In vivo exploration of repolarization instability at the level of the ventricular action potential

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In vivo exploration of repolarization instability at the level of the ventricular action potential

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Abstract of Thesis

Accurate prediction of individuals at risk of ventricular arrhythmia and sudden cardiac death remains a major challenge. Beat-to-beat variability of ventricular action potential duration (APD) has long been proposed as a potential mechanism of arrhythmogenesis, however, studies examining the dynamics of this metric in humans are lacking and to date have been dependent largely on assessment of the QT interval. We hypothesised that increased beat-to-beat variability of APD would be seen in patients experiencing ventricular arrhythmia and that autonomic tone would play a significant role in the modulation of beat-to-beat variability of APD. These hypotheses were tested in a series of human experiments.

In patients with heart failure and implanted cardiac resynchronization therapy defibrillator devices we demonstrated for the first time that increased left ventricular beat-to-beat variability of APD was associated with an increased risk of ventricular tachyarrhythmia.

Subsequently, through invasive electrophysiology studies we compared the behavior of local right and left ventricular APD with global body surface QT intervals. These results highlighted that although the body surface QT interval is representative of global repolarization, subtle regional inhomogeneity of repolarization would be missed by QT interval assessment alone.

Finally, a sequence of experiments in patients with heart failure and patients with structurally normal hearts demonstrated for the first time the ability of a sympathetic stimulus to increase the beat-to-beat variability of human ventricular APD, and the

potentially important role of beta-adrenergic blocking agents in the suppression of this increase.

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Declaration

I confirm the work within this thesis is my own and I have appropriately acknowledged the work of others.

Dr Bradley R. Porter

Acknowledgments

During this PhD, I have been extremely fortunate to work alongside many talented individuals across a wide range of disciplines. First and foremost, I would like to thank my supervisors, Professor Jaswinder Singh Gill and Professor Reza Razavi for their guidance, encouragement, patience and wisdom.

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I would like to thank Dr Simon Claridge, Dr Justin Gould, Dr Ben Sieniewicz and Dr Baldeep Sidhu for help with patient recruitment and data collection, and who were always there for support and as a sounding board of ideas.

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Last, and by no means least, I would like to thank my wife, Alice, for steering me through the difficult times, and to my daughter, Florence, who helps me keep things in perspective.

Thesis outline

Chapter 1 provides an introduction into the burden of ventricular arrhythmia and discusses potential mechanisms of arrhythmogenesis, in particular the beat-to-beat variability of the ventricular action potential.

Chapter 2 explores the potential of the ventricular action potential as a risk marker for patients at risk of ventricular tachyarrhythmia.

Chapter 3 takes the most widely accepted surface marker of repolarization (the QT interval) and compares this to the localized ventricular action potential duration.

Chapter 4 assesses the impact of autonomic modulation by means of a Valsalva maneuver on the beat-to-beat variability of the ventricular action potential duration in patients with heart failure. It addresses the impact of beta-adrenergic blockade on repolarization instability.

Chapter 5 examines in greater detail the frequency domains of action potential duration oscillation. In particular this looks closer at the impact of an autonomic stimulus on the low frequency oscillation (sympathetic) of the ventricular action potential duration.

Chapter 6 explores the impact of beta-adrenergic blockade on the low frequency oscillation of the ventricular action potential in normal hearts and its potential impact on the beat-to-beat variability of repolarization.

Chapter 7 is a synthesis and summary of the previous chapter findings. It draws the final conclusions and details suggestions for future development of the use of beat-to-beat variability of the ventricular action potential duration in the clinical setting.

Abbreviations

APD – action potential duration

ARI – activation-recovery interval

ARIV – activation-recovery interval variability

ARIVi – activation-recovery interval variability index

AT – activation time

BP – blood pressure

CRT-D – cardiac resynchronization therapy defibrillator

dP/dt_{max} – maximum rate of systolic pressure increase

HF – high frequency power

ICD – implantable cardioverter-defibrillator

ICM – ischaemic cardiomyopathy

LF – low frequency power

LV – left ventricular

nHF – normalized high frequency power

NICM – non-ischaemic cardiomyopathy

nLF – normalized low frequency power

nSD – normalized standard deviation

NYHA – New York Heart Association

RV – right ventricular

RT – repolarization time

SBP – systolic blood pressure

SD – standard deviation

VF – ventricular fibrillation

VT – ventricular tachycardia

Chapter 1: Introduction

1.1 General Introduction

Sudden cardiac death from cardiac arrhythmia claims the lives of over 70,000 people per year in the UK, and approximately 300,000 people per year in the USA. The majority of these deaths are due to ventricular tachyarrhythmia (Zheng et al., 2001; Papadakis et al., 2009).

Direct current cardioversion of ventricular tachyarrhythmia is highly effective at restoring normal cardiac rhythm, and the introduction of the implantable cardioverter-defibrillator (ICD) stands as one of the major advances in clinical cardiology over the last 30 years (Moss et al., 1996; AVID Investigators 1997; Buxton et al., 1999; Moss et al., 2002). However, preventative medical therapies have been disappointing. Both the CAST trial (C. A. S. T. (CAST) Investigators, 1989) and the SWORD trial (Waldo et al., 1996) were stopped early due to an excess of deaths in the antiarrhythmic assigned cohort, and the sudden cardiac death in heart failure (SCD-HeFT) trial showed amiodarone to be equivalent to placebo and inferior to ICD therapy in preventing death for patients with heart failure (Bardy et al., 2005). To date, only beta-adrenergic receptor blockade has demonstrated a benefit in preventing sudden death in randomized clinical trials (Hjalmarson et al., 2000).

Whilst ICD implantation is effective, patient selection is complex and accurate prediction of individuals at risk of ventricular arrhythmia and sudden cardiac death remains a major challenge (Køber et al., 2016). Attempts to provide reliable indicators of sudden cardiac death has fueled one of the most active areas of investigation in arrhythmology during recent decades (Goldberger et al., 2014). The increasing appreciation of the long-term risk of complications from ICD implantation, i.e. device infection and inappropriate

therapy delivery (Tolosana et al., 2009; Sakhuja et al., 2009; Duncan et al., 2010), again emphasises the need for improvement in our assessments of patient risk.

Approximately 50% of sudden cardiac deaths occur in individuals without prior documented history of heart disease, but with most of these sufferers living with undiagnosed ischaemic heart disease (Myerburg et al., 1992). As a consequence, the most effective prevention of sudden cardiac death in the general population has been through application of risk score charts and subsequent control of risk factors associated with the development of ischaemic heart disease (Lloyd-Jones et al., 2004; Graham et al., 2017).

Several studies (Friedlander et al., 1998; Jouven et al., 1999; Dekker et al., 2006; Kaikkonen et al., 2006; Bezzina et al., 2010) have demonstrated evidence that there is a genetic predisposition to die suddenly. The Paris prospective study demonstrated evidence that a parental history of sudden death was associated with an increased relative risk of sudden death (Jouven et al., 1999). Similarly results from the Framingham study demonstrated an increased relative risk in the context of a family history of sudden cardiac death (Friedlander et al., 1998). Dekker et al highlighted that familial sudden death occurred more frequently in individuals resuscitated from primary VF than in controls (Dekker et al., 2006). This has led the way for studies searching for single nucleotide polymorphisms that predict sudden cardiac death in the general population. Although results are promising these tests are not yet ready for the clinical setting (Bezzina et al., 2010; Cupples et al., 2011).

Spanning more than two decades investigators have searched for metrics to predict sudden cardiac death. In patients with ischaemic heart disease, numerous non-invasive metrics demonstrated early promise, among them, heart rate variability, baroreflex

sensitivity, QT interval dispersion, and microvolt T-wave alternans. However, none of these have since gone on to influence clinical practice. Furthermore, invasive assessment of electrophysiology has been repeatedly demonstrated to have little if any clinical role in risk stratification (Buxton et al., 2000). As a consequence, risk stratification tools developed to predict ventricular tachyarrhythmia have resorted to using a general assessment of contractile function (left ventricular ejection fraction) as the best predictor to guide the use of ICD's for primary prevention of sudden cardiac death (Moss et al., 1996; Moss et al., 2002; Bardy et al., 2005). The Danish Study to Assess the Efficacy of ICDs in Patients with Non-ischemic Systolic Heart Failure on Mortality (DANISH) has recently highlighted the shortcomings of the use of this metric alone (Køber et al., 2016).

Where assessment of electrical function has shown promise is in the use of dynamic markers. The potential for risk stratification of individuals at risk of ventricular tachyarrhythmia using markers of beat-to-beat variability of repolarization has been demonstrated using both surface and intracardiac QT intervals (Atiga et al., 1998; Haigney et al., 2004; Tereshchenko et al., 2009; Hinterseer et al., 2010; Oosterhoff et al., 2011; Tereshchenko et al., 2011). These findings pointing to instability of repolarization as a key factor would be in keeping with a cellular mechanism such as proposed by Johnson et al (Johnson et al., 2013), who also observed dissociation between action potential duration (APD) variability and basic APD under certain conditions. Spontaneous beat-to-beat fluctuation in repolarization is an inherent property of ventricular myocardium (Baumert et al., 2016), and although influenced by cycle length (Boyett and Jewell, 1978) is largely due to variation in APD (Zaniboni et al., 2000). An assessment of beat-to-beat variability at the level of the ventricular APD in vivo, and what this represents clinically, is required.

The presented work attempts to translate basic measurements of cardiac electrophysiology to clinical arrhythmias, concentrating at the level of the ventricular APD in the human heart in vivo.

1.2 The cardiac action potential and its ionic contributions

The cardiac action potential was first described in 1880 (Burdon-Sanderson and Page, 1882). Its distinctive spike-and-plateau shape results from its ionic basis; first described by Hodgkin and Huxley (Hodgkin and Huxley, 1952) with further redefinition over time (Noble, 1962; Noble, 2007), its sharp activation allows rapid conduction and activation and the relatively drawn-out plateau allows for contraction of cardiac muscle (Grant, 2009). **Figure 1-1** demonstrates the phases of the cardiac ventricular action potential and the contributing ionic currents.

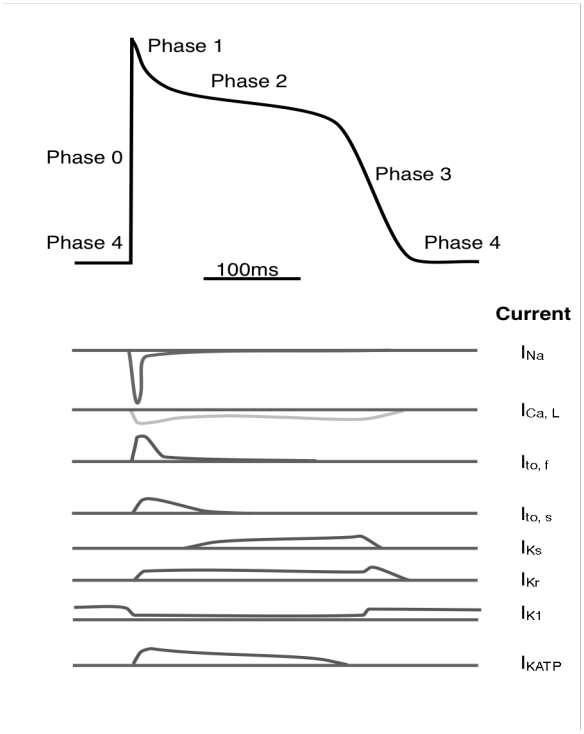


Figure 1-1 The normal cardiac ventricular action potential in humans

Currents are shown with their molecular correlates.

In the human heart the cardiac action potential has five distinct phases (0 to 4). A minor depolarization brings the membrane potential to threshold, opening the voltage-activated sodium channels (I_{Na}) (Kunze, 2004) and allowing sodium ions to diffuse down their electrochemical gradient from the extracellular space, across the membrane and into the cell. Through a positive feedback loop further sodium channels open, and depolarization of the membrane proceeds until the sodium Nernst potential is reached or inactivation of the channels occurs. The resultant fast sodium current is responsible for the rapid upstroke of the action potential (phase 0).

In phase 1, fast and slow transient outward potassium currents ($I_{to,f}$ and $I_{to,s}$) are activated causing early rapid repolarization. A prolonged plateau (phase 2) then ensues due to a balance between the inward currents of the voltage-gated L-type calcium channel ($I_{Ca,L}$) and sodium-calcium exchanger (I_{NCX}), and the outward currents of the voltage-gated

rapid, slow delayed rectifier potassium channels (I_{Kr}, I_{Ks}, respectively) and inward rectifying potassium channel (and I_{K1}) (Carmeliet, 2017). A large difference between the membrane potential and the potassium Nernst potential maintains high potassium efflux during the plateau phase. As calcium channels inactivate, the outward potassium currents begin to dominate, and further repolarization brings the membrane potential towards the potassium equilibrium potential (phase 3).

Following full repolarization, the cardiac cell membrane potential returns to its resting, non-excited state (phase 4), maintaining a voltage gradient of between -80 to -64 mV (Amos et al., 1996). This resting state is maintained by the inward rectifier potassium current (I_{K1}) and the ATP-regulated potassium current ($I_{K,ATP}$). Restoration of the membrane potential marks the end of cellular refractoriness, and the cell will once again respond to electrical stimuli (Pond and Nerbonne, 2001; Noble, 2007).

The above generalisation of cellular electrophysiology introduces the ionic basis of cardiac cellular activation and repolarization. Variation in the action potential is seen between species, between cardiac chambers, and in vitro has been demonstrated to vary between cell layers. The ionic nature of the cardiac action potential can be influenced by insults e.g. cellular ischaemia or disturbances in the cellular ionic environment, and also by physiological changes e.g. changes in autonomic tone or cardiac rate (Chien Suu and Surawicz, 1976). This highlights an important aspect of the cardiac action potential; the cardiac action potential is dynamic.

1.3 Beat-to-beat variability of repolarization and arrhythmogenesis

Beat-to-beat variability of APD may be arrhythmogenic either by the development of early or delayed afterdepolarizations (Johnson et al., 2013) or by enhanced dispersion of repolarization facilitating re-entry. Common to both of these modes of arrhythmogenesis is the concept of interactions between activation and repolarization.

1.3.1 Early and delayed afterdepolarization and triggered activity

Afterdepolarizations are depolarizations triggered by one or more preceding action potentials, which prematurely activate cardiac tissues. This is known as triggered activity (Cranefield, 1977; January et al., 2012).

Early afterdepolarization can develop before full repolarization in phase 2 or 3 of the cardiac action potential. They can be associated with prolonged or shortened APDs. Prolonged APDs occur when the inward current is greater in amplitude than the outward current. This can result from increases in the late sodium current (I_{Na}), the voltage-gated L-type calcium channel current ($I_{Ca,L}$), the sodium-calcium exchanger (I_{NCX}), or decreases in the repolarizing potassium currents (I_{Kr}, I_{Ks}, I_{K1}) (January and Riddle, 1989; Szabo et al., 1994; Xie et al., 2013). Shortened APDs permit normal calcium release from the sarcoplasmic reticulum which can in turn activate the sodium-calcium exchanger (I_{NCX}) and result in late early afterdepolarizations in phase 3 of the action potential (Patterson et al., 2006). Whether due to prolonged or shortened APDs, if the early afterdepolarization causes a significant change in membrane potential then activation of the late sodium current (I_{Na}) occurs and results in triggered activity. This type of triggered activity is

thought to explain the arrhythmogenesis observed in heart failure and long QT syndromes (Maruyama et al., 2011).

Delayed afterdepolarizations occur after full repolarization during stage 4 of the cardiac action potential. They were originally described as oscillatory afterpotentials and are observed under conditions of intracellular calcium overload (Guinamard et al., 2004). It is suspected that high levels of intracellular calcium results in spontaneous calcium release from the sarcoplasmic reticulum which in turn activates calcium-sensitive currents responsible for membrane depolarization. As with early afterdepolarizations, if the depolarization produced by the delayed afterdepolarization is significant, late sodium current (I_{Na}) activation occurs, resulting in triggered activity. Delayed afterdepolarizations are considered important in the arrhythmogenesis seen in catecholaminergic polymorphic ventricular tachycardia and other conditions causing calcium overload e.g. digitalis toxicity (Priori et al., 2001).

