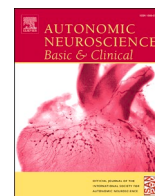


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## Aberrant autonomic pattern during the post-exercise recovery phase in long QT syndrome patients

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## ABSTRACT

**Objectives:** It is well-established that the autonomic nervous system (ANS) plays a central role in arrhythmogenesis. During and after exercise the ANS is particularly active, and since long QT syndrome (LQTS) patients have an increased risk of lethal arrhythmias during physical activity, it is important to investigate the autonomic function in these patients. In this study we investigate the ANS response during and after exercise in LQTS patients and healthy age and sex matched controls.

**Methods:** Forty-four genotype-verified adult LQTS patients and forty-four healthy age- and sex-matched controls performed a submaximal bicycle exercise stress test. Heart rate recovery (HRR) and heart rate variability (HRV) were analyzed from registered electrocardiogram (ECG) and vector electrocardiogram (VCG) recordings collected throughout rest, exercise and in the post-exercise phase.

**Results:** LQTS patients had a slower HRR than controls at 1- and 4-min post-exercise ( $p < 0.001$ ). During the post-exercise phase, LQTS patients had a lower total power ( $p < 0.001$ ), low frequency power ( $p < 0.001$ ) and high frequency power ( $p < 0.001$ ) than controls. In the same phase, LQTS patients off betablocker (BB) treatment showed a lower high frequency power ( $p = 0.01$ ) and different low frequency/high frequency ratio ( $p = 0.003$ ) when comparing with LQTS patients on BB treatment.

**Conclusions:** The parasympathetic effect on both HRR and HRV after exercise appears depressed in this LQTS patient cohort compared to healthy controls. This indicates an aberrant ANS response during the post-exercise phase which might be compensated by BB treatment. Our findings emphasize the importance of performing further investigations to identify the role of the ANS in LQTS arrhythmogenesis.

## 1. Introduction

Congenital long QT syndrome (LQTS) is an inherited cardiac ion channelopathy resulting in prolonged cardiac repolarization and consequently an increased risk for life-threatening cardiac events, such as arrhythmias and sudden cardiac death (SCD) (Schwartz et al., 1975). Many studies have demonstrated that the autonomic nervous system (ANS) is involved in LQTS, for example, it is known that an increase in

sympathetic activity often triggers arrhythmias, and that beta-adrenergic blocking and left cardiac denervation are effective treatments (Liu et al., 2005; Moss et al., 2000).

Heart rate variability (HRV) is a frequently used method to monitor the balance between sympathetic-parasympathetic interaction of the ANS. Abnormalities in HRV during daily activity and night-time sleep have been found in studies of LQTS patients (Morita et al., 1996; Porta et al., 2015; Shamsuzzaman, 2003). Despite the well-known fact that

**Abbreviations:** ANOVA, analysis of variance; ANS, autonomic nervous system; BB treatment, treatment with betablockers; BMI, body mass index; ECG, electrocardiogram; HF, high frequency; HR, heart rate; HRR, heart rate recovery; HRV, heart rate variability; ICD, implantable cardioverter defibrillator; LF, low frequency; LQT1, long QT syndrome type 1; LQT2, long QT syndrome type 2; LQTS, long QT syndrome; PTOT, total power; QTC, corrected QT time; RPM, revolutions per minute; RR interval, the interval between two consecutive R-peaks; SCD, sudden cardiac death; VCG, vector electrocardiogram;  $W_{max}$ , maximal workload;  $W_{pred}$ , predicted maximal workload.

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exercise triggers cardiac symptoms and events in LQTS patients, there have been few investigations regarding ANS modulation of the heart rate (HR) during and after exercise in these patients (Crotti et al., 2012).

Electrocardiographic (ECG) exercise stress test is an easily accessible, non-invasive and commonly used clinical method for assessment of arrhythmias during cardiovascular stress. When LQTS is suspected, this method can be used in the diagnostic process to reveal corrected QT time (QTc) prolongation in the post-exercise phase (Schwartz score) (Schwartz and Crotti, 2011). However, the fact that exercise is one of the major triggers for arrhythmias in LQTS patients, especially in patients with LQTS type 1, indicates that this method could provide additional valuable information regarding ANS regulation during physical activity.

HR is controlled by a balance between the sympathetic and parasympathetic nervous systems. During exercise, HR increases initially due to rapid vagal withdrawal, increasing exercise intensity and subsequent sympatho-adrenal activation. Heart rate recovery (HRR) reflects the immediate decrease in HR upon exercise termination, with a significantly impaired HRR predicting overall mortality in both cardiovascular and non-cardiovascular diseases (Qiu, 2017; Peçanha et al., 2014). Assessment methods reflecting autonomic modulation of HR, such as HRV and HRR, are thus suitable for examining patients with LQTS both during and after exercise.

The aim of this study was to investigate whether subjects with LQTS show a different autonomic pattern during exercise and in the post-exercise phase compared to controls, by analyzing HRV and HRR.

## 2. Methods

### 2.1. Study design and participants

Forty-six subjects with genetically confirmed LQTS (35 LQT1 and 11 LQT2) attending regular cardiology follow-up at Umeå University Hospital, were recruited. Two LQT2 patients were excluded due to frequent ventricular arrhythmias that lead to premature termination of exercise testing. The remaining 44 LQTS patients Holter ECG and vector electrocardiogram (VCG) registrations were included in this study. The genotypes were ascertained by molecular genetic analysis at the Department of Clinical Genetics according to current clinical practices for molecular genetic diagnostics. Patients were divided into symptomatic and asymptomatic subgroups based on documented history of cardiac events in the patients' medical record (syncope, cardiac arrest, ventricular tachyarrhythmia). QTc interval duration at rest: <450 ms or >450 ms and on-going betablocker (BB) therapy was noted. The LQTS patients were compared with a healthy control group consisting of one-to-one age- and sex-matched subjects, recruited from the same region. All study participants were invited for submaximal bicycle exercise testing, including Holter ECG and VCG. Informed consent was obtained from all subjects. The study was approved by the Regional Ethical Review Board (Umeå University) and complies with the declaration of Helsinki.

