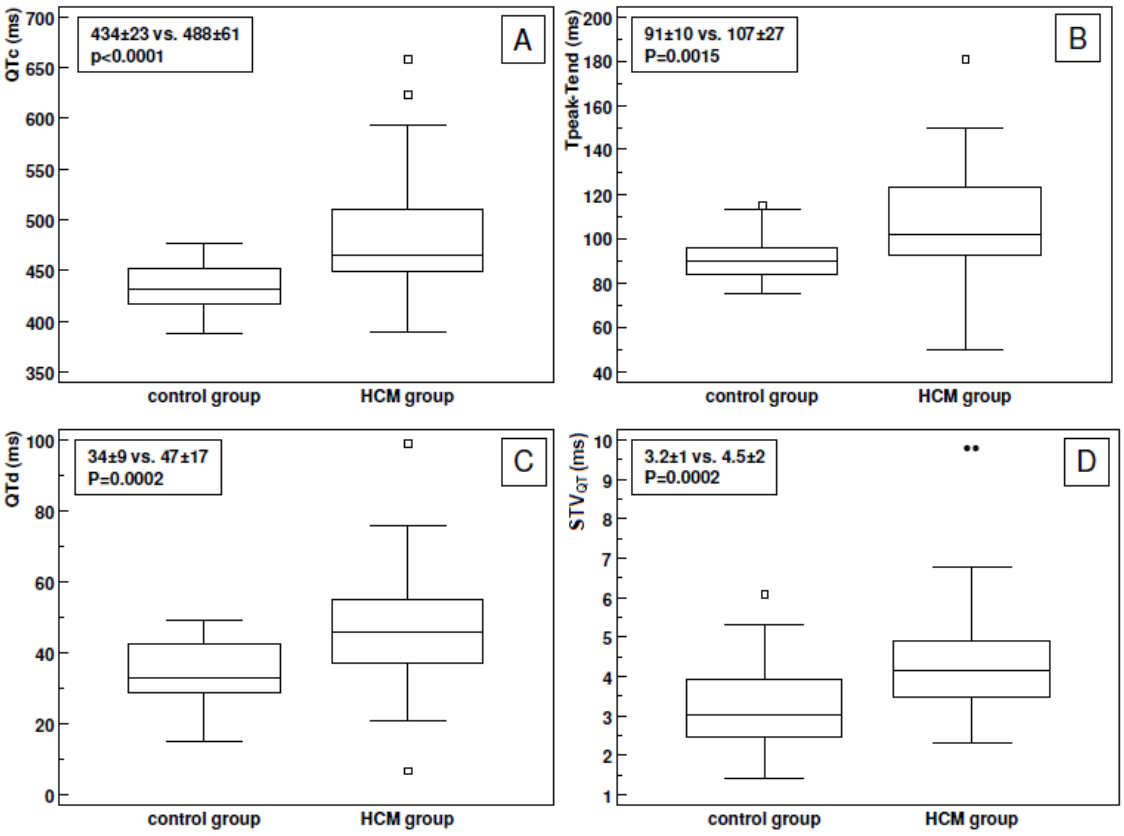
	Control	HCM	Relative difference (%)
RR (ms)	**867 ± 119**	**947 ± 140***	**9.2**
PQ (ms)	**152 ± 17**	**166 ± 30***	**8.5**
QRS (ms)	**96 ± 7**	**112 ± 17*****	**15.6**
QT (ms)	**401 ± 24**	**473 ± 66*****	**18.0**
QTc (ms) Bazett	**434 ± 23**	**488 ± 61*****	**13.0**
QTc (ms) Fridericia	**422 ± 20**	**483 ± 60*****	**14.7**
QTc (ms) Framingham	**423 ± 19**	**481 ± 60*****	**14.5**
QTc (ms) Hodges	**420 ± 18**	**482 ± 60*****	**15.0**
QTd (ms)	**34 ± 9**	**47 ± 17****	**37.1**
Tpeak-Tend (ms)	**91 ± 10**	**107 ± 27***	**18.9**
STV_{QT} (ms)	**3.2 ± 1**	**4.5 ± 2****	**40.6**

Values are represented as mean ± SD and relative difference between the two groups in %.

Abbreviations: HCM: hypertrophic cardiomyopathy; QTc: frequency corrected QT interval (calculated by the Bazett's, Fridericia, Framingham and Hodges formulas); QTd: QT dispersion; Tpeak-Tend: duration of the T wave from the peak to the end; $STV_{QT:}$ short-term beat-to-beat temporal variability of the QT interval.

*p < 0.05, **p < 0.001, ***p < 0.0001 *vs* control. n = 37 in each group.

Figure 7. Box and whisker plots illustrating significant differences between patients with HCM and controls



Values are represented as mean ± SD.

A) the frequency corrected QT interval (QTc, calculated by the Bazett's formula); **B**) the duration of the T wave from the peak to the end (Tpeak-Tend); **C**) QT dispersion (QTd); **D**) short-term beat-to-beat temporal variability of QT interval (STV_{QT}). The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outside (open squares) and far out values (filled dots) which are displayed as separate points. n = 37 in each group.

4.2.3. Correlation of repolarization parameters in HCM patients

Correlation between different repolarization parameters, QT dispersion and short-term QT variability are given in Table 6. The QTc prolongation correlated significantly with the prolongation of the Tpeak-Tend interval, but not the QRS width, indicating that the QTc prolongation was, at least in part, due to the prolongation of the terminal phase of the T wave. Short-term QT variability showed a relatively strong correlation with the QTc prolongation

and with, to a lesser extent, with the Tpeak-Tend interval. The QT dispersion did not correlate with any of the repolarization parameters.

Table 6. Correlation of repolarization parameters in HCM patients

	QRS	Tpeak-Tend	QTd	STV_{QT}
QTc	**0.284**	**0.527*** **	**- 0.013**	**0.616*** **
Tpeak-Tend	**0.299**	**–**	**0.018**	**0.378***
QTd	**0.253**	**–**	**–**	**- 0.228**

Values are represented as Pearson correlation coefficient.

Abbreviations: HCM: hypertrophic cardiomyopathy; QTc: frequency corrected QT interval (calculated by the Bazett's formula); QTd: QT dispersion; Tpeak-Tend: duration of the T wave from the peak to the end; STV_{QT}: short-term beat-to-beat temporal variability of the QT interval.

*p < 0.05, ***p < 0.001 *vs* control. n = 37 in each group.

4.2.4. Correlation between repolarization parameters and echocardiographic parameters in HCM patients

Correlation between ECG repolarization parameters and echocardiography parameters in HCM patients showed no correlation between these parameters except for a weak correlation between short-term QT variability and left ventricular end-systolic diameter or left ventricular ejection fraction (data not shown).

4.2.5. Correlation between repolarization parameters and indices of left ventricular hypertrophy determined by cardiac MRI technique in HCM patients

Correlation between repolarization parameters and indices of left ventricular hypertrophy (maximal left ventricular wall thickness and left ventricular mass, measured by cardiac magnetic resonance imaging) with or without normalization for body surface area are shown in Table 7. Degree of correlation increased with normalization in almost all comparisons. Short-term QT variability showed significant, albeit modest correlation, with both un-normalized and normalized indices of left ventricular hypertrophy (LVmax; LVmax BSA and LVM BSA, Figures 8 and 9). Tpeak-Tend interval also correlated significantly with some of

the hypertrophy parameters, but showed no significant correlation to the most reliable hypertrophy parameter, i. e. LVM indexed for BSA.

Table 7. Correlation between repolarization parameters and morphologic parameters of hypertrophy in HCM patients

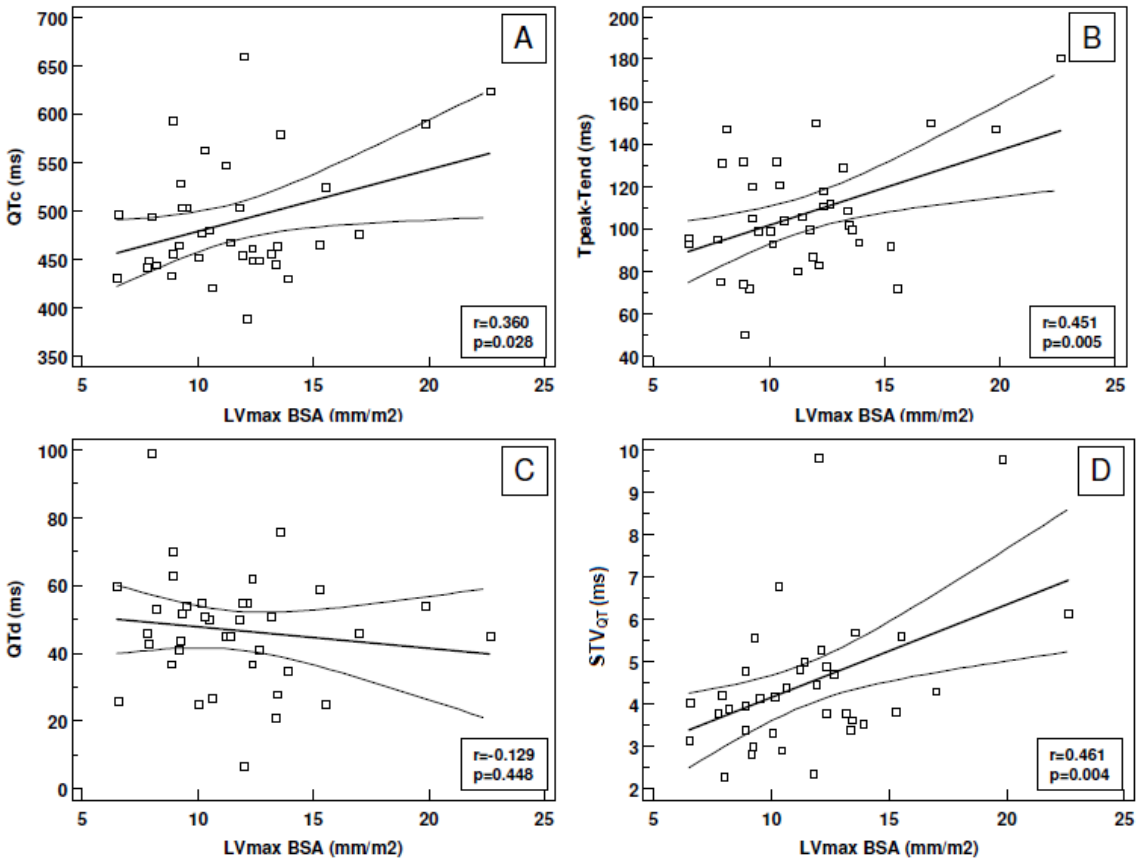
	QTc	Tpeak-Tend	QTd	STV_{QT}
IVS (mm)	**0.099**	**0.344***	**- 0.144**	**0.285**
LVmax (mm)	**0.216**	**0.450****	**- 0.238**	**0.381***
LVmax BSA (mm/m^2)	**0.360***	**0.451****	**- 0.129**	**0.461****
LVM (g)	**0.037**	**0.241**	**- 0.128**	**0.273**
LVM BSA (g/m^2)	**0.195**	**0.348**	**- 0.116**	**0.455***

Values are represented as Pearson correlation coefficient.

Abbreviations: HCM: hypertrophic cardiomyopathy; QTc: frequency corrected QT interval (calculated by the Bazett's formula); Tpeak-Tend: duration of the T wave from the peak to the end; QTd: QT dispersion; STV_{QT}: short-term beat-to-beat temporal variability of the QT interval; IVS: interventricular septum thickness; LVmax: maximal left ventricular wall thickness; LVmax BSA: maximal left ventricular wall thickness normalized for body surface area; LVM: left ventricular mass; LVM BSA: left ventricular mass normalized for body surface area.

*$p < 0.05$, **$p < 0.01$ *vs* control. n = 37 in each group.

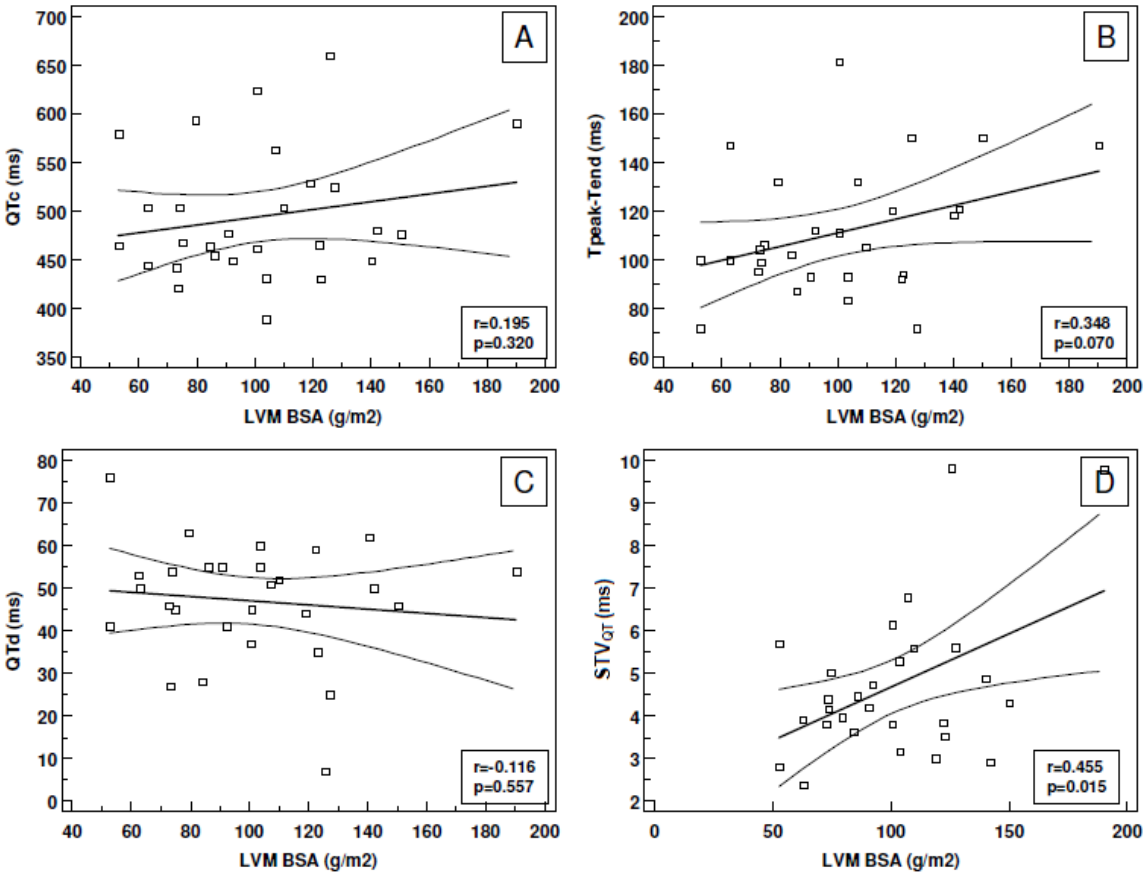
Figure 8. Scatter plot illustrating the correlation between short-term variability of QT interval and morphologic parameters of hypertrophy in HCM patients



Values are represented as Pearson correlation coefficient (r).

Correlation between maximal left ventricular wall thickness normalized for body surface area (LVmax BSA) and **A**) the frequency corrected QT interval (QTc, calculated by the Bazett's formula); **B**) the duration of the T wave from the peak to the end (Tpeak-Tend); **C**) QT dispersion (QTd); **D**) short-term beat-to-beat temporal variability of QT interval (STV_{QT}).

Figure 9. Scatter plot illustrating the correlation between left ventricular mass normalized for body surface area and repolarization parameters



Values are represented as Pearson correlation coefficient (r).

Scatter plot illustrating the correlation between left ventricular mass normalized for body surface area (LVM BSA) and **A**) the frequency corrected QT interval (QTc; calculated by the Bazett's formula); **B**) the duration of the T wave from the peak to the end (Tpeak-Tend); **C**) QT dispersion (QTd); **D**) short-term beat-to-beat temporal variability of QT interval (STV_{QT}).

4.3. Short-term variability of QT interval in patients with acromegaly

4.3.1. Clinical data of acromegalic patients and control subjects

In 30 acromegalic patients studied, body weight and mean body mass index (BMI) were significantly higher ($p < 0.001$ for both parameters) than those in age- and sex-matched volunteers (Table 8). Mean systolic blood pressure did not differ significantly between control subjects and acromegalic patients receiving standard care and treatment, however, acromegalic patients had higher diastolic blood pressure ($p < 0.05$). The incidence of high blood pressure was 7/30 in control and 13/30 in acromegaly groups during the actual measurements. Average serum glucose and glycosylated hemoglobin (HbA1c) values were

also similar in both groups; incidences of diabetes were 0/30 and 1/30 in control and acromegaly groups, respectively. Incidence of impaired glucose tolerance was 0/30 in control and 4/30 in acromegalic subjects. Significant differences were seen in serum hGH ($p = 0.0028$) and IGF-1 ($p = 0.0013$) levels between acromegalic and control groups. There was no significant difference in nadir value of hGH during oral glucose tolerance test (OGTT) between active (3.40 ± 2.10 ng/ml, n = 17) and inactive (1.80 ± 1.86 ng/ml, n = 13) acromegalic subgroups. However, significantly higher average hGH (7.00 ± 6.73 ng/ml *vs* 2.03 ± 2.86 ng/ml, $p = 0.0180$) and IGF-1 (501.3 ± 359.6 ng/ml *vs* 198.5 ± 79.1 ng/ml, $p = 0.0060$) concentrations were measured in active acromegalic subgroup compared to inactive one.

Table 8. Clinical data of acromegalic patients and age-matched control subjects

	Control	Acromegaly
Age (years)	**51.3 ± 7.6**	**55.7 ± 10.4**
Weight (kg)	**68.9 ± 14.7**	**87.7 ± 19.3****
Height (cm)	**165.1 ± 10.5**	**168.9 ± 8.2**
BMI (kg/m^2)	**25.1 ± 3.7**	**30.6 ± 5.3****
Systolic BP (mmHg)	**126.9 ± 13.4**	**133.2 ± 17.7**
Diastolic BP (mmHg)	**75.5 ± 8.5**	**82.7 ± 12.4***
0 min glucose (mmol/l)	**5.04 ± 0.52**	**5.40 ± 0.71**
120 min glucose (mmol/l)	**5.30 ± 1.30**	**6.30 ± 2.53**
HbA1c (%)	**5.70 ± 0.50**	**5.90 ± 0.74**
hGH nadir following OGTT (ng/ml)	**1.02 ± 1.42**	**2.72 ± 2.13***
IGF-1 (ng/ml)	**151.0 ± 51.4**	**370.1 ± 311.8***
IGF-1 x ULN	**0.50 ± 0.33 x ULN**	**1.66 ± 1.59 x ULN****

Values are represented as mean ± SD.

Abbreviations: BMI: body mass index; BP: blood pressure; HbA1c: glycosylated hemoglobin; hGH: human growth hormone; OGTT: oral glucose tolerance test; IGF-1: insulin-like growth factor-1; ULN: upper limit of normal value.

*$p < 0.05$, **$p < 0.001$ *vs* control. n = 30 in each group.

4.3.2. Echocardiographic measurements in study subjects

There were significant differences between the two groups in the echocardiographic dimensions. Patients with acromegaly exhibited significantly higher values in left ventricular end-diastolic and end-systolic diameter and in interventricular septum thickness, left ventricular posterior wall thickness compared to age-matched controls (Table 9). These results were not unexpected and were supportive of the presence of myocardial hypertrophy in the acromegalic patients and could be related to the duration and activity of the disease. However, no significant difference was detected in the echocardiographic parameters measured between active and inactive acromegaly subgroups (EF: 66.7 ± 7.4% *vs* 67.9 ± 6.4%, LVEDD: 52.6 ± 4.9 mm *vs* 52.7 ± 6.1 mm, LVESD: 32.1 ± 5.9 mm *vs* 32.5 ± 4.3 mm, IVS: 10.6 ± 1.3 mm *vs* 11.8 ± 3.0 mm, PW: 10.5 ± 1.3 mm *vs* 11.2 ± 1.6 mm, respectively).

Table 9. Echocardiographic parameters in patients with acromegaly and age-matched controls

	Control	Acromegaly
EF (%)	**70.6 ± 5.4**	**67.2 ± 6.9***
LVEDD (mm)	**48.0 ± 3.9**	**52.6 ± 5.4***
LVESD (mm)	**29.1 ± 4.4**	**32.3 ± 5.2***
IVS (mm)	**8.8 ± 0.7**	**11.1 ± 2.2****
PW (mm)	**8.9 ± 0.7**	**10.8 ± 1.4****

Values are represented as mean ± SD.

Abbreviations: EF: ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; IVS: interventricular septum thickness; PW: posterior wall thickness.

*$p < 0.05$, **$p < 0.0001$ *vs* control. n = 30 in each group.

4.3.3. Electrocardiographic parameters in study subjects

Comparison of the two groups (acromegalic patients *vs* control) revealed no significant differences in heart rate, the PQ, QRS and QT intervals and the QT dispersion. In order to reliably assess the duration of ventricular repolarization and to minimize the influence of changing heart rate on the QT interval, frequency corrected QT interval (QTc) was performed by the Bazett's, Fridericia, Framingham and Hodges formulas. QTc values calculated with all

the four formulas showed no significant differences between acromegalic patients and controls. However, the Tpeak-Tend interval was significantly increased in acromegalic patients compared to controls (Table 10). Electrocardiographic parameters tended to be shorter in active acromegaly subgroup compared to the data measured in inactive subgroup (RR: 859.8 ± 134.5 ms *vs* 901.0 ± 178.2 ms, not significant (NS), QT: 392.7 ± 28.5 ms *vs* 412.0 ±29.5 ms, NS; QTc Bazett's: 425.0 ± 16.0 ms *vs* 436.8 ±20.3 ms, NS; QTc Fridericia: 413.7 ± 15.6 ms *vs* 428.1 ±16.6 ms, p = 0.0220; QTc Framingham: 414.3 ± 14.9 ms *vs* 427.3 ±17.8 ms, p = 0.0376; QTc Hodges: 412.5 ± 15.4 ms *vs* 426.9 ±16.7 ms, p = 0.0209; Tpeak-Tend: 86.0 ± 15.7 ms *vs* 84.7 ±11.0 ms, NS, respectively).