1.3.2 Enhanced dispersion of repolarization facilitating re-entry

The pioneering work of George Mines (Mines, 1914) established the concept of re-entry and to this day remains influential in contemporary understanding of arrhythmogenesis. Re-entry arises when an action potential reactivates a region that has recovered from refractoriness and subsequently results in a propagating wave of activation (Janse and Wit, 1989). Increased beat-to-beat variability of APD creates enhanced dispersion of repolarization allowing propagation of action potentials from areas where they are maintained to areas where they are abolished (Krishnan and Antzelevitch, 1993; Di Diego and Antzelevitch, 1993; Lukas and Antzelevitch, 1996).

1.3.3 Arrhythmogenesis in the clinical setting

There remains a gap in understanding between the cellular electrophysiology described above and the temporal promotion of arrhythmia, i.e. why should an individual develop a specific arrhythmia at a particular time? In some cases, a clear systemic upset can be held responsible e.g. myocardial ischaemia, electrolyte imbalance or pharmacological toxicity. However, at the onset of many clinical arrhythmias no specific acute structural or functional change is ever identified. A prime suspect for the apparent randomness of events is the autonomic nervous system. By causing transient changes in the excitable properties of cardiac tissue the autonomic nervous system is thought to play a key role in the modulation of cellular activation and repolarization and therefore in both the promotion and suppression of clinical arrhythmia.

1.4 The autonomic nervous system

A wealth of evidence points to the importance of the autonomic nervous system on clinical arrhythmogenesis. The CAST trial (C. A. S. T. (CAST) Investigators, 1989) demonstrated a reduction in arrhythmic death in patients post myocardial infarction treated with beta-blockade, and both the Multicenter Unsustained Tachycardia Trial (MUST) (Buxton et al., 1999; Ellison et al., 2002) and the Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF) (Hjalmarson et al., 2000) showed decreased mortality in those on beta-blockade. Inhibition of cardiac beta-receptors has remained a mainstay of current antiarrhythmic therapy (Priori et al., 2015).

Again, evidence of the influence of the autonomic nervous system on arrhythmogenesis is highlighted by observations of increased arrhythmia occurrence coinciding with major psychological stress. Examples include natural disasters (Voridis et al., 1983; Trichopoulos et al., 1983; Huang et al., 2001; Leor et al., 2002), war (Meisel et al., 1991), terrorist attacks (Steinberg et al., 2004), sport (Wilbert-Lampen et al., 2008) and the phenomenon of Voodoo death (Samuels, 1997).

In the laboratory, animal work has demonstrated increased arrhythmogenicity under induced stress (Parker et al., 1987, 1990). In the clinical setting in humans, the impact of psychometric tests (including mental arithmetic and anger recall) on cardiac repolarization at the level of the body surface ECG has been well studied (Lampert et al., 2000, 2005, 2009; Abisse et al., 2011), and increased periods of stress have been associated with increased ICD anti-tachycardia therapies in patients with ischaemic heart disease (Lampert et al., 2002). Similarly, in patients with known long QT syndromes, a history of frequent stressful life events has been associated with an increased frequency of ventricular arrhythmia (Hintsa et al., 2010).

Studies into the effects of sympathomimetic agents on cardiac electrophysiology have been conducted in animals (Mantravadi et al., 2007) and humans (Taggart et al., 2003). Observations include shortening of APD and steepening of the APD restitution curve. Animal work has also described the impact of direct sympathetic and vagus nerve stimulation on the cardiac APD (Ng et al., 2007). This study demonstrated again how sympathetic stimulation steepened the APD restitution curve but also importantly how it decreased both the effective refractory period and VF threshold. Vagus nerve stimulation had the opposite effect. Work in dogs has also highlighted the beneficial effects of vagal

stimulation in the reduction of VF witnessed during acute coronary occlusion (Myers et al., 1974).

Despite these large volumes of work, major questions remain on the precise impact of autonomic modulation on the beat-to-beat variability of APD in humans in vivo.

1.5 Conclusion and hypotheses

Prediction of individuals at risk of ventricular arrhythmias is imperfect. Electrophysiological risk predictors are numerous but a risk predictor at the level of the ventricular APD has not previously been demonstrated. Furthermore, studies translating human APD to body surface electrophysiology could help examine the weaknesses of electrophysiological risk predictors.

Whilst beat-to-beat variability of ventricular APD has been proposed as a mechanism of arrhythmogenesis, no studies of the dynamics of this metric exist in man. In particular, data is lacking on the impact of autonomic modulation on the beat-to-beat variability of APD in humans in vivo.

The body of this work presented addresses the following major hypotheses:

1) Increased beat-to-beat variability of APD will be seen in patients experiencing ventricular arrhythmia.
2) The behaviour of APD dynamics and body surface repolarization dynamics will be similar.

3) Autonomic modulation will impact beat-to-beat variability of APD in humans.

4) This impact will be suppressed by beta-adrenergic blockade

Chapter 2: Action potential duration variability as a predictor of ventricular tachyarrhythmia

This section has been adapted from *Left ventricular activation-recovery interval variability predicts spontaneous ventricular tachyarrhythmia in heart failure patients* (Porter et al., 2018a).

2.1 Introduction

Accurate prediction of individuals at risk of ventricular tachyarrhythmia and sudden cardiac death remains a major challenge. Exaggerated beat-to-beat variability of repolarization is known to be associated with arrhythmogenesis in animal models (Thomsen et al., 2004; Gallacher et al., 2007; Abi-Gerges, Valentin, and Pollard, 2009; Jacobson, Carlsson, and Duker, 2011) and humans (Atiga et al., 1998; Haigney et al., 2004; Tereshchenko et al., 2009; Hinterseer et al., 2010; Tereshchenko et al., 2011; Oosterhoff et al., 2011) and has been proposed as a potential risk marker.

The activation-recovery interval (ARI) is well validated (Haws and Lux, 1990; Coronel et al., 2006; Potse et al., 2009). In vivo it can be obtained from pacing leads in ambulatory patients, invasively during electrophysiology studies, and more recently has been derived from non-invasive cardiac electrophysiology mapping techniques (Andrews et al., 2017). As such it is readily available for the assessment of ventricular repolarization and therefor a potential adjunct in the prediction of patients at risk of ventricular tachyarrhythmia. Recent animal studies have demonstrated significant increases in the beat-to-beat variability of ARI prior to the onset of Torsades de pointes and have highlighted its potential for integration into implantable cardiac devices to monitor arrhythmia risk (Wijers et al., 2018).

This chapter explores the potential of the ventricular action potential as a risk marker for patients at risk of ventricular tachyarrhythmia. We have recorded left ventricular (LV) unipolar electrograms, while pacing from the right ventricular (RV) lead to maintain a constant cycle length in patients with heart failure. From these electrograms we have calculated ARI variability (ARIV). We hypothesized that higher baseline ARIV would be seen in patients experiencing ventricular tachycardia/fibrillation (VT/VF) during follow-up.

2.2 Methods

2.2.1 Ethical Approval

The study was approved by the local research ethics committee and conformed to the Declaration of Helsinki (latest revision: 64th WMA General Assembly) standard. Informed consent was obtained in writing from all subjects.

2.2.2 Study population and data acquisition

We retrospectively analyzed the prospectively collected data of 43 consecutive patients who underwent electrogram recordings to study basic ARI within a heart failure population. The study enrolled patients with St. Jude Medical cardiac resynchronization therapy defibrillator (CRT-D) devices for primary or secondary indications of sudden cardiac death. Patients of either sex, >18 years of age and undergoing CRT-D follow-up at our institution were eligible. During a routine follow-up visit LV unipolar electrogram recordings were made via the device programmer (Merlin, St. Jude Medical Inc., St Paul, MN). Effects of heart rate variability on repolarization dynamics were removed by establishing fixed cycle length with steady-state pacing (DDD-RV for sinus rhythm or VVI-RV for atrial fibrillation) (Bueno-Orovio et al., 2014). A constant rate of 10 beats above the patient's intrinsic heart rate was chosen with a minimum adaptation period of 2 minutes (Franz et al., 1988). A 30 second recording of LV unipolar electrogram was made using the device programmer at a sampling frequency of 512 Hz and extracted for off-line analysis (Hanson et al., 2014; Chen et al., 2016). **Figure 2-1** shows examples of raw digital unipolar electrograms. Occurrence of ventricular tachyarrhythmia therapy with either ATP or shock therapy was assessed by CRT-D checks and served as the endpoint. Programming of the CRT-D device was based on clinical evaluation of the

attending electrophysiologist. CRT-D interrogation data of recorded events was evaluated by an electrophysiologist blinded to the outcome of the LV unipolar electrogram data.

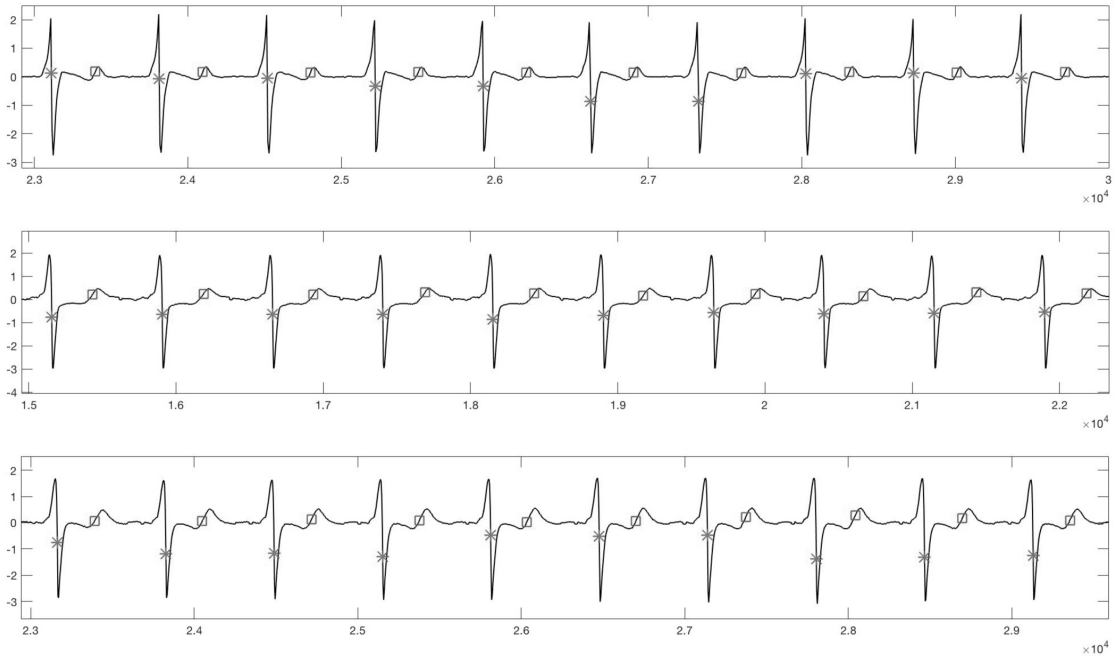


Figure 2-1. Left ventricular unipolar electrograms

Unipolar electrograms recorded from the left ventricular lead of 3 separate patients demonstrating local activation (star) and repolarization (square).

2.2.3 Repolarization variability analysis

Raw digital LV unipolar electrogram traces were analysed off-line using custom built MATLAB software (MathWorks Inc, Natick, Mass). Recordings were separately low pass filtered at both 80 and 30 Hz for calculation of activation times (ATs) and repolarization times (RTs), respectively. The choice of two separate frequencies for AT and RT calculation allowed us to maintain the sharp activation gradients required to identify ATs, whilst also successfully preserving the morphology of the slower T-wave to identify RTs. Consecutive ARIs were calculated by identifying AT and RT for each beat using the Wyatt method (Wyatt et al., 1981; Coronel et al., 2006; Potse et al., 2009;

Hanson et al., 2012; Hanson et al., 2014). Automated identification of ATs and RTs removed any observer variability. **Figure 2-1** shows examples of the identification of ATs and RTs and the resultant ARI across various morphologies of unipolar electrogram. ARIV over the full 30s recording was then computed as.

$$ARIV = \frac{\sum_{i=1}^{n_{beats}-1} |ARI_{i+1} - ARI_i|}{(\sqrt{2} \times n_{beats})}$$

where n beats is the number of beats contained within the 30s period (Pueyo et al., 2016a). To account for the possibility that the magnitude of beat-to-beat changes may depend on the intrinsic ARI duration we introduced the ARIV index. The ARIV index provides a normalized value of the ARIV relative to the mean ARI duration for each patient. The ARIV index was computed as.

$$ARIV\ index = \frac{1}{ARI_{mean}} \times \frac{\sum_{i=1}^{n_{beats}-1} |ARI_{i+1} - ARI_i|}{(\sqrt{2} \times n_{beats})}$$

$$\text{where} \quad ARI_{mean} = \frac{\sum_{i=1}^{n_{beats}} ARI_i}{n_{beats}}$$

2.2.4 Statistical Analysis

Results are presented as mean±standard deviation for normally distributed variables and as median and interquartile range (IQR) for non-normally distributed variables. The independent-samples t-test was used to compare normally distributed continuous variables; otherwise the Mann-Whitney U test was used. Categorical variables were

compared using Fisher's exact test. ROC analysis was performed using Youden's index to determine the variable cut-off levels with optimal sensitivity and specificity for the endpoint. The estimated cutoff values were retrospectively used to reclassify and dichotomize the study subjects into high and low-risk categories. Kaplan-Meier survival analysis was used to address our hypothesis testing the association between increased ARIV and probability of first appropriate defibrillator therapy for VT/VF. Cox proportional hazards analyses were performed separately for each variable of interest (Mean ARI, ARIV and ARIV index). A P value of <0.05 was considered to be statistically significant for all tests. All statistical analyses were performed using SPSS (IBM Switzerland, Switzerland) and Prism (GraphPad Software Inc., California, USA).

2.3 Results

2.3.1 Data eligibility

A total of 43 ambulatory heart failure patients underwent unipolar electrogram recordings. Of these, six patients were excluded from the ARIV analysis: two due to a >15% ectopy burden during recordings, three due to significant electrogram fractionation (**Figure 2-2A**), one due to absence of a well-defined T-wave such that no positive gradient could be identified during repolarization (**Figure 2-2B**). ARIV analysis was performed in the remaining 37 patients. T-wave morphology remained constant and there were no AV conducted beats throughout the recordings. The median RV pacing rate used during LV unipolar electrogram recordings was 85 bpm (IQR, 80 to 95). As expected, significant correlation was seen between the pacing rate and mean ARI (r = -0.725, $p < 0.001$). However, there was no correlation between mean ARI and ARIV (r = 0.045, $p = 0.792$), nor the pacing rate and ARIV (r = -0.150, $p = 0.377$).