### 2.2. ECG monitoring

Both Holter ECG and VCG recordings were obtained during exercise testing for analysis of HR, HRR, HRV and QTc. Electrode noise and release are common issues effecting heart rhythm recordings during exercise testing. For these reasons we chose to complete full registrations with two independent systems to ensure optimal data quality and failsafe recording throughout testing. VCG was recorded using the Coronet II system (Ortivus AB, Danderyd, Sweden), with a sampling rate of 500 Hz. VCG electrodes were placed according to the lead system described by Frank (1956). Holter recordings were performed using 3 lead SpaceLab Holter monitor units (SpaceLabs Healthcare, Snoqualmie, WA, USA), with a sampling rate of 100 Hz and built-in software automated R-wave detection. Analyses were mainly performed using the VCG recordings, however Holter recordings were also used when the

quality of the VCG recordings were insufficient. To increase accuracy of RR-interval detection, all analyzed Holter ECGs were interpolated using splines and resampled at 500 Hz, and R-wave detection was adjusted based on the location of peaks in the interpolated ECGs. Custom-made software was used in post processing to automatically calculate HRR and HRV indices, and to determine QT intervals and the average QTc intervals. Bazett's formula was used to correct QT time for HR (Bazett, 1997).

### 2.3. Exercise testing

All subjects performed a semi-supine bicycle exercise test using a bicycle ergometer (General Electric, model 900, Ergoline GmbH, Bitz, Germany). Holter ECG and VCG recordings were obtained continuously; starting at the resting phase prior to the exercise test and ending 5 min into post-exercise recovery. The participants commenced exercise by peddling at a constant rate (between 55 and 65 rpm) with an increasing workload every 2 min. The subjects were encouraged to cycle until a feeling of fatigue and thereafter an additional 2 min at this effort level in order to reach at least a submaximal exercise performance. The predicted maximal workload ( $W_{pred}$ ) was determined based on the subject's age, sex, height and the maximum workload ( $W_{max}$ ) as a percent of  $W_{pred}$ , was calculated according to Brudin et al. (2014). Any symptoms or relevant comments from the subject during testing were recorded, as well as the reason for cessation of exercise, Watts achieved and peak RPMs.

### 2.4. Analysis of heart rate variability

Analysis of the beat-to-beat variation of the RR intervals (HRV) was performed using power spectrum analysis. HRV indices were determined from one-minute sequences during rest, at peak exercise and during post-exercise. All segments analyzed were manually edited prior to analysis in order to exclude premature beats, noise and other aberrant heartbeats. Power spectrum analysis of HRV was performed using autoregressive modelling with 30 parameters. RR intervals were converted to HR, interpolated using cubic splines and then resampled at 5 Hz. Finally, the mean HR was removed. The total power (PTOT) was calculated, as well as the power in the low-frequency region (LF, 0.04–0.15 Hz), and the high-frequency region (HF, 0.15–0.50 Hz). HRV data was expressed in  $ms^2$  and logarithmically transformed (base 10). All HRV analyses were performed using Matlab version. 2019a (MathWorks Inc., Natick, MA, USA).

### 2.5. Statistical analyses

Data was reported as mean  $\pm$  standard deviation (SD). Comparisons between groups at specific time points were performed using Student *t*-tests. Additionally, statistical analyses were performed using analysis of variance (ANOVA) for repeated measurements with time, group and their interaction as variables. Intra-subject effects were evaluated using the Huynh-Feldt correction. The analysis was performed in two steps: firstly, changes from rest to peak exercise, and secondly, changes during the 5 min of post-exercise phase compared to peak exercise.

A significant interaction between time and group variables indicates that the pattern of change over time was different for each group. If the analysis included two time points, e.g. the comparison of rest and peak exercise data, post-hoc testing was performed using group-wise *t*-tests at each time point. However, in the analysis of the data from the 5 min post-exercise, post-hoc tests were performed by comparing confidence intervals. A *p*-value <0.05 was considered as statistically significant. Statistical analysis was performed using IBM SPSS Statistics v26 (IBM Corp, Armonk, NY, USA).

### 3. Results

#### 3.1. Participants

Initially forty-six LQTS patients were recruited for assessment, however two registrations were excluded due to ventricular arrhythmias during exercise. The remaining 44 LQTS patient registrations were included in this study. Twenty-three of the 44 subjects were men (52%), the mean age of the LQTS patients was  $41 \pm 16$  years (range 18–72), 35 had genetically verified LQT1 and 9 LQT2 (Table 1). Eighteen were classified as symptomatic (41%). Twenty LQTS patients were currently on betablocker treatment (45%) (Table 1). The LQTS subjects were one-to-one matched to control subjects according to their age and sex. None of the controls were on medication which could influence HR response or exercise capacity.

#### 3.2. Exercise test

There were no significant differences in HR during rest or at peak exercise between LQTS patients and controls (rest:  $67.5 \pm 13.8$  beats/min vs  $66.5 \pm 12.4$  beats/min,  $p = 0.71$ ; peak exercise:  $127.6 \pm 16.8$  beats/min vs  $125.6 \pm 9.0$  beats/min,  $p = 0.47$ ). Patients off-betablockers had higher HR than patients on betablockers, both at rest and at peak exercise (rest:  $71.8 \pm 15.1$  beats/min vs  $62.5 \pm 10.5$  beats/min,  $p = 0.04$ ; peak exercise:  $133.6 \pm 15.0$  beats/min vs  $120.5 \pm 16.4$  beats/min,  $p = 0.01$ ). There were also significant differences in HR during peak exercise between symptomatic and asymptomatic patients (see supplementary materials for further details regarding HR in different subgroups of LQTS patients).