Table 10. Electrocardiographic parameters in patients with acromegaly and age-matched controls

	Control	**Acromegaly**
RR (ms)	**840.0 ± 75.0**	**877.6 ± 153.4**
PQ (ms)	**158.2 ± 17.7**	**158.0 ± 17.3**
QRS (ms)	**92.2 ± 6.5**	**95.3 ± 8.4**
QT (ms)	**389.3 ± 16.5**	**401.1 ± 30.0**
QTc (ms) Bazett	**425.6 ± 17.3**	**430.1 ± 18.6**
QTc (ms) Fridericia	**413.1 ± 14.5**	**419.9 ± 17.4**
QTc (ms) Framingham	**414.0 ± 13.7**	**419.9 ± 17.2**
QTc (ms) Hodges	**410.4 ± 13.8**	**418.7 ± 17.3**
QTd (ms)	**36.6 ± 10.2**	**38.2 ± 13.2**
Tpeak-Tend (ms)	**80.0 ± 10.3**	**85.5 ± 13.6***
STV_{QT} (ms)	**3.02 ± 0.80**	**4.23 ± 0.10****

Values are represented as mean ± SD.

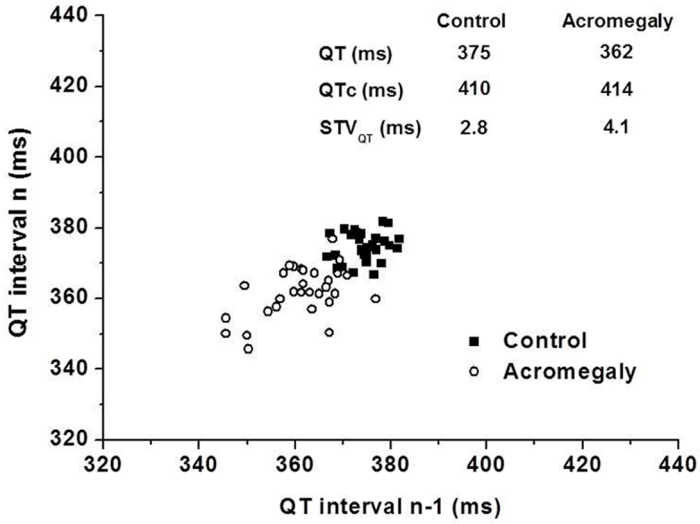
Abbreviations: QTc: frequency corrected QT interval (calculated by the Bazett's, Fridericia, Framingham and Hodges formulas); QTd: QT dispersion; Tpeak-Tend: duration of the T wave from the peak to the end; STV_{QT}: short-term beat-to-beat temporal variability of QT interval.

*p < 0.05, **p < 0.001 *vs* control. n = 30 in each group.

4.3.4. Short-term beat-to-beat temporal variability of the QT intervals

To characterize the instability of cardiac ventricular repolarization, the short-term beat-to-beat variability of the QT interval was calculated in acromegalic patients and age-matched controls. As individual representative examples (Poincaré plots, Figure 10) and grouped average data show STV_{QT} was significantly increased by 36% in acromegalic patients compared to controls (4.23 ± 0.10 ms *vs* 3.02 ± 0.80 ms, $p < 0.001$) (Figure 11). STV_{QT} values did not differ significantly between active (4.16 ± 0.89 ms) and inactive (4.33 ± 1.22 ms) acromegalic patient subgroups. There was no difference between acromegalic subjects treated with antihypertensive drugs (4.33 ± 0.95 ms, n = 18) and normotensive acromegalic patients (4.10 ± 1.16 ms, n = 12). We could not find any significant correlation between the STV_{QT} values and the left ventricular hypertrophy parameters in acromegaly patients or in the subgroups of active and inactive patients (data not shown).

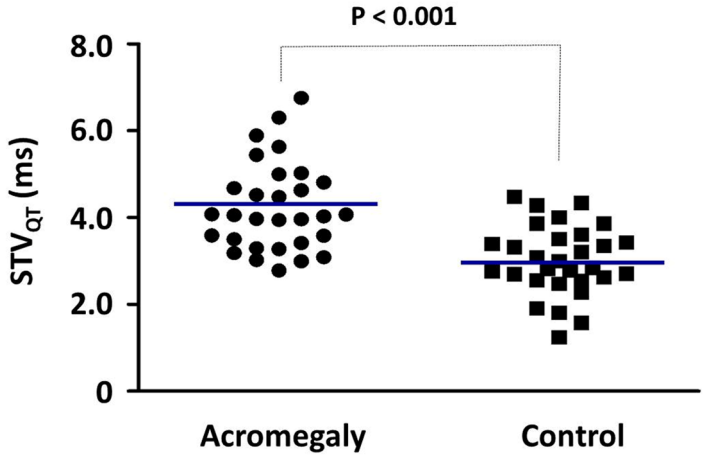
Figure 10. Representative Poincaré plots of a control individual and a patient with acromegaly



Note the larger area covered by data points obtained in the acromegalic patient illustrating increased short-term variability of the QT interval.

Abbreviations: QTc: frequency corrected QT interval (calculated by the Bazett's formula); STV_{QT}: short-term beat-to-beat temporal variability of the QT interval.

Figure 11. Short-term beat-to-beat temporal variability of the QT interval (STV_{QT}) in acromegalic and matched control patients



Individual values measured in n = 30 patients in each group are presented, the blue lines indicate mean values, $p < 0.001$.

Abbreviation: STV_{QT}: short-term beat-to-beat temporal variability of the QT interval.

4.3.5. Autonomic function

Standard cardiovascular reflex tests indicated significant deteriorations in Valsalva ratio ($p = 0.0015$), 30/15 ratio ($p = 0.0143$), and AN score ($p = 0.0023$) in patients with acromegaly, however, no significant differences in systolic blood pressure response after standing up, and diastolic blood pressure response after sustained handgrip were detected between the two groups (Table 11). AN score was significantly lower in active acromegaly subgroup, than in inactive group (2.1 ± 1.7 *vs* 3.9 ± 2.2; $p = 0.0260$), whereas other autonomic functions measured did not differ significantly in our two acromegalic subgroups (heart rate variation during deep breathing: 15.5 ± 6.5 1/min *vs* 11.9 ± 7.95 1/min; Valsalva ratio: 1.50 ± 0.26 *vs* 1.40 ± 0.29; 30/15 ratio: 1.10 ± 0.13 *vs* 1.10 ± 0.29; systolic blood pressure fall after standing up: 8.6 ± 11.8 mmHg *vs* 9.8 ± 6.2 mmHg; diastolic blood pressure increase after handgrip: 19.1 ± 8.2 mmHg *vs* 15.4 ± 8.2 mmHg; respectively). There was no significant difference between the values of autonomic parameters measured in acromegalic subjects treated with antihypertensive drugs and normotensive patients with acromegaly.

Table 11. Autonomic neuropathy parameters of acromegalic patients and age-matched control subjects

	Control	Acromegaly
Heart rate variation during deep breathing (1/min)	**17.20 ± 6.44**	**14.00 ± 7.22**
Valsalva ratio	**1.70 ± 0.34**	**1.40 ± 0.28***
30/15 ratio	**1.20 ± 0.18**	**1.10 ± 0.21***
Systolic BP fall after standing up (mmHg)	**6.40 ± 6.77**	**9.10 ± 9.66**
Diastolic BP increase after sustained handgrip (mmHg)	**20.00 ± 9.22**	**17.50 ± 8.2**
AN score	**1.50 ± 1.28**	**2.90 ± 2.11***

Values are represented as mean ± SD.

Abbreviations: 30/15 ratio: immediate heart rate response to standing; BP: blood pressure; AN: autonomic neuropathy.

*$p < 0.05$ *vs* control. n = 30 in each group.

4.3.6. Correlation of serum hGH and IGF-1 x ULN levels with cardiovascular data and autonomic neuropathy parameters

Pearson coefficient values indicated that neither hGH nor IGF-1 x ULN hormone level correlated with STV_{QT} or any other ECG parameters measured (Table 12). However, serum hGH concentration negatively correlated with diastolic blood pressure ($p = 0.0326$), thickness of posterior wall of left ventricle ($p = 0.0333$), and AN score ($p = 0.0131$), whereas IGF-1 x ULN levels positively correlated with Valsalva ratio ($p = 0.0087$).

Table 12. Correlation of serum average hGH and IGF-1 x ULN level of acromegalic patients with cardiovascular data and autonomic neuropathy parameters

	Serum average hGH level (ng/ml)		Serum IGF-1 x ULN level	
	Pearson r	**p value (two-tailed)**	**Pearson r**	**p value (two-tailed)**
Systolic BP (mmHg)	**- 0.2294**	***0.2313***	**0.3206**	***0.0900***
Diastolic BP (mmHg)	**- 0.3978**	***0.0326****	**0.1256**	***0.5161***
EF (%)	**0.3170**	***0.0878***	**-0.1735**	***0.3593***
LVEDD (mm)	**- 0.2911**	***0.1186***	**-0.1680**	***0.3749***
LVESD (mm)	**- 0.3507**	***0.0574***	**-0.1076**	***0.5716***

IVS (mm)	**- 0.2714**	***0.1469***	**0.0388s**	***0.8387***
PW (mm)	**- 0.3897**	***0.0333****	**0.1048**	***0.5815***
RR (ms)	**- 0.1204**	***0.5262***	**- 0.1045**	***0.5826***
PQ (ms)	**- 0.1968**	***0.2974***	**- 0.0284**	***0.8817***
QRS (ms)	**- 0.0127**	***0.9468***	**- 0.3023**	***0.1044***
QT (ms)	**- 0.2032**	***0.2815***	**- 0.2360**	***0.2092***
QTc (ms) Bazett	**- 0.1090**	***0.5663***	**- 0.2084**	***0.2690***
QTc (ms) Fridericia	**- 0.1992**	***0.2914***	**- 0.2919**	***0.1175***
QTc (ms) Framingham	**- 0.1834**	***0.3320***	**- 0.2690**	***0.1506***
QTc (ms) Hodges	**- 0.2154**	***0.2530***	**- 0.2975**	***0.1103***
QTd (ms)	**0.1562**	***0.4099***	**0.1758**	***0.3526***
Tpeak-Tend (ms)	**- 0.0917**	***0.6298***	**- 0.0788**	***0.6791***
STV_{QT} (ms)	**- 0.3401**	***0.0659***	**- 0.0924**	***0.6272***
Heart rate variation during deep breathing (1/min)	**0.2300**	***0.2390***	**- 0.1267**	***0.5206***
Valsalva ratio	**0.1340**	***0.4967***	**0.4864**	***0.0087****
30/15 ratio	**0.2386**	***0.2307***	**0.0167**	***0.9342***
Systolic BP fall after standing up (mmHg)	**- 0.3617**	***0.0586***	**- 0.2206**	***0.2593***
Diastolic BP increase after sustained handgrip (mm Hg)	**0.2421**	***0.2146***	**- 0.0762**	***0.6998***
AN score	**- 0.4714**	***0.0131****	**- 0.2077**	***0.2987***

Values are represented as Pearson correlation coefficient (r) and p values.

Abbreviations: hGH: human growth hormone; IGF-1: insulin-like growth factor-1; ULN: upper limit of normal value; BP: blood pressure; EF: ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; IVS: interventricular septum thickness; PW: posterior wall thickness; QTc: frequency corrected QT interval (calculated by the Bazett's, Fridericia, Framingham and Hodges formulas); QTd: QT dispersion; Tpeak-Tend: duration of the T wave from the peak to the end; STV_{QT}: short-term beat-to-beat temporal variability of QT interval; 30/15 ratio: immediate heart rate response to standing; AN: autonomic neuropathy.

*$p < 0.05$ for correlation. n = 30 for each number of XY pairs.

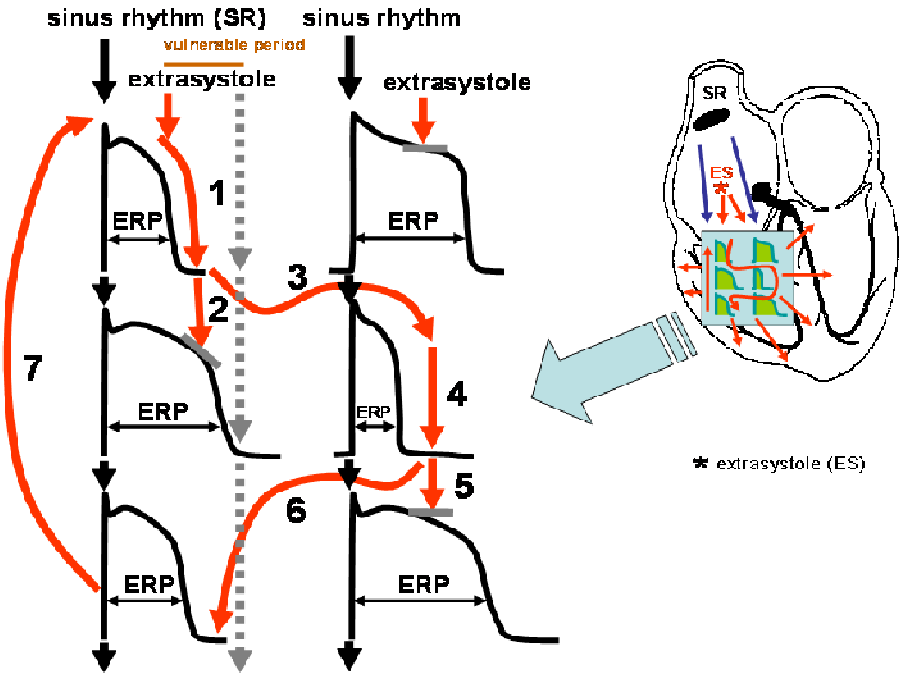
5. Discussion

5.1. Electrophysiological background

In series of animal experiments, in dogs with chronic AV block, myocardial hypertrophy and downregulation of potassium channels, most notably of the slow component of the delayed rectifier potassium current (I_{Ks}), develop [Vos et al. 1998; Nuss et al. 1999; Volders et al. 1999; Li et al. 2002; Rose et al. 2005]. These animals are more susceptible to lethal ventricular arrhythmias subjected to various challenges [Vos et al. 1998]. I_{Ks} has been identified as a key component in the somewhat redundant repolarizing capacity of the myocardium, termed repolarization reserve [Roden 1998; Varró et al. 2000; Roden and Yang 2005; Roden 2006; Jost et al. 2007]. Repolarization reserve refers to the heart's compensating ability for loss or impaired function of one or more potassium currents critical for normal repolarization [Roden 1998]. Impaired repolarization reserve does not necessarily lead to clinically manifest repolarization abnormalities on the ECG but makes the heart more susceptible to arrhythmia development [Roden 1998; Roden and Yang 2005; Varró and Papp 2006].

It is well established that there are marked transmural and regional differences in the expression of cardiac transmembrane ion channels, including potassium channels, that create some spatial heterogeneity of repolarization already in normal circumstances [Antzelevitch and Fish 2001; Gaborit et al. 2007]. These differences in repolarization, also called dispersion and/or heterogeneity of repolarization, can be significantly enhanced by impaired repolarization reserve, thus creating an *arrhythmia substrate* [Varró and Baczkó 2010]. It should be emphasized that the creation of an arrhythmia substrate, i.e. the increased repolarization heterogeneity following repolarization prolongation, is not enough in itself to precipitate arrhythmias. A *trigger* extrasystole critically timed to the vulnerable period that can travel re-entry paths is also required for arrhythmia induction. Enhanced repolarization heterogeneity results in longer vulnerable periods and with more frequent extrasystoles the chance for serious arrhythmia generation is greater (Figure 12) [Varró and Baczkó 2010]. Development of TdP via this mechanism was demonstrated by Akar and co-workers [Akar et al. 2002] in dog ventricular wedge preparations, where increased transmural heterogeneity of repolarization was found and TdP incidence depended on both bradycardia and administration of an I_{Kr} blocker.

Figure 12. Proposed mechanism of arrhythmia development due to increased repolarization heterogeneity



The illustration is represented by exaggerated action potential duration differences.

In the normal setting, sinus impulses (black arrows) travel via physiological pathways. Early extrasystoles („triggers") propagate in directions (red arrows) where propagation is not blocked by refractoriness and where action potentials are in their vulnerable periods (1, 3, 4, 6), while conduction is blocked in refractory cells (2 and 5). The extra stimulus can travel back to its site of origin, creating a re-entry pattern (7). From Varró and Baczkó 2010, with permission.

Abbreviations: SR: sinus rhythm; ERP: effective refractory period, ES: extrasystole.

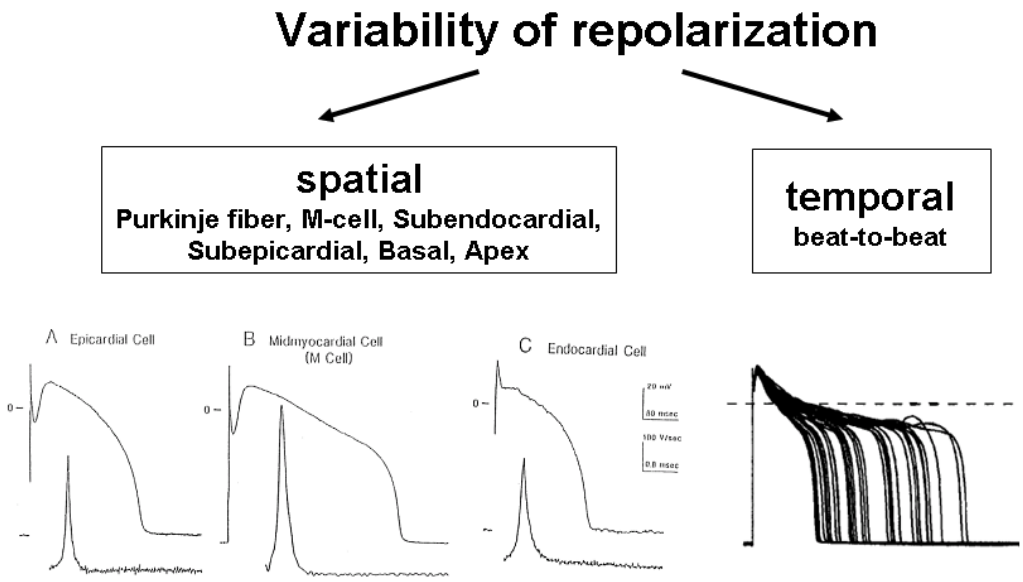
The downregulation of repolarizing potassium currents, including the I_{K1},I_{to},I_{Kr} and I_{Ks} has also been shown both in animal models and patients with heart failure, leading to prolonged repolarization manifested as QT prolongation on the surface ECG [Beuckelmann et al. 1993; Tomaselli and Marban 1999; Li et al. 2004] and increased dispersion of repolarization with concomitant increase in the incidence of serious ventricular arrhythmias [Tomaselli et al. 1994; Akar and Rosenbaum 2003; Tamargo et al. 2004; Jost et al. 2007]. Increased action potential prolongation favors increased Ca^{2+} influx that in turn can facilitate delayed afterdepolarization (DAD) and arrhythmia development [Bers et al. 2006]. Prolongation of repolarization can also precipitate serious ventricular re-entry type

arrhythmias via promoting early afterdepolarization (EAD) generation [Zeng and Rudy 1995; Michael et al. 2009; Farkas and Nattel 2010]. It might be plausible that myocardial hypertrophy, whatever the underlying cause, may lead to potassium channel downregulation and may result in decreased repolarization reserve and increased propensity for arrhythmias including Torsades de Pointes, a characteristic arrhythmia that can degenerate into ventricular fibrillation and culminate in sudden cardiac death.

The reliable identification of patients at risk for serious ventricular arrhythmia and sudden cardiac death remains elusive. Accumulating evidence suggests that QT interval prolongation alone cannot reliably predict the development of TdP since cardiac repolarization reserve may be reduced without significant changes in the duration of cardiac repolarization. A number of clinical studies [Hinterseer et al. 2008; Hinterseer et al. 2009; Hinterseer et al. 2010] and data from *in vivo* animal experiments using species that are electrophysiologically relevant for humans in regard of ventricular repolarization [Thomsen et al. 2005; Lengyel et el. 2007; Thomsen et al. 2007; Hanton et al. 2008] as well as *in vitro* studies [So et al. 2008; Abi-Gerges et al. 2010; Szentandrássy et al. 2015] strongly suggest that the short-term variability of the duration of repolarization (i.e. QT interval on the ECG) may be a better novel parameter to predict serious ventricular arrhythmias. These studies found that increased STV_{QT} correlated with elevated incidence of lethal ventricular arrhythmias and sudden cardiac death. Therefore, based on these studies and the present results, the elevated temporal beat-to-beat variability may indicate a larger repolarization instability and an increased propensity for ventricular arrhythmias.