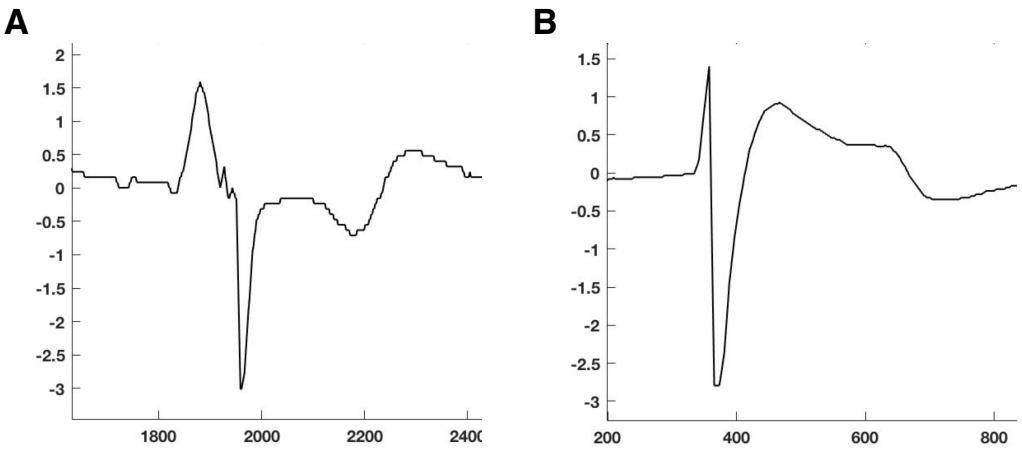


Figure 2-2. Pitfalls of ARI analysis

*Two pitfalls of ARIV analysis: (**A**) fractionation, (**B**) neutral gradient during repolarization.*

2.3.2 Study population

Of those eligible for ARIV analysis (**Table 2-1**), 30 were men (81.1%) and 7 women (18.9%) who had undergone CRT-D implantation for primary (29 patients, 78.4%) or secondary (8 patients, 21.6%) prevention of sudden cardiac death. The patients were enrolled in the study in median time 6.9 months after CRT-D implantation (range, 5.3 to 31.9 months). At the time of data acquisition, no patients had decompensated heart failure. All patients had electrolytes within ranges unexpected to disturb repolarization prior to unipolar electrogram recordings (sodium 138.1 ± 3.2 mEq/L, potassium 4.7 ± 0.5 mEq/L). During follow-up no patients were initiated on class I or III antiarrhythmic agents, nor underwent coronary intervention/VT ablation prior to meeting the study endpoint or before conclusion of study follow-up.

Table 2-1. Baseline characteristics of patients with and without subsequent appropriate ICD therapy for VT/VF.

NYHA, New York Heart Association.

Variables	No VT/VF event (n=26)	VT/VF events at follow-up (n=11)	*P* value
Age (IQR), years	68 (63 - 77)	63 (52 - 66.5)	0.059
Male, n (%)	19 (73.1)	11 (100)	0.080
Ischemic cardiomyopathy, n (%)	12 (46.2)	4 (36.4)	0.723
Ejection fraction ± SD, %	38.9 ± 11.7	26 ± 11.2	**0.004**
NYHA class ≥ 2, n (%)	16 (61.5)	9 (81.8)	0.279
Secondary prevention ICD, n (%)	6 (23.1)	2 (18.2)	1
Diabetes mellitus, n (%)	8 (30.8)	4 (36.4)	1
Hypertension, n (%)	8 (30.8)	5 (45.5)	0.465
Atrial fibrillation, n (%)	6 (23.1)	4 (36.4)	0.442
Beta-blockade, n (%)	21 (80.8)	11 (100)	0.295
ACE inhibitor, n (%)	24 (92.3)	11 (100)	1
Aldosterone antagonists, n (%)	15 (57.7)	4 (36.4)	0.295
Digoxin, n (%)	4 (15.4)	3 (27.3)	0.403
Amiodarone, n (%)	3 (11.5)	0 (0)	0.540
Biventricular pacing percentage (IQR), %	99 (97 - 99)	98 (94 - 99)	0.377
Pacing rate for unipolar electrogram recording (IQR), bpm	80 (80 - 95)	90 (82.5 - 92.5)	0.780

Comparing patients with ischemic and non-ischemic cardiomyopathy, there was no difference in mean ARI (257.69 ± 26.6 ms vs. 251.21 ± 35.27 ms, $p = 0.554$), ARIV (3.44 ± 1.32 ms vs. 2.67 ± 0.98 ms, $p = 0.055$), nor the ARIV index (1.37 ± 0.6% vs. 1.07 ± 0.36%, $p = 0.115$). LV ejection fraction (LVEF) showed no correlation with mean ARI

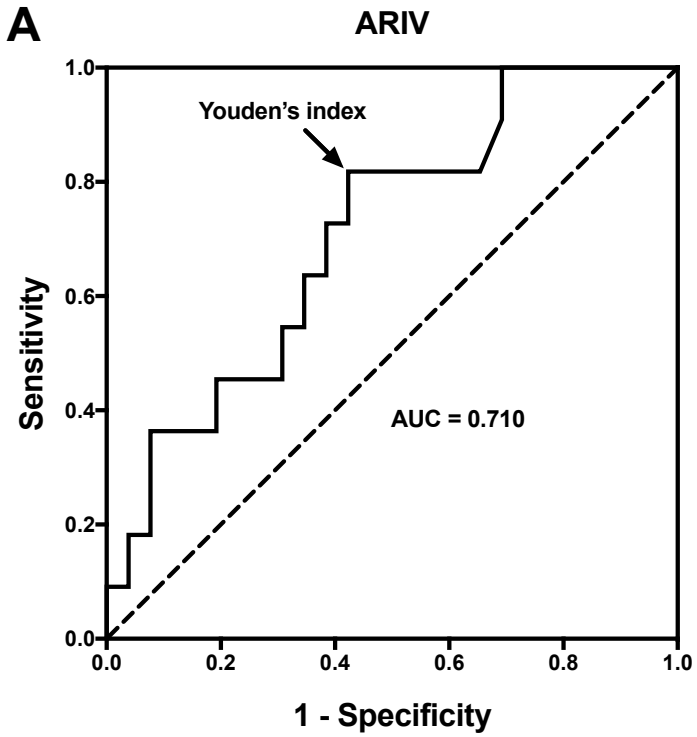
(r_s = 0.021, p = 0.901), ARIV (r_s = 0.020, p = 0.907) nor the ARIV index (r_s = 0.039, p = 0.818). Between patients with primary and secondary prevention indications for CRT-D there was no difference in mean ARI (257.34 ± 32.35 ms vs. 241.97 ± 26.99 ms, p = 0.207). Differences in ARIV approached significance (2.83 ± 1.21 ms vs. 3.62 ± 0.94 ms, p = 0.051), and a significantly higher ARIV index was seen in the secondary prevention group (1.12 ± 0.5 % vs. 1.49 ± 0.34 %, p = 0.021). 23 of the patients were CRT responders and 14 non-responders (a CRT responder was defined as a ≥5% improvement in LVEF from pre-implant). Between responders and non-responders there was no observed difference in mean ARI (253.26 ± 35.24 ms vs. 255.26 ± 25.61 ms, p = 0.865), ARIV (2.94 ± 1.14 ms vs. 3.11 ± 1.3 ms, p = 0.699) nor ARIV index (1.18 ± 0.48 % vs. 1.23 ± 0.53 %, p = 0.817).

2.3.3 Implantable cardioverter-defibrillator therapy

Following LV unipolar electrogram recordings, a mean follow-up of 23.6 ± 13.6 months took place. During follow-up 11 patients of 37 reached the endpoint of appropriate ICD therapy for VT/VF. ATP was attempted and successful in 9 patients with VT. One patient with VT had successful rescue shock therapy. One patient experienced VF with successful shock therapy. One patient died from heart failure before reaching the endpoint. **Table 2-1** shows a comparison of clinical characteristic of patients with and without subsequent appropriate ICD therapy for VT/VF**.**

ARIV was significantly greater in patients with subsequent VT/VF events vs. those without VT/VF events (3.55 ± 1.3 ms vs. 2.77 ± 1.09 ms, p = 0.047). The ARIV index was also significantly greater in patients with subsequent VT/VF events vs. those without VT/VF events (1.43 ± 0.5 % vs. 1.1 ± 0.47 %, p = 0.036). No observed difference between groups was found in mean ARI (249.34 ± 27.91 % vs. 256 ± 33.31 %, p = 0.618).

Receiver operating characteristic (ROC) curve analysis (**Figure 2-3**) suggested cut-off levels for ARIV of ≥2.52 ms with 82% sensitivity (95% CI, 48–98%) and 58% specificity (95% CI, 37-77%) (AUC 0.71; 95% CI, 0.53-0.89; $p = 0.046$) and ARIV index of ≥1.14% with 64% sensitivity (95% CI, 31-89%) and 65% specificity (95% CI, 44-83%) (AUC 0.72; 95% CI, 0.55-0.9; $p = 0.036$) to dichotomize into high/low risk for the endpoint of appropriate ICD therapy.



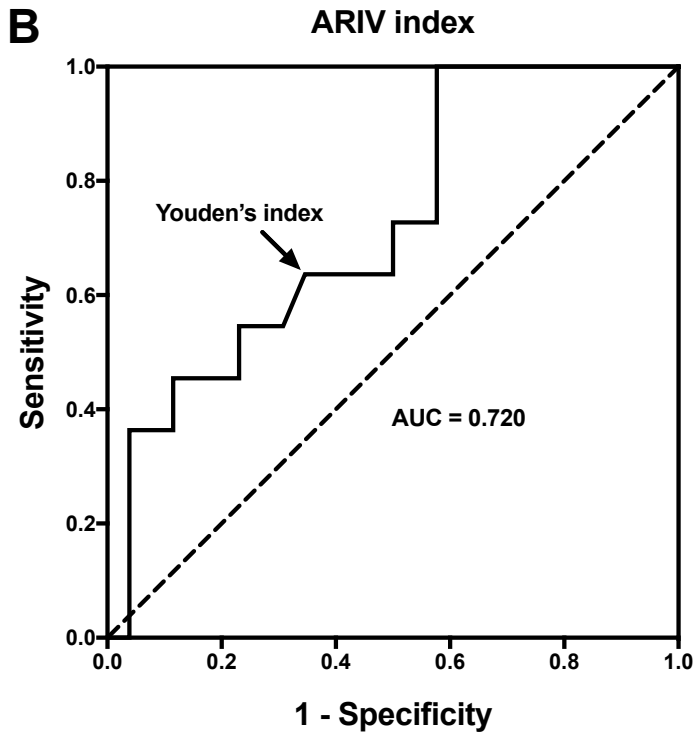


Figure 2-3. Receiver operating characteristic analysis for (A) ARIV and (B) ARIV index to predict VT/VF

Optimal cut-off levels determined by Youden's index.

Table 2-2 shows the clinical characteristics of patients with ARIV dichotomized at high and low risk of VT/VF. When comparing subjects in the high-risk group for ARIV, 45% experienced an episode of VT/VF by 3 years, compared with 11.8% in the low-risk group. **Figure 2-4A** demonstrates the separation of the Kaplan-Meier curves at the variable cut-off for ARIV (Mantel-Cox log-rank test, $p = 0.028$). When comparing subjects in the high-risk group for ARIV index, 43.8% experienced an episode of VT/VF by 3 years, compared with 19.0% in the low-risk group. **Figure 2-4B** demonstrates the separation of the Kaplan-Meier curves at the variable cut-off for ARIV index (Mantel-Cox log-rank test, $p = 0.079$).

Table 2-2. Clinical characteristics of patients with ARIV dichotomized as per ROC suggested optimal cut-off values.

NYHA, New York Heart Association.

Variables	**ARIV low-risk (n=17)**	**ARIV high-risk (n=20)**	***P* value**
Age (IQR), years	68 (62 - 76)	66 (56 - 75)	0.279
Male, n (%)	12 (70.6)	18 (90)	0.212
Ischemic cardiomyopathy, n (%)	5 (29.4)	11 (55)	0.185
Ejection fraction ± SD, %	35.5 ± 13.6	34.8 ± 12.5	0.857
NYHA class ≥ 2, n (%)	10 (58.8)	15 (75)	0.482
Secondary prevention ICD, n (%)	1 (5.9)	7 (35)	**0.048**
Diabetes mellitus, n (%)	7 (41.2)	5 (25)	0.482
Hypertension, n (%)	2 (11.8)	11 (55)	**0.014**
Atrial fibrillation, n (%)	6 (35.3)	4 (20)	0.460
Beta-blockade, n (%)	14 (82.4)	18 (90)	0.644
ACE inhibitor, n (%)	16 (94.1)	19 (95)	1
Aldosterone antagonists, n (%)	11 (64.7)	8 (40)	0.191
Digoxin, n (%)	5 (29.4)	2 (10)	0.212
Amiodarone, n (%)	0 (0)	3 (15)	0.234
Biventricular pacing percentage ± SD, %	99 (98 - 99)	98 (94 - 99)	0.368
Pacing rate for unipolar electrogram recording ± SD, bpm	90 (80 - 100)	80 (80 - 90)	0.148

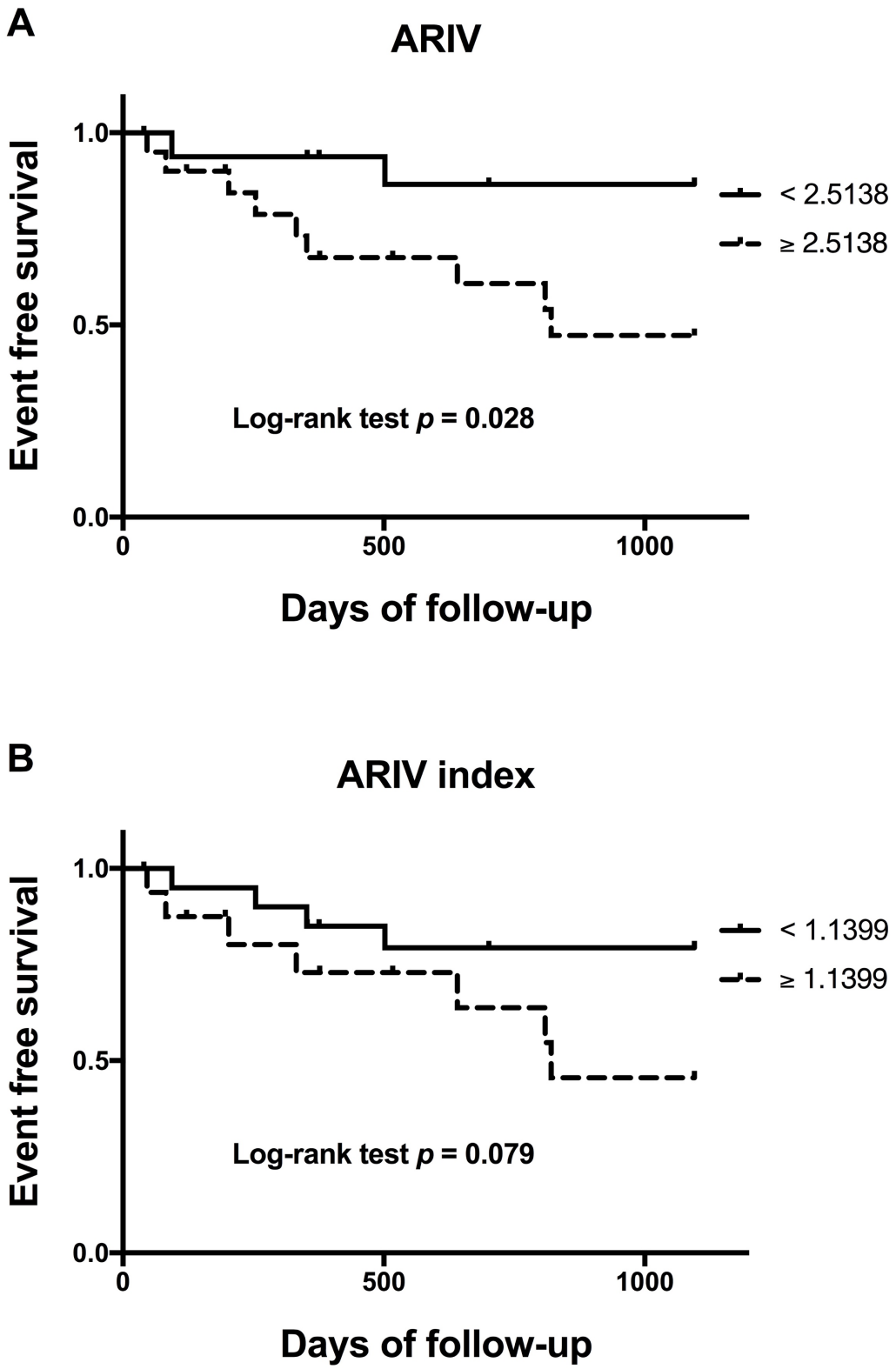


Figure 2-4. Kaplan-Meier survival curves

*Kaplan-Meier curves for freedom from VT/VF events in patients dichotomized by receiver operating characteristic curve analysis–derived optimal cut-off values for (**A**) activation-recovery interval variability (ARIV) and (**B**) ARIV index.*

Mean ARI, ARIV and the ARIV index were tested separately in the multivariate Cox proportional-hazards regression model for all VT/VF events, with the significant clinical covariate LVEF. Low LVEF remained a significant predictor of appropriate ICD therapy for VT/VF in all models tested. Mean ARI was not predictive (HR, 0.997; 95% CI, 0.977-1.017; $p = 0.758$). ARIV (HR, 1.623; 95% CI, 1.1-2.393; $p = 0.015$) and the ARIV index (HR, 3.256; 95% CI, 1.222-8.676; $p = 0.018$) were independent predictors of VT/VF (**Figure 2-5**). After exclusion of patients with secondary indications for ICD therapy both ARIV (HR, 1.518; 95% CI, 1.009-2.285; $p = 0.045$) and the ARIV index (HR, 2.87; 95% CI, 1.033-7.975; $p = 0.043$) remained independent predictors of VT/VF. After exclusion of patients on amiodarone both ARIV (HR, 1.625; 95% CI, 1.114-2.371; p = 0.012) and the ARIV index (HR, 3.259; 95% CI, 1.262-8.411; p = 0.015) remained independent predictors of VT/VF.