There was no significant difference in percent of predicted Wmax between the LQTS group and controls ( $61.9\% \pm 11.0\%$  vs  $56.9\% \pm 14.3\%$ ,  $p = 0.07$ ). There was also no significant difference in percent of predicted Wmax between patients off- and on-betablockers ( $61.5\% \pm 10.8\%$  vs  $62.4\% \pm 11.4\%$ ,  $p = 0.79$ ). During the early phase of exercise testing, one of the included LQT2 patients had a period of self-terminating ventricular bigeminy. Two ICD-treated LQT2 patients had intermittent ventricular bigeminy during exercise, and therefore had testing prematurely terminated. Consequently, these two patients were excluded from the study.

#### 3.3. QTc

QTc was calculated from the ECG recordings and showed that LQTS patients had a significantly longer QTc interval than controls at rest and 4 min after peak exercise (Table 2). No difference was found between QTc interval duration in patients on and off-betablockers. Symptomatic LQTS patients presented with a longer QTc interval at rest than asymptomatic LQTS patients ( $454 \pm 24$  vs  $479 \pm 45$ ,  $p = 0.02$ ). LQT1 patients had longer QTc intervals than LQT2 patients after exercise. The LQTS patients were divided into groups based on resting QTc < 450 ms or > 450 ms, during the post-exercise phase the difference in QTc interval between the two groups was maintained.

**Table 1**  
Summary of control subject and LQTS patient characteristics.

	Controls	LQT1	LQT2
Total number	44	35	9
Age years mean (range)	40 (18–72)	40 (18–72)	45 (21–67)
Male/female	23/21	18/17	5/4
BMI mean (range)	24.1 (18.9–35.6)	24.5 (18.7–33.0)	26.7 (21.7–34.1)
Beta blocker treatment yes/no	0	13/22	7/2
Symptoms yes/no	0/44	13/22	5/4
Both beta blocker treatment and symptoms yes/no	0/44	9/26	5/4
ICD treatment yes/no	0/44	0/35	0/9

**Table 2**

QTc at rest and 4 min after exercise.

	LQTS (N = 44)	Controls (N = 44)
QTc rest	$464 \pm 36$	$410 \pm 28^*$
QTc 4 min	$480 \pm 34$	$421 \pm 26^*$
	LQTS off-BB (N = 24)	LQTS on BB (N = 20)
QTc rest	$459 \pm 27$	$471 \pm 44$
QTc 4 min	$484 \pm 35$	$476 \pm 34$
	LQTS asymp (N = 26)	LQTS symp (N = 18)
QTc rest	$454 \pm 24$	$479 \pm 45^*$
QTc 4 min	$480 \pm 31$	$481 \pm 40$
	LQT1 (N = 35)	LQT2 (N = 9)
QTc rest	$467 \pm 39$	$457 \pm 18$
QTc 4 min	$488 \pm 33$	$451 \pm 29^*$
	LQTS QTc < 450 ms (N = 16)	LQTS QTc > 450 ms (N = 28)
QTc rest	$435 \pm 14$	$482 \pm 33^*$
QTc 4 min	$469 \pm 30$	$487 \pm 36^*$

N = number of subjects. QTc = corrected QT time reported in ms.

\*  $p < 0.05$ .

#### 3.4. Heart rate changes

Fig. 1 shows the mean HR in LQTS patients and controls during the post exercise phase. The LQTS subjects showed a higher HR over a longer period, i.e., a generally slower HRR compared to healthy controls. When comparing all LQTS patients with controls, the ANOVA for repeated measurements of HR during the post exercise phase showed a significant effect in the variables of Time ( $p < 0.001$ ) and Group ( $p = 0.003$ ), as well as the interaction between the two variables ( $p < 0.001$ ). Thus, the pattern of changes in HR was different in the two groups and there was a significantly higher HR in LQTS patients during all parts of the post exercise phase, whereas no difference was found during peak exercise (as shown above). There were also significant differences in HR during the post exercise phase between LQTS patients on or off beta-blocker treatment ( $p = 0.01$ ), and between asymptomatic and symptomatic patients ( $p = 0.01$ ), but not between the other subgroups of patients (Fig. 1 and Table S0, supplementary materials).

Table 3 shows HRR 1 min after exercise and 4 min after exercise. LQTS patients presented with a slower HRR at both 1 min and 4 min after exercise. The corresponding HRR patterns are graphically illustrated in Fig. S1, supplementary materials. There were no significant overall differences in HRR between subgroups of patients (Table 3). However, the group of patients with QTc > 450 ms showed a tendency towards a slower HRR compared to patients with QTc < 450 ms during the first minute into the post-exercise phase ( $p = 0.08$ ) (Table S1, supplementary materials).