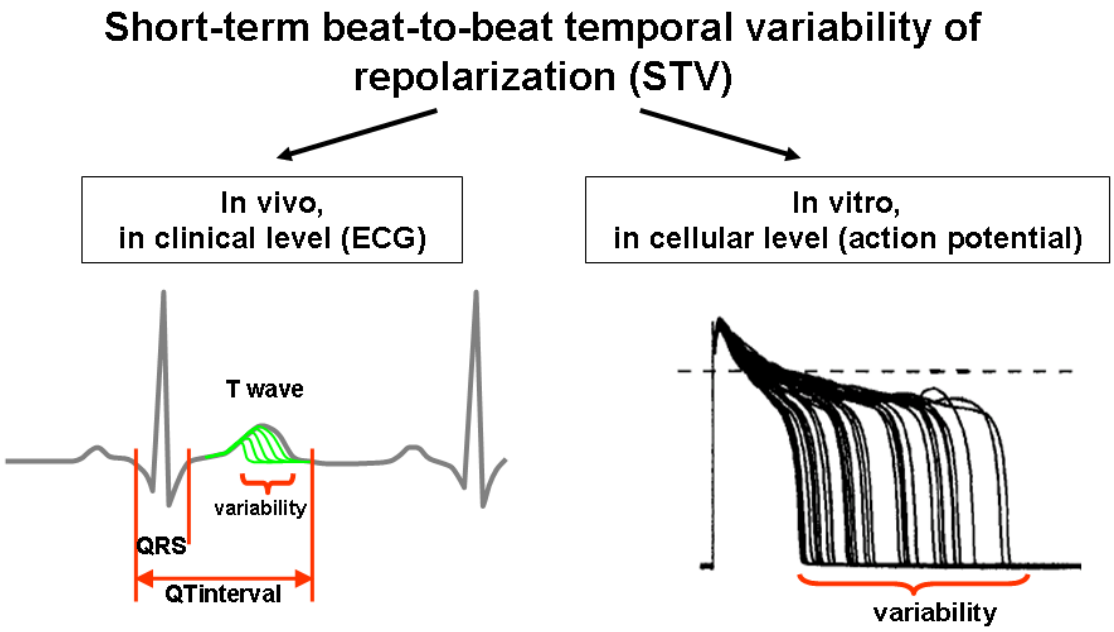
The heterogeneity or variability of ventricular repolarization can be characterised by *spatial* (transmural) or *temporal* (beat-to-beat) dispersion of the action potential duration (APD) (Figure 13). The spatial or transmural heterogeneity of repolarization is achieved by different electrophysiological properties (different APDs) of myocardial cells (e.g. Purkinje fibers, M-cells, epi- or endocardial cells). Clinically, on surface ECG, QT dispersion (QTd; the range measured as the maximum – minimum QT interval in all leads of the 12-lead ECG) is considered to be an indirect measurement of spatial heterogeneity or dispersion of ventricular repolarization. The variability of cardiac repolarization can also be determined as temporal or beat-to-beat variability, which means beat-to-beat alternation of the APD measured *in vitro* in a certain myocardial region. Clinically, the short-term beat-to-beat variability (STV) can be defined as the alternation (variability) of several (in the present study 30) consecutive QT intervals measured in a certain lead (in the present study in lead II) of surface ECG (Figure 14).

Figure 13. Difference between spatial and temporal dispersion of repolarization



With permission of Varró A.

Figure 14. Short-term beat-to-beat temporal variability (STV) of repolarization, as a novel measurable parameter of repolarization reserve



With permission of Varró A.

Abbreviations: STV: short-term beat-to-beat temporal variability; ECG: electrocardiogram.

5.2. Short-term variability of the QT interval in professional soccer players

In competitive athletes, the cardiovascular system adapts to chronic physical exercise by the development of ‘‘athlete’s heart’’, characterized by lower resting heart rate (increased vagal tone), increased ventricular mass (hypertrophy) and volume to meet the increased demand.

It should be noted that autonomic and cardiac electrophysiological changes in dogs with chronic AV block [Volders et al. 1999] possibly do not exactly mirror those developing in athlete’s heart, however, few animal experimental data are available on the effect of endurance exercise training on cardiac hypertrophy and electrophysiology in species that are highly relevant to human, i.e. not in mice and rats. Some of these studies observed slowed heart rate, prolonged QT interval and ECG signs of cardiac hypertrophy in such animals [Constable et al. 1994; Constable et al. 2000]. Whether a ventricular electrical remodeling leading to decreased repolarization reserve develops in these animals is not known, however, it has been speculated that in top endurance athletes, downregulation of potassium channels might occur [Hart 2003].

Based on autopsy findings, hypertrophic cardiomyopathy (HCM) is the most common cause of SCD in young athletes [Basavarajaiah et al. 2008]. This familial malformation leads to cardiac hypertrophy, cardiomegaly and interstitial fibrosis, and it is due to mutations identified in a number of sarcomeric genes [Maron 2002]. In athletes, it is quite difficult to distinguish normal compensatory cardiac hypertrophy from HCM, and only following a 2-3 month sports activity-free period can echocardiographic studies reliably identify HCM, since this hypertrophy is irreversible [Calderón Montero et al. 2007; Williams et al. 2009]. There are a number of other cardiac diseases and pathologies that have been associated with SCD in athletes, including arrhythmogenic right ventricular cardiomyopathy, congenital coronary artery anomalies, myocarditis, commotio cordis, aortic stenosis, Wolff-Parkinson-White and Brugada syndromes [Basso et al. 2007; Pigozzi and Rizzo 2008], however, these are mostly identified upon autopsy.

As mentioned above, there are two main prerequisites for the development of TdP chaotic ventricular tachycardia and consequent fatal ventricular fibrillation: an *arrhythmia substrate* (prolonged repolarization, spatial and temporal inhomogeneity of repolarization creating re-entry paths) and a *trigger* (extrasystole in the vulnerable period) for the initiation of the arrhythmic event [Varró and Baczkó 2010]. Increased vagal tone in athletes lowers heart rate that favors prolonged repolarization and increased inhomogeneity [Farkas et al. 2008]. The possible potassium channel downregulation due to myocardial hypertrophy also prolongs repolarization and reduces repolarization reserve. Theoretically, in this scenario a

number of conditions, compounds and dietary constituents can precipitate such events of sudden cardiac death (as recently reviewed by Varró and Baczkó) [Varró and Baczkó 2010]. These may include serum electrolyte changes (e.g. hypokalemia when fluid intake is not adequate), food and drinks containing flavonoids with hERG (human ether-à-go-go-related gene) inhibitory effects [Zitron et al. 2005], medications with various degree of hERG and other potassium channel blocking properties. These factors can create and enhance the arrhythmia substrate in athletes, while elevated intracellular cyclic adenosine monophosphate (cAMP) levels due to increased sympathetic discharge may contribute to trigger extrasystole generation via increased pacemaker (I_f) current [DiFrancesco and Borer 2007] and/or increased L-type calcium current [Sperelakis et al. 1996].

In conclusion, the short-term temporal variability of the QT interval is elevated in professional soccer players, which, according to our present knowledge, might indicate increased repolarization instability even without any underlying cardiac disease. In our study, some soccer players exhibited greatly increased STV_{QT} even when compared to other players, suggesting that it may be beneficial to screen athletes for elevated repolarization instability by adding the relatively low cost STV_{QT} determination to routine ECG examinations. Individual athletes with large STV_{QT} could be then subjected to more detailed and sophisticated examinations (e.g. evaluation of possible mutations in potassium channel protein encoding genes) to carefully evaluate their vulnerability to ventricular arrhythmias and sudden cardiac death. Importantly, our results further support the inclusion of ECG in preparticipation athlete screening expertly worked out by Corrado et al. [Corrado et al. 2008], with the notion that calculation of STV_{QT} could also be added to the ECG evaluation in case our findings can be confirmed in a broader athlete population. It is important to emphasize that no arrhythmias were observed among soccer players in this study and further, more comprehensive investigations are needed to establish whether the higher STV_{QT} relates to higher arrhythmia propensity in this population.

Study limitations

For purely practical and logistic reasons echocardiographic assessment of all soccer players and all controls were not performed in this study. However, our echocardiography data randomly performed on 23 soccer players and 23 controls support the findings of a number of previous studies showing that endurance athletes, including soccer players, as part of the cardiovascular system's physiological response to long-term intense physical training, develop athlete's heart that features myocardial hypertrophy [Shapiro 1984; Scharhag et al.

2002; Abernethy et al. 2003; Atchley and Douglas 2007; Paolo and Pelliccia 2007]. In our study, professional soccer players from the first division were enlisted who participated in rigorous endurance training schedule for years based on international standards. We also found they had significantly decreased resting heart rate and consequent prolongation of the QT interval, both are characteristics of athlete's heart. Based on the above it is assumed that the echocardiography data are representative for their respective groups.

Since the duration of repolarization is cycle length dependent, variability in the RR interval could influence QT variability. Based on our results, the influence of STV_{RR} on STV_{QT} cannot be ruled out, however, in professional soccer players STV_{RR} was reduced and was similar to control values after the competitive game while STV_{QT} remained significantly higher; strongly suggesting that STV_{QT} was increased irrespective of changes in STV_{RR}. The short-term variability of the monophasic action potential was found to be partially influenced by pacing cycle length and was moderately decreased at faster cycle lengths in anesthetized dogs with chronic AV block characterized by marked bradycardia and myocardial hypertrophy [Thomsen et al. 2005].

5.3. Short-term variability of the QT interval and correlation with parameters of left ventricular hypertrophy in patients with hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy is characterized by morphological and structural changes, including left ventricular hypertrophy, myocardial fibrosis, myofiber disarray, and small vessel disease among them, that may represent an *arrhythmogenic substrate* of the disease [Maron 2002]. Remodeling in HCM is a progressive process [Olivotto et al. 2012] and a very recent study highlighted a close correlation between the development of adverse remodeling and increased risk for SCD in HCM patients [Vriesendorp et al. 2014]. In chronic heart failure, structural remodeling is accompanied by electrical remodeling that includes profound changes in the expression of voltage gated depolarizing and repolarizing ionic currents and exchangers resulting in decreased cardiomyocyte repolarizing capacity [Nattel et al. 2007]. This decreased repolarizing capacity can be brought about by an increase in depolarizing currents (Na^+ and Ca^{2+}) and decreased potassium channel densities (particularly I_{K1}, I_{to} and I_{Ks}), resulting in action potential prolongation manifested as QT prolongation on the ECG [Beuckelmann et al. 1993; Tomaselli and Marban 1999; Li et al. 2004].

A particularly interesting observation is the increase in slowly inactivating, late sodium current ($I_{Na,late}$), that has been shown to prolong repolarization in heart failure and also to contribute to arrhythmogenesis [Valdivia et al. 2005]. Most interestingly, these elements of

arrhythmogenic electrical remodeling have not only been described in congestive heart failure and pathologies leading to cardiac hypertrophy, but a very recent study identified similar changes in cardiomyocytes isolated from HCM patients [Coppini et al. 2013]. The decreased repolarization capacity due to HCM leads to markedly impaired repolarization reserve [Varró and Baczkó 2011] and increased arrhythmia susceptibility in HCM, where even drugs or dietary constituents with only mild repolarization inhibitory effects can provoke serious ventricular arrhythmias and SCD.

QT variability has been previously shown to be increased in patients with HCM. The normalized QT variability index (QTVI), measured as described by Berger et al, was shown to be higher in HCM patients than in controls [Atiga et al. 2000], and the greatest abnormality was detected in patients with malignant HCM mutations (i.e. Arg403Gln mutation of the beta myosin heavy chain gene). In a recent paper [Magri et al. 2014] several myocardial repolarization parameters, including normalized QT variability (QTVN) and normalized QT variability index (QTVI) were shown to be associated with the presence and extent of late gadolinium enhancement (LGE) detected on cardiac magnetic resonance in patients with hypertrophic cardiomyopathy. Both QTVN and QTVI were higher in patients with LGE. Among other parameters, the extent of LGE and sudden cardiac death risk factor burden (the number of traditional risk factors for sudden cardiac death) predicted QTVI. Of note, left ventricular mass index was also associated with QTVN. However, QTVI or QTVN provide a measure of overall QT variability measured during the whole duration of the ECG recording and does not take into account beat-to-beat variations, which might be equally, or even more important.

In our work, STV_{QT} showed correlation with different indices of LV hypertrophy. Myocardial hypertrophy is an inherent feature of HCM, the magnitude of which is shown to be related to adverse cardiac events, including sudden cardiac death, in patients with HCM [Spirito et al. 2000]. Indeed, pronounced myocardial hypertrophy, defined as left ventricular wall thickness >30 mm is an independent predictor for SCD in HCM, and a prophylactic ICD implantation for primary SCD prevention is suggested in such cases by current clinical guidelines [Gersh et al. 2011]. Left ventricular mass, measured by MRI, might be an even stronger predictor for such adverse events, as markedly increased LV mass index was proved to be more sensitive with regard to HCM-related death, than maximal wall thickness [Olivotto et al. 2008]. It is of note that ECG voltage parameters, indicating the magnitude of myocardial hypertrophy, also correlates with adverse events in HCM [Ostman-Smith et al. 2010].

Study limitations

The study was not designed to assess link between QT variability and increased risk of sudden cardiac death. With this regard, it would be necessary to prove that increased STV_{QT} is directly linked to SCD risk in HCM. Initially, it would be important to show whether STV_{QT} correlates with other established parameters defining increased SCD risk (occurrence of syncope, abnormal blood pressure response during exercise, non-sustained ventricular tachycardia (NSVT) on 24-hour Holter recording, etc.). Further, a direct association of STV_{QT} with SCD should be tested in a large patient cohort with HCM in a multivariate analysis.

5.4. Short-term variability of QT interval in patients with acromegaly

Cardiac rhythm abnormalities have been demonstrated by electrocardiogram and Holter studies in acromegaly [Colao et al. 2004; Rhee and Pearce 2009]. Resting electrocardiological changes included left axis deviation, increased QT intervals, septal Q-waves, ST-T wave depression, and late potentials in acromegalic patients [Rodrigues et al. 1989; Herrmann et al. 2001]. Atrial and ventricular ectopic beats, paroxysmal atrial fibrillation, paroxysmal supraventricular tachycardia, sick sinus syndrome, bundle branch block, and ventricular tachycardia were seen during physical exercise [Colao et al. 2004; Rhee and Pearce 2009]. The severity of ventricular arrhythmias correlated with increases in left ventricular mass and the frequency of ventricular premature complexes increased with the duration of acromegaly [Kahaly et al. 1992]. Fatti et al. [Fatti et al. 2006] detected abnormally long QTc interval before treatment in one-quarter of 30 acromegalic patients in a retrospective study.

Acromegalic cardiomyopathy is frequently present at diagnosis and the majority of patients with acromegaly meet echocardiographic criteria for left ventricular hypertrophy [Rhee and Pearce 2009]. A possible reason is that acromegalic patients are sometimes diagnosed only after longer duration (7–10 years) of the disease. No significant difference in left ventricle hypertrophy was observed between active and inactive acromegalic patients in our study, which may indicate that adequate treatment of acromegaly could not turn back the process. Cardiac performance of acromegalic patients during physical exercise depends on left ventricular diastolic function under resting condition [Spinelli et al. 2003]. Ciulla et al. [Ciulla et al. 1999] found elevated myocardial echoreflectivity and increased QTd in acromegalic patients and explained these changes by long-term, blood pressure-independent cardiac hypertrophy and prolonged exposure to high serum concentrations of hGH and IGF-1.

Patients with acromegaly may also develop congestive heart failure, the ratio was less than 3% (10 of 330 consecutive patients) in a study performed in 2 centers [Bihan et al.

2004]. Recent studies indicated that I_{Ks}, I_{Kr}, I_{K1} and I_{to} potassium channels were down-regulated [Kääb et al. 1998; Näbauer and Kääb 1999; Li et al. 2002] and the persistent or slowly-inactivating sodium current was also increased in chronic heart failure [Valdivia et al. 2005]. Additionally, acromegalic patients could also develop coronary heart disease and most patients have systemic complications affecting the Framingham risk score [Bogazzi et al. 2007]. hGH receptor antagonist therapy improved the score and reduced the risk for coronary heart diseases [Berg et al. 2010]. It should be noted that myocardial fibrosis occurring in acromegaly [Lie 1980] can also contribute to the underlying *arrhythmia substrate* in the heart due to disturbances in conduction.

Our observations indicate deterioration in autonomic function assessed by standard cardiovascular reflex tests in acromegalic patients. AN score was significantly worse in inactive acromegalic patients and there was no apparent difference between acromegalic subgroups in other autonomic parameters measured, which may suggest that these neuropathy parameters are long-term consequences of acromegaly and cannot be reverted by the control of the disease. Among the tests primarily reflecting parasympathetic functions, the Valsalva ratio and 30/15 ratio were significantly decreased in acromegaly, whereas heart rate variation during deep breathing was not changed significantly. The tests demonstrating sympathetic activity, such as systolic blood pressure fall after standing up and diastolic blood pressure increase after sustained handgrip, did not change significantly in acromegalic patients. These reflex tests indicate a moderate parasympathetic dysfunction in our study, which could represent a predisposition to proarrhythmic activity in acromegalic patients. Increased risk of sudden cardiac death and ventricular arrhythmia has been associated with decreased parasympathetic and increased sympathetic activity [Lahiri et al. 2008]. Parasympathetic activation has been considered as antiarrhythmic regarding the development of ventricular fibrillation in pathological settings; for a recent review see [Shen and Zipes 2014].

In conclusion, STV_{QT} is increased in patients with acromegaly while more conventional parameters of ventricular repolarization were unchanged. STV_{QT} values did not differ between active and inactive acromegalic patients and did not correlate with actual serum concentrations of hGH and IGF-1. These observations may suggest that elevated short-term beat-to-beat variability is a consequence of the disease and not related directly to current treatment or condition of the patient. The elevated STV_{QT} suggests instability of ventricular repolarization and may be an early indicator of increased liability to arrhythmia in patients with acromegaly. Further prospective clinical studies are needed to identify individual risk for

ventricular arrhythmias in acromegalic patients.

Study limitations

It is important to note that in the present study the duration of acromegaly from the diagnosis can be defined (10–30 years), however the exact onset of the disease is not determinable and furthermore the duration since the remission in the inactive acromegalics is also not known. Therefore the real exposure time of increased hGH level before the diagnosis and effective treatment of the disease is not known and our active and inactive patients groups can be heterogenous in this regard. Moreover, the actual hormone levels used for correlation calculations with echocardiography and other cardiovascular parameters do not necessarily correspond to the duration of the disease. Because of our unexpected negative correlation between the hGH level and the posterior wall thickness, further echocardiographical studies are warranted to examine the relationship between hGH and IGF-1 levels and echocardiographic parameters in a larger series of acromegalic patients. A prospective study on newly diagnosed acromegaly patients could answer the question whether effective treatment would have any time-related effects on the changes in STV_{QT} variability and autonomic cardiovascular functions.

6. New observations and conclusions

1. The main and novel finding of this study is that short-term beat-to-beat temporal variability of the QT interval (STV_{QT}) is significantly increased in professional soccer players compared to age-matched healthy volunteers. The increased STV_{QT} was accompanied by a prolonged QT, and a lengthened frequency corrected QT interval calculated by Fridericia and Hodges formulas in these athletes.

2. In this study we also found that all ECG repolarization parameters, including frequency corrected QT interval, QT dispersion, short-term beat-to-beat temporal variability of QT interval and the duration of the T wave from the peak to the end (Tpeak-Tend) were significantly increased in patients with hypertrophic cardiomyopathy. STV_{QT} exhibited the largest relative increase among the different parameters and also showed the best correlation with indices of left ventricular hypertrophy, i.e. maximal left ventricular wall thickness or magnetic resonance imaging derived left ventricular mass, indexed or unindexed for body surface area.

3. Although a connection between acromegaly and increased cardiovascular morbidity and mortality was established previously, this study is the first to demonstrate increased short-term beat-to-beat temporal variability of the QT interval in acromegalic patients. There was no significant difference between STV_{QT} values measured in clinically and biochemically active acromegalic patients and those in inactive patients, which may suggest that elevated STV_{QT} is related to the presence of acromegaly and not to the efficacy of the treatments applied.

4. In this study, we showed that a novel parameter of repolarization instability, the short-term beat-to-beat variability of the QT interval, is increased in cardiac hypertrophy initiated by different clinical conditions: competitive sport training, hypertrophic cardiomyopathy or acromegaly.

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I.

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Increased Short-Term Variability of the QT Interval in Professional Soccer Players: Possible Implications for Arrhythmia Prediction

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Abstract

Background: Sudden cardiac death in competitive athletes is rare but it is significantly more frequent than in the normal population. The exact cause is seldom established and is mostly attributed to ventricular fibrillation. Myocardial hypertrophy and slow heart rate, both characteristic changes in top athletes in response to physical conditioning, could be associated with increased propensity for ventricular arrhythmias. We investigated conventional ECG parameters and temporal short-term beat-to-beat variability of repolarization (STV_{QT}), a presumptive novel parameter for arrhythmia prediction, in professional soccer players.

Methods: Five-minute 12-lead electrocardiograms were recorded from professional soccer players (n = 76, all males, age 22.0±0.61 years) and age-matched healthy volunteers who do not participate in competitive sports (n = 76, all males, age 22.0±0.54 years). The ECGs were digitized and evaluated off-line. The temporal instability of beat-to-beat heart rate and repolarization were characterized by the calculation of short-term variability of the RR and QT intervals.

Results: Heart rate was significantly lower in professional soccer players at rest (61±1.2 vs. 72±1.5/min in controls). The QT interval was prolonged in players at rest (419±3.1 vs. 390±3.6 in controls, $p<0.001$). QTc was significantly longer in players compared to controls calculated with Fridericia and Hodges correction formulas. Importantly, STV_{QT} was significantly higher in players both at rest and immediately after the game compared to controls (4.8±0.14 and 4.3±0.14 vs. 3.5±0.10 ms, both $p<0.001$, respectively).

Conclusions: STV_{QT} is significantly higher in professional soccer players compared to age-matched controls, however, further studies are needed to relate this finding to increased arrhythmia propensity in this population.