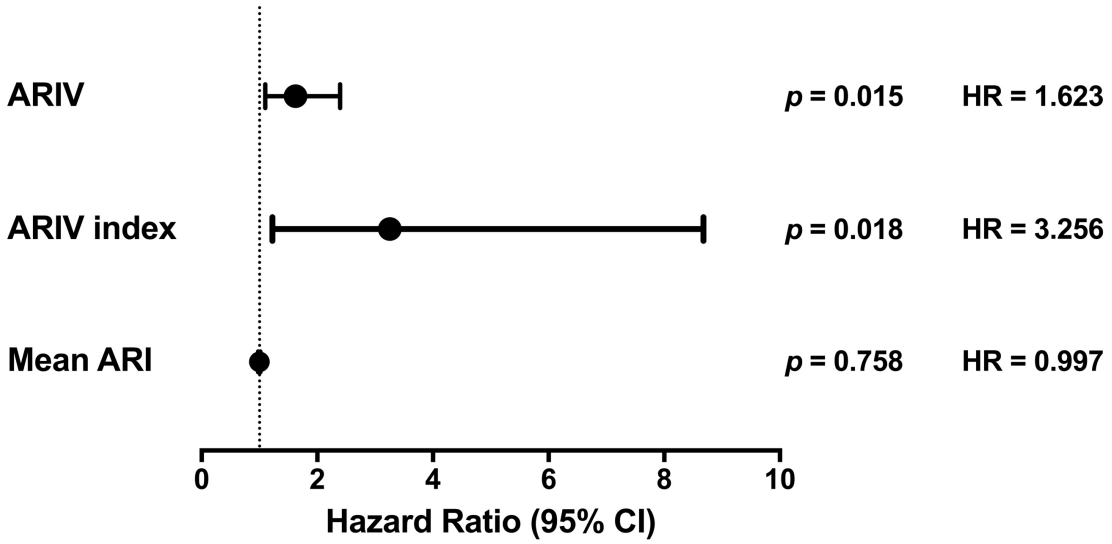


Figure 2-5. Predictors of ventricular tachyarrhythmia

Hazard ratios (adjusted for left ventricular ejection fraction) for the association of mean activation-recovery interval (ARI), ARI variability (ARIV) and ARIV index with appropriate implantable cardioverter-defibrillator therapy for ventricular tachycardia or ventricular fibrillation. CI = confidence interval.

2.4 Discussion

To our knowledge, the work in this chapter demonstrates for the first time, an association between increased LV beat-to-beat variability of repolarization and risk of ventricular tachyarrhythmia. The main findings were: 1) increased ARIV was associated with an independent risk for VT/VF; 2) increased ARIV index (ARIV normalized to mean ARI) remained an independent predictor for VT/VF; 3) there was no association between mean ARI and VT/VF risk.

2.4.1 Relation to prior work on repolarization variability

The potential for stratification of individuals at risk of ventricular tachyarrhythmia by means of repolarization instability has been demonstrated with QT intervals from the surface ECG and RV intracardiac electrograms (Atiga et al., 1998; Haigney et al., 2004; Tereshchenko et al., 2009; Hinterseer et al., 2010; Tereshchenko et al., 2011; Oosterhoff et al., 2011). Our findings of higher values of beat-to-beat variability of ARI in heart failure patients experiencing VT/VF extends these observations to the level of the ventricular APD and to the assessment of LV beat-to-beat variability of repolarization. The ROC analysis found ARIV to be more sensitive than the ARIV index in the prediction of ICD therapies. This was highlighted again in the Kaplan-Meier analysis showing less separation in the curves for ARIV index when compared to ARIV. These results would suggest that use of beat-to-beat variability of ARI to assess risk of VT/VF is more reliable without adjustment for basic ARI.

A major component of QTV is heart rate variability and both the QT interval and APD are strongly cycle length dependent (Boyett and Jewell, 1978; Zaza et al., 1991). As the

majority of QTV studies occurred in the absence of controlled cycle length it is technically challenging to separate heart rate driven QTV from actual fluctuations in the QT interval. RV pacing to obtain cycle length control as employed in our study removes the component of beat-to-beat variability of repolarization due to heart rate variability.

It is accepted that the QT interval in a given ECG lead measures the interval between the earliest depolarization and latest repolarization as projected onto the axis of that lead (Baumert et al., 2016). Given its spatial heterogeneity the use of multi-lead ECG recordings to assess QTV has been suggested but warrants further investigation (Baumert et al., 2016). The in vivo dispersion of ARIV and correlations to various body surface ECG repolarization indices should be studied and could be invaluable in our understanding of both QTV and ARIV.

2.4.2 Relation to prior work on basic APD and QT interval measurements

In the present study, basic ARI in heart failure patients was not predictive of VT/VF events. This is consistent with several studies reporting QT variability as a stronger predictor of arrhythmia than QT prolongation (Thomsen et al., 2004; Haigney et al., 2004; Gallacher et al., 2007; Hinterseer et al., 2010; Jacobson et al., 2011). These findings pointing to instability of repolarization as a key factor would be in keeping with a cellular mechanism such as proposed by Johnson et al (Johnson et al., 2013), who also observed dissociation between APD variability and basic APD under certain conditions.

Shortening of basic APD occurs in responders to CRT, whilst lengthening of basic APD occurs in non-responders (Chen et al., 2016). In vivo electrical remodelling in heart

failure at the level of beat-to-beat variability of APD needs to be studied prospectively and may offer insight into the impact of CRT on ventricular tachyarrrhythmias.

2.4.3 Mechanisms of beat-to-beat variability of repolarization

Several mechanisms have been proposed for the cellular basis of beat-to-beat variability of APD. Its apparently random nature suggests the involvement of a stochastic process. Stochastic variation of fast sodium current (I_{Na}), L-type calcium current (I_{CaL}), transient outward current (I_{to}), rapid delayed rectifier (I_{kr}) and slow delayed rectifier (I_{ks}) potassium currents has been shown to influence beat-to-beat variability of APD (Tanskanen et al., 2005; Heijman et al., 2013; Pueyo et al., 2016a) with considerable interdependence between individual channels (Pueyo et al., 2016a). Spontaneous calcium release from the sarcoplasmic reticulum exhibits beat-to-beat variability and in the presence of calcium overload has been shown experimentally and in silico to generate beat-to-beat variability of APD (Johnson et al., 2013). This mechanism was due to spontaneous calcium release from the sarcoplasmic reticulum in late diastole reducing the subsequent calcium transient and hence reducing I_{CaL} deactivation and prolonging the APD. However, the extent to which these effects seen in isolated cells may be operative in the whole heart where cells are well coupled is uncertain due to electrotonic interaction between cells (Zaniboni et al., 2000). Nevertheless, under conditions of calcium overload or reduced repolarization reserve, the effect of stochasticity on channel behavior may be enhanced suggesting that these effects may become operative in pathological conditions. Beat-to-beat variability of APD may be arrhythmogenic either by the development of early or delayed afterdepolarizations (Johnson et al., 2013) or by enhanced dispersion of repolarization facilitating re-entry.

2.4.4 Clinical implications and future work

Risk stratification of patients at high risk of sudden cardiac death remains a major challenge. In view of the multiple mechanisms involved it is unlikely that a single test would prove sufficient and that a combination of clinical characteristics with a selection of stratification tools may be more appropriate (Dagres and Hindricks, 2013). In this context, our study builds on the body of evidence highlighting the potential for assessment of baseline beat-to-beat variability of repolarization to form part of the risk stratification tool.

Wijers et al (Wijers et al., 2018) have highlighted the significance of the temporal behavior of ARI prior to the onset of ventricular tachyarrhythmia in dogs. Furthermore, this work demonstrated comparable short-term variability of ARI between RV and LV. The potential for automated continuous real-time monitoring of ARIV offers a novel future application for ICDs. However, the optimal recording location is unknown and further work is needed to compare ARIV across multiple simultaneous recording sites within the heart. Paroxysmal atrial arrhythmias may result in variable ventricular filling in biventricular paced patients and as such their influence on the ventricular ARI within a CRT population should be studied.

2.4.5 Limitations

The study population was relatively small and as a single tertiary centre study the patient group may not be representative of the usual CRT-D population. These results should be validated in a larger multicenter prospective study of a primary prevention ICD indication cohort. As ischemia testing was not conducted as part of the protocol we are unable to determine the influence of ischemia on beat-to-beat variability of ARI. Our observations

are confined to a single LV epicardial site. Regional variation of the electrophysiological properties throughout the ventricular myocardium makes it possible that other regions may have demonstrated differing results. Short and long-term variation in ARIV should be studied in order to determine the optimal duration and frequency of recordings for its use as a predictor of ventricular tachyarrhythmia (Guduru et al., 2013). Whilst strategies to analyze fractionated electrograms have been proposed (Ellis et al., 1996), a clear consensus in their interpretation does not exist. In the context of the assessment of beat-to-beat variability of repolarization this could prove a challenge. Furthermore, the presence of a high ectopy burden or the lack of a gradient to define repolarization time could exclude some patients altogether. In our study 14% of patients were excluded due to these limitations thus highlighting an area for future work.

2.5 Conclusions

In patients with heart failure, increased ARIV is associated with increased risk of spontaneous VT/VF. These results accord with observations in QTV and extend observations to assessment of LV beat-to-beat variability of repolarization and specifically to the level of ventricular APD. Our findings are supportive of the possible utility of beat-to-beat variability of repolarization as an adjunct to risk stratification of patients at risk of ventricular arrhythmia.

Chapter 3: QT interval tracks temporal behavior of ventricular action potential duration at single and multiple intracardiac sites during steady-state adaptation

3.1 Introduction

The QT interval of the body surface ECG is widely used as a non-invasive measure of the time interval between the onset of ventricular activation and the end of ventricular repolarization. It is commonly used clinically to identify and characterize a number of proarrhythmic states related to APD. The temporal behavior of APD is well known to be an important predictor of arrhythmias. In animal models and in humans these are usually recordings obtained from a single or a limited number of selected ventricular sites (Thomsen et al., 2004; Gallacher et al., 2007; Abi-Gerges et al., 2010; Jacobson et al., 2011). However, information on the relation of these single site recordings of APD to the QT interval in the body surface ECG is scant. Several studies have reported a good correspondence between RV electrograms and the body surface ECG (Paz et al., 2006; Sandhu et al., 2008; Tereshchenko et al., 2009). However, none of these provide a specific measure of APD. Studies of the relationship of local ventricular APD recordings in humans are therefore important to underpin experimental models and to facilitate extrapolation to the in situ beating human heart.

We have recorded ARIs from unipolar electrograms, an established validated measure of APD (Haws and Lux, 1990; Coronel et al., 2006; Potse et al., 2009), from 10 RV and 10 LV endocardial sites in patients with normal ventricles. APD (ARI) values from the 20 sites were compared singly and in various combinations with QT intervals from simultaneous body surface lead II ECGs during RV pacing. In order to obtain a range of APD and QT values we studied a two-minute adaptation period following an abrupt change by RV pacing.

3.2 Methods

3.2.1 Ethical approval

The study was approved by the ethics committee of Guy's and St Thomas' Hospitals and conformed to the standards set by the Declaration of Helsinki (latest revision: 59th WMA General Assembly). All patients gave written informed consent.

3.2.2 Subjects

Studies were performed in 12 patients (10 males, 2 females, age 41 - 70, median 62) during the course of routine clinical radiofrequency ablation procedures for atrial fibrillation (AF). Three patients had paroxysmal AF and 9 persistent AF. All patients had normal ventricular function and were otherwise apparently healthy. No subject was known to have ventricular scar or disordered conduction due to bundle branch abnormality. The studies were conducted in the cardiac catheterization suite at St Thomas' Hospital prior to the routine clinical procedure in the un-sedated state as described previously (Taggart et al., 2003; Hanson et al., 2009). Cardioactive medications were discontinued for 5 days prior to the study.

3.2.3 Measurements

Synchronous measurements were made of the unipolar electrograms and body surface lead II ECG sampled at 1200Hz and analyzed off-line. Unipolar electrograms were measured using two decapolar electrode catheters (St Jude Medical (St. Paul, MN, USA) 6F Livewire^{TM} Steerable Catheter model 401915 with 2-5-2mm spacing, 35 mm total span). One electrode catheter was introduced from the femoral vein into the LV (via an

atrial trans-septal approach) and positioned on the infero-posterior endocardial wall in a base-apex orientation. The other electrode catheter was introduced into the RV and positioned on the anterior septal wall in a base-apex orientation. The electrode arrays were positioned over the mid and lower third of the LV and RV endocardial wall. Both electrodes were referenced to a large skin surface electrode (100 x 150 mm) on the abdomen at the level of the naval such that distance to each individual electrode was considered to be approximately equal. The position of the recording and pacing electrodes are shown in **Figure 3-1**. Cine imaging fluoroscopy was used to verify secure positioning of the catheters throughout the cardiac and respiratory cycles. This has previously been established in detail for catheters in these positions (Hanson et al., 2012).

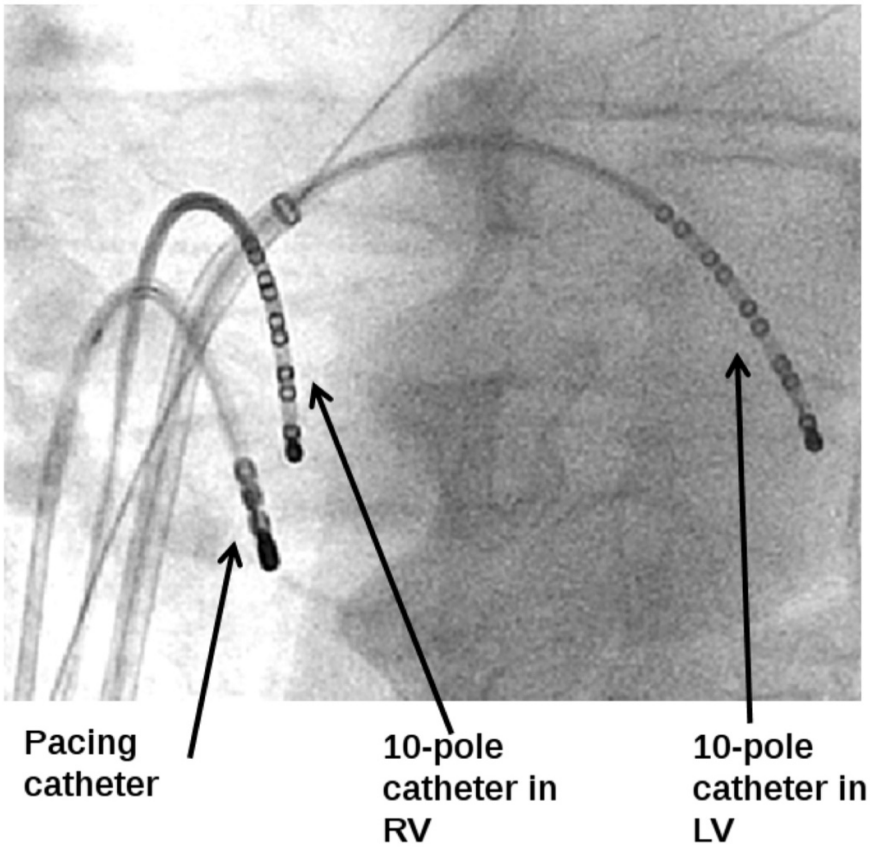


Figure 3-1. Fluoroscopic image showing the position of the two 10-pole recording catheters located in the left ventricle (LV) and right ventricle (RV)

3.2.4 Protocol

Subjects were paced from the RV apex using a Biotronik (Berlin, Germany) stimulator (model UHS 3000) at 2 x diastolic threshold and 2 ms pulse width, at a cycle length >20 beats per minute (bpm) faster than the intrinsic AF rate (median 500ms) to avoid breakthrough intrinsic beats. The first 2-minute period following initiation of pacing was used for comparison of QT with ARI as a model previously described in the literature (Franz et al., 1988). Synchronous recordings of lead II ECG, RV and LV unipolar electrograms were made.