#### 3.5. Heart rate variability

##### 3.5.1. Rest and peak exercise

Fig. 2 shows HRV in LQTS patients and controls. No statistically significant differences in PTOT or HF were found between LQTS patients and controls during rest or at peak exercise (Table 4). However, a significant interaction between the variables Group and Time was found for LF and LF/HF, indicating that the pattern of changes in LF ( $p = 0.007$ ) and LF/HF ( $p < 0.001$ ) were different between the two groups. Post-hoc

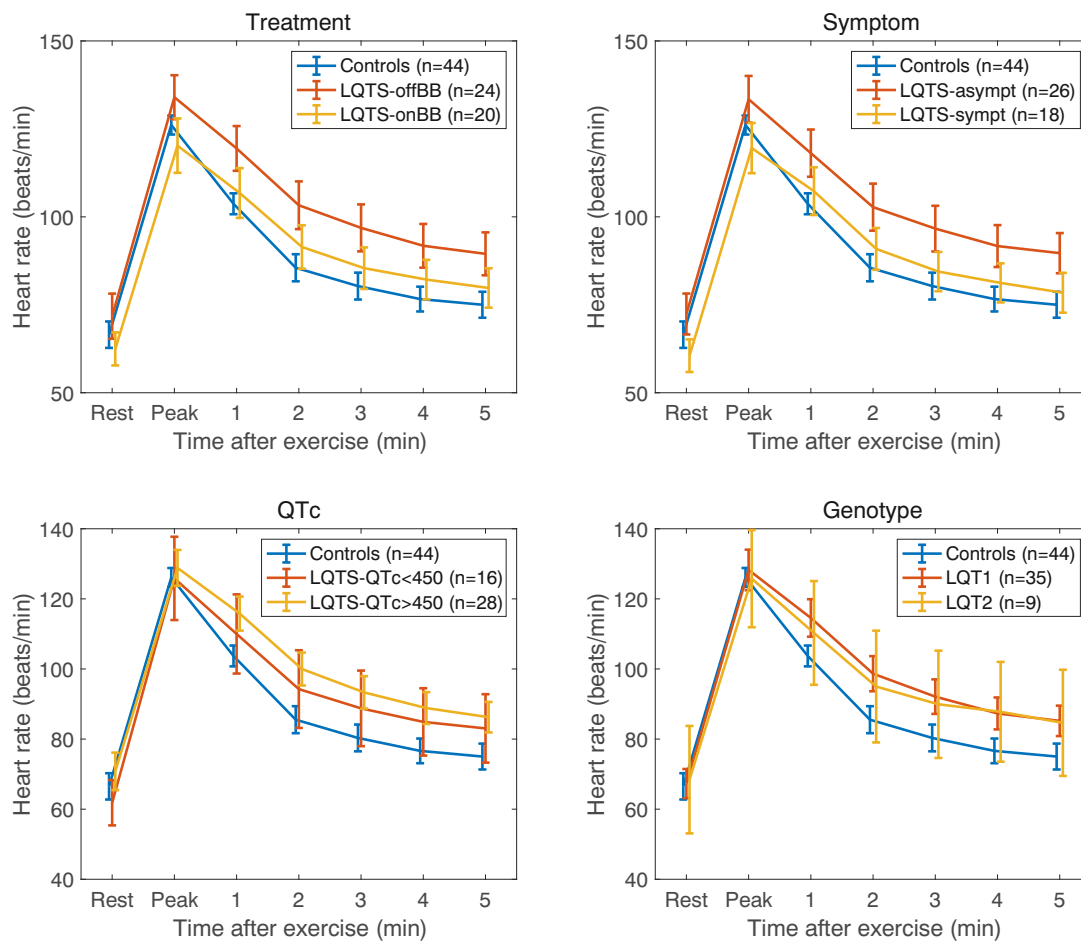


Fig. 1. Heart rate at rest (pre-exercise), at peak exercise and during the post exercise phase in different subgroups of LQTS patients compared to controls. Data is presented as group means and whiskers represents 95% CI. LQTS (long QT syndrome patients).

Table 3  
Heart rate recovery (HRR) after exercise in LQTS patients and controls.

	N	HRR <sub>1min</sub> = HR <sub>max</sub> -HR <sub>1min</sub>	HRR <sub>4min</sub> = HR <sub>max</sub> -HR <sub>4min</sub>	ANOVA p-values		
				Time	Time × Group	Group
Controls	44	33.6 ± 10.6*	49.9 ± 11.6*	<0.001	0.26	<0.001*
LQTS	44	23.4 ± 7.3*	41.6 ± 9.8*			
Off-betablock	24	23.9 ± 6.2	43.4 ± 9.1	<0.001	0.22	0.27
On betablock	20	22.7 ± 8.5	39.4 ± 10.3			
Asymptomatic	26	24.1 ± 6.5	42.7 ± 8.9	<0.001	0.75	0.35
Symptom	18	22.3 ± 8.3	40.0 ± 11.0			
QTc < 450	16	25.1 ± 9.5	42.3 ± 10.0	<0.001	0.49	0.45
QTc > 450	28	22.4 ± 5.6	41.6 ± 9.8			
LQT1	35	22.6 ± 6.8	41.9 ± 10.4	<0.001	0.06	0.72
LQT2	9	26.2 ± 8.6	40.3 ± 6.8			

N = number of subjects.

\* p < 0.05.

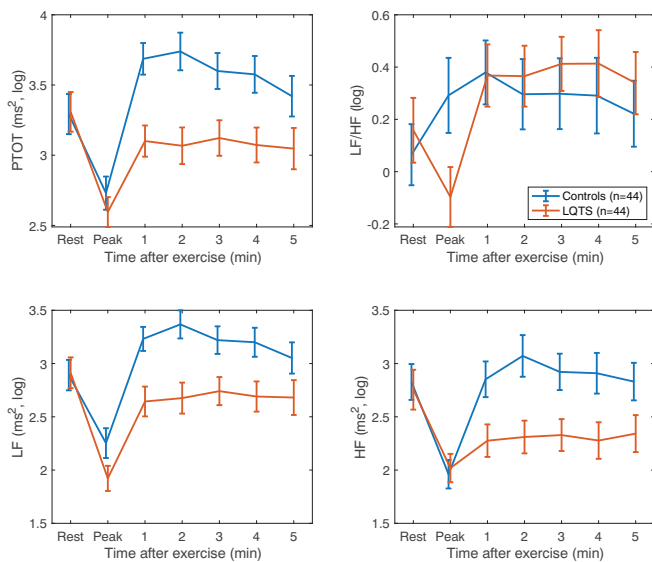
tests supported this finding and revealed that LQTS patients had a lower LF (p < 0.001) and LF/HF (p < 0.001) at peak exercise than controls (Table 4).