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Introduction

Sports activities are undoubtedly beneficial that improve quality of life and life expectancy, however, a number of tragic athletic field deaths have been reported in recent years, attracting widespread media attention. A significant amount of these cases involved elite professional soccer players [1–3]. Sudden death among young athletes is rare (1:50 000–1:100 000), however, it is still 2–4 times more frequent than in age-matched controls [4]. Although a number of congenital and acquired cardiac diseases have been identified to be in the background of sudden cardiac death in athletes (for a recent review see Pigozzi and Rizzo) [5], approximately 5% of SCD cases in athletes no structural abnormalities were detected in the heart upon autopsy, that is the heart appeared completely normal [6,7]. The exact mechanism of SCD in these cases is not established and the cause is mostly attributed to ventricular fibrillation. In case of inconclusive autopsy findings, an ischemic origin of SCD is often suspected without hard evidence. In young athletes, SCD usually does not happen at peak performance, but during warmup, after training, or during a relatively inactive period of a competitive game, and ischemia specific signs on the ECG or proof of myocardial infarction is rarely found during or following these events. In addition, regular training is considered to lead to cardiac preconditioning, one of the most powerful cardioprotective (antiarrhythmic and antiischemic) mechanisms, that would

significantly increase the chance for survival during these episodes [8,9]. Therefore, as a cause myocardial ischemia in sudden cardiac death of young (<35 years) competitive athletes seems unlikely. Importantly, the scenario is quite different in older (>35 years) athletes, where ischemia is an important contributor to SCD, as reviewed by Pigozzi and Rizzo [5]. It should be noted that blunt trauma to the chest and concomitant cardiac contusion suffered during a game or training can also lead to electrocardiographic abnormalities [10] and sudden cardiac death [11].

Physical conditioning in competitive athletes induces cardiovascular adaptation including lower resting heart rate (increased vagal tone) and increased cardiac mass (hypertrophy) and volume as a consequence of increased demand on the cardiovascular system, called "athlete's heart", a physiological compensatory mechanism that reverses in most cases following the termination of sports activities [12]. Echocardiography studies show that myocardial hypertrophy develops following long-term sports activities [12–14]. The largest increase in left ventricular cavity and wall thickness (>75%) was measured in cyclists, cross-country skiers, rowers, football players, and water polo players, while weight lifters, fencers, and wrestlers exhibited smaller changes (<50%) [15].

Myocardial hypertrophy in pathological settings in humans [16–18] and in animal models, especially in the chronic atrioventricular (AV) block dog model [19] and heart failure models [20–22], has been shown to cause electrophysiological remodeling where the expression of different ion channels, including potassium channels critical for repolarization, and exchangers is altered. In particular, the detected downregulation of different potassium channels (i.e. I_{Ks}, I_{Kr} and I_{K1}) in the chronic AV block dog model has been associated with increased incidence of serious ventricular arrhythmias probably due to decreased repolarization reserve [19–23].

Furthermore, the duration of repolarization is cycle length dependent and low heart rate in athletes leads to prolonged repolarization. These changes can also be associated with increased propensity for ventricular arrhythmias, including Torsades de Pointes (TdP). It is conceivable that prolonged repolarization, increased spatial dispersion of repolarization and a possibly impaired repolarization reserve due to myocardial hypertrophy-induced downregulation of potassium currents might represent increased risk for the development of ventricular arrhythmias, including TdP that can degenerate into VF and lead to sudden cardiac death in athletes.

In theory, if athletes with no apparent structural cardiac abnormalities but with increased susceptibility for cardiac arrhythmias could be identified, current screening methods could be improved to further decrease the incidence of sudden cardiac death in young athletes. However, current techniques for the reliable prediction of TdP and other, potentially fatal ventricular arrhythmias remain unsatisfactory. Based on recent evidence, in addition to the prolonged QTc interval, the short-term variability (STV) of repolarization can probably more reliably predict the development of TdP both in humans [24] and in animal models with decreased repolarization reserve [25,26], and short-term variability of repolarization can increase when no noticable changes in the duration of cardiac repolarization are observed.

Since elevated STV of the QT interval (STV_{QT}) has been associated with latent repolarization disorders and increased suspectibility to serious ventricular arrhythmias in LQT patients and patients with dilated cardiomyopathy [27,28], the aim of this study was to compare conventional ECG parameters as well as the short-term beat-to-beat temporal variability of the RR and QT intervals of professional soccer players to age-matched controls who do not participate in competitive sports.

Methods

Ethics Statement

The studies described here were carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and were approved by the Scientific and Research Ethical Committee of the Medical Scientific Board at the Hungarian Ministry of Health (ETT-TUKEB), under ethical approval No. 4987-0/2010-1018EKU (338/PI/010). All subjects have given written informed consent of the study.

Study Subjects

The study population consisted of 76 male professional soccer players from the Hungarian Premier League (ages 16 to 39, mean 22.0±0.61 years; weight 76.2±0.95 kg, BMI 23.2±0.18 kg/m^2), and 76 male, age-matched healthy control sedentary subjects who did not participate in sports activities (age 15 to 39, mean 22.0±0.54 years; weight 77±1.7 kg, BMI 23.3±0.48 kg/m^2). Professional soccer players or age-matched controls were excluded from the study if they exhibited an excessive number (>5%) of ectopic atrial or ventricular beats, were in a rhythm other than normal sinus rhythm, had repolarization abnormalities (i.e. early repolarization pattern, T wave inversion and complete LBBB or RBBB), had a permanent pacemaker or had any other disorder such as serious retinopathy, symptomatic cardiac and/or pulmonary disease, acute metabolic disease, had excessive noise on the electrocardiographic signal that precluded analysis of the ECG waveform, were on any medication likely to affect the investigated parameters or consumed significant amount of food within 3 hours or drank alcohol, coffee or smoked within 10 hours. All of the control individuals and soccer players were of European descent.

Electrocardiography

Five-minute 12-lead electrocardiograms (lead II) were recorded from professional soccer players and age-matched healthy human volunteers using Cardiosys H-01 software (Experimetria Ltd., Budapest, Hungary) in the supine position. The ECGs were digitized and stored on a computer for later analysis. The RR, QT intervals were measured using automated algorithms as the average of 30 beats, the frequency corrected QT interval (QTc) was calculated using Bazett's ($QTc = QT/\sqrt{RR}$), Fridericia ($QTc = QT/[RR/1000]^{1/3}$), Framingham ($QTc = QT+[0.154 * \{1000-RR\}]$) and the Hodges formulas ($QTc = QT+1.75 * [\{60\,000/RR\}-60]$).

In athletes, baseline ECG recordings were taken before a competitive soccer game (Hungarian Premier League) and also approximately 20 minutes after the end of the game in the dressing room.

The calculation of the short-term beat-to-beat variability of repolarization was chosen since it is a relatively simple method that has been suggested as a future screening tool; moreover it has been shown in animal studies [25,26] and in certain patient populations [27,28] to reliably predict increased arrhythmia propensity.

Using 30 consecutive beats, RR and QT intervals were plotted against their respective previous interval and Poincaré plots were constructed as described previously [23]. The instability of beat-to-beat heart rate and repolarization were characterized by the short-term variability (STV) of the RR and QT intervals, and were calculated using the following formula: $STV = \sum|D_{n+1}-D_n|(30\times\sqrt{2})^{-1}$, where D is the duration of the RR or QT intervals. This calculation defines the STV as the mean distance of points perpendicular to the line of identity in the Poincaré plot.

Echocardiography

Echocardiographic measurements were performed at rest on 23 professional soccer players and 23 age-matched controls using a Dornier AI 4800 (Germany) echocardiograph with a 2.5 MHz transducer. Two-dimensionally guided M-mode recordings were obtained parasternally in accordance with the recommendations of the American Society of Echocardiography [29]. Measurements were carried out as described previously [30]. For purely logistic reasons, not all control individuals and soccer players were subjected to echocardiography.

Statistics

Body weight, BMI, age and ECG interval data are expressed as means ± standard error of the mean (S.E.M.). Comparisons between controls and soccer players were made using the unpaired Student's *t*-test. ECG parameters of athletes before and after the game were compared by one-way analysis of variance (ANOVA) followed by a paired *t*-test. A p value of <0.05 was considered significantly different. Statistical analyses were performed using Statistica for Windows (version 9).

Results

Echocardiography measurements in study subjects

Professional soccer players exhibited significantly higher values in interventricular septum, left ventricular posterior wall thickness and in left ventricular internal diameter during diastole compared to age-matched controls (Table 1). These results were not unexpected and were supportive of the presence of athlete's heart in these professional soccer players.

Heart rate, QT and QTc intervals in study subjects

The development of athlete's heart in response to long-term physical conditioning is characterized by increased vagal tone. As expected, the RR intervals in soccer players were significantly longer before the game compared to age-matched volunteers (Fig. 1A). Consequently, the heart rate of professional soccer players were lower compared to the control group before the game (Fig. 1B). However, after the soccer game the heart rates of athletes were higher than in controls (Fig. 1B).

The duration of cardiac repolarization is cycle length dependent where slower heart rates lead to prolonged repolarization. Accordingly, significantly longer QT intervals and were measured in soccer players before the game (Fig. 2). However, after the game these differences in QT intervals were not observed, since heart rates of athletes were similar to controls, while the QT intervals in soccer players were significantly shorter than before the game (Fig. 2.).

Table 1. Echocardiographic parameters in professional soccer players and age matched controls.

	IVSd (mm)	LVPWd (mm)	LVIDd (mm)	LVIDs (mm)
Controls	9.0±0.31	9.1±0.9	48.1±0.95	31.9±0.96
Soccer players	10.2±0.20**	9.9±0.14**	50.6±0.80*	33.3±0.66

IVSd: interventricular septum thickness during diastole; LVPWd: left ventricular posterior wall thickness; LVIDd, LVIDs: left ventricular internal diameter during diastole and systole; n = 23 in each group,
*$p<0.05$,
**$p<0.01$ vs. control.
doi:10.1371/journal.pone.0018751.t001

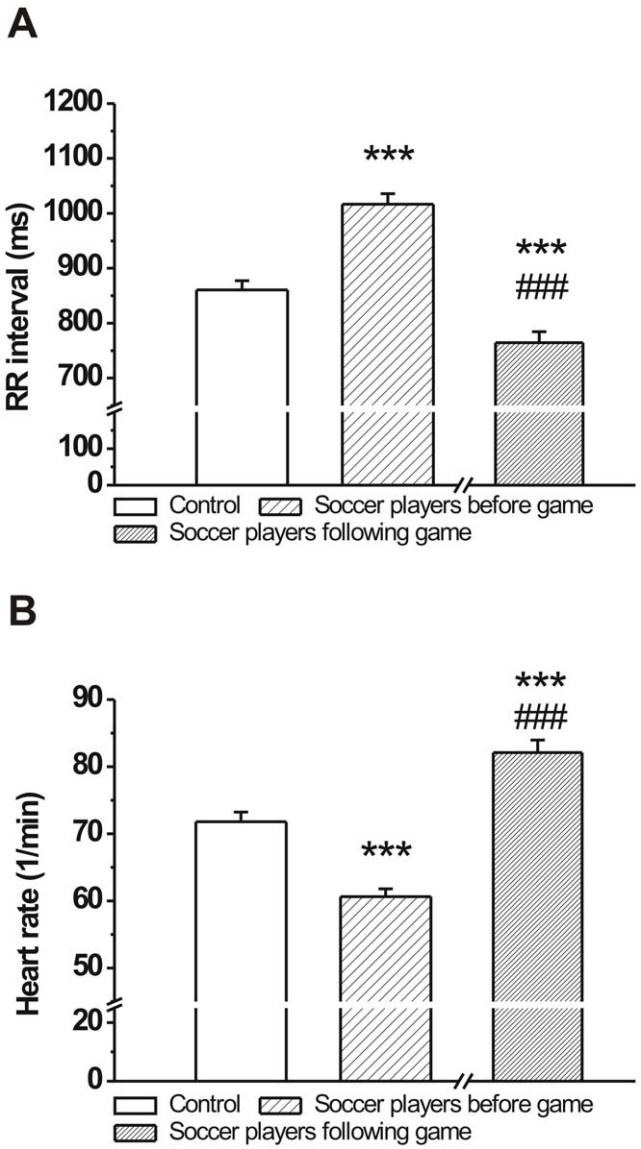

Figure 1. The RR interval and heart rate of age-matched controls and professional soccer players before and following a competitive game. A: The RR interval was significantly longer (**A**) and heart rate was significantly lower (**B**) in soccer players before the game compared to controls (n = 76 persons/group; ***$p<0.001$ vs. age-matched control; Means ± S.E.M.; ###$p<0.001$ vs. before game values).
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In order to reliably assess the duration of ventricular repolarization and to minimize the influence of changing heart rate on the QT interval, it is necessary to carry out frequency correction of the QT interval. However, recent work has shown that Bazett and Fridericia correction formulas described over 90 years ago [31,32] overestimate changes in QT interval [33]. Therefore, in this study, to calculate the frequency corrected QT interval (QTc) we also used Framingham and Hodges correction formulas shown to alter the accuracy of QT interval changes due to heart rate in a smaller degree [33]. In this regard, QTc calculated using the Bazett and Framingham formulae were not different in athletes before the game compared to controls (Fig. 3A and D), while QTc values calculated with the other two formulas were significantly longer in players before the game (Fig. 3B and D). In addition, QTc was significantly prolonged in soccer players

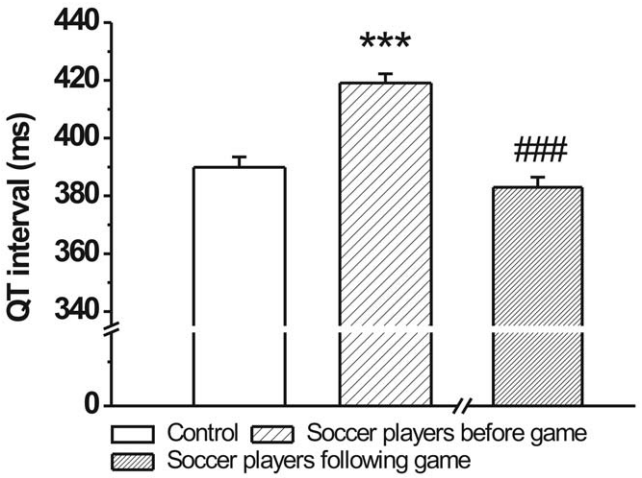

Figure 2. The QT intervals of age-matched controls and professional soccer players before and following a competitive game. The QT interval was significantly longer in soccer players before the game (n = 76 persons/group; ***$p<0.001$ vs. age-matched control; Means ± S.E.M.; ###$p<0.001$ vs. before game values).
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following the game compared to control values calculated with all correction formulae in the present study (Fig. 3A–D). Only QTc calculated with the Bazett formula showed a large and significant prolongation in soccer players after the game compared to pre-game values (Fig. 3A–D).

Short term beat-to-beat variability of the RR and QT intervals

To characterize the instability of cardiac ventricular repolarization, the short-term beat-to-beat variability of the QT interval was calculated in professional soccer players and age-matched controls. Since it is reasonable to assume that STV_{QT} can be, at least in part, influenced by the short-term variability of the RR interval, the STV_{RR} was also calculated in both groups. Soccer players before the competitive game exhibited a significantly larger STV_{RR} compared to controls, however, this difference disappeared after the game, when their heart rates were similar to controls (Fig. 4A).

As individual representative examples (Poincaré plots) and grouped average data show, the short-term beat-to-beat variability of the QT interval was significantly higher in soccer players compared to controls (Fig. 4B and C). Importantly, and unlike the STV_{RR}, the STV_{QT} was still significantly higher in soccer players compared to controls but was also reduced after the game compared to pre-game values (Fig. 4C). Histograms on Figures 5 and 6 show the distribution of QT interval and STV_{QT} values within the control and soccer player groups, respectively. The histograms clearly exhibit a shift to the right in the distribution of both QT intervals and STV_{QT} in soccer players before the competitive game compared to controls (Figs. 5A and 6A). However, while the distribution of QT intervals show a similar pattern in soccer players to controls following the game (Fig. 5B), the distribution pattern of STV_{QT} largely remained unchanged after the game (Fig. 6B). These results suggest that the increased STV_{QT} in soccer players is very unlikely caused by the prolonged QT interval itself in these athletes.

In six players before the game the STV_{QT} was markedly larger than the average STV_{QT} in the soccer player group (9.7, 7.2, 7.0, 7.0, 6.7, 6.7 and group average was 4.8 ± 0.14 ms; Fig. 6.). Since increased STV_{QT} has been associated with increased proarrhythmic risk in certain patient populations, the player who had 9.7 ms STV_{QT} was contacted and the measurement was repeated to yield a heart rate of 47/min and an STV_{QT} of 5.0 ms, however, before the repeated measurement he had been injured for 2 months. Whether this smaller (but still higher and close to the group average) STV_{QT} on repeated measurement was due to the well-known de-training phenomenon in an athlete who had been out of training due to injury was unclear.

Discussion

The main and novel finding of this study is that short-term beat-to-beat variability of the QT interval is significantly increased in professional soccer players compared to age-matched healthy volunteers. The increased STV_{QT} was accompanied by a prolonged QT, and a lengthened Fridericia and Hodges QT_c interval in these athletes.

In competitive athletes, the cardiovascular system adapts to chronic physical exercise by the development of ''athlete's heart'', characterized by lower resting heart rate (increased vagal tone), increased ventricular mass (hypertrophy) and volume to meet the increased demand.

In a reasonable animal experimental analogue for athlete's heart, in dogs with chronic AV block, myocardial hypertrophy and downregulation of potassium channels, most notably of the slow component of the delayed rectifier potassium current (I_{Ks}), develops [34]. These animals are more susceptible to lethal ventricular arrhythmias subjected to various challenges [19]. I_{Ks} has been identified as a key component in the somewhat redundant repolarizing capacity of the myocardium, termed repolarization reserve [35,36]. Repolarization reserve refers to the heart's compensating ability for loss or impaired function of one or more potassium currents critical for normal repolarization [37]. Impaired repolarization reserve does not necessarily lead to clinically manifest repolarization abnormalities on the ECG but makes the heart more susceptible to arrhythmia development [37–39]. The downregulation of repolarizing potassium currents, including the I_{K1}, I_{to}, I_{Kr} and I_{Ks} has also been shown both in animal models and patients with heart failure, leading to prolonged repolarization, increased dispersion of repolarization with concomitant increase in the incidence of serious ventricular arrhythmias [36,40–42]. It might be plausible that myocardial hypertrophy, whatever the underlying cause, may lead to potassium channel downregulation and may result in decreased repolarization reserve and increased propensity for arrhythmias including Torsades de Pointes, a characteristic arrhythmia that can degenerate into ventricular fibrillation and culminate in sudden cardiac death. It should be noted that autonomic and cardiac electrophysiological changes in dogs with chronic AV block possibly do not exactly mirror those developing in athlete's heart, however, few animal experimental data are available on the effect of endurance exercise training on cardiac hypertrophy and electrophysiology in species that are highly relevant to human, i.e. not in mice and rats. Some of these studies observed slowed heart rate, prolonged QT interval and ECG signs of cardiac hypertrophy in such animals [43,44]. Whether a ventricular electrical remodeling leading to decreased repolarization reserve develops in these animals is not known, however, it has been speculated that in top endurance athletes, downregulation of potassium channels might occur [45].

There are two main prerequisites for the development of TdP chaotic ventricular tachycardia and consequent fatal ventricular fibrillation: an arrhythmia substrate (prolonged repolarization,

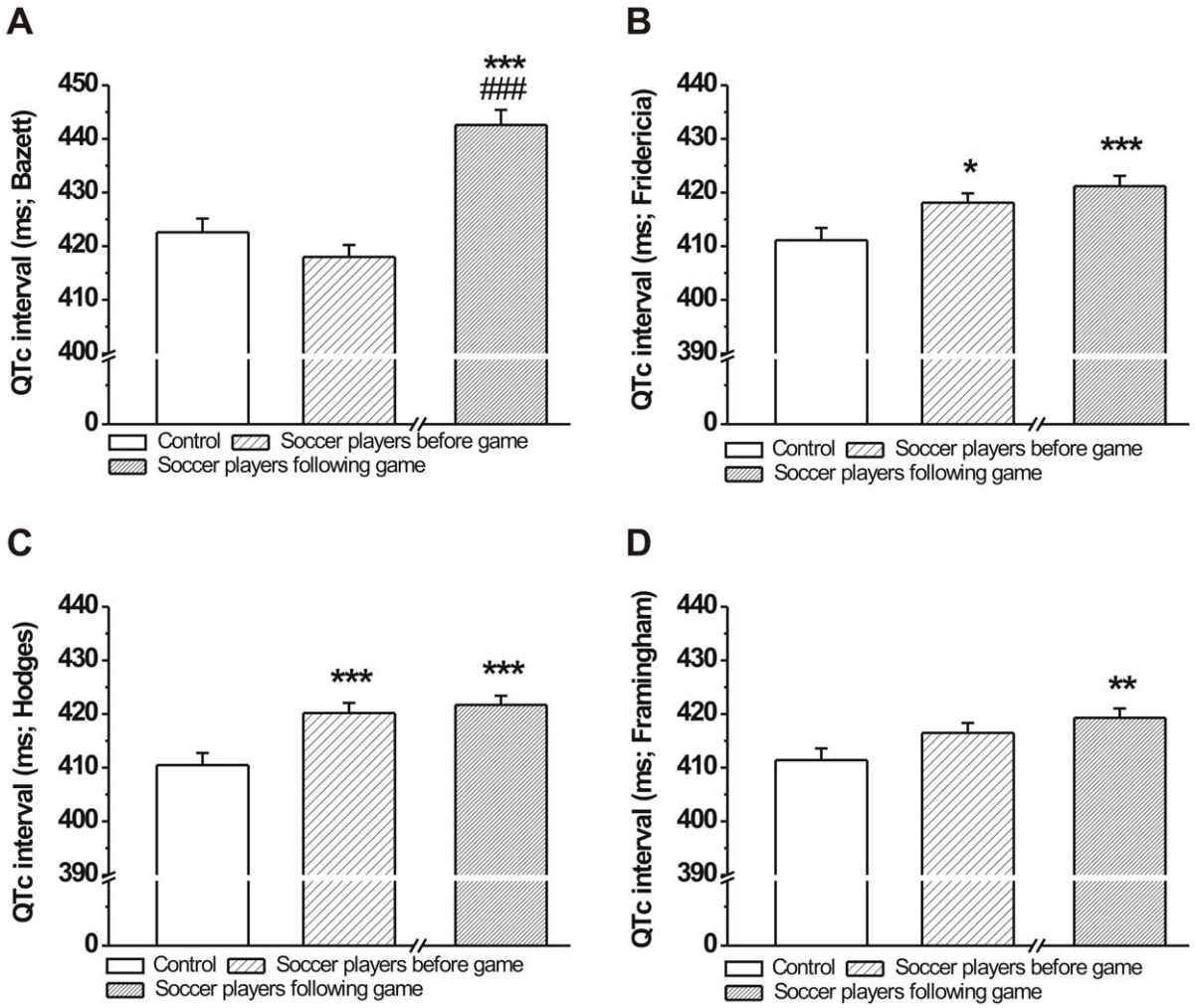

Figure 3. Frequency corrected QT interval of age-matched controls and professional soccer players before and following a competitive game. QTc interval calculated with the Bazett formula was not different in soccer players before the game and was significantly prolonged after the game (**A**). QTc values calculated with the Fridericia and Hodges formulae but not the Framingham formula showed significant difference between groups before the game, and none of the three calculations yielded any difference between before and after game values in soccer players (**B**, **C** and **D**; n = 76 persons/group; Means ± S.E.M.; *p<0.05; **p<0.01; ***p<0.001 vs. age-matched control; ###p<0.001 vs. before game values).
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spatial and temporal inhomogeneity of repolarization creating re-entry paths) and a trigger (extrasystole in the vulnerable period) for the initiation of the arrhythmic event. Increased vagal tone in athletes lowers heart rate that favors prolonged repolarization and increased inhomogeneity. The possible potassium channel down-regulation due to myocardial hypertrophy also prolongs repolarization and reduces repolarization reserve. In some athletes, loss of function mutations of repolarizing potassium channels and/or gain of function mutations of sodium channels may be present. In these individuals with impaired repolarization reserve, additional, most likely moderate potassium channel blocking effects can provoke TdP arrhythmias that, in some cases lead to ventricular fibrillation. Theoretically, in this scenario a number of conditions, compounds and dietary constituents can precipitate such events of sudden cardiac death (as recently reviewed by Varró and Baczkó) [46]. These may include serum electrolyte changes (e.g. hypokalemia when fluid intake is not adequate), food and drinks containing flavonoids with HERG inhibitory effects [47], medications with various degree of HERG and other potassium channel blocking properties. In this regard, the celecoxib has been shown to block Kv2.1 channels [48]. Non-steroid antiinflammatory drugs are used by athletes very often and in large doses to treat sports injuries. These factors can create and enhance the arrhythmia substrate in athletes, while elevated intracellular cAMP levels due to increased sympathetic disharge may contribute to trigger extrasystole generation via increased pacemaker (I_f) current [49] and/or increased L-type calcium current [50].