3.2.5 Data analysis

Ventricular APDs at each recording site were estimated from the unipolar electrogram by measuring ARIs using the Wyatt method (Wyatt et al., 1981). This method has been validated in theoretical, computational and experimental studies (Millar et al., 1985; Haws and Lux, 1990; Coronel et al., 2006; Potse et al., 2009; Western et al., 2015). According to this method, activation is measured at the moment of minimum dV/dt of the QRS complex of the unipolar electrogram and repolarization at the moment of maximum dV/dt of the T-wave. The relationship between APD and ARI has been illustrated previously. In this work, ARI was measured semi-automatically using a custom algorithm written in MATLAB (MathWorks Inc, Natick, Mass). QT was measured from the lead II surface ECG. The R-wave peak was detected using a derivative-threshold algorithm with parabolic fitting on the R apex (Baumert et al., 2012; Porta et al., 2015). T-wave end was determined using the tangent method (Xue and Reddy, 1998). An example of ARI adaption over the two-minute period is shown in **Figure 3-2**.

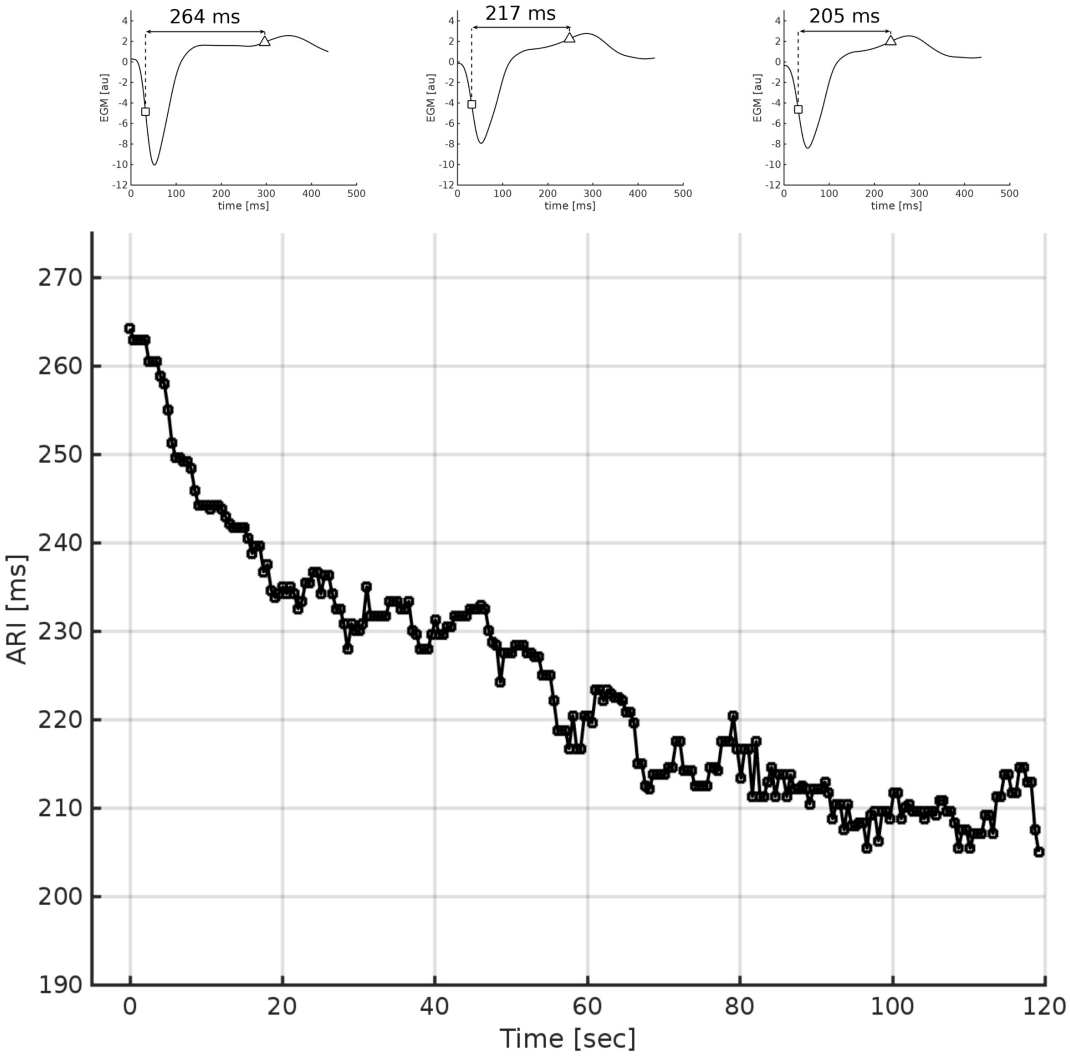


Figure 3-2. An example of ARI adaption over the two-minute period with individual ARIs highlighting its gradual shortening.

Beat-by-beat combined RV and LV ARI ($ARI_{Combined}$ 20 electrodes), RV ARI ($ARI_{RV,}$ 10 electrodes) and LV ARI ($ARI_{LV,}$ 10 electrodes) were determined by averaging the ARI for each beat using data from all the corresponding electrograms.

Heuristic-based screening was used to identify and discount any cases where the T-wave (ECG and unipolar electrogram) was indistinct or corrupt. To establish evenly-sampled series, any beats for which ARI and/or QT measurement could not be determined were

replaced by linear interpolation between the surrounding beats. If these surrogate beats constituted more than 15% of any series, the series was rejected.

3.2.6 Statistical Analysis

Results are presented as mean±standard deviation for normally distributed variables and as median and interquartile range (IQR) for nonnormally distributed variables. The Shapiro-Wilk test was used to determine normality for continuous variables. Continuous variables were then compared using the paired t test or Wilcoxon matched-pairs signed rank test for related observations depending on normality. Correlation between variables was expressed using the Spearman correlation coefficient (r_s). Linear regression was used to assess the relationship in magnitude between continuous variables. A *P* value of <0.05 was considered to be statistically significant for all tests. All statistical analyses were performed using SPSS Statistics version 24 (IBM Switzerland, Zurich, Switzerland) and Prism version 7 software (GraphPad Software Inc., La Jolla, California, USA).

3.3 Results

3.3.1 Study population and data eligibility

In one patient, there were no RV electrogram recordings. A total of 230 electrograms were available. Of these, 38 where the T-wave was indistinct or corrupt were excluded. The remaining 192 electrograms were included in the analysis.

3.3.2 QT interval / endocardial ARI (APD) relationship

Lead II QT demonstrated highly significant positive correlations with RV and LV endocardial ARI (r_s of 0.786±0.253 and 0.795±0.287 respectively). In order to enable comparisons to be made between QT and ARI values at each electrode site, recordings were made during a 2-minute period following a pacing induced abrupt alteration in cycle length. The adaptation of APD, due to the cycle length dependence of APD, thereby providing a range of values as both QT and ARI shortened to a new steady state. An example is shown for one patient in **Figure 3-3** demonstrating QT vs. ARI values at all 10 RV and all 10 LV electrode recording sites. For all electrodes the slope of the regression line was close to unity.

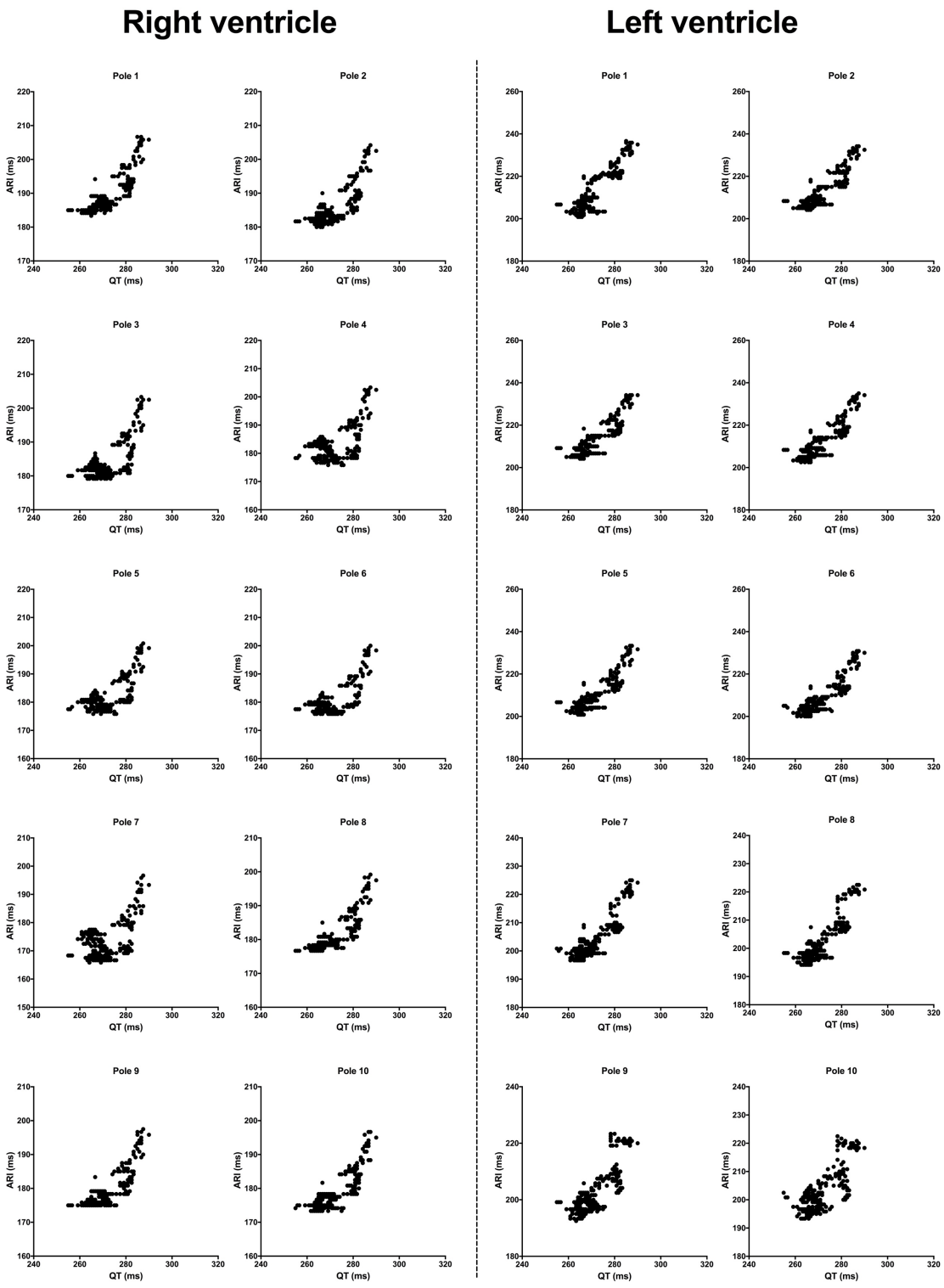


Figure 3-3. Example of one patient's scatter plots showing the strong correlations between body surface lead II QT vs. ARI values at all 10 right ventricular and left ventricular electrode recording sites.

To confirm that the strength of the correlation in temporal behavior was not dependent on signal quality the signal-to-noise ratios of both the body surface ECG and the endocardial signals were calculated. There was no relationship between the strength of QT to ARI correlations with the signal-to-noise ratio of the electograms (r_s=-0.252, p=0.426), nor the signal-to-noise ratio of the surface ECG (r_s=0.336, p=0.283).

Combining electrogram data demonstrated that the correlation coefficient was strongest for QT vs $ARI_{Combined}$ (r_s=0.88±0.116, all p<0.001), intermediate for QT vs. ARI_{RV} (r_s=0.822±0.236, all p<0.001) and QT vs. ARI_{LV} (r_s=0.818±0.3, one case p=ns, all others p<0.001), and slightly less for QT vs. ARI when compared to the average correlation coefficient of individual unipolar electrode sites (r_s=0.807±0.2). $ARI_{Combined}$ correlated significantly more with lead II QT than the average single electrogram (p=0.019). Data for all patients is summarized in **Figure 3-4**.

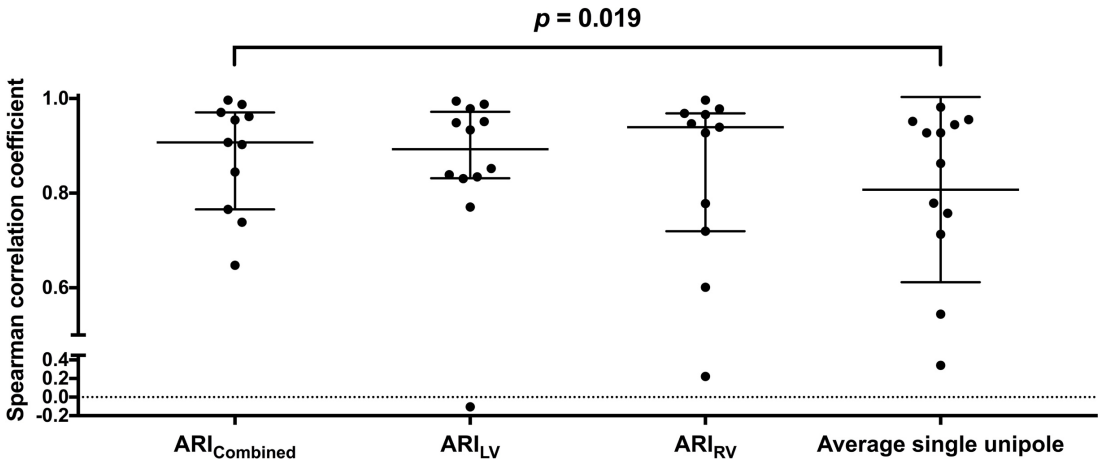


Figure 3-4. Demonstration of the improvement in correlation strength between QT and ARI as ARI is derived from increasing electrogram recording sites.

Highlighting how lead II QT is representative of global repolarization. ARICombined, mean beat-by-beat ARI from all poles (LV & RV); ARI_{LV}, mean beat-by-beat ARI from all LV sites; ARI_{RV}, mean beat-by-beat ARI from all RV sites.

For an inter-patient assessment, near steady state values of QT and ARI, obtained by averaging over the last 30 seconds of the 2-minute rate adaptation period, were compared. Mean steady state paced QT was 266±24.54 ms, mean ARI_{RV} was 194.6±21.96 ms, and mean ARI_{LV} was 199.4±23.4 ms. Inter-patient QT correlated significantly with mean steady state ARI_{RV} (r_s=0.611, p=0.025) and mean steady state ARI_{LV} (r_s=0.522, p=0.042).

3.3.3 Magnitude of change of ARI and QT

The linear regression slope was used to assess the relationship in magnitude (ms change in ARI for every ms change in QT) between body surface QT and endocardial ARI. **Figure 3-5** demonstrates this relationship for all LV and RV electrograms. The beat-to-beat change in magnitude of the LV ARI closely followed the beat-to-beat change in body surface QT intervals with a 1.02±0.03 ms change in LV ARI for every 1 ms change in QT. The beat-to-beat change in magnitude of the RV ARI was less than that seen in the beat-to-beat change in body surface QT with a 0.76±0.06 ms change in RV ARI for every 1 ms change in body surface QT. When comparing LV 1 to RV 1, LV 2 to RV 2, etc., the mean beat-to-beat change in LV ARI was significantly higher than that of RV ARI (p<0.001) for each ms change in QT.