### 3.5.2. Post-exercise phase

All LQTS patients presented with significantly lower PTOT, LF and HF (p < 0.001) during the post-exercise phase compared to controls

(Fig. 2, Table 5), where the shape of the corresponding variation over time also was different in each group. The LQTS patients also showed a significantly different pattern in the LF/HF ratio compared to controls (p < 0.001), with a markedly increased LF/HF ratio after cessation of exercise (Fig. 2, Table 5).

There were significant differences in HF (p = 0.01) and LF/HF (p = 0.003) when comparing LQTS patients on or off BB treatment (Fig. 3 and



**Fig. 2.** HRV in LQTS patients compared to controls. Data is presented as group means and whiskers represents 95% CI for mean. *PTOT* total power, *LF* power of low-frequency component, *HF* power of high frequency component, *LF/HF* the ratio between LF and HF.

**Table 4**

Comparison between HRV in LQTS patients and controls at rest and peak exercise.

	Rest and peak exercise				
	Time	Time × LQTS group	Group	Post-hoc Rest	Post-hoc Peak
PTOT	<0.001	0.23	0.36	–	–
LF	<0.001	0.007*	0.03	0.83	<0.001*
HF	<0.001	0.40	0.93	–	–
LF/HF	0.81	<0.001*	0.03*	0.27	<0.001*

P-values derived from ANOVA for repeated measurements for the changes from rest to peak exercise. Post-hoc testing was only performed for variables with significant interactions between Time and Group variables. *PTOT* total power, *LF* power of low-frequency component, *HF* power of high frequency component, *LF/HF* the ratio between LF and HF.

\* p < 0.05.

**Table 5**

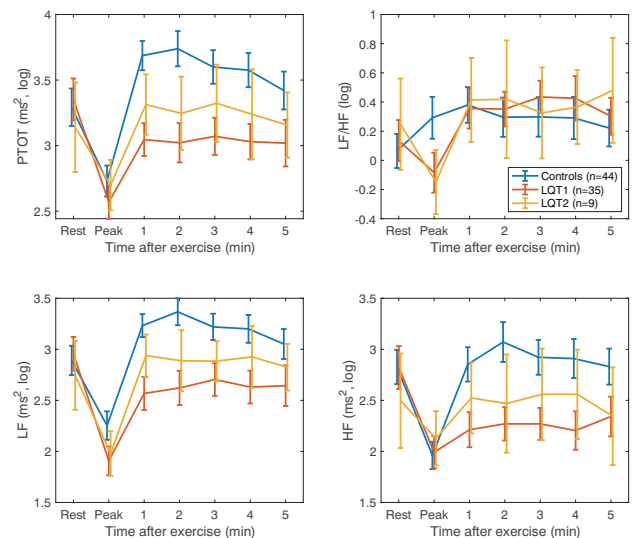
Comparison between HRV in LQTS patients and controls during post-exercise.

	Post-exercise		
	Time	Time × Grp	Grp
PTOT	<0.001	<0.001*	<0.001*
LF	<0.001	0.003*	<0.001*
HF	<0.001	<0.001*	<0.001*
LF/HF	<0.001	<0.001*	0.77

P-values derived from ANOVA for repeated measurements for the changes during post-exercise. *PTOT* total power, *LF* power of low-frequency component, *HF* power of high frequency component, *LF/HF* the ratio between LF and H.

\* p < 0.05.

Table S2, supplementary materials). A similar pattern was noted when comparing symptomatic and asymptomatic LQTS patients, but the differences were not significant (Fig. S3, supplementary materials). No significant differences were seen on analysis of HRV parameters in the other subgroups of LQTS patients (Figs. S4–S5, supplementary materials). However, there was a tendency for LQT1 patients to have a lower overall HRV (*PTOT*, *LF* and *HF*) throughout the duration of the post-



**Fig. 3.** HRV in LQTS patients according to treatment. Data is presented as group means and whiskers represents 95% CI for mean. *PTOT* total power, *LF* power of low-frequency component, *HF* power of high frequency component, *LF/HF* the ratio between LF and HF.

exercise phase, compared to LQT2 patients. Significant differences in overall HRV (*PTOT*, *LF* and *HF*) during post-exercise were found between controls and all subgroups of LQTS patients. Significant differences in *LF/HF* after exercise were only found between controls and LQTS patients off BB treatment (ANOVA p < 0.001 for Group × Time, p < 0.05 for post-hoc tests) (Fig. S2, supplementary materials).

**4. Discussion**

This study demonstrates that patients with LQTS show an aberrant pattern of cardiac autonomic response during the post-exercise recovery phase compared to healthy controls. Changes both in HRR and HRV indicate that the reactivation of the parasympathetic system is affected.

HRR is often described as having two phases, one fast, immediate phase mediated by parasympathetic reactivation, and one slow secondary phase attributed to both progressive parasympathetic reactivation and sympathetic withdrawal (Peçanha et al., 2014; Coote, 2010). This study shows that the LQTS patients, regardless of BB treatment, have a slower HRR than healthy controls. The difference is seen directly after exercise cessation, indicating that the parasympathetic reactivation is affected. Previous studies have shown that slow HRR following exercise is associated with an increased risk of overall mortality in healthy individuals, as well as in individuals with cardiovascular diseases (Qiu, 2017; Watanabe et al., 2001; Jolly et al., 2011; Cole et al., 1999) where some authors claim it may be primarily arrhythmic (Kizilbash et al., 2007).