The reliable identification of patients at risk for serious ventricular arrhythmia and sudden cardiac death remains elusive. Accumulating evidence suggests that QT interval prolongation alone cannot reliably predict the development of TdP since cardiac repolarization reserve may be reduced without siginificant changes in the duration of cardiac repolarization. A number of clinical studies [27,28,51] and data from *in vivo* animal experiments using species that are electrophysiologically relevant for humans in regard of ventricular repolarization [26,52–54] as well as *in vitro* studies [55,56] strongly suggest that the short-term variability of the duration of repolarization (i.e. QT interval on the ECG) may

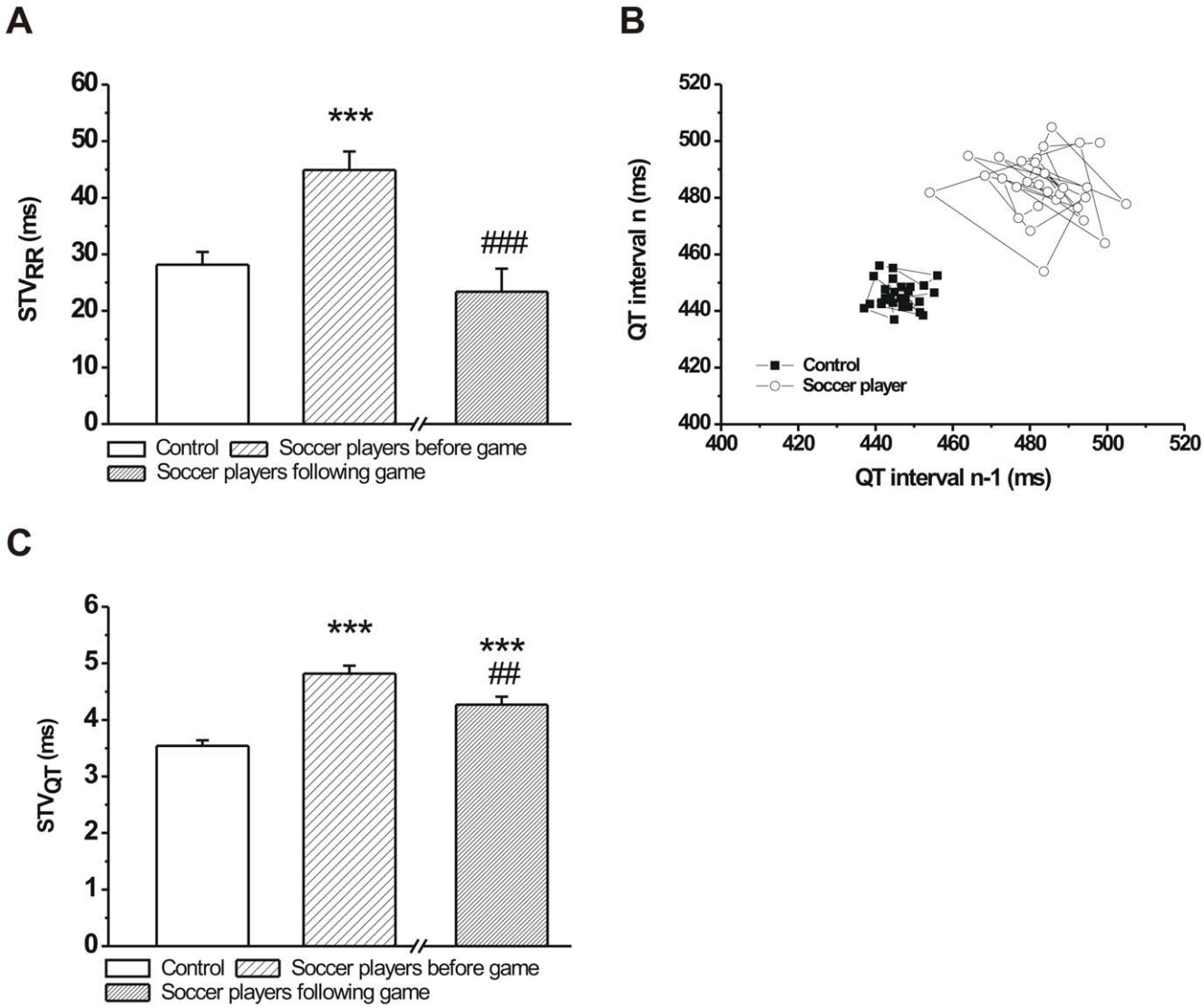

Figure 4. Short-term beat-to-beat temporal variability of the RR (STV_{RR}) and QT (STV_{QT}) intervals in age-matched controls and professional soccer players before and following a competitive game. Soccer players had a significantly higher STV_{RR} compared to controls before the game. STV_{RR} was similar to controls in soccer players immediately after the game (**A**). Poincaré plots illustrating short-term temporal variability of the QT interval at rest in a control individual and in a professional soccer player before the game. Note the shift of QT values to the right and upwards in the soccer player indicating QT prolongation and the increased scattering of QT interval values in the soccer player demonstrating increased beat-to-beat variability of the QT interval (**B**). Short-term QT variability was significantly higher in soccer players both before and after the game compared to controls but also decreased in players compared to pre-game values (**C**). (n = 76 persons/group; Means ± S.E.M.; ***$p<0.001$ vs. age-matched control; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ vs. before game values).
doi:10.1371/journal.pone.0018751.g004

be a better novel parameter to predict serious ventricular arrhythmias. These studies found that increased STV_{QT} correlated with elevated incidence of lethal ventricular arrhythmias and sudden cardiac death. Importantly, the patients and experimental animals in all of these studies had narrowed repolarization reserve, albeit due to different mechanisms, ranging from pharmacological inhibition of repolarizing potassium channels to downregulation of potassium currents during electrical remodeling and including mutations in ion channels leading to congenital long QT syndromes. Therefore, based on these studies and the present results, the elevated temporal beat-to-beat variability in competitive soccer players may indicate a larger repolarization instability and an increased propensity for ventricular arrhythmias. Notably, physical deconditioning in trained athletes with no cardiac structural abnormalities decreased the incidence and complexity of ventricular tachyarrhytmias [57].

Study limitations

For purely practical and logistic reasons echocardiographic assessment of all soccer players and all controls were not performed in this study. However, our echocardiography data randomly performed on 23 soccer players and 23 controls support the findings of a number of previous studies showing that endurance athletes, including soccer players, as part of the cardiovascular system's physiological response to long-term intense physical training, develop athlete's heart that features myocardial hypertrophy [12–14,58,59]. In our study, professional soccer players from the first division were enlisted who participated in

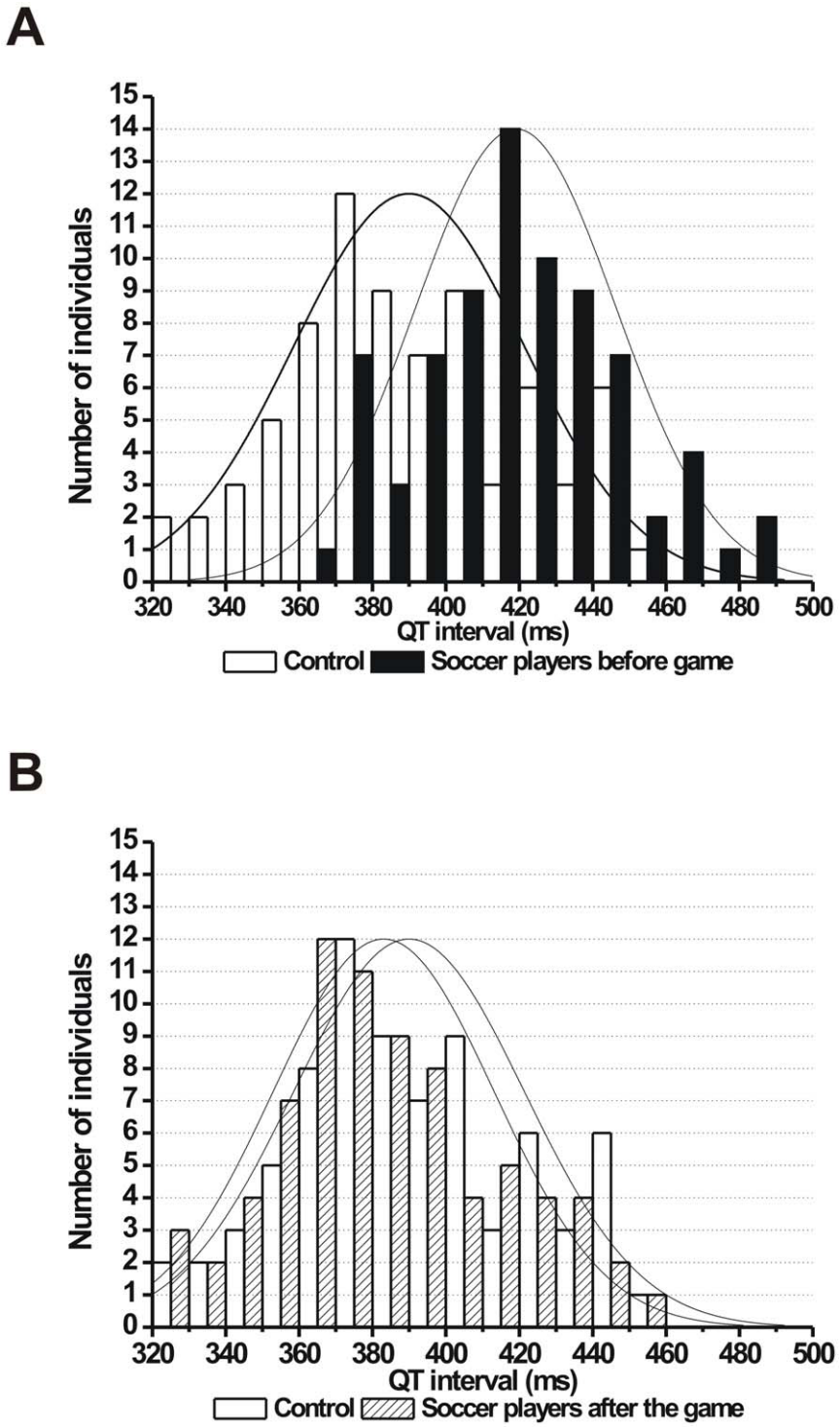

Figure 5. Histograms showing the distribution of the QT interval. (**A**) Controls (empty bars) and soccer players before game (full bars) and (**B**) controls (empty bars) and soccer players after the game (hashed bars). Bin size is 10 ms. (n = 76 persons/group).
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rigorous endurance training schedule for years based on international standards. We also found they had significantly decreased resting heart rate and consequent prolongation of the QT interval, both are characteristics of athlete's heart. Based on the above it is assumed that the echocardiography data are representative for their respective groups.

Since the duration of repolarization is cycle length dependent, variability in the RR interval could influence QT variability. Based on our results, the influence of STV_{RR} on STV_{QT} cannot be ruled out, however, in professional soccer players STV_{RR} was reduced and was similar to control values after the competitive game while STV_{QT} remained significantly higher,

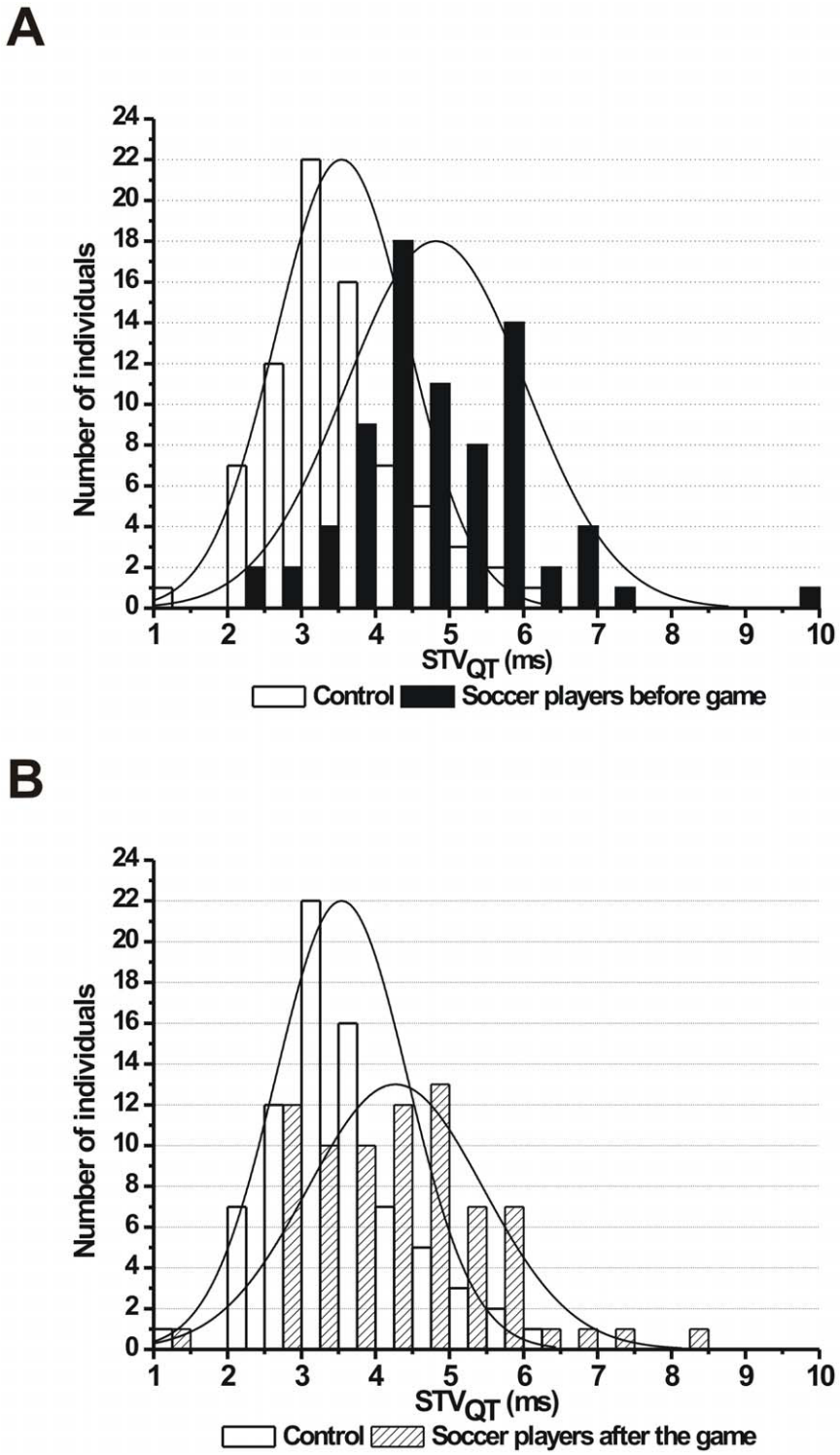

Figure 6. Histograms showing the distribution of short-term beat-to-beat variability of the QT interval (STV$_{QT}$). (**A**) Controls (empty bars) and soccer players before game (full bars) and (**B**) controls (empty bars) and soccer players after the game (hashed bars). Bin size is 0.5 ms. (n = 76 persons/group).
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strongly suggesting that STV_{QT} was increased irrespective of changes in STV_{RR}. The short-term variability of the monophasic action potential was found to be partially influenced by pacing cycle length and was moderately decreased at faster cycle lengths in anesthetized dogs with chronic AV block characterized by marked bradycardia and myocardial hypertrophy [52].

We found elevated STV_{QT} in the present study in soccer players, who were chosen as subjects of the study since the different degrees of cardiac hypertrophy found in athletes of

different sports [15] may have significant influence on cardiac repolarization, and sudden cardiac death associated with sports activity has been reported most commonly in Europe among soccer players [60]. However, the changes in STV_{QT} can be different in other sports, depending on type, intensity and duration of various training schedules used in different sports. Further studies are needed to confirm whether STV_{QT} elevation is uniformly present in other endurance athletes.

Conclusions

In conclusion, the short-term variability of the QT interval is elevated in professional soccer players, which, according to our present knowledge, might indicate increased repolarization instability even without any underlying cardiac disease. Based on the available literature, decreased repolarization reserve due to downregulation of certain repolarizing potassium currents associated with myocardial hypertrophy may underlie these changes. As clinical and animal studies illustrate, increased STV_{QT} may be more predictive for the development of serious ventricular arrhythmias than conventional ECG parameters, such as the prolongation of the QT_c interval. In our study, some soccer players exhibited greatly increased STV_{QT} even when compared to other players, suggesting that it may be beneficial to screen athletes for elevated repolarization instability by adding the relatively low cost STV_{QT} determination to routine ECG examinations. Individual athletes with large STV_{QT} could be then subjected to more detailed and sophisticated examinations (e.g. evaluation of possible mutations in potassium channel protein encoding genes) to carefully evaluate their vulnerability to ventricular arrhythmias and sudden cardiac death. Importantly, our results further support the inclusion of ECG in preparticipation athlete screening expertly worked out by Corrado *et al.* [61], with the notion that calculation of STV_{QT} could also be added to the ECG evaluation in case our findings can be confirmed in a broader athlete population. It is important to emphasize that no arrhythmias were observed among soccer players in this study and further, more comprehensive investigations are needed to establish whether the higher STV_{QT} relates to higher arrhythmia propensity in this population. This study also warrants the investigation of STV_{QT} in top athletes with various training levels and in a larger number of athletes preferably taking part in different types of sports activities to enable investigators to make a direct link between STV_{QT}, arrhythmia susceptibility and sudden cardiac death in top competitive athletes.

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Author Contributions

Conceived and designed the experiments: CL MT TW JGP AV PH. Performed the experiments: CL AO ZsK EB ET AU GP. Analyzed the data: AO ZK AU EB IB. Contributed reagents/materials/analysis tools: GP TW PH AV. Wrote the paper: IB AV CL. Drafted and edited the manuscript: IB CL PH AV JGP.

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Short-term beat-to-beat variability of the QT interval is increased and correlates with parameters of left ventricular hypertrophy in patients with hypertrophic cardiomyopathy

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Abstract

Stratification models for prediction of sudden cardiac death (SCD) are inappropriate in patients with hypertrophic cardiomyopathy (HCM). We investigated conventional ECG repolarization parameters and the beat-to-beat short-term QT interval variability (QT-STV), a new parameter of proarrhythmic risk, in 37 patients with HCM (21 males, average age 48±15 years). Resting ECGs were recorded for 5 minutes and the frequency corrected QT interval (QTc), QT dispersion (QTd), beat-to-beat short-term variability of QT interval (QT-STV) and the duration of terminal part of T waves (Tpeak-Tend) were calculated. While all repolarization parameters were increased significantly in patients with HCM compared to controls (QTc: 488±61 vs. 434±23 ms, $p<0.0001$; QT-STV: 4.5±2 vs. 3.2±1 ms, $p=0.0002$; Tpeak-Tend duration: 107±27 vs. 91±10 ms, $p=0.0015$; QTd: 47±17 vs. 34±9 ms, $p=0.0002$), QT-STV had the highest relative increase (+41%). QT-STV also showed the best correlation with indices of left ventricular hypertrophy, i.e. maximal left ventricular (LV) wall thickness normalized for body surface area (BSA; $r=0.461$, $p=0.004$) or LV mass (determined by cardiac magnetic resonance imaging) normalized for BSA ($r=0.455$, $p=0.015$). In summary, beat-to-beat QT-STV showed the most marked increase in patients with HCM and may represent a novel marker which merits further testing for increased SCD risk in HCM.