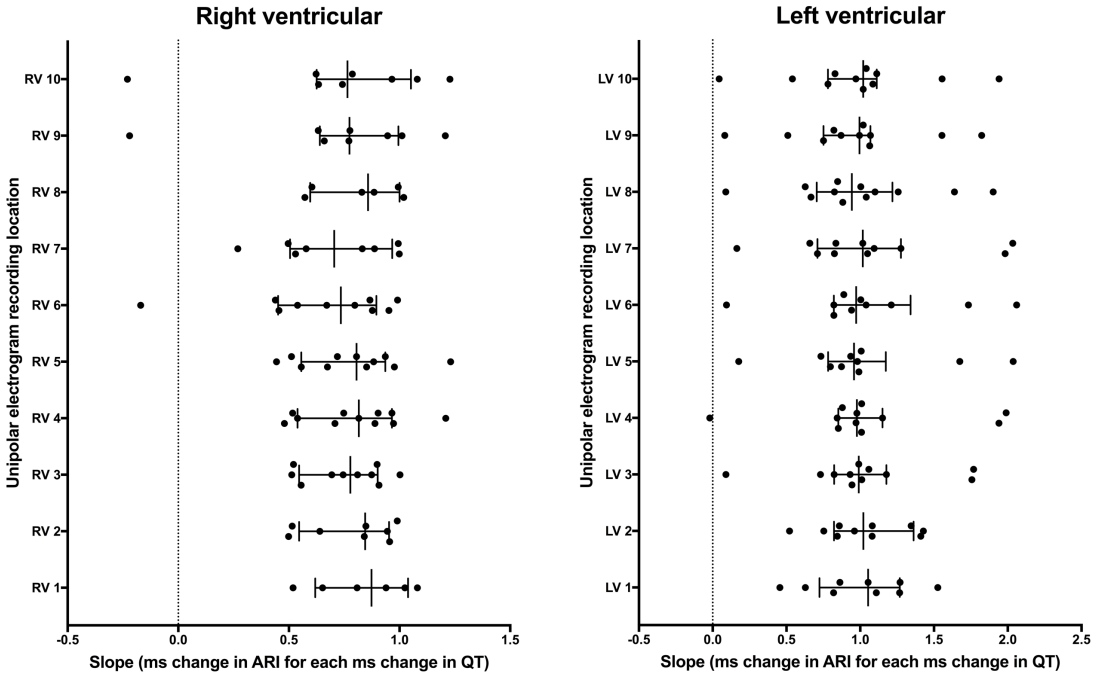


Figure 3-5. Slope results from the linear regression analysis showing the relationship in magnitude (ms change in ARI for every ms change in QT) between body surface lead II QT and ARI at all 10 right ventricular and left ventricular endocardial electrode recording sites.

3.4 Discussion

To our knowledge, this is the first study to investigate the in vivo relationship between body surface QT intervals and intracardiac ARIs in humans during steady-state adaptation. We studied patients undergoing routine electrophysiological procedures with normal ventricles. Body surface ECG lead II was recorded together with intracardiac unipolar electrograms from 10 RV and 10 LV sites from which ARIs were derived as a conventional surrogate for APD. The main findings were: (1) the temporal behavior of the body surface ECG lead II measured over a range of cycle lengths correlated with the temporal behavior of local APD measured as ARIs at 10 RV and 10 LV sites; (2) The correlation was strongest for QT vs. all LV and RV sites combined, intermediate for QT vs. LV and QT vs. RV, and slightly less but still significant for QT vs. individual electrode sites; (3) The magnitude of beat-to-beat change in QT intervals were near equal to the magnitude of beat-to-beat change in LV ARI; (4) The magnitude of beat-to-beat change in RV ARI was lower than that seen in the magnitude of beat-to-beat change in body surface QT and LV ARI.

Relatively little information has been available to date on the relationship of ARI measurements of ventricular APD to the QT interval of the body surface ECG. Several studies have demonstrated a good correspondence between repolarization waveforms derived from endocardial signals with the body surface ECG (Paz et al., 2006; Sandhu et al., 2008; Tereshchenko et al., 2009). In patients with implantable cardioverter defibrillators (ICD), T-wave amplitude obtained from a variety of different electrode combinations between tip, can and coil correlated well with T-wave alternans on the body surface ECG (Paz et al., 2006). In patients undergoing routine electrophysiological procedures recordings obtained from a RV quadripolar catheter during pacing induced

alternans showed a good correspondence between T-wave alternans in unipolar electrograms and microvolt T-wave alternans in the ECG (Sandhu et al., 2008). In ICD patients QT variability index in the ECG correlated well with measurements derived from both near field and far field RV electrograms recorded from the device (Tereshchenko et al., 2009). In the present study we have used ARI calculated from a unipolar electrogram which is an established validated surrogate measure of APD (Millar et al., 1985; Haws and Lux, 1990; Coronel et al., 2006; Potse et al., 2009; Western et al., 2015).

It must be emphasized that this study does not in any way attempt to characterize the exact relationship between the body surface lead II QT interval and the local intracardiac APD. Such a comparison would require multiple global recording sites together with highly complex computation in relation to measurements of cardiac/torso geometry and has been the subject of extensive study. In the present studies we show a good correspondence between changes in local single site and multiple site recordings of endocardial APD measured as ARI, and changes in the QT interval in lead II of the body surface ECG in patients with normal ventricles. A number of factors might modify our results. Factors which increase the contribution of ventricular activation time to the QT interval or alter the sequence of activation might be expected to reduce the degree of correspondence with APD that we observe. Conversely factors which increase the contribution of APD to the QT interval might be expected to increase it. The presence of pathology and increased electrophysiological inhomogeneity might similarly be expected to reduce the degree of correspondence between QT and endocardial APD.

Our results may be particularly pertinent to beat-to-beat QT interval variability in the body surface ECG (QTV). Enhanced QTV is strongly associated with increased arrhythmia risk and has been advocated for ICD risk stratification (Baumert et al., 2016).

At a constant heart rate QTV is generally considered to comprise beat-to-beat variation in ventricular activation and APD (Baumert et al., 2016). Investigation of underlying mechanisms has usually focused on APD using monophasic action potentials in animal models, ARIs in humans and transmembrane recordings in single cells and wedge preparations (Thomsen et al., 2004; Nánási et al., 2017; Porter et al., 2018b). Several mechanisms have been identified which enhance beat-to-beat variability of APD including spontaneous sarcoplasmic calcium release, stochastic behavior of ion channels and stretch activated channels (Tanskanen et al., 2005; Johnson et al., 2013; Stams et al., 2016). Our results demonstrating a good correspondence between changes in APD and changes in QT interval help underpin the extrapolation of mechanistic research on APD to clinical QT measurements.

In our study, whilst the temporal behavior of the body surface QT interval followed both the LV ARI and RV ARI, the magnitude of beat-to-beat change was closer between the QT interval and the LV ARI than the RV ARI. Following a pacing induced rate increase it has been shown that LV ARI adapts more slowly than RV ARI (Bueno-Orovio et al., 2012), and has been proposed as a potential pro-arrhythmic mechanism (Bueno-Orovio et al., 2014). In our analysis, more marked adaption in magnitude was seen in the LV than the RV. A combination of slower adaption and greater change in magnitude in the LV over RV would lead to considerable heterogeneity under times of heart rate acceleration.

In dogs, higher beat-to-beat variability of ARI was demonstrated in the LV than the RV (Wijers et al., 2018), with our results echoing this in humans. A proposed explanation is the difference in distribution of repolarizing ion channels; in the RV there are more transient outward (I_{to}) and slow delayed rectifier (I_{ks}) potassium currents. This has important implications in the use of beat-to-beat variability of ARI clinically as whilst

increases in beat-to-beat variability of ARI have been seen prior to ventricular arrhythmia in dogs (Wijers et al., 2018), it is possible that some site specificity may exist with regard to beat-to-beat variability of ARI as a risk predictor. Longitudinal studies are needed to assess the temporal behavior of ARI across recording locations and its relation to ventricular arrhythmia in humans.

3.4.1 Clinical implications

Our study provides evidence that the temporal behavior of the ECG QT interval correlates with the temporal behavior of local APD and supports extrapolation of mechanistic insight derived from APD measurements to the clinical application of the QT interval. Importantly, misadaptation of APD and repolarization to changing cycle length (such as in premature beats) are well known to affect stability of the electrophysiology. Our results support the clinical application of the temporal dynamics of the QT interval for assessing cardiac electrophysiology as the temporal behavior of the QT interval correlates well with that of local APD during a sudden change in cycle length.

3.4.2 Translational outlook

Numerous indices of repolarization from both the body surface ECG and intracardiac electrograms have been demonstrated to be predictive of risk of ventricular arrhythmia. None of these markers have been 100% sensitive. This is likely to be in part due to discreet localized regional variation not detected on the global QT interval or the single more localized electrogram. Pathologies resulting in localized injury may not have repolarization abnormalities detected within the global or localized recording and may require assessment of repolarization within or around the pathological tissue to improve sensitivity. Furthermore, recent work has demonstrated the novel potential application of

real time assessment of arrhythmic risk by continuous monitoring of repolarization from implanted devices. Further exploration of inhomogeneity within the diseased heart is needed to help determine the optimal recording site/s.

3.4.3 Limitations

Recordings were obtained using decapolar catheters on the endocardium of both the RV and LV and whilst location was similar for all patients this is an approximation and the precise anatomical position could vary. Furthermore, recordings were limited to 20 endocardial sites and whereas our results are interpreted as being representative of the endocardium it is possible that other locations may have given different results.

3.5 Conclusions

In patients with normal ventricles changes in APD (measured as ARI) at single or multiple RV and LV endocardial sites correlated strongly with changes in QT interval in ECG lead II. The correlation was strongest for all endocardial RV and LV sites combined, intermediate for all RV and all LV sites and slightly less but significant for individual sites. The magnitude of beat-to-beat change in QT interval was near equal with that of LV APD (ARI) whilst RV APD demonstrated smaller changes. These results may be important in integrating the clinical application of QT interval with mechanistic insight based on APD electrophysiology.

Chapter 4: Autonomic Modulation of Beat-to-Beat Variability of Ventricular Action Potential Duration in patients with heart failure

This section has been adapted from *Autonomic modulation in patients with heart failure increases beat-to-beat variability of ventricular action potential duration* (Porter et al., 2017).

4.1 Introduction

Exaggerated beat-to-beat variability of repolarization is strongly associated with pro-arrhythmia and is modulated by sympathetic activity (Shen and Zipes, 2014; Baumert et al., 2016). Beat-to-beat variability of repolarization is an intrinsic property of cardiac myocytes and can be observed at all levels from the ventricular action potential in the single cardiac cell to the QT interval in the ECG (Tereshchenko et al., 2010; Heijman et al., 2013; Zaniboni et al., 2017). A growing body of experimental and computational work is providing insight into the potential mechanisms underlying beat-to-beat variability of repolarization and the modulatory role of sympathetic stimulation at the level of the ventricular APD (Heijman et al., 2013; Johnson et al., 2013; Pueyo et al., 2016a, 2016b). However, information on ventricular APD behaviour in humans at present relies largely on QT interval measurements from the body surface ECG (Piccirillo et al., 2001; Piccirillo et al., 2006; Malik, 2008; Porta et al., 2011). While QT measurements have provided a great deal of valuable information, direct extrapolation to APD is not possible from these global recordings. Furthermore, such measurements are usually made with uncontrolled cycle length which complicates interpretation in view of the strong cycle length dependence of APD (Boyett and Jewell, 1978).

In this chapter we explore the impact of an autonomic stimulus on the beat-to-beat variability of left ventricular APD in patients with heart failure and CRT. Studies were

performed during controlled cycle length through right ventricular pacing, and both with and without beta-adrenergic blockade.

4.2 Methods

4.2.1 Ethical Approval

The study was approved by the West London Ethics Committee and conformed to the standards set by the Declaration of Helsinki (latest revision: 64th WMA General Assembly). Informed consent was obtained in writing from all subjects.

4.2.2 Subjects

Studies were performed in 11 ambulatory heart failure patients (all male, age 58–76) who were recipients of a CRT-D device (Quadra Assura MP^{TM} CRT-D, St. Jude Medical). Patient characteristics are shown in **Table 4-1**.

Table 4-1. Patient characteristics.

ICM, Ischaemic cardiomyopathy; NICM, Non-ischaemic cardiomyopathy.

Subject	Age (years)	Gender	Aetiology	NYHA Class	Ejection fraction (%)	Bisoprolol dose
1	75	M	ICM	1	34	10mg
2	75	M	ICM	2	33	10mg
3	58	M	NICM	2	44	nil
4	64	M	ICM	3	19	3.75mg
5	61	M	ICM	2	22	7.5mg
6	64	M	NICM	2	41	5mg
7	69	M	ICM	2	40	5mg
8	65	M	NICM	3	35	1.25mg
9	76	M	NICM	1	63	nil
10	71	M	NICM	2	42	7.5mg
11	70	M	ICM	2	15	nil
Range	58-76			1-3	15-63	
Mean ± SD	68 ± 6				35 ± 13	

4.2.3 Physiological Recordings

Heart rate was fixed by constant RV pacing using their implanted CRT device. The rate was chosen as the minimum rate required to maintain continuous capture at a fixed cycle length. A minimum adaptation period of 10 min took place prior to any recordings. The cycle length remained constant throughout the entire study and the same cycle length was chosen when studies were repeated on beta-blockade. The LV epicardial lead was used to record unipolar electrograms, sampled at 512 Hz (**Figure 4-1**). The pacing programmer used to alter the pacing settings in this study allowed storage of five separate recordings of 30s duration.

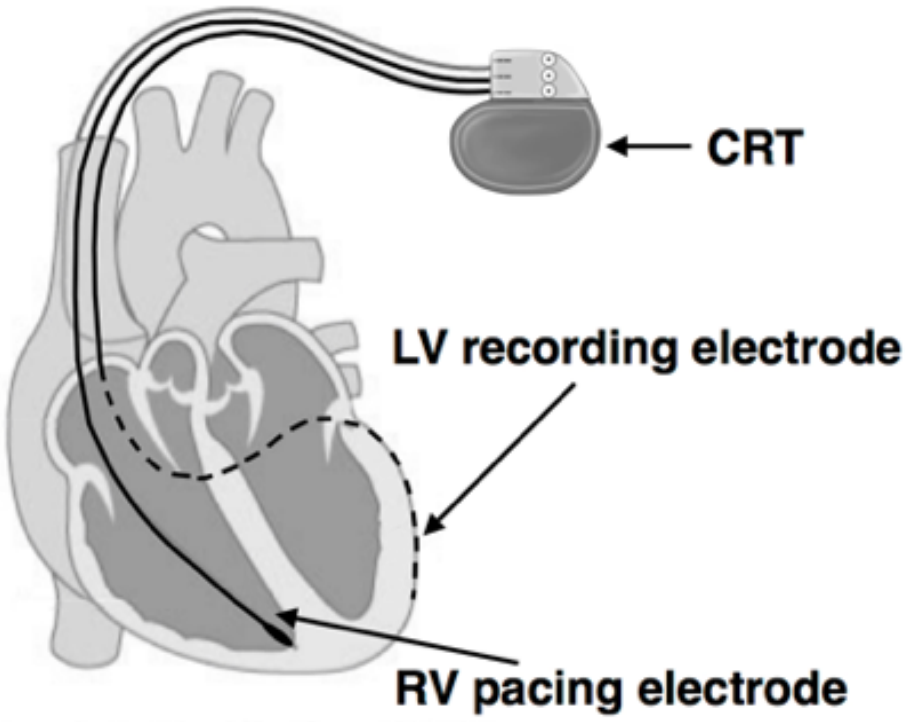


Figure 4-1. Exporting left ventricular unipolar electrograms

Biventricular pacing device (CRT) programmed to pace from the right ventricular lead (solid line) and record a local electrogram from the left ventricular lead (dashed line).