The main focus of this study was on the difference between healthy controls and LQTS patients, nonetheless we conducted several subgroup analyses to rule out possible confounders. In the LQTS patient group we found no significant differences in HRR between genotypes, QTC or BB treatment status. Theoretically, one could assume that BB treatment during exercise testing should have no effect on reintroduction of parasympathetic influence upon exercise cessation, and thereby no effect on HRR. This has been shown in a study using exercise stress echocardiography, where HRR in subjects with normal echocardiographic findings was not affected by BB treatment status (Karnik et al., 2008). The same results regarding HRR and BB treatment were found in a study by Myers et al. (2007). In our study assessing HRR in both healthy controls and LQTS patients, BB treatment status had no significant impact on HRR. These findings are in agreement with findings published by Crotti et al. in which the authors found reduction in HRR

post-exercise was not affected by BB treatment (Crotti et al., 2012; Lieve et al., 2020).

Moreover, we did not find any difference in HRR between asymptomatic and symptomatic patients. Therefore, this study could not verify the findings made by Crotti et al., who reported that a more rapid HRR following exercise was correlated to symptomatic LQT1, independent of BB treatment (Crotti et al., 2012). Lieve et al. also identified a correlation between a more rapid HRR and symptomatic patients in a different ion channel disease, catecholaminergic polymorphic ventricular tachycardia (CPVT) (Lieve et al., 2020). When comparing these findings to our study, it should be considered that our subgroups were relatively small (25 symptomatic and 19 asymptomatic patients) and that the groups consisted of a mixture of LQTS genotypes. Additionally, in the study performed by Lieve et al. the authors exercised their patients to a much higher intensity. Consequently, this makes comparison of the findings difficult. However, our findings regarding slow HRR in LQTS patients compared to controls in the post-exercise phase is still a relevant and interesting finding as slow HRR reflects impaired parasympathetic reactivation after exercise cessation. This finding was further reinforced by the HRV data gathered in the post-exercise phase.

Normally, HRV measures are often observed to decrease during exercise with incremental Watt increases, reaching near-zero at a moderate intensity (range of 120–180 beats per minutes) and remaining substantially unchanged with further increases in exercise intensity (Karapetian et al., 2008; Sales et al., 2011; Tulppo et al., 1996). Upon exercise cessation, HR and HRV demonstrate a time-dependent recovery, eventually return to pre-exercise levels (Stanley et al., 2013). After exercise, HR regulation is gradually shifted back to predominantly parasympathetic control both by progressive parasympathetic reactivation and sympathetic withdrawal (Michael et al., 2017).

During recovery the cardiac autonomic process occurs in reverse (Michael et al., 2017) and this pattern could be seen in our controls. However, when comparing LQTS subjects with controls, a significant difference was found in all HRV indices. Interestingly, LQTS patients off betablocker (BB) treatment showed a lower HF compared to LQTS patients on BB treatment, which may indicate an even more affected parasympathetic reactivation. The LF/HF ratio in the LQTS patient group also showed a strikingly different pattern compared to that of the controls, with an increased LF/HF ratio predominantly due to the lack of increase in HF. It appears that the parasympathetic reactivation in LQTS subjects is not strong enough to slow down the HR and thus the increased sympathetic activity during exercise remains dominant. One may speculate that the imbalance between parasympathetic-sympathetic interactions, represented in the data by an alteration in the LF/HF ratio, explains the repolarization pattern and the lengthening of the QT interval in the recovery phase.

As previously mentioned, HRV measures normally decrease with gradually increasing exercise intensity in a healthy person, and at a HR between 120 and 180 beats per minute (bpm) the power of the different HRV components will be very low or close to zero (before logarithmic transformation). Our subjects were exercised to an average HR of 126 bpm which is considered to be a submaximal exercise intensity level, and consequently as expected, we could see a marked reduction in all HRV indices during exercise. When comparing LQTS patients with control subjects a significant difference was found in LF, where the LQTS patients had a greater reduction at peak exercise, whereas no difference was found in HF or HR at peak exercise. Thus, the reduction in LF was the main contributor to the difference in the LF/HF ratio at peak exercise which could be interpreted as autonomic imbalance. Previous studies on healthy subjects have shown a range of conflicting results regarding the LF/HF ratio during exercise (Saito and Nakamura, 1995; Hautala et al., 2003; Casties et al., 2006; Yamamoto et al., 1991), therefore making it hard to interpret the LF/HF ratio during exercise, even in healthy subjects.

#### 4.1. Genotype

In this study a total of 44 LQTS patients were included, of which 35 had type 1 and 9 had type 2 LQTS. No significant differences in HRV indices were found between the two genotypes, but in Fig. S5 and Table S5 it can be noted that there is a tendency for type 2 patients to have a lower LF and HF. However, since the LQTS type 2 group is small and has a larger proportion of symptomatic and BB treated patients, this tendency is difficult to evaluate.

#### 4.2. Betablocker treatment

Currently, there is very limited and contradictory data regarding BB treatment and its effect on HRV. Results in a study by Tankeu et al. indicate that HRV parameters remain unchanged after introduction of the betablocker propranolol (Tankeu et al., 2018). On the other hand, Chen et al. showed that affected HRV parameters caused by sleep-deprivation, could normalize during treatment with metoprolol, indicating a positive effect of betablocker treatment (Chen et al., 2012). This supports our results showing a significantly higher HF post-exercise in LQTS patients on BB treatment compared to patients off BB treatment (Fig. 3, Table S2). Due to the lower HF in LQTS patients off BB treatment, the LF/HF ratio post-exercise is higher which may represent an autonomic imbalance that could be prevented by BB treatment.