Key words: hypertrophic cardiomyopathy, repolarization, QT prolongation, QT dispersion, Tpeak-Tend interval, short-term beat-to-beat variability of the QT interval, left ventricular hypertrophy, sudden cardiac death

Introduction

Hypertrophic cardiomyopathy (HCM) is a common inherited cardiac disease with a prevalence of one in 500 in the general population (Maron et al. 1995), characterized by marked but variable left ventricular hypertrophy and myocardial fibrosis (Gersh et al. 2011). HCM is associated with lethal ventricular arrhythmias (Gersh et al. 2011; Maron 2002; Wigle et al. 1995), and it is the most common cause of sudden cardiac death (SCD) in young individuals (Decker et al. 2009; Maron 2010) and in competitive athletes younger than 35 years (Maron et al. 2009). The reliable assessment of SCD risk in individual HCM patients and the identification of patients for implantable cardioverter defibrillator (ICD) implantation, the most effective intervention for SCD prevention, are critically important. Currently models for SCD risk stratification uses non-invasive conventional clinical markers including family history of SCD, unexplained recent syncope, multiple repetitive non-sustained ventricular tachycardia identified on ambulatory ECG, abnormal blood pressure response upon exercise and massive (≥30mm thickness) LV hypertrophy (Gersh et al. 2011; Maron 2010; Monserrat et al. 2003; Spirito et al. 2009; Spirito et al. 2000). Additional risk modifier factors that help making the decision regarding ICD implantation include marked left ventricular outflow tract obstruction, high risk genotype involving multiple sarcomere mutations, extensive and diffuse late gadolinium enhancement on MRI, apical aneurism in the left ventricle, and coronary artery disease (Maron 2010). However, all established SCD risk factors exhibit a low positive predictive value and the current SCD risk assessment algorithm in HCM is still considered incomplete and hampered by lack of sufficient evidence for all elements (McKeown and Muir 2013). This notion is supported by SCD events in HCM patients who were not considered to be at high risk for SCD (Maron et al. 2008).

ECG parameters have been studied for their utility as prognostic non-invasive markers in SCD risk assessment in HCM. The prolongation of the frequency corrected QT interval

(QTc) and increased QTc dispersion (characterizing spatial repolarization heterogeneity) were observed in patients with HCM (Buja et al. 1993; Dritsas et al. 1992; Yi et al. 1998). However, QTc prolongation and QTc dispersion have been found not to be predictive for SCD in HCM patients (Maron et al. 2001; Yi et al. 1998). The Tpeak-Tend interval, another ECG parameter representing spatial (including transmural) dispersion of repolarization (Antzelevitch 2001), has been shown to more reliably predict the development of Torsades des Pointes (TdP) polymorphic ventricular tachycardia in congenital (Schwartz et al. 2001) and acquired long QT syndromes than QTc prolongation or increased QT dispersion (Yamaguchi et al. 2003). The prolongation of the Tpeak-Tend interval has been associated with SCD in the Oregon Sudden Unexpected Death Study (Panikkath et al. 2011). In a small number of HCM patients with a cardiac troponin I gene mutation, the Tpeak-Tend interval was associated with SCD (Shimizu et al. 2002). In addition to increased spatial dispersion of repolarization, increased temporal variability of cardiac ventricular repolarization has been associated with increased propensity for ventricular arrhythmias and SCD in patients with different cardiovascular pathologies (Atiga et al. 1998; Haigney et al. 2004; Piccirillo et al. 2007). The beat-to-beat short-term temporal variability of the QT interval (QT-STV) has been suggested as a novel ECG parameter for more reliable prediction of development of serious ventricular arrhythmias both in experimental animal and clinical studies [for a current review see (Varkevisser et al. 2012)]. However, according to our knowledge, the characterization of QT-STV in patients with HCM has not been addressed in the literature so far.

Therefore, the aim of the present study was to compare conventional ECG parameters of repolarization and QT-STV in HCM patients and age matched healthy volunteers, and to investigate whether there was a relationship between QT-STV and the morphologic parameters of cardiac hypertrophy, proved to be predictors of higher SCD risk, obtained by cardiac echocardiography and MRI in patients with HCM.

Methods

Ethics Statement

The studies described here were carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and were approved by the Scientific and Research Ethical Committee of the Medical Scientific Board at the Hungarian Ministry of Health (ETT-TUKEB), under ethical approval No. 4987-0/2010-1018EKU (338/PI/010).

Study Subjects

Thirty-seven consecutive patients with hypertrophic cardiomyopathy (HCM) were enrolled into the study. The diagnosis of HCM was based on established diagnostic criteria (Gersh et al. 2011). Among the 37 HCM patients, 24 patients were taking beta blockers and 8 patients were taking verapamil as first line therapy. Three patients were taking cardiac medications known to prolong QT interval (two were taking amiodarone and one was taking propafenone). None of the patients were on any other drug therapy with known QT-interval prolonging effect.

A total of 37 age- and gender-matched healthy volunteers (mean age 43±12 years, males/females 21/16) without a history or evidence of heart disease was enrolled in the study as controls. Body mass index was significantly lower in the control group (25±4 kg/m^2 vs. 28±6, p=0.007, see Table 1). All of the control individuals and HCM patients were of Caucasian origin.

Electrocardiography

12-lead electrocardiograms were continuously recorded for 5 min at rest, in the supine position to obtain signals with the least amount of motion artefact. In all leads the ECG signals were digitized at 2000 Hz sampling rate with a multichannel data acquisition system

(Cardiosys A01 software, Experimetria Ltd., Budapest, Hungary; MDE Heidelberg GMBH, Heidelberg, Germany) connected to a PC and stored for later off-line analysis.

Out of the repolarization parameters we analyzed the frequency corrected QT interval (QTc) using Bazett's (QTc = QT/√RR), Fridericia (QTc = QT/[RR/1000]1/3), Framingham (QTc = QT + [0.154 * {1000-RR}]) and the Hodges formulas (QTc = QT + 1.75 * [60 000/RR-60]), the QT dispersion (QTd), the PQ and QRS intervals, the duration of terminal part of T waves (Tpeak-Tend) and the short-term variability of QT interval (QT-STV).

The RR, QT intervals and duration of the T wave from the peak to the end (Tpeak-Tend) intervals were measured automatically in 30 consecutive beats (minimum number of intervals needed for variability measurements), were checked by the investigator and manually corrected if needed and were calculated as the average of 30 beats. Heart rate correction of QT intervals was performed by the Bazett's, Fridericia, Framingham and Hodges formulas, and QTc interval duration was defined as the mean duration of all QTc intervals measured. As no statistical difference was noted between different correction methods with regard to corrected QT interval, QTc corrected with the Bazett's formula was used for further comparisons. The PQ and QRS intervals were measured as the average of 15 consecutive beats. All measurements were carried out using lead II and in case of excessive noise in lead II, lead V5.

To characterize the temporal instability of beat-to-beat repolarization, Poincare' plots of the QT intervals were constructed, where each QT value is plotted against its former value. QT-STV was calculated using the following formula: $QT\text{-}STV=\sum|D_{n+1}-D_n|/(30x\sqrt{2})$, where D represents the duration of the QT intervals. This calculation defines the QT-STV as the mean distance of points perpendicular to the line of identity in the Poincaré plot.

Patients were excluded if they had history and/or clinical documentation of significant comorbidity [e.g. known coronary artery disease, severe obstructive lung disease, pulmonary

embolism, primary pulmonary hypertension, valvular heart disease, pericardial disease, moderate–severe renal failure (serum creatinine >2 mg/dl), moderate–severe anemia (hemoglobin <11 g/dl)]. All patients with complete left bundle branch block, with rhythm other than normal sinus rhythm (e.g. atrial fibrillation, pacemaker-dependent rhythm), with excessive (>5%) ectopic atrial or ventricular beats or with excessive noise on the electrocardiographic signal that precluded analysis of the ECG waveform, were also excluded. Patients were instructed not to consume significant amount of food within 3 hours, to drink alcohol, coffee or to smoke within 10 hours.

Echocardiography

All HCM patients and controls underwent transthoracic echocardiographic examination. Two-dimensional echocardiographic images were obtained by a commercially available Toshiba Powervision 8000 echocardiography equipment, in a number of cross-sectional planes using standard imaging positions to determine standard morphological and functional parameters [left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), ejection fraction (EF), left atrial diameter (LA), resting left ventricular outflow tract (LVOT) peak gradient]. Maximal left ventricular wall thickness (LVmax) was defined as the largest wall thickness of the left ventricle at any left ventricular segment. LVmax was also normalized for body surface area (LVmax BSA). Echocardiographic parameters for the HCM group are shown in Table 1.

Cardiac MRI

In all HCM patients, cardiac magnetic resonance imaging (MRI) was carried out to determine the left ventricular mass (LVM). MRI assessments were performed in supine position with the head first on a commercially available 1.5T scanner (Signa Excite HDxT, GE Medical

Systems) using a phased-array body coil. Sequential gradient-echo short-axis cine images (base to apex; slice thickness: 8 mm; field of view: 43 mm; matrix: 224x224; repetition time: 100 milliseconds) covering the entire LV were acquired during breath hold after normal expiration. Three long-axis images (2-, 3- and 4-chamber views) were also acquired. The acquisition was triggered by ECG. The gradient-echo short-axis images were used to measure LVM by planimetry of the manually defined endocardial and epicardial borders on each short-axis image covering the entire LV. The measurement was performed in both end diastole and end systole to enable calculation of LV ejection fraction. Papillary muscles were not included in the LVM. LVM was also normalized for body surface area (LVM BSA).

Statistics

All data are expressed as mean±standard deviation (SD). Comparisons between HCM patients and controls for the study variables were done using the independent samples Student's *t* test for normally distributed parameters. Normal distribution was verified by the Kolmogorov-Smirnov test. Degree of association between two variables was expressed by the Pearson correlation coefficient (r). The statistical analyses were performed using the MedCalc software package (ver. 14.12.0). Statistical significance was accepted at the $p<0.05$ level.

Results

1. Electrocardiographic parameters in HCM patients and controls

Comparison of ECG parameters between HCM patients and controls are presented in Table 2. Patients with HCM exhibited significantly increased RR, PQ and QRS intervals. QTc was significantly prolonged in HCM patients (Figure 1, A), regardless of the method used for QTc correction (using Bazett's, Fridericia, Framingham or the Hodges formulas). There was no difference between QTc intervals in the HCM group obtained by the different correction methods. The terminal part of the T wave, the Tpeak-Tend interval was also markedly longer in HCM patients (Figure 1, B). QT dispersion (Figure 1, C) and short-term QT variability was also markedly increased in patients with HCM (Figure 1, D). The largest relative increase among the different parameters was seen with regard to short-term QT variability with a relative increase of 41%. Differences between the HCM and control groups remained highly significant when we excluded patients taking QT prolonging drugs (amiodarone or propafenone, n=3) from the comparisons. BMI or obesity status did not correlate with either of the repolarization parameters.

2. Correlation of repolarization parameters in HCM patients

Correlation between different repolarization parameters, QT dispersion and short-term QT variability are given in Table 3. The QTc prolongation correlated significantly with the prolongation of the Tpeak-Tend interval, but not the QRS width, indicating that the QTc prolongation was, at least in part, due to the prolongation of the terminal phase of the T wave. Short-term QT variability showed a relatively strong correlation with the QTc prolongation and with, to a lesser extent, with the Tpeak-Tend interval. The QT dispersion did not correlate with any of the repolarization parameters.

3. Correlation between repolarization parameters and echocardiographic parameters in HCM patients

Correlation between ECG repolarization parameters and echocardiography parameters in HCM patients showed no correlation between these parameters except for a weak correlation between short-term QT variability and left ventricular end-systolic diameter or left ventricular ejection fraction (data not shown).

4. Correlation between repolarization parameters and indices of left ventricular hypertrophy in HCM patients

Correlation between repolarization parameters and indices of left ventricular hypertrophy (maximal left ventricular wall thickness and left ventricular mass, measured by cardiac magnetic resonance imaging) with or without normalization for body surface area are shown in Table 4. Degree of correlation increased with normalization in almost all comparisons. Short-term QT variability showed significant, albeit modest correlation, with both un-normalized and normalized indices of left ventricular hypertrophy (LVmax; LVmax BSA and LVM BSA, Figures 2 and 3). Tpeak-Tend interval also correlated significantly with some of the hypertrophy parameters, but showed no significant correlation to the most reliable hypertrophy parameter, i. e. LVM indexed for BSA.

Discussion

In this study we showed that all ECG repolarization parameters, including frequency corrected QT interval (QTc), QT dispersion (QTd), beat-to-beat short-term variability of QT interval (QT-STV) and the duration of terminal part of T waves (Tpeak-Tend) were significantly increased in patients with HCM. QT-STV showed the largest relative increase among the different parameters and also showed the best correlation with indices of left ventricular hypertrophy, i.e. maximal left ventricular wall thickness or MRI derived LV mass, indexed or unindexed for body surface area.

IIypertrophic cardiomyopathy is characterized by morphological and structural changes, including left ventricular hypertrophy, myocardial fibrosis, myofiber disarray, and small vessel disease among them, that may represent an arrhythmogenic substrate of the disease (Maron 2002). Remodeling in HCM is a progressive process (Olivotto et al. 2012) and a very recent study highlighted a close correlation between the development of adverse remodeling and increased risk for SCD in HCM patients (Vriesendorp et al. 2014). In chronic heart failure, structural remodeling is accompanied by electrical remodeling that includes profound changes in the expression of voltage gated depolarizing and repolarizing ionic currents and exchangers resulting in decreased cardiomyocyte repolarizing capacity [for a comprehensive review see (Nattel et al. 2007)]. This decreased repolarizing capacity can be brought about by an increase in depolarizing currents (Na^+ and Ca^{2+}) and decreased potassium channel densities (particularly I_{K1}, I_{to} and I_{Ks}), resulting in action potential prolongation manifested as QT prolongation on the surface ECG (Beuckelmann et al. 1993; Li et al. 2004; Tomaselli and Marban 1999). Increased action potential prolongation favors increased Ca^{2+} influx that in turn can facilitate delayed afterdepolarization (DAD) and arrhythmia development (Bers et al. 2006). Prolongation of repolarization can also precipitate serious ventricular re-entry type

arrhythmias via promoting early afterdepolarization (EAD) generation (Michael et al. 2009; Zeng and Rudy 1995). A particularly interesting observation is the increase in slowly inactivating, late sodium current ($I_{Na,late}$), that has been shown to prolong repolarization in heart failure and also to contribute to arrhythmogenesis (Valdivia et al. 2005). Most interestingly, these elements of arrhythmogenic electrical remodeling have not only been described in congestive heart failure and pathologies leading to cardiac hypertrophy, but a very recent study identified similar changes in cardiomyocytes isolated from HCM patients (Coppini et al. 2013). The decreased repolarization capacity due to HCM leads to markedly impaired repolarization reserve (Varro and Baczko 2011) and increased arrhythmia susceptibility in HCM, where even drugs or dietary constituents with only mild repolarization inhibitory effects can provoke serious ventricular arrhythmias and SCD.

Impaired repolarization reserve and temporal repolarization instability can be characterized by the calculation of the short-term beat-to-beat variability of the QT interval (QT-STV) which characterizes differences in the duration of QT intervals in consecutive heart beats. This parameter has emerged as a novel parameter for assessing pro-arrhythmia risk in arrhythmogenic cardiac diseases and has been shown to be a more reliable estimate and predictor of pro-arrhythmic risk associated with impaired repolarization reserve as opposed to more conventional ECG parameters of repolarization (Berger 2003; Varkevisser et al. 2012). A number of animal experimental and clinical studies (Hinterseer et al. 2009; Hinterseer et al. 2010; Lengyel et al. 2007; Thomsen et al. 2004; van Opstal et al. 2001) found that QT-STV was increased and showed a better correlation with subsequent arrhythmias than repolarization prolongation in animals or patients with decreased repolarization reserve later exhibiting serious ventricular arrhythmias and/or SCD.

QT variability has been previously shown to be increased in patients with HCM. The normalized QT variability index (QTVI), measured as described by Berger et al, was shown to be higher in HCM patients than in controls (Atiga et al. 2000), and the greatest abnormality was detected in patients with malignant HCM mutations (i.e. Arg403Gln mutation of the beta myosin heavy chain gene). In a recent paper (Magri et al. 2014) several myocardial repolarization parameters, including normalized QT variability (QTVN) and QT variability index (QTVI) were shown to be associated with the presence and extent of late gadolinium enhancement (LGE) detected on cardiac magnetic resonance in patients with hypertrophic cardiomyopathy. Both QTVN and QTVI were higher in patients with LGE. Among other parameters, the extent of LGE and sudden cardiac death risk factor burden (the number of traditional risk factors for sudden cardiac death) predicted QTVI. Of note, left ventricular mass index was also associated with QTVN. However, QTVI or QTVN provide a measure of overall QT variability measured during the whole duration of the ECG recording and does not take into account beat-to-beat variations, which might be equally, or even more important.

In our work, QT-STV showed correlation with different indices of LV hypertrophy. Myocardial hypertrophy is an inherent feature of HCM, the magnitude of which is shown to be related to adverse cardiac events, including sudden cardiac death, in patients with HCM (Spirito et al. 2000). Indeed, pronounced myocardial hypertrophy, defined as left ventricular wall thickness >30 mm is an independent predictor for SCD in HCM, and a prophylactic ICD implantation for primary SCD prevention is suggested in such a cases by current clinical guidelines (Gersh et al. 2011). Left ventricular mass, measured by MRI, might be an even stronger predictor for such adverse events, as markedly increased LV mass index was proved to be more sensitive with regard to HCM-related death, than maximal wall thickness (Olivotto et al. 2008). It is of note that ECG voltage parameters, indicating the magnitude of myocardial hypertrophy, also correlates with adverse events in HCM (Ostman-Smith et al. 2010).

In summary, we have provided evidence that among ECG repolarization parameters, the beat-to-beat short-term variability of QT interval showed a pronounced increase in patients with HCM. QT-STV also correlated with indices of left ventricular hypertrophy, known to be associated with SCD in HCM. We conclude that QT-STV may represent a novel candidate non-invasive marker which should be tested for increased SCD risk in patients with HCM.

Study limitations:

The study was not designed to assess link between QT variability and increased risk of sudden cardiac death risk. With this regard, it would be necessary to prove that increased QT-STV is directly linked to SCD risk in HCM. Initially, it would be important to show whether QT-STV correlates with other established parameters defining increased SCD risk (occurrence of syncope, abnormal blood pressure response during exercise, NSVT on 24-hour Holter recording, etc.). Further, a direct association of QT-STV with SCD should be tested in a large patient cohort with HCM in a multivariate analysis.

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Conflict of interest

None.

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Table 1. The main demographical, clinical and echocardiographic parameters of the patients with HCM and controls.

	Control	HCM
n	**37**	**37**
Sex (male/female)	**21/16**	**21/16**
Age (year)	**43 ± 12**	**48 ± 15**
BMI (kg/m^2)	**25 ± 4**	**28 ± 6***
BMI ≥30/≥25/<25 kg/m^2 (n)	**5/13/19**	**11/16/10**
NYHA class 0/1/2/3 (n)	**19/18/0/0**	**0/5/26/6****
EF (%)	**68 ± 6**	**69 ± 9**
LVEDD (mm)	**48 ± 4**	**46 ± 7**
LVESD (mm)	**30 ± 4**	**27 ± 7***
IVS (mm)	**9 ± 1**	**20 ± 6*****
PW (mm)	**9 ± 1**	**11 ± 2*****

Note: Values are represented as mean ± SD. Values are considered statistically significantly different at $p<0.05$ (*), $p<0.001$ (***) or $p<0.0001$ (****) compared with the control group. HCM, hypertrophic cardiomyopathy; BMI, body mass index; NYHA, New York Heart Association; EF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IVS, interventricular septum thickness; PW, posterior wall thickness.

Table 2. Electrocardiographic parameters in HCM patients and controls.

	Control	HCM	Relative difference (%)
RR (ms)	**867 ± 119**	**947 ± 140***	**9.2**
PQ (ms)	**152 ± 17**	**166 ± 30***	**8.5**
QRS (ms)	**96 ± 7**	**112 ± 17*****	**15.6**
QT (ms)	**401 ± 24**	**473 ± 66*****	**18.0**
QTc (ms) Bazett	**434 ± 23**	**488 ± 61*****	**13.0**
QTc (ms) Fridericia	**422 ± 20**	**483 ± 60*****	**14.7**
QTc (ms) Framingham	**423 ± 19**	**481 ± 60*****	**14.5**
QTc (ms) Hodges	**420 ± 18**	**482 ± 60*****	**15.0**
QTd (ms)	**34 ± 9**	**47 ± 17****	**37.1**
Tpeak-Tend (ms)	**91 ± 10**	**107 ± 27***	**18.9**
QT-STV (ms)	**3.2 ± 1**	**4.5 ± 2****	**40.6**

Note: Values are represented as mean ± SD and relative difference between the two groups in %. Values are considered statistically significantly different at $p<0.05$ (*), $p<0.001$ (**), $p<0.0001$ (***) compared with the control group. n = 37 in each group. HCM, hypertrophic cardiomyopathy; QTc, heart rate correction of QT intervals (calculated by the Bazett's, Fridericia, Framingham and Hodges formulas); QTd, QT dispersion; Tpeak-Tend, duration of the T wave from the peak to the end; QT-STV, beat-to-beat short-term temporal variability of the QT interval.

Table 3. Correlation of repolarization parameters in HCM patients.