Arterial blood pressure was measured non-invasively using a finger cuff (Finometer pro, Finapres Medical Systems B.V., Amsterdam, The Netherlands). The signals were digitized by the MP150 System using AcqKnowledge software (Goleta, CA) and sampled at 1 kHz.

In addition, sympathetic nerve activity (SNA) recordings were made using a novel method from a pair of bipolar skin electrodes in the Lead II position as by Doytchinova et al. (Jiang et al., 2015; Doytchinova et al., 2017) . In this method the typical low-pass filters applied during the recording of the body surface ECG are removed and the raw

unfiltered signals are obtained. This allows application of separate filters at the analysis stage to remove the ECG signal and reduce signal noise as described later. The signals were digitized by the MP150 System using AcqKnowledge software (Goleta, CA) and sampled at 10 kHz.

4.2.4 Protocol 1

Recordings were made with the subject seated upright. Physiological recordings were made simultaneously using multiple recording channels. Beta-adrenergic blocking agents (bisoprolol) were discontinued for 5 days prior to the study, which was sufficient wash-out time for all drugs in this population (see **Table 4-1** for patient-specific dosage).

Subjects were asked to perform the Valsalva maneuver (forced expiration against a fixed resistance (Doytchinova et al., 2017)) for 10 s. Physiological recordings were made continuously before, during and after the procedure. Following a 10 min recovery period a second Valsalva maneuver was performed and recordings were made as per the first.

4.2.5 Protocol 2

Eight subjects returned to repeat the same protocol as above but without discontinuing their beta-blockers (Bisoprolol in all 8). Three patients could not be included as beta-blockers were not part of their regular medication.

4.2.6 Analysis of Data

ARI Analysis

Each 30s output from the CRT device was digitized and truncated to form one long data sequence of ~150 s in length. Any possible overlap between successive 30s sequences was found by searching for matching traces at the start/end of successive traces and was removed. Raw, digitized unipolar electrogram traces were then lowpass filtered at 80 Hz which removed high-frequency noise but maintained the sharp activation gradients required to identify activation times. **Figure 4-2** shows the fidelity of the unipolar electrogram traces, showing both the raw trace (top) and the filtered trace (bottom). **Figure 4-3A** shows an example of the digitized unipolar electrogram trace during a 20 s period of rest showing multiple beats.

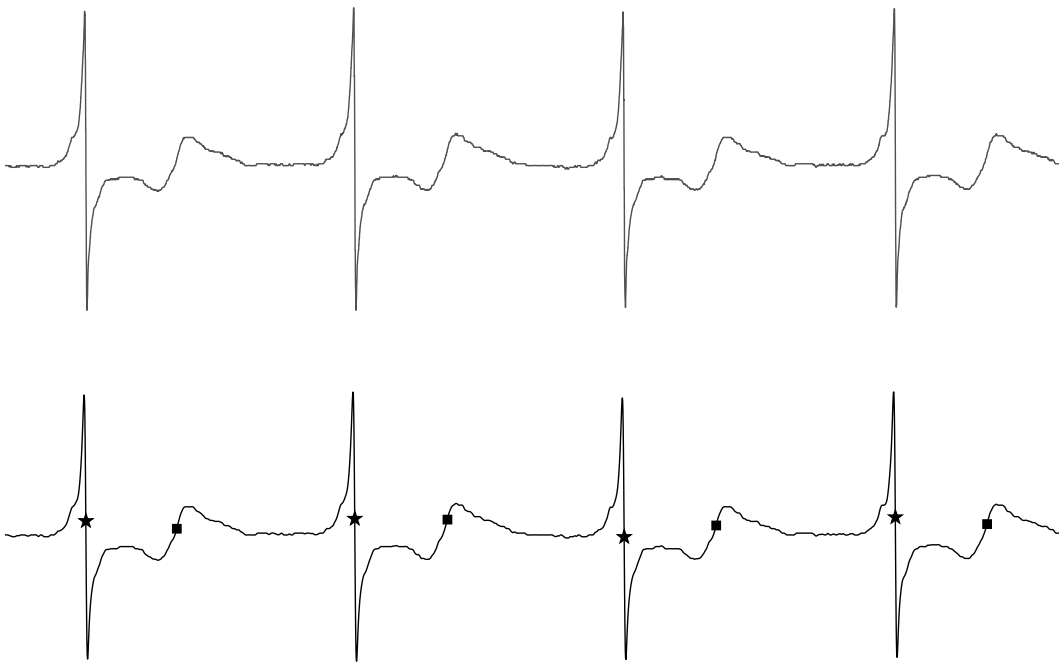


Figure 4-2: Fidelity of the unipolar electrogram traces

Unipolar electrograms recorded from the left ventricular lead demonstrating the good quality of the signals. Both the raw trace (top) and filtered trace (bottom) is shown demonstrating the lack of any significant distortion of the signal by filtering. The moment of local activation (star) and of local repolarization (square) are shown.

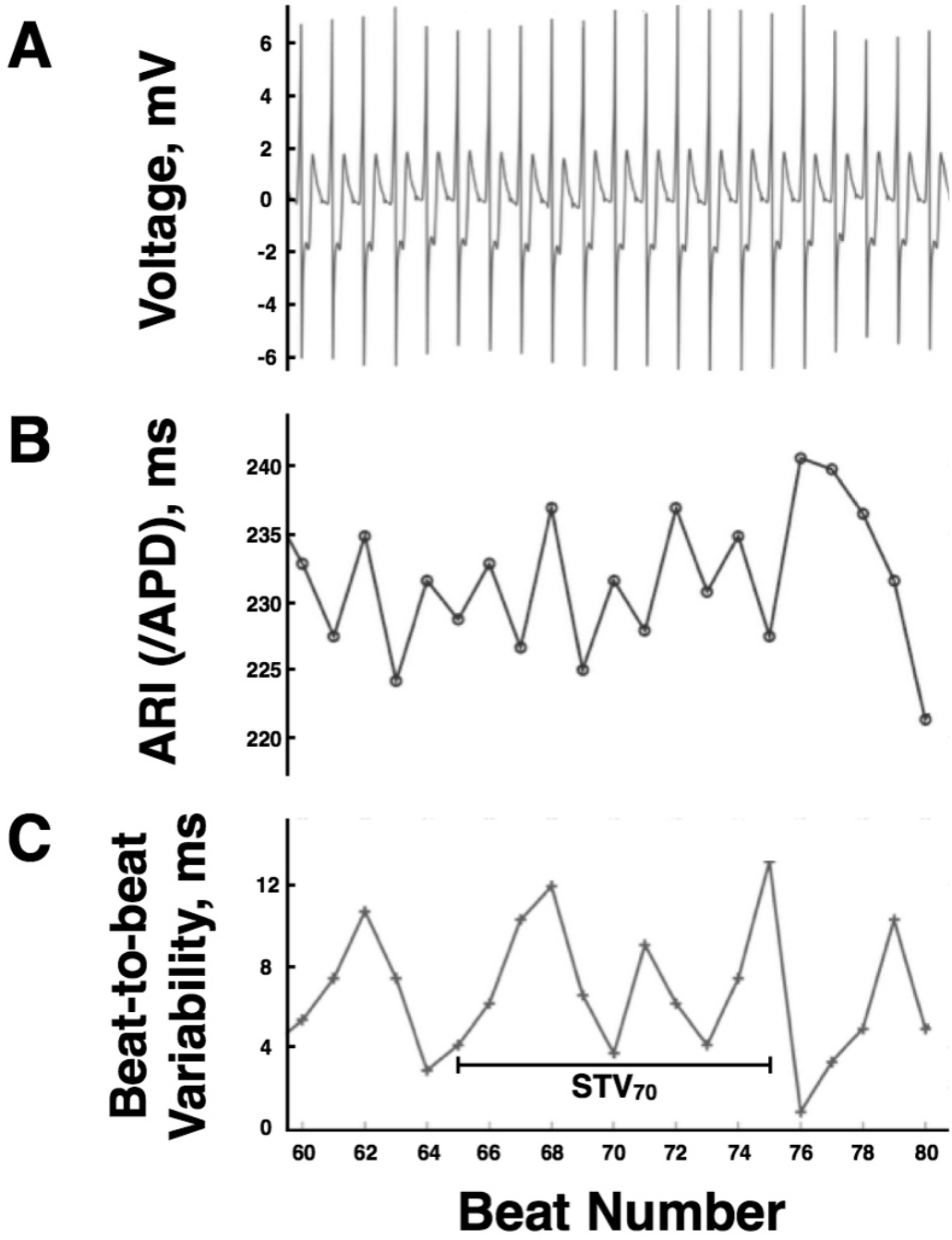


Figure 4-3. Calculation of beat-to-beat variability

A: *Example of the digitized electrogram trace during a 20 second period of rest.* ***B:*** *Computed activation-recovery interval (ARI) values for the same electrogram trace.* ***C:*** *Magnitude of the beat-to-beat variation in ARIs during the example 20 second period of rest. APD = action potential duration, STV = short-term variability*

Activation times (ATs) were defined as the point of maximum negative downslope of the unipolar electrogram following the R-wave. Repolarization time (RT) was defined using the Wyatt method as the point of maximum upslope of the T-wave (Wyatt et al., 1981; Coronel et al., 2006; Potse et al., 2009; Hanson et al., 2014). ARIs for each (*i*th) beat were then computed as.

$$ARIi = RTi - ATi$$

Figure 4-2 shows the identification of the AT (star) and RT (square) for a single ARI. **Figure 4-3B** shows the computed ARI values for the corresponding unipolar electrogram trace in **Figure 4-3A**. The magnitude of the mean beat-to-beat change in ARI during resting recordings was in line with previous studies (Hanson et al., 2014). **Figure 4-3C** shows the corresponding magnitude of the beat-to-beat variation in ARIs during the example 20 s period of rest.

To quantify the variation in ARIs between beats and examine how this may change during sympathetic stimulation, short-term variability (STV) of ARIs was computed, as previously defined (Johnson et al., 2013). Specifically, a 10-beat moving window was defined throughout the recordings and STV at the beat *i*th computed as.

$$STV_i = \frac{\sum_{j=-5}^{5} |ARI_{i+j+1} - ARI_{i+j}|}{n\sqrt{2}}$$

where ARI_i is the ARI of the ith beat and n is the number of beats. A schematic representation is shown in **Figure 4-3C**.

Blood Pressure Data

Raw BP signals were filtered using a lowpass filter of 40 Hz. Systolic peaks were then located in the filtered BP traces. Systolic BP during the Valsalva was then computed by visually identifying the different phases of the systolic BP signal as described by Palamarchuk et al (Palamarchuk et al., 2016). Pressure recovery time (PRT) and systolic overshoot from baseline were calculated allowing assessment of established indices of sympathetic activity (Vogel et al., 2005).

A typical blood pressure response to the Valsalva maneuver is shown in **Figure 4-4**. The subject exhales forcibly against a fixed resistance for 10 s which increases intrathoracic and abdominal pressures thereby impeding venous return, reducing ventricular filling, and reducing LV systolic pressure. An initial increase in blood pressure occurs mainly due to the direct effect of increased intrathoracic pressure (Phase 1). This is followed by a fall in blood pressure due to impeded venous return which reaches a plateau (early phase II) or increases slightly due to reflex sympathetically mediated peripheral vasoconstriction and increased inotropic state of the myocardium (late phase II). Following release of the forced expiration intrathoracic pressure returns to normal and refilling of the pulmonary vascular bed results in a further transient fall in blood pressure (Phase III). As venous return and ventricular filling are restored blood pressure rises and may overshoot due to persisting increased sympathetic tone (Phase IV).

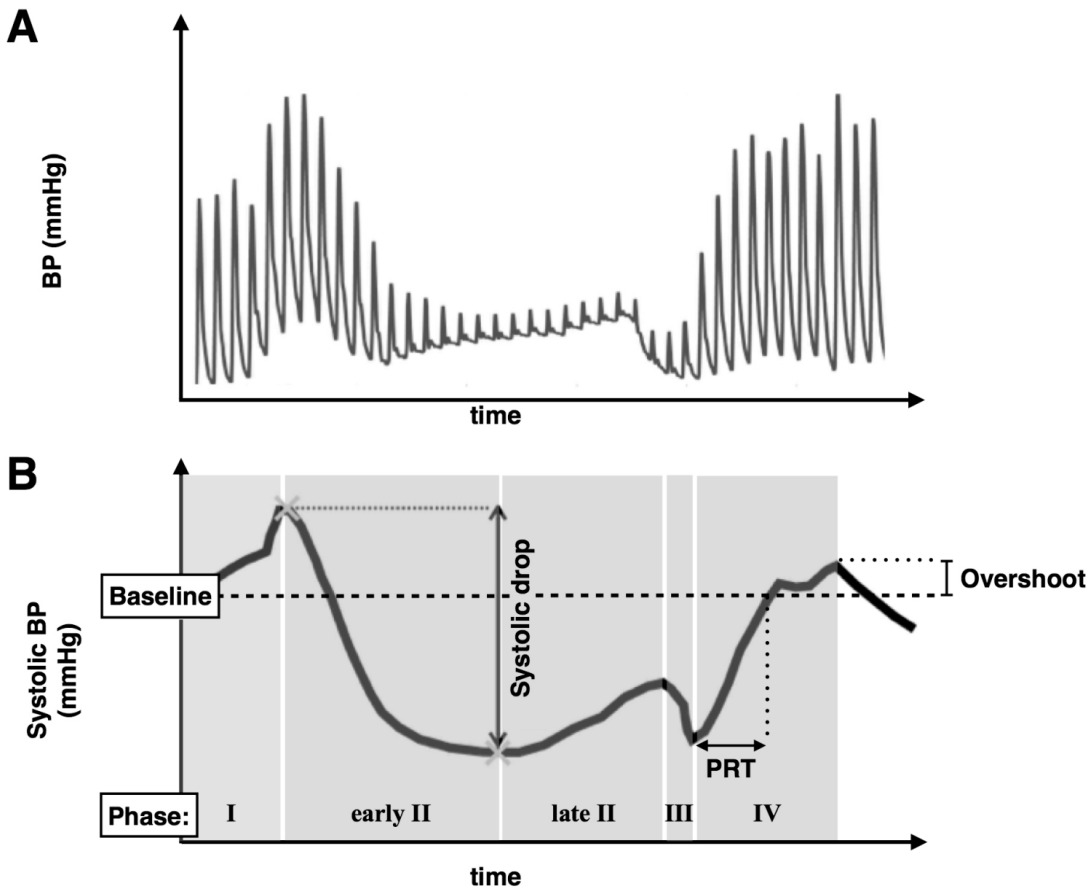


Figure 4-4. Typical blood pressure response to the Valsalva maneuver

A: *Typical blood pressure response during a Valsalva maneuver.* ***B:*** *Systolic blood pressure trace demonstrating phases of the Valsalva, blood pressure drop between phase I and II, pressure recovery time (PRT) and phase IV systolic overshoot.*

Sympathetic Nerve Activity

Raw SNA signals were bandpass filtered between 500 and 1,000 Hz as in Doytchinova et al. (Doytchinova et al., 2017), to remove noise from muscle nerve activity and hence improve signal to noise ratio. In contrast to Doytchinova et al. (Doytchinova et al., 2017), patients in this study were paced from the CRT devices. Consequently, much larger pacing artifacts were present in the SNA which were only attenuated (and not fully removed) by the bandpass filtering. More aggressive filtering in the lower range was seen to significantly lower the SNA trace itself, increasing signal-to-noise. Thus, in the cases

where residual ECG signal was still present in the signals after filtering, the numerical contribution of the ECG was estimated and removed from the computations involving SNA values. Specifically, this involved not including SNA data within ±20 ms of the pacing spikes.

Changes in SNA during sympathetic stimulation were quantified as in Doytchinova et al. (Doytchinova et al., 2017) by computing average values of (filtered) SNA signals occurring within a given temporal window (SNA_{av}). Here, SNA_{av} values were computed over a 20 s window during the Valsalva and during a corresponding 20 s period of rest.