#### 4.3. QTc groups

LQTS patients were divided into two groups based on the duration of the QTc at rest. The cut-off duration for QTc was set at 450 ms based on the Schwartz score criteria. Sixteen patients had a short QTc (<450 ms), and 28 patients a prolonged QTc > 450 ms. As expected, the patients with a prolonged QTc interval displayed a more severe phenotype. We also found no significant differences between these two groups regarding HRV (Fig. S4). The patients with a QTc > 450 ms tended to have lower HRV indices generally, however this was not statistically significant.

#### 4.4. Symptoms

We encountered several problems when comparing symptomatic and asymptomatic patient groups, with the main issue was the small population sample sizes (Table 1). In our cohort 41% (18/44) of the LQTS patients had experienced symptoms during their life and 59% (26/44) had not. Another problem was the fact that several asymptomatic patients had been treated pre-symptomatically with BB treatment since childhood. Furthermore 14/44 LQTS patients were symptomatic and treated with BB. All these facts combined complicate and interfere with the interpretation of the results gained.

No significant differences were found in any of the HRV indices when comparing symptomatic and asymptomatic patient groups (Fig. 3, Table S3). However, an interesting trend in HF was noticed, in which HF increased more slowly and peak at 2 min into the post-exercise phase in symptomatic patients, compared with asymptomatic patients, which showed their HF peak at 1-min into the post-exercise phase. Even though this difference was not significant, this pattern might indicate that symptomatic patients have weaker vagal reactivation directly after exercise cessation. If this could be confirmed in larger studies with more homogenous subgroups, the method of HRV measurements in exercise test may be used to identify patients at higher risk of adverse events such as SCD.

#### 4.5. Possible underlying mechanisms

Autonomic imbalance appears to be involved in different kinds of cardiac arrhythmias, and the fact that the sympathetic division of the ANS has an impact on LQTS is well-established. However, the

underlying mechanisms responsible of this phenomenon have been sparsely explored. In a recent study by Winbo et al. the authors revealed that an increased excitability in patient-derived LQT1 sympathetic neurons indicating an arrhythmogenic potential even in LQTS neurons (Winbo, 2021). Nevertheless, the underlying mechanisms regarding the parasympathetic role in LQTS remains unexplored. In this study we found that LQTS patients show a vagal impairment post-exercise which agrees with the well-known fact that a combination of sympathetic overactivity and vagal impairment are powerful negative prognostic indicators for arrhythmias (Habecker et al., 2016). Even though this study does not reveal any specific indicators for the underlying mechanism behind the low parasympathetic onset post-exercise, the autonomic imbalance demonstrated could be strong explanatory factor for arrhythmogenesis in LQTS.

Neuromodulation therapy, such as betablocker treatment and surgical ablation of stellate ganglion affecting the sympathetic ANS, has been shown to have good results and are clinically important and achievable treatment options. Interventions in the vagal part of ANS are still in the experimental phase but increasing knowledge about the autonomic balance and the parasympathetic ANS in LQTS is essential to enable the development of future neuromodulation therapies.

#### 4.6. Limitations and strengths

Posture has been shown to affect HRV recovery, where a more upright posture has been shown to slow down the HRV recovery (Barak et al., 2010). However, in this study participants in both study groups had the same semi-supine position.

The size of the genotype groups differed considerable (35 LQT1 and 9 LQT2), which may contribute to difficulties in comparing HRV parameters with respect to genotype.

Since exercise intensity is individual and highly dependent on the subject's current exercise fitness, exercise capacity and participation effort, there will always be some limitations in the interpretation of results gained using this method. However, when calculating the study subject's maximum workload in percent of predicted maximal workload, no significant difference was found between the groups regarding exercise capacity obtained.

## 5. Conclusion

In this study, we found that our LQTS patients showed a different pattern of autonomic reactivation during the post-exercise recovery phase when compared to healthy controls. We could reveal an impaired parasympathetic effect on both HRR and HRV in this LQTS cohort which was even more pronounced in patients off BB treatment. These findings may indicate imbalance of the ANS which could play a role in arrhythmogenesis. Future studies with larger cohorts of patients are needed to validate this method and will show if new therapeutic options could stabilize this delicate balance and protect against exercise induced arrhythmias in these patients.

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## Declaration of competing interest

None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.autneu.2021.102897>.