	QRS	Tpeak-Tend	QTd	QT-STV
QTc	**0.284**	**0.527*** **	**- 0.013**	**0.616*** **
Tpeak-Tend	**0.299**	–	**0.018**	**0.378***
QTd	**0.253**	–	–	**- 0.228**

Note: Values are represented as Pearson correlation coefficient. Values are considered statistically significantly different at $p<0.05$ (*), $P<0.001$ (***); n = 37. HCM, hypertrophic cardiomyopathy; QTc, heart rate correction of QT intervals (calculated by the Bazett's formula); QTd, QT dispersion; Tpeak-Tend, duration of the T wave from the peak to the end; QT-STV, beat-to-beat short-term temporal variability of the QT interval.

Table 4. Correlation between repolarization parameters and morphologic parameters of hypertrophy in HCM patients.

	QTc	Tpeak-Tend	QTd	QT-STV
IVS (mm)	**0.099**	**0.344***	**- 0.144**	**0.285**
LVmax (mm)	**0.216**	**0.450****	**- 0.238**	**0.381***
LVmax BSA (mm/m^2)	**0.360***	**0.451****	**- 0.129**	**0.461****
LVM (g)	**0.037**	**0.241**	**- 0.128**	**0.273**
LVM BSA (g/m^2)	**0.195**	**0.348**	**- 0.116**	**0.455***

Note: Values are represented as Pearson correlation coefficient. Values are considered statistically significantly different at $p<0.05$ (*), $p<0.01$ (**); n = 37. HCM, hypertrophic cardiomyopathy; QTc, heart rate correction of QT intervals (calculated by the Bazett's formula); Tpeak-Tend, duration of the T wave from the peak to the end; QTd, QT dispersion; QT-STV, beat-to-beat short-term temporal variability of the QT interval; IVS, interventricular septum; LVmax, maximal left ventricular wall thickness; LVmax BSA, maximal left ventricular wall thickness normalized for body surface area; LVM, left ventricular mass; LVM BSA, left ventricular mass normalized for body surface area.

Figure captions

Figure 1. Box and whisker plots illustrating significant differences between patients with HCM and controls with regard to A) the frequency corrected QT interval (QTc); B) the duration of terminal part of T waves (Tpeak-Tend); C) QT dispersion (QTd); D) beat-to-beat short-term variability of QT interval (QT-STV). The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outside (open squares) and far out values (filled dots) which are displayed as separate points.

Figure 2. Scatterplot illustrating the correlation between maximal left ventricular wall thickness normalized for body surface area (LVmax BSA) and A) the frequency corrected QT interval (QTc); B) the duration of terminal part of T waves (Tpeak-Tend); C) QT dispersion (QTd); D) beat-to-beat short-term variability of QT interval (QT-STV).

Figure 3. Scatterplot illustrating the correlation between left ventricular mass normalized for body surface area (LVM BSA) and A) the frequency corrected QT interval (QTc); B) the duration of terminal part of T waves (Tpeak-Tend); C) QT dispersion (QTd); D) beat-to-beat short-term variability of QT interval (QT-STV).

III.

PLOS ONE

RESEARCH ARTICLE

Increased Short-Term Beat-To-Beat Variability of QT Interval in Patients with Acromegaly

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Abstract

Cardiovascular diseases, including ventricular arrhythmias are responsible for increased mortality in patients with acromegaly. Acromegaly may cause repolarization abnormalities such as QT prolongation and impairment of repolarization reserve enhancing liability to arrhythmia. The aim of this study was to determine the short-term beat-to-beat QT variability in patients with acromegaly. Thirty acromegalic patients (23 women and 7 men, mean age ±SD: 55.7±10.4 years) were compared with age- and sex-matched volunteers (mean age 51.3±7.6 years). Cardiac repolarization parameters including frequency corrected QT interval, PQ and QRS intervals, duration of terminal part of T waves (T_{peak}-T_{end}) and short-term variability of QT interval were evaluated. All acromegalic patients and controls underwent transthoracic echocardiographic examination. Autonomic function was assessed by means of five standard cardiovascular reflex tests. Comparison of the two groups revealed no significant differences in the conventional ECG parameters of repolarization (QT: 401.1±30.6 ms *vs* 389.3±16.5 ms, corrected QT interval: 430.1±18.6 ms *vs* 425.6±17.3 ms, QT dispersion: 38.2±13.2 ms *vs* 36.6±10.2 ms; acromegaly *vs* control, respectively). However, short-term beat-to-beat QT variability was significantly increased in acromegalic patients (4.23 ±1.03 ms *vs* 3.02±0.80, $P<0.0001$). There were significant differences between the two groups in the echocardiographic dimensions (left ventricular end diastolic diameter: 52.6 ±5.4 mm *vs* 48.0±3.9 mm, left ventricular end systolic diameter: 32.3±5.2 mm *vs* 29.1±4.4 mm, interventricular septum: 11.1±2.2 mm *vs* 8.8±0.7 mm, posterior wall of left ventricle: 10.8±1.4 mm *vs* 8.9±0.7 mm, $P<0.05$, respectively). Short-term beat-to-beat QT variability was elevated in patients with acromegaly in spite of unchanged conventional parameters of ventricular repolarization. This enhanced temporal QT variability may be an early indicator of increased liability to arrhythmia.

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Introduction

Hypertension, left ventricular hypertrophy, asymmetric septal hypertrophy, cardiomyopathy, and congestive heart failure are well-known cardiovascular complications of acromegaly caused by pituitary tumors [1]. Excessive secretion of growth hormone and IGF-1 can result in major structural and functional changes in cardiac system and arrhythmias, hypertension, and valvular heart disease are present in up to 60% of patients by the time of the diagnosis of acromegaly [1]. Clinical data suggest that a specific cardiomyopathy develops in acromegaly associated with life-threatening dysrhythmias [2]. Complex morphological and functional remodeling may be partially reversed by effective control of growth hormone and IGF-1 concentrations [2]. Moreover, acromegaly can also be associated with cardiovascular diseases contributing to increased mortality among patients [1,2]. Effective control of acromegaly with pegvisomant, a GH receptor antagonist, led to a significant improvement of Framingham risk score, and reduced the likelihood for development of coronary heart diseases, too [3].

Electrocardiographic studies also indicated cardiac rhythm abnormalities in patients with acromegaly [2,4,5]. Dysrhythmias, atrioventricular conduction delay and sick sinus syndrome were reported in sudden death in acromegalic heart disease [6]. Rodrigues *et al.* [7] found arrhythmias in 41% of 34 patients with acromegaly, thirteen patients had frequent ventricular extrasystoles and there were long periods of asymptomatic ventricular bigeminy in one patient. Both prevalence and severity of ventricular arrhythmia were significantly higher in acromegalic patients compared to controls, and the frequency of ventricular premature complexes increased with duration of acromegaly [8]. Higher incidence of late potential positivity, QT interval prolongation and higher QT dispersion in acromegaly patients might explain the increased susceptibility to sudden cardiac deaths from ventricular tachyarrhythmias [9]. Herrmann *et al.* [10] detected late potentials, a predictor of ventricular dysrhythmias in a signal-averaged electrocardiogram, in 56% of patients with active acromegaly (n = 16) and 6% of well-controlled patients (n = 32) and speculated that late potentials might indicate myocardial remodeling in acromegaly. In another study, the occurrence of late potentials were 22.9% in acromegalic *vs* 2.9% in control patients ($P<0.001$; n = 70 in both groups) and a significant association with premature ventricular complexes were seen by means of 24-h Holter ECG recording [11]. Maffei *et al.* [11] also described that one case of sudden cardiac death occurred during the observation period, and this acromegalic patient had late potentials, left ventricular hypertrophy, Lown 4 premature ventricular complexes, and non-sustained ventricular tachycardia.

The identification of patients with risk for serious ventricular arrhythmia and sudden cardiac death could be important during the diagnosis and treatment of acromegaly. Fatti *et al.* [12] described that octreotide, a somatostatin analogue, could improve abnormally prolonged QT interval in acromegalic patients. Treatment with GH receptor antagonist Pegvisomant for 6-month and 18-month (long-term) also improved rhythm abnormalities in 13 patients suffering from acromegaly [13]. However, QT interval prolongation alone cannot reliably predict the development of ventricular arrhythmias including the chaotic ventricular tachycardia, Torsades de Pointes (TdP), since cardiac repolarization reserve may be reduced even without significant changes in the duration of cardiac repolarization [14]. The short-term variability of the duration of repolarization (STV_{QT}) [15] might be a better parameter to predict serious ventricular arrhythmias and sudden cardiac death, as it has been suggested by both animal experimental work [16–19] and recent clinical studies [20–23]. On the basis of these observations, Varkevisser *et al.* [24] suggested that beat-to-beat STV_{QT} could be superior to QT interval prolongation in identifying patient populations at risk for ventricular arrhythmias and might be able to accurately predict individual risk. The aim of the present study was to determine beat-to-beat QT variability in patients with acromegaly.

Methods

Patient Population

Patients with acromegaly who are followed at the 1st Department of Internal Medicine in Szeged, Hungary, were eligible for this study. Patients were excluded if they had excessive (>5%) ectopic atrial or ventricular beats, were in a rhythm other than normal sinus, had repolarization abnormalities (i.e. early repolarization pattern, T wave inversion and complete left bundle branch block or right bundle branch block), had a permanent pacemaker or any other disorders such as serious retinopathy, symptomatic cardiac and pulmonary disease, acute metabolic disease, had excessive noise on the electrocardiographic signal that precluded analysis of the ECG waveform, were on any medication likely to affect the investigated ECG parameters or consumed significant amount of food within 3 hours or drank alcohol, coffee or smoked within 10 hours.

We studied 30 acromegalic patients, 7 males and 23 females with the age of 55.7 ± 10.4 years (all values presented are mean ± SD). A total of 30 age- and sex-matched volunteers (mean age 51.3 ± 7.6 years) without a history or evidence of heart disease were enrolled in the study as controls. All of the control individuals and acromegaly patients were of Caucasian origin. Acromegalic patient group was also divided to subgroups on the basis of medical examinations and serum diagnostic tests performed (hGH rhythm, IGF-1 level, HbA1c concentration, oral glucose tolerance test). Active acromegalic subgroup included acromegalic patients before hypophysectomy or with remnant hormonally active tumor after hypophysectomy (n = 14), as well as treated acromegalic patients with high serum IGF-1 levels in spite of long-acting somatostatin analogue octreotide or lanreotide therapy received (n = 3). Inactive acromegalic subgroup included acromegalic patients after successful hypophysectomy (n = 6) and treated acromegalic patients with an age-sex-appropriate normal IGF-1 and/or random GH < 1 ng ml^{-1} and/or nadir GH after OGTT < 0.4 ng ml^{-1} during bromocriptine, pegvisomant, or long-acting somatostatin analogue octreotide treatments (n = 7). There was no significant age difference between the active and inactive patients (56.4 ± 11.5 vs 54.8 ± 9.2 years, respectively; $P = 0.69$). In acromegaly group, there were 18 hypertensive patients receiving therapy (for details see S1 Table) and 12 normotensive subjects, whereas volunteers in control group did not receive antihypertensive treatment.

The studies described here were carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and were approved by the Scientific and Research Ethical Committee of the Medical Research Council at the Hungarian Ministry of Health (ETT-TUKEB), under ethical approval No. 4987-0/2010-1018EKU (338/PI/010). All subjects have given written informed consent of the study.

Data Collection and Analysis

12-lead electrocardiograms were continuously recorded for 5 min at rest, in the supine position to obtain signals with the least amount of motion artefact. In all leads the ECG signals were digitized at 2000 Hz sampling rate with a multichannel data acquisition system (Cardiosys-H1 software, Experimetria Ltd, Budapest, Hungary) connected to a personal computer and stored for later off-line analysis.

Out of the repolarization parameters we analyzed the frequency corrected QT interval (QTc) using Bazett's ($QTc = QT/\sqrt{RR}$), Fridericia ($QTc = QT/[RR/1000]1/3$), Framingham ($QTc = QT + [0.154 * \{1000\text{-}RR\}]$) and the Hodges formulas ($QTc = QT + 1.75 * [60\ 000/RR\text{-}60]$), the QT dispersion (QTd), the PQ and QRS intervals, the duration of terminal part of T waves (T_{peak}-T_{end}) and the short-term variability of QT interval (STV_{QT}).

The RR and QT intervals and duration of the T wave from the peak to the end (T_{peak}-T_{end}) intervals were measured automatically in 30 consecutive beats (minimum number of intervals needed for variability measurements), were checked by the same expert investigator of the team for all ECGs and manually corrected if needed and were calculated as the average of 30 beats. QTc interval duration was defined as the mean duration of all QTc intervals measured. The PQ and QRS intervals were measured as the average of 15 consecutive beats. All measurements were carried out using limb lead II and in case of excessive noise in limb lead II and lead V5.

To characterize the temporal instability of beat-to-beat repolarization, Poincaré plots of the QT intervals were constructed, where each QT value is plotted against its former value. STV_{QT} was calculated using the following formula: $STV_{QT} = \Sigma|QT_{n+1}\text{-}QT_n|/(30x\sqrt{2})$, where QT represents the duration of the QT interval. This calculation defines the STV as the mean distance of points perpendicular to the line of identity in the Poincaré plot and relies on previous mathematical analysis (25).

All acromegalic patients and controls also underwent transthoracic echocardiographic examination performed by the single observer blinded to subject data for all participants. Two-dimensional echocardiographic images were obtained by Toshiba Powervision 8000 echocardiography equipment, in a number of cross-sectional planes using standard transducer positions to determine standard morphological and functional parameters.

Autonomic function was assessed by means of five standard cardiovascular reflex tests: the heart rate (HR) responses to deep breathing and to standing up (30/15 ratio), the Valsalva maneuver, the systolic blood pressure response to standing up, and the diastolic pressure change during a sustained handgrip. A score was created to express the severity of autonomic neuropathy (AN), based on the results of the five tests (normal: 0, borderline: 1, abnormal: 2). The total score was in the interval of 0 to 10.

Fasting venous blood samples were obtained from each patient and controls for the determination of serum glucose, blood urea nitrogen, creatinine, sodium and potassium levels. GH and IGF-1 were measured by chemiluminescent immunoassay (IMMULITE 1000 Immunoassay System, Siemens. GH measurement comparator: Recombinant 98/574; detection limit: 0.01 ng ml^{-1}; intra-assay coefficients of variation: 6.0%; interassay coefficients of variation: 6.2%. IGF-1 measurement comparator: WHO IRP 87/517; detection limit: 20.0 ng ml^{-1}; intra-assay coefficients of variation: 5.0%; interassay coefficients of variation: 9.0%).

Statistical Analysis

All data are expressed as mean±SD. Comparisons between acromegalic patients and controls for the study variables were done using the unpaired Student's *t* test for normally distributed parameters, nonparametric Mann-Whitney U test for non-normal distributions, and linear regression for revealing correlations. The statistical analyses were performed using the SPSS 16.0 software package. Statistical significance was accepted at the $P<0.05$ level.

Results

Clinical data of acromegalic patients and control subjects

In 30 acromegalic patients studied, body weight and mean body mass index (BMI) were significantly higher ($P<0.001$ for both parameters) than those in age- and sex-matched volunteers (Table 1). Mean systolic blood pressure did not differ significantly between control subjects and acromegalic patients receiving standard care and treatment, however, acromegalic patients had higher diastolic blood pressure ($P<0.05$). The incidence of high blood pressure was 7/30 in control and 13/30 in acromegaly groups during the actual measurements. Average serum glucose and HbA1c values were also similar in both groups; incidences of diabetes were 0/30 and

Table 1. Clinical data of acromegalic patients and age-matched control subjects.

	Control	Acromegaly
Age (years)	51.3 ± 7.6	55.7 ± 10.4
Weight (kg)	68.9 ± 14.7	87.7 ± 19.3**
Height (cm)	165.1 ± 10.5	168.9 ± 8.2
BMI (kg m^{-2})	25.1 ± 3.7	30.6 ± 5.3**
Systolic BP (mmHg)	126.9 ± 13.4	133.2 ± 17.7
Diastolic BP (mmHg)	75.5 ± 8.5	82.7 ± 12.4*
0 min glucose (mmol l^{-1})	5.04 ± 0.52	5.40 ± 0.71
120 min glucose (mmol l^{-1})	5.30 ± 1.30	6.30 ± 2.53
HbA1c (%)	5.70 ± 0.50	5.90 ± 0.74
hGH nadir following OGTT (ng ml^{-1})	1.02 ± 1.42	2.72 ± 2.13*
IGF-1 (ng ml^{-1})	151.0 ± 51.4	370.1 ± 311.8*
IGF-1 x ULN	0.50 ± 0.33 x ULN	1.66 ± 1.59 x ULN**

Abbreviations: BMI: body mass index; BP: blood pressure; HbA1c: glycosylated hemoglobin; hGH: human growth hormone; IGF-1: insulin-like growth factor-1; OGTT: oral glucose tolerance test; ULN: upper limit of normal value; n = 30 in each group,

*P<0.05,

**P<0.001 *vs* controls.

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1/30 in control and acromegaly groups, respectively. Incidence of impaired glucose tolerance was 0/30 in control and 4/30 in acromegalic subjects. Significant differences were seen in serum hGH (P = 0.0028) and IGF-1 (P = 0.0013) levels between acromegalic and control groups. There was no significant difference in nadir value of hGH during oral glucose tolerance test (OGTT) between active (3.40 ± 2.10 ng/ml) and inactive (1.80 ± 1.86 ng/ml) acromegalic subgroups. However, significantly higher average hGH (7.00 ± 6.73 ng/ml *vs* 2.03 ± 2.86 ng/ml, P = 0.0180) and IGF-1 (501.3 ± 359.6 ng/ml *vs* 198.5 ± 79.1 ng/ml, P = 0.0060) concentrations were measured in active acromegalic subgroup compared to inactive one.

Echocardiography measurements in study subjects

There were significant differences between the two groups in the echocardiographic dimensions. Patients with acromegaly exhibited significantly higher values in left ventricular end diastolic and end systolic diameter and in interventricular septum, left ventricular posterior wall thickness compared to age-matched controls (Table 2). These results were not unexpected and

Table 2. Echocardiographic parameters in patients with acromegaly and age-matched controls.

	Control	Acromegaly
EF (%)	70.6 ± 5.4	67.2 ± 6.9*
EDD (mm)	48.0 ± 3.9	52.6 ± 5.4*
ESD (mm)	29.1 ± 4.4	32.3 ± 5.2*
IVS (mm)	8.8 ± 0.7	11.1 ± 2.2**
PW (mm)	8.9 ± 0.7	10.8 ± 1.4**

Abbreviations: EF: ejection fraction; EDD: left ventricular end diastolic diameter; ESD: left ventricular end systolic diameter; IVS: interventricular septum; PW: posterior wall of left ventricle; n = 30 in each group

*P<0.05

**P<0.0001 *vs* controls

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were supportive of the presence of myocardial hypertrophy of in the acromegalic patients and could be related to the duration and activity of the disease. However, no significant difference was detected in the echocardiographic parameters measured between active and inactive acromegaly subgroups (EF: 66.7 ± 7.4% *vs* 67.9 ± 6.4%, EDD: 52.6 ± 4.9 mm *vs* 52.7 ± 6.1 mm, ESD: 32.1 ± 5.9 mm *vs* 32.5 ±4.3 mm, IVS: 10.6 ± 1.3 mm *vs* 11.8 ± 3.0 mm, PW: 10.5 ± 1.3 mm *vs* 11.2 ± 1.6 mm, respectively).

Electrocardiographic parameters in study subjects

Comparison of the two groups (acromegalic patients *vs* control) revealed no significant differences in heart rate, the PQ, QRS and QT intervals and the QT dispersion. In order to reliably assess the duration of ventricular repolarization and to minimize the influence of changing heart rate on the QT interval, frequency correction of the QT interval (QTc) was performed by the Bazett, Fridericia, Framingham and Hodges formulas. QTc values calculated with all the four formulas showed no significant differences between acromegalic patients and controls. However, the T_{peak}-T_{end} interval was significantly increased in acromegalic patients compared to controls (Table 3). Electrocardiographic parameters tended to be shorter in active acromegaly subgroup compared to the data measured in inactive subgroup (RR: 859.8 ± 134.5 ms *vs* 901.0 ± 178.2 ms, not significant (NS), QT: 392.7 ± 28.5 ms *vs* 412.0 ±29.5 ms, NS; QTc Bazett: 425.0 ± 16.0 ms *vs* 436.8 ±20.3 ms, NS; QTc Friderica: 413.7 ± 15.6 ms *vs* 428.1 ±16.6 ms, P = 0.0220; QTc Framingham: 414.3 ± 14.9 ms *vs* 427.3 ±17.8 ms, P = 0.0376; QTc Hodges: 412.5 ± 15.4 ms *vs* 426.9 ±16.7 ms, P = 0.0209; T_{peak}—T_{end}: 86.0 ± 15.7 ms *vs* 84.7 ±11.0 ms, NS, respectively).

Short-term beat-to-beat variability of the QT intervals

To characterize the instability of cardiac ventricular repolarization, the short-term beat-to-beat variability of the QT interval was calculated in acromegalic patients and age-matched controls. As individual representative examples (Poincaré plots, Fig 1) and grouped average data show STV_{QT} was significantly increased by 36% in acromegalic patients compared to controls

Table 3. ECG parameters in patients with acromegaly and age-matched controls.