Statistical Analysis

For all subjects mean values of Systolic BP, SNA, and STV were averaged across both Valsalva maneuvers. Change following stimulus and comparison between off and on beta-blocker was assessed using two-tailed, paired, *t*-tests. Results were considered significant at $P \leq 0.05$.

4.3 Results

In the absence of beta-blockade mean baseline systolic blood pressure prior to the Valsalva was 129.6 ± 19.2 mmHg. Mean systolic blood pressures during each phase of the Valsalva were: Phase I = 152.3 ± 23.3 mmHg, Early Phase II = 92.7 ± 25.9 mmHg, Late Phase II = 97.5 ± 25.7 mmHg, Phase III = 65.9 ± 17.3 mmHg, Phase IV 142.8 ± 23.5 mmHg. Analysis of indices of sympathetic function demonstrated a mean overshoot in phase IV from baseline of 13.2 ± 11.1 mmHg and mean PRT of 5.26 ± 4.08 s.

Without beta-blockade all 11 participants demonstrated an increase in mean STV of ARI following the Valsalva. Mean STV increased from 4 ± 1.22 ms at rest to 5.25 ± 0.9 ms ($p < 0.01$). Mean SNA increased from a resting value of 2.3 ± 1–3.7 ± 1.7 uV ($p < 0.01$). Individual blood pressure responses and changes in STV of ARI are presented in **Table 4-2**.

Table 4-2. Individual systolic blood pressure responses and changes in short-term variability (STV) of activation-recovery intervals.

Subject	Bisoprolol	Baseline (mmHg)	Max phase I (mmHg)	Early phase II (mmHg)	Late phase II (mmHg)	Phase III (mmHg)	Phase IV (mmHg)	Systolic drop (mmHg)	Rise during Phase II (mmHg)	Overshoot from baseline (mmHg)	PRT (seconds)	Rest STV (ms)	Valsalva STV (ms)
1	OFF	114.7	144.8	78.1	85.3	74	128.2	66.7	6.8	13.5	2.99	2.86	6.41
1	ON	130.5	164.4	102.7	101.3	71	135.1	61.7	0	4.6	8.485	4.27	4.89
2	OFF	155.1	181.2	129.1	136	59.3	159.4	52.1	6.9	4.3	5.07	1.58	4.79
2	ON	126.2	139.6	111.7	113.7	78.5	124.5	27.9	2	0	4.895	2.69	4.14
3	OFF	158.4	186.4	125.8	125.1	83.3	175	60.6	0	16.6	2.79	4.04	4.66
4	OFF	137.5	153.3	117.4	120.5	84.5	145.5	35.9	3.1	8	2.715	3.48	3.82
4	ON	112	122.2	101.2	101.5	84.5	120.5	21	0.3	8.5	3.48	2.8	3.84
5	OFF	125.3	132	90.3	103.2	79.1	142.4	41.7	12.9	17.1	2.32	4.5	4.51
5	ON	125.5	131.6	65.2	66.4	48.8	115.9	66.4	1.2	0	6.56	4.7	4.15
6	OFF	132.5	175.1	88.6	88.5	54.5	167	86.5	0	34.5	3.585	4.13	5.97
6	ON	126.3	150.2	75	86.5	67.5	134.5	75.2	11.5	8.2	6.2	4.84	6.01
7	OFF	103.8	123	72	73	44.6	108.1	51	1	4.3	2.45	4.84	5.55
7	ON	96.5	128	97.6	97.3	51.7	103.6	30.4	0	7.1	5.43	4.49	3.76
8	OFF	126	147.5	75.2	87.1	67.5	133.5	72.3	11.9	7.5	6.735	5.62	6.7
8	ON	145.4	174.6	81.2	81.5	61.6	149	93.4	0.3	3.6	7.53	6.05	6.27
9	OFF	100.7	114	42.4	45.4	30.5	100.5	71.6	3	0	13.31	5.23	5.42
10	OFF	148.2	156.9	106.2	110.2	80.4	156.4	50.7	4	8.2	12.86	2.83	4.39
10	ON	166.4	188.9	116.4	119.6	90.6	161.5	72.5	3.2	0	11.835	3.39	3.87
11	OFF	123.5	161.5	94.1	98.3	67.5	154.9	67.4	4.2	31.4	3.045	4.95	5.53

A sub-group of eight participants additionally undertook repeated studies during established beta-blockade (Bisoprolol in all 8). Direct comparison of phases of the blood pressure response and indices of beta-adrenergic function are shown in **Table 4-3**.

Table 4-3. Off and on bisoprolol comparison showing mean systolic blood pressure by phases of the Valsalva, indices of beta-adrenergic function, and changes in short-term variability (STV) of activation-recovery intervals.

	OFF beta-blocker	ON beta-blocker	*P* value
Baseline (mmHg)	130.4 ±16.8	128.6 ±20.8	0.8
Max phase I (mmHg)	151.7 ±19.7	149.9 ±24	0.86
Early phase II (mmHg)	94.6 ±20.9	93.9 ±18.2	0.92
Late phase II (mmHg)	100.5 ±20	96 ±17.3	0.56
Phase III (mmHg)	68 ±14.1	19 ±15	0.82
Phase IV (mmHg)	142.6 ±19.1	130.6 ±18.6	0.13
Systolic drop (mmHg)	57.1 ±16.8	56.1 ±26.3	0.89
Rise during Phase II (mmHg)	5.8 ±4.7	2.3 ±3.9	0.22
Overshoot from baseline (mmHg)	12.2 ±10	4 ±3.7	**0.05**
PRT (seconds)	4.84 ±3.58	6.8 ±2.55	**0.04**
Increase in STV (ms)	1.54 ±1.29	0.46 ±0.78	**0.02**

Both beta-adrenergic measures of the Valsalva (systolic overshoot in Phase IV and PRT) demonstrated a statistically significant difference off compared to on bisoprolol ($p = 0.05$ and 0.04 respectively) (**Figure 4-5**). This is in keeping with a reduced beta-adrenergic response to the Valsalva when beta-blocked. In the absence of beta-blockade mean STV following the Valsalva increased from 3.73 ± 1.3 ms at rest to 5.27 ± 1.04 ms ($p = 0.01$). When repeated on established bisoprolol mean STV of ARI demonstrated a smaller magnitude of increase from 4.15 ± 1.14 ms at rest to 4.62 ± 1 ms ($p = 0.14$) following the Valsalva, which is also noted to be statistically non-significant. **Figure 4-6** demonstrates changes in STV of ARI off and on bisoprolol. Mean rest SNA off beta-blockers was 2.4

± 1 uV which increased to 4.4 ± 1.9 uV (p = 0.04). When repeated on bisoprolol mean rest SNA was 1.9 ± 0.8 uV and increased to 2.6 ± 0.8 uV (p = 0.05).

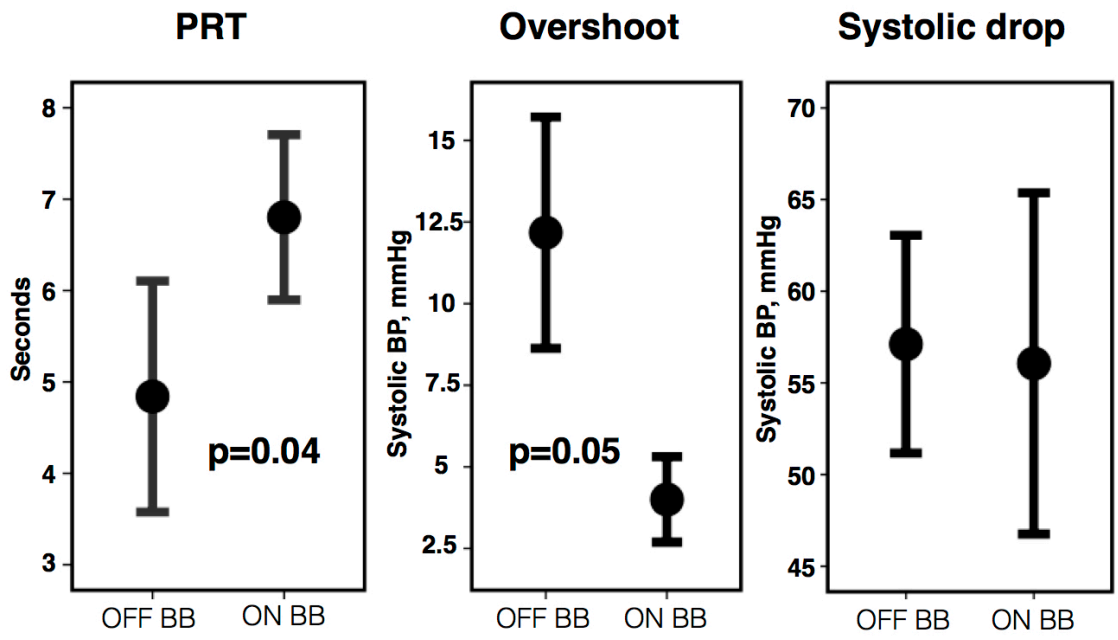


Figure 4-5: Beta-adrenergic measures of the Valsalva off and on beta-blockade

Pressure recovery time (PRT), phase IV systolic overshoot and systolic drop from Phase I to II off vs. on bisoprolol (BB). Error bars represent ± 1 standard error of the mean.

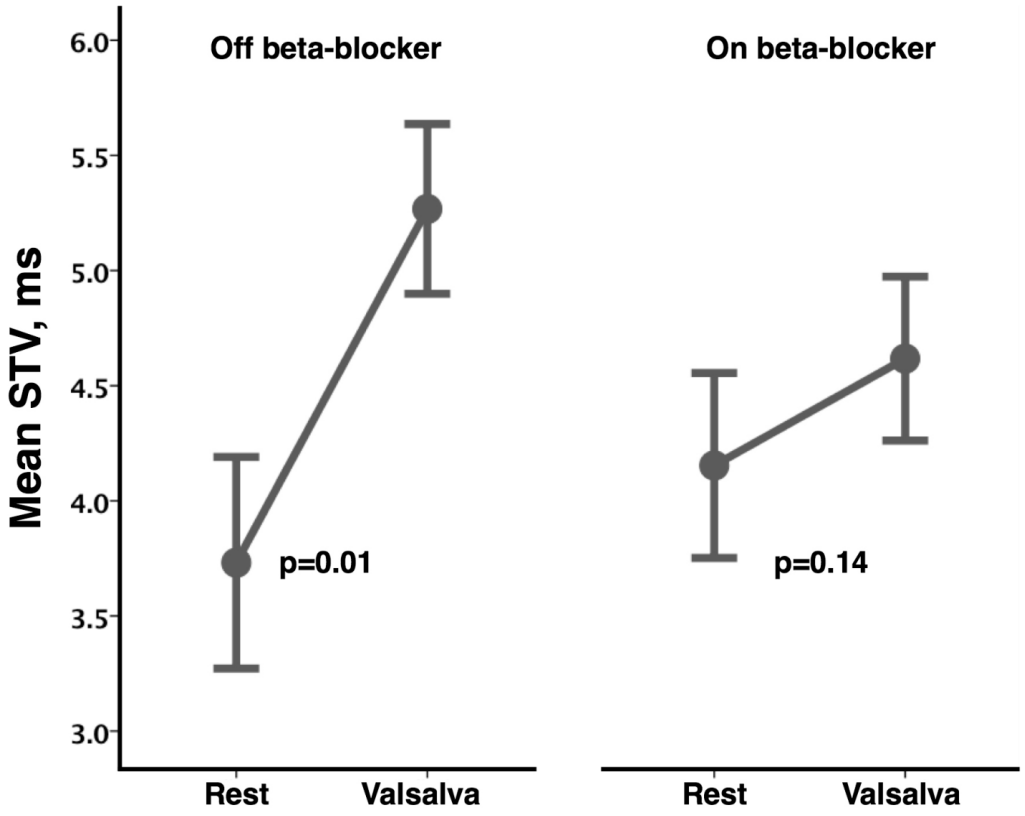


Figure 4-6. Effect of beta-blockade on Valsalva induced increase in beat-to-beat variability of APD

Mean short-term variability (STV) of activation-recovery intervals at rest and following Valsalva, off and on bisoprolol. Error bars represent ± 1 standard error of the mean.

Any relative reductions in the magnitude of the changes when on (compared to when off) beta-blockers were specifically analyzed by performing a direct pair-wise comparison of changes from rest during the Valsalva. Following the Valsalva a significantly greater increase in STV of ARI was seen when off beta-blockade (1.54 ± 1.29 ms) compared to when on bisoprolol (0.46 ± 0.78 ms) (p = 0.02). Although the observed increases in SNA were higher whilst off beta-blocker (2 ± 1.1 uV) compared to on bisoprolol (0.7 ± 0.4 uV) this was not statistically significant (p = 0.07). **Figure 4-7** demonstrates corresponding changes in STV of ARI, and SNA.

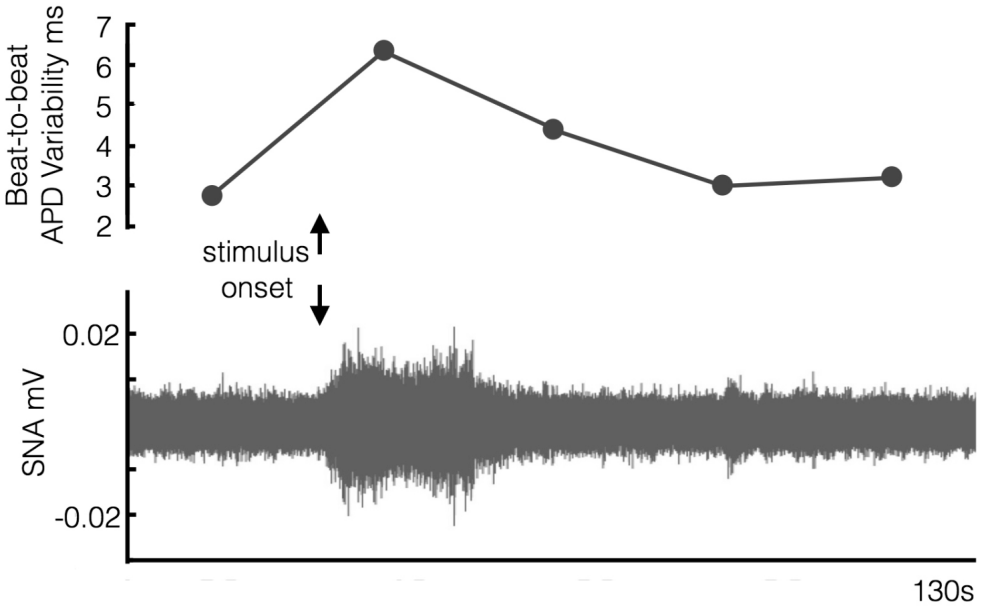


Figure 4-7. Example of corresponding increases in beat-to-beat variability of ventricular action potential duration and sympathetic nerve activity

SNA = sympathetic nerve activity, stimulus onset = start of Valsalva.

4.4 Discussion

The studies in this chapter employed a novel methodology whereby ventricular APD was measured directly from the epicardium (as ARIs) in ambulatory patients with heart failure during an autonomic challenge induced by the Valsalva maneuver. The main findings were (1) The Valsalva was associated with an increase in beat-to-beat variability of APD; (2) The haemodynamic indices were consistent with increased sympathetic activity as is characteristic of the Valsalva; (3) The Valsalva increased SNA in skin recordings in keeping with a recent validation study (Doytchinova et al., 2017); (4) Beta-blockade with bisoprolol reduced but did not eliminate the increase in beat-to-beat variability of APD.

The Valsalva maneuver is an established method of increasing sympathetic activity whereby forced expiration impedes venous return resulting in a reduction in ventricular pressure and volume, and a baroreflex increase in sympathetic activity (Booth et al., 1962; Korner et al., 1976; Smith et al., 1987). Sympathetic activity has been shown to be greatly enhanced during the strain phase of the Valsalva maneuver in healthy control subjects in studies using microneurographical recordings