## References

- Barak, O.F., et al., 2010. Heart rate variability before and after cycle exercise in relation to different body positions. *J. Sports Sci. Med.* 9 (2), 176–182.
- Bazett, H.C., 1997. An analysis of the time-relations of electrocardiograms. *Ann. Noninvasive Electrocardiol.* 2 (2), 177–194.
- Brudin, L., Jorfeldt, L., Pahlm, O., 2014. Comparison of two commonly used reference materials for exercise bicycle tests with a Swedish clinical database of patients with normal outcome. *Clin. Physiol. Funct. Imaging* 34 (4), 297–307.
- Casties, J.F., Mottet, D., Le Gallais, D., 2006. Non-linear analyses of heart rate variability during heavy exercise and recovery in cyclists. *Int. J. Sports Med.* 27 (10), 780–785.
- Chen, W.-R., et al., 2012. Protective effect of metoprolol on arrhythmia and heart rate variability in healthy people with 24 hours of sleep deprivation. *J. Interv. Card. Electrophysiol.* 36 (3), 267–272.
- Cole, C.R., et al., 1999. Heart-rate recovery immediately after exercise as a predictor of mortality. *N. Engl. J. Med.* 341 (18), 1351–1357.
- Coote, J.H., 2010. Recovery of heart rate following intense dynamic exercise. *Exp. Physiol.* 95 (3), 431–440.
- Crotti, L., et al., 2012. Vagal reflexes following an exercise stress test: a simple clinical tool for gene-specific risk stratification in the long QT syndrome. *J. Am. Coll. Cardiol.* 60 (24), 2515–2524.
- Frank, E., 1956. An accurate, clinically practical system for spatial vectorcardiography. *Circulation* 13 (5), 737–749.
- Habecker, B.A., et al., 2016. Molecular and cellular neurocardiology: development, and cellular and molecular adaptations to heart disease. *J. Physiol.* 594 (14), 3853–3875.
- Hautala, A.J., et al., 2003. Short-term correlation properties of R-R interval dynamics at different exercise intensity levels. *Clin. Physiol. Funct. Imaging* 23 (4), 215–223.
- Jolly, M.A., Brennan, D.M., Cho, L., 2011. Impact of exercise on heart rate recovery. *Circulation* 124 (14), 1520–1526.
- Karapetian, G.K., Engels, H.J., Gretebeck, R.J., 2008. Use of heart rate variability to estimate LT and VT. *Int. J. Sports Med.* 29 (8), 652–657.
- Karnik, R.S., et al., 2008. The effect of beta-blockade on heart rate recovery following exercise stress echocardiography. *Prev. Cardiol.* 11 (1), 26–28.
- Kizilbash, M.A., et al., 2007. The association of heart rate recovery immediately after exercise with coronary artery calcium: the coronary artery risk development in young adults study. *Clin. Auton. Res.* 17 (1), 46–49.
- Lieve, K.V.V., et al., 2020. Heart rate recovery after exercise is associated with arrhythmic events in patients with catecholaminergic polymorphic ventricular tachycardia. *Circ. Arrhythm. Electrophysiol.* 13 (3), e007471.
- Liu, T., et al., 2005. Sex modulates the arrhythmogenic substrate in prepubertal rabbit hearts with long QT 2. *J. Cardiovasc. Electrophysiol.* 16 (5), 516–524.
- Michael, S., Graham, K.S., Davis, G.M.O., 2017. Cardiac autonomic responses during exercise and post-exercise recovery using heart rate variability and systolic time intervals—a review. *Front. Physiol.* 8, 301.
- Morita, H., Yamanari, H., Ohe, T., 1996. Evaluation of autonomic nervous activity in patients with congenital long QT syndrome by an analysis of RR variability. *Jpn. Circ. J.* 60 (10), 742–748.
- Moss, A.J., et al., 2000. Effectiveness and limitations of -blocker therapy in congenital long-QT syndrome. *Circulation* 101 (6), 616–623.
- Myers, J., et al., 2007. Comparison of the chronotropic response to exercise and heart rate recovery in predicting cardiovascular mortality. *Eur. J. Cardiovasc. Prev. Rehabil.* 14 (2), 215–221.
- Peçanha, T., Silva-Júnior, N.D., Forjaz, C.L., 2014. Heart rate recovery: autonomic determinants, methods of assessment and association with mortality and cardiovascular diseases. *Clin. Physiol. Funct. Imaging* 34 (5), 327–339.
- Porta, A., et al., 2015. Autonomic control of heart rate and QT interval variability influences arrhythmic risk in long QT syndrome type 1. *J. Am. Coll. Cardiol.* 65 (4), 367–374.
- Qiu, S., et al., 2017. Heart rate recovery and risk of cardiovascular events and all-cause mortality: a meta-analysis of prospective cohort studies. *J. Am. Heart Assoc.* 6 (5), e005505.

- Saito, M., Nakamura, Y., 1995. Cardiac autonomic control and muscle sympathetic nerve activity during dynamic exercise. *Jpn. J. Physiol.* 45 (6), 961–977.
- Sales, M.M., et al., 2011. Noninvasive method to estimate anaerobic threshold in individuals with type 2 diabetes. *Diabetol. Metab. Syndr.* 3 (1), 1.
- Schwartz, P.J., Crotti, L., 2011. QTc behavior during exercise and genetic testing for the long-QT syndrome. *Circulation* 124 (20), 2181–2184.
- Schwartz, P.J., Periti, M., Malliani, A., 1975. The long Q-T syndrome. *Am. Heart J.* 89 (3), 378–390.
- Shamsuzzaman, A.S.M., 2003. Sympathetic nerve activity in the congenital long-QT syndrome. *Circulation* 107 (14), 1844–1847.
- Stanley, J., Peake, J.M., Buchheit, M., 2013. Cardiac parasympathetic reactivation following exercise: implications for training prescription. *Sports Med.* 43 (12), 1259–1277.
- Tankeu, A.T., et al., 2018. Effect of propranolol on heart rate variability in hyperthyroidism. *BMC Res Notes* 11 (1), 151.
- Tulppo, M.P., et al., 1996. Quantitative beat-to-beat analysis of heart rate dynamics during exercise. *Am. J. Phys.* 271 (1 Pt 2), H244–H252.
- Watanabe, J., et al., 2001. Heart rate recovery immediately after treadmill exercise and left ventricular systolic dysfunction as predictors of mortality: the case of stress echocardiography. *Circulation* 104 (16), 1911–1916.
- Winbo, A., et al., 2021. Functional hyperactivity in long QT syndrome type 1 pluripotent stem cell-derived sympathetic neurons. *Am. J. Physiol. Heart Circ. Physiol.* 321 (1), H217–H227.
- Yamamoto, Y., Hughson, R.L., Peterson, J.C., 1991. Autonomic control of heart rate during exercise studied by heart rate variability spectral analysis. *J. Appl. Physiol.* (1985) 71 (3), 1136–1142.