	Control	Acromegaly
RR (ms)	840.0 ± 75.0	877.6 ± 153.4
PQ (ms)	158.2 ± 17.7	158.0 ± 17.3
QRS (ms)	92.2 ± 6.5	95.3 ± 8.4
QT (ms)	389.3 ± 16.5	401.1 ± 30.0
QTc (ms) Bazett	425.6 ± 17.3	430.1 ± 18.6
QTc (ms) Fridericia	413.1 ± 14.5	419.9 ± 17.4
QTc (ms) Framingham	414.0 ± 13.7	419.9 ± 17.2
QTc (ms) Hodges	410.4 ± 13.8	418.7 ± 17.3*
QTd (ms)	36.6 ± 10.2	38.2 ± 13.2
T_{peak}-T_{end} (ms)	80.0 ± 10.3	85.5 ± 13.6
STV_{QT} (ms)	3.02 ± 0.80	4.23 ± 0.10**

Abbreviations: QTc: frequency corrected QT interval; QTd: QT dispersion; STV_{QT}: short-term variability of QT interval; n = 30 in each group,

*P<0.05

**P<0.001 *vs* controls.

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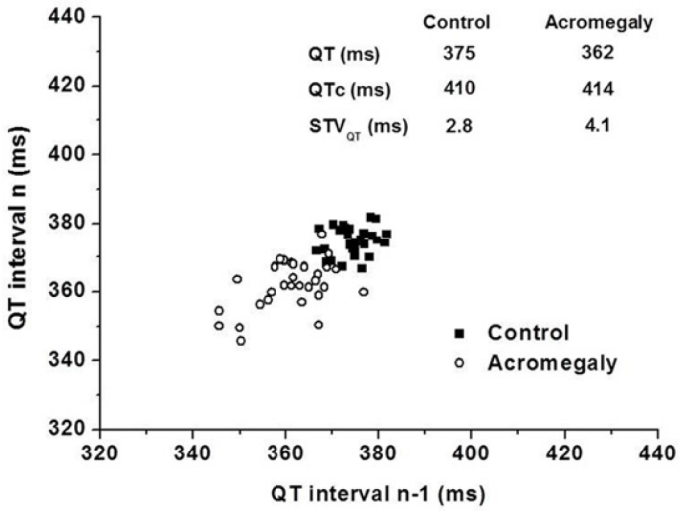

Fig 1. Representative Poincaré plots of a control individual and a patient with acromegaly. Note the larger area covered by data points obtained in the acromegalic patient illustrating increased short-term variability of the QT interval. Abbreviations: QTc, corrected QT interval by Bazett formula; STV_{QT}, short-term variability of the QT interval.

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(4.23 ± 0.10 ms *vs* 3.12 ± 0.80, $P<0.0001$) (Fig 2). STV_{QT} values did not differ significantly between active (4.16 ± 0.89 ms) and inactive (4.33 ± 1.22 ms) acromegalic patient subgroups. There was no difference between acromegalic subjects treated with antihypertensive drugs (4.33 ± 0.95 ms, n = 18) and normotensive acromegalic patients (4.10 ± 1.16 ms, n = 12). We could not find any significant correlation between the STV_{QT} values and the left ventricular hypertrophy parameters in acromegaly patients or in the sub-groups of active and inactive patients (data not shown).

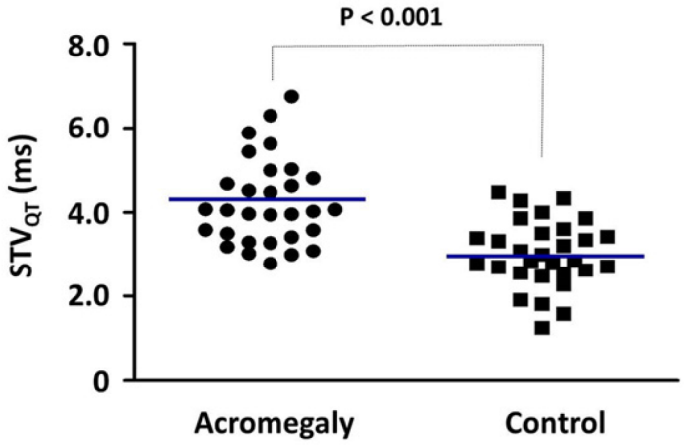

Fig 2. Short-term beat-to-beat temporal variability of the QT interval (STV_{QT}) in acromegalic and matched control patients. Individual values measured in n = 30 patients in each group are presented, the blue lines indicate mean values, $P<0.001$.

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Table 4. Autonomic neuropathy parameters of acromegalic patients and age-matched control subjects.

	Control	Acromegaly
Heart rate variation during deep breathing (1/min)	17.20 ± 6.44	14.00 ± 7.22
Valsalva ratio	1.70 ± 0.34	1.40 ± 0.28*
30/15 ratio	1.20 ± 0.18	1.10 ± 0.21*
Systolic BP fall after standing up (mm Hg)	6.40 ± 6.77	9.10 ± 9.66
Diastolic BP increase after sustained handgrip (mm Hg)	20.00 ± 9.22	17.50 ± 8.2
AN score	1.50 ± 1.28	2.90 ± 2.11*

Abbreviations: AN, autonomic neuropathy; BP, blood-pressure; 30/15 ratio, immediate heart rate response to standing; n = 30 in each group,

*$P<0.05$

**$P<0.001$ *vs* controls.

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Autonomic function

Standard cardiovascular reflex tests indicated significant deteriorations in Valsalva ratio ($P = 0.0015$), 30/15 ratio ($P = 0.0143$), and AN score ($P = 0.0023$) in patients with acromegaly, however, no significant differences in systolic blood pressure response after standing up, and diastolic blood pressure response after sustained handgrip were detected between the two groups (Table 4). AN score was significantly lower in active acromegaly subgroup, than in inactive group (2.1 ± 1.7 *vs* 3.9 ± 2.2; $P = 0.0260$), whereas other autonomic functions measured did not differ significantly in our two acromegalic subgroups (heart rate variation during deep breathing: 15.5 ± 6.5 min^{-1} *vs* 11.9 ± 7.95 min^{-1}; Valsalva ratio: 1.50 ± 0.26 *vs* 1.40 ± 0.29; 30/15 ratio: 1.10 ± 0.13 *vs* 1.10 ± 0.29; Systolic blood pressure fall after standing up: 8.6 ± 11.8 mmHg *vs* 9.8 ± 6.2 mmHg; Diastolic blood pressure increase after handgrip: 19.1 ± 8.2 mmHg *vs* 15.4 ± 8.2 mmHg; respectively). There was no significant difference between the values of autonomic parameters measured in acromegalic subjects treated with antihypertensive drugs and normotensive patients with acromegaly.

Correlation of serum hGH and IGF-1 x ULN levels with cardiovascular data and autonomic neuropathy parameters

Pearson coefficient values indicated that neither hGH nor IGF-1 x ULN hormone level correlated with STV_{QT} or any other ECG parameters measured (Table 5). However, serum hGH concentration negatively correlated with diastolic blood pressure ($P = 0.0326$), thickness of posterior wall of left ventricle ($P = 0.0333$), and AN score ($P = 0.0131$), whereas IGF-1 x ULN levels positively correlated with Valsalva ratio ($P = 0.0087$).

Discussion

Although a connection between acromegaly and increased cardiovascular morbidity and mortality has been established previously, this study is the first to demonstrate increased beat-to-beat short-term variability of the QT interval in acromegalic patients. There was no significant difference between STV_{QT} values measured in clinically and biochemically active acromegalic patients and those in inactive patients, which may suggest that elevated STV_{QT} is related to the presence of acromegaly and not to the efficacy of the treatments applied. STV_{QT} is a novel ECG parameter that, according to experimental [16–18] and clinical [20–23] data, more reliably predicts the development of serious ventricular arrhythmia compared to conventional

Table 5. Correlation of serum average hGH and IGF-1 x ULN level of acromegalic patients with cardiovascular data and autonomic neuropathy parameters.

	Serum average hGH level (ng ml^{-1})		Serum IGF-1 x ULN level	
	Pearson r	*P value (two-tailed)*	Pearson r	*P value (two-tailed)*
Systolic BP (mmHg)	- 0.2294	*0.2313*	0.3206	*0.0900*
Diastolic BP (mmHg)	- 0.3978	*0.0326**	0.1256	*0.5161*
EF (%)	0.3170	*0.0878*	-0.1735	*0.3593*
EDD (mm)	- 0.2911	*0.1186*	-0.1680	*0.3749*
ESD (mm)	- 0.3507	*0.0574*	-0.1076	*0.5716*
IVS (mm)	- 0.2714	*0.1469*	0.0388	*0.8387*
PW (mm)	- 0.3897	*0.0333**	0.1048	*0.5815*
RR (ms)	- 0.1204	*0.5262*	- 0.1045	*0.5826*
PQ (ms)	- 0.1968	*0.2974*	- 0.0284	*0.8817*
QRS (ms)	- 0.0127	*0.9468*	- 0.3023	*0.1044*
QT (ms)	- 0.2032	*0.2815*	- 0.2360	*0.2092*
QTc (ms) Bazett	- 0.1090	*0.5663*	- 0.2084	*0.2690*
QTc (ms) Fridericia	- 0.1992	*0.2914*	- 0.2919	*0.1175*
QTc (ms) Framingham	- 0.1834	*0.3320*	- 0.2690	*0.1506*
QTc (ms) Hodges	- 0.2154	*0.2530*	- 0.2975	*0.1103*
QTd (ms)	0.1562	*0.4099*	0.1758	*0.3526*
T_{peak}-T_{end} (ms)	- 0.0917	*0.6298*	- 0.0788	*0.6791*
STV_{QT} (ms)	- 0.3401	*0.0659*	- 0.0924	*0.6272*
Heart rate variation during deep breathing (1/min)	0.2300	*0.2390*	- 0.1267	*0.5206*
Valsalva ratio	0.1340	*0.4967*	0.4864	*0.0087**
30/15 ratio	0.2386	*0.2307*	0.0167	*0.9342*
Systolic BP fall after standing up (mm Hg)	- 0.3617	*0.0586*	- 0.2206	*0.2593*
Diastolic BP increase after sustained handgrip (mm Hg)	0.2421	*0.2146*	- 0.0762	*0.6998*
AN score	- 0.4714	*0.0131**	- 0.2077	*0.2987*

Abbreviations: 30/15 ratio, immediate heart rate response to standing; AN, autonomic neuropathy; BP: blood pressure; EDD: left ventricular end diastolic diameter; EF: ejection fraction; ESD: left ventricular end systolic diameter; hGH: human growth hormone; IGF-1: insulin-like growth factor-1; ULN: upper limit of normal value; IVS: interventricular septum; PW: posterior wall of left ventricle; QTc: frequency corrected QT interval; QTd: QT dispersion; STV_{QT}: short-term variability of QT interval; n = 30 for each number of XY pairs,

*P<0.05 for correlation.

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ECG parameters of repolarization. STV_{QT} values may be used to help predict individual risk for arrhythmia and sudden cardiac death in patients with acromegaly, however, the efficacy of this approach could only be confirmed during prospective clinical studies.

Cardiac rhythm abnormalities have been demonstrated by electrocardiogram and Holter studies in acromegaly [4,5]. Resting electrocardiological changes included left axis deviation, increased QT intervals, septal Q-waves, ST-T wave depression, and late potentials in acromegalic patients [7,10]. Atrial and ventricular ectopic beats, paroxysmal atrial fibrillation, paroxysmal supraventricular tachycardia, sick sinus syndrome, bundle branch block, and ventricular tachycardia were seen during physical exercise [4,5]. The severity of ventricular arrhythmias correlated with increases in left ventricular mass and the frequency of ventricular premature complexes increased with the duration of acromegaly [8]. Fatti *et al.* [12] detected abnormally long QTc interval before treatment in one-quarter of 30 acromegalic patients in a retrospective study. Octreotide, a somatostatin analogue, was shown to reduce QT intervals [12], and reduce the number of ventricular premature complexes in acromegalic patients [26].

Acromegalic cardiomyopathy is frequently present at diagnosis and the majority of patients with acromegaly meet echocardiographic criteria for left ventricular hypertrophy [5]. A possible reason is that acromegalic patients are sometimes diagnosed only after longer duration (7–10 years) of the disease. No significant difference in left ventricle hypertrophy was observed between active and inactive acromegaly patients in our study, which may indicate that adequate treatment of acromegaly could not turn back the process. Cardiac performance of acromegalic patients during physical exercise depends on left ventricular diastolic function under resting condition [27]. Ciulla *et al.* [28] found elevated myocardial echoreflectivity and increased QTd in acromegalic patients and explained these changes by long-term, blood pressure-independent cardiac hypertrophy and prolonged exposure to high serum concentrations of hGH and IGF-1. Baykan *et al.* [29] analyzed echocardiographic parameters by tissue and two-dimensional Doppler imaging in acromegalic patients and found that GH level positively correlated with interventricular septum thickness. Our observations regarding these parameters were unexpectedly different. Myocardial hypertrophy in relevant animal models has been shown to result in electrophysiological remodeling where the expression of potassium channels critical for repolarization and repolarization reserve (such as I_{Ks}), is significantly reduced, creating an arrhythmia substrate of increased spatial heterogeneity and temporal instability of repolarization and leading to increased arrhythmia susceptibility in the heart [14,30 32]. Patients with acromegaly may also develop congestive heart failure, the ratio was less than 3% (10 of 330 consecutive patients) in a study performed in 2 centers [33]. Recent studies indicated that I_{Ks}, I_{Kr}, I_{K1}, and I_{to} potassium channels were down-regulated [34–36] and the persistent or slowly-inactivating sodium current was also increased in chronic heart failure [37]. Additionally, acromegalic patients could also develop coronary heart disease and most patients have systemic complications affecting the Framingham risk score [38]. GH receptor antagonist therapy improved the score and reduced the risk for coronary heart diseases [3]. In acromegalic patients, increased stiffness of ascending aorta was described [39] and ambulatory arterial stiffness indexes might have an important role in predicting cardiovascular risk [40]. Several mechanisms have been implicated in the development of ventricular arrhythmias in the settings of myocardial ischemia and myocardial infarction [41]. The surviving ventricular myocytes in the border zone next to the infarcted area play a particularly important role in the development of arrhythmias [42,43]. In these cells, a consistent downregulation of different potassium channels has been found, including I_{to} [42], I_{K1} [44], I_{Kr} and I_{Ks} [45]. The QT variability index, among the first ECG parameters used to characterize temporal variability of repolarization, has been shown to more reliably predict myocardial ischemia and myocardial infarction associated serious ventricular arrhythmia development compared to more conventional ECG parameters [15,46,47]. It should be noted that myocardial fibrosis occurring in acromegaly [48] can also contribute to the underlying arrhythmia substrate in the heart due to disturbances in conduction.

Animal studies support the cardiovascular findings of clinical observations on acromegalic patients. Overexpression of bovine GH gene increased cardiac mass, induced hypertrophy of left ventricle, and deteriorated cardiac systolic function in adult female transgenic mice [49]. The long-term exposure to high serum GH concentration also resulted in impaired high-energy phosphate metabolism and mitochondrial ultrastructural changes in the heart muscle of mice [49]. Bovine GH transgenic mice also developed a salt-resistant form of hypertension and structural narrowing of the resistance vasculature [50].

Our observations indicate deterioration in autonomic function assessed by standard cardiovascular reflex tests in acromegalic patients. AN score was significantly worse in inactive acromegalic patients and there was no apparent difference between acromegalic subgroups in other autonomic parameters measured, which may suggest that these neuropathy parameters are long-term consequences of acromegaly and cannot be reverted by the control of the disease.

Among the tests primarily reflecting parasympathetic functions, the Valsalva ratio and 30/15 ratio were significantly decreased in acromegaly, whereas heart rate variation during deep breathing was not changed significantly. The tests demonstrating sympathetic activity, such as systolic blood pressure fall after standing up and diastolic blood pressure increase after sustained handgrip, did not change significantly in acromegalic patients. These reflex tests indicate a moderate parasympathetic dysfunction in our study, which could represent a predisposition to proarrhythmic activity in acromegalic patients. Increased risk of sudden cardiac death and ventricular arrhythmia has been associated with decreased parasympathetic and increased sympathetic activity [51]. Parasympathetic activation has been considered as antiarrhythmic regarding the development of ventricular fibrillation in pathological settings; for a recent review see [52]. There are conflicting data published about the cardiac autonomic functions in patients with acromegaly [53–57]. Dural *et al.* [53] provided evidence of sympathovagal imbalance due to sympathetic hypertone in acromegalic patients. Acromegaly was significantly associated with cardiac autonomic dysfunction independent from the presence of hypertension or diabetes mellitus [53]. Comunello *et al.* [54] analyzed 24 h frequency domain heart rate variability and found a correlation between reduced sympathovagal balance and pathological conditions, such as diabetes or hypertension in acromegalic patients. Chemla *et al.* [55] found that 10±6 months successful treatment of acromegaly could increase parasympathetic modulation and decrease sympathetic modulation of the night time heart variability and this effect was unrelated to changes in sleep apnea status. In contrast to our observations, sympathovagal imbalance due to increased vagal tone was demonstrated as a new risk factor for arrhythmias and syncope in acromegalic patients with left ventricle hypertrophy, although with normal heart rate, normal QT interval, and normal ejection function [56]. High frequency bands in orthostatism, but not in clinostatism, were higher in acromegalic patients than in normal subjects [56]. However, Seravalle *et al.* [57] have recently detected significantly decreased adrenergic tone through direct recording of muscle sympathetic nerve activity in newly diagnosed acromegalic patients with insulin resistance, but without cardiac hypertrophy.

Determination of beat-to-beat STV_{QT} is an intensively investigated new and non-invasive method for assessment of proarrhythmic risk [14,24]. QT interval measurements provide physiological information regarding the duration of cardiac repolarization. However, simple QT interval measurements are not always reliable in arrhythmic risk prediction. Ventricular repolarization is governed by a fine balance of inward and outward ionic currents. Under normal conditions impairment of one type of outward potassium channels is not likely to cause excessive QT prolongation, since other types of potassium channels provide sufficient repolarizing capacity. This was termed as repolarisation reserve [58,59]. Temporal STV_{QT} proved to be a more sensitive predictor of Torsades de Pointes ventricular tachycardia development than conventional QT parameters, such as QT and rate corrected QT intervals or the spatial QT interval dispersion, in case of experimentally impaired repolarization reserve [17,18,60]. There are numerous examples for the association between different pathophysiological conditions and attenuated repolarization reserve caused by electrophysiological remodelling. In addition, in experimental studies ventricular hypertrophy and chronic heart failure (CHF) were associated with decreased repolarisation reserve and/or high incidence of proarrhythmic events [17,61–63]. The significance and sensitivity of STV_{QT} as a predictor for electrical remodelling and proarrhythmia has recently been confirmed in clinical conditions in connection with CHF [22]. Increased STV_{QT} in the context of moderate CHF may reflect a latent repolarization disorder and increased susceptibility to sudden death in patients with dilated cardiomyopathy, which is not identified by a prolonged QT interval. In this study, increased STV_{QT} was the strongest indicator with an odds ratio of 1.52 (95% confidence interval 1.20 to 2.07, $P = 0.007$) for a history of documented ventricular tachycardia [22].

Varkevisser *et al.* [24] has recently reviewed the studies in which beat-to-beat STV_{QT} was a better indicator than QT interval prolongation for identification of healthy subjects or patients at risk for ventricular arrhythmias. In this regard, we have recently demonstrated that professional soccer players with hypertrophied hearts had increased STV_{QT} both in resting conditions and after exercise [64]. Significantly increased baseline STV_{QT} was able to identify patients with diminished repolarization reserve exhibiting drug-induced Torsades de Pointes [20] and those with inherited long QT syndrome [21]. Increase in STV_{QT} and prolongation of QT interval were observed in patients receiving cardiotoxic doxorubicin therapy [65]. A prospective clinical trial, the EUTrigTreat clinical study, was completed to investigate arrhythmogenic risk factors, including beat-to-beat variability of repolarization in sudden cardiac death risk stratification in patients with implantable cardioverter defibrillator [66]. Similar, prospective trials may elucidate the benefit of the use of STV_{QT} in other patient populations including acromegaly.

Increased temporal instability of cardiac repolarization characterized by elevated STV_{QT} in pathological situations, including acromegaly, can refer to impairment of repolarization reserve and increased propensity for arrhythmias [14]. In this setting, even relatively weak inhibition of potassium channels by seemingly harmless medications and/or dietary constituents may lead to sudden and unexpected excessive QT prolongation and development of Torsades de Pointes ventricular tachycardia [14].

Limitations of the study: It is important to note that in the present study the duration of acromegaly from the diagnosis can be defined (10–30 years), however the exact onset of the disease is not determinable and furthermore the duration since the remission in the inactive acromegalics is also not known. Therefore the real exposure time of increased hGH level before the diagnosis and effective treatment of the disease is not known and our active and inactive patients groups can be heterogenous in this regard. Moreover, the actual hormone levels used for correlation calculations with echocardiography and other cardiovascular parameters do not necessarily correspond to the duration of the disease. Because of our unexpected negative correlation between the GH level and the posterior wall thickness, further echocardiographical studies are warranted to examine the relationship between GH and IGF-1 levels and echocardiographic parameters in a larger series of acromegalic patients. A prospective study on newly diagnosed acromegaly patients could answer the question whether effective treatment would have any time-related effects on the changes in STV_{QT} variability and autonomic cardiovascular unctions.

In conclusion, STV_{QT} is increased in patients with acromegaly while more conventional parameters of ventricular repolarization were unchanged. STV_{QT} values did not differ between active and inactive acromegalic patients and did not correlate with actual serum concentrations of hGH and IGF-1. These observations may suggest that elevated short-term beat-to-beat variability is a consequence of the disease and not related directly to current treatment or condition of the patient. The elevated STV_{QT} suggests instability of ventricular repolarization and may be an early indicator of increased liability to arrhythmia in patients with acromegaly. Further prospective clinical studies are needed to identify individual risk for ventricular arrhythmias in acromegalic patients.

Supporting Information

S1 Table. Clinical data of acromegalic patients. Abbreviations: BMI: body mass index; hGH: human growth hormone; IGF-1: insulin-like growth factor-1; OGTT: oral glucose tolerance test; ULN: upper limit of normal value.
(DOC)

Author Contributions

Conceived and designed the experiments: AO ÉCs ZsV AN AV CsL. Performed the experiments: AO ÉCs CsC HG SM ZsV CsL. Analyzed the data: AO TTV AN IB TF CsL. Contributed reagents/materials/analysis tools: TTV AN TF TW JGP AV CsL. Wrote the paper: AO ÉCs ZsV AN IB JGP AV CsL.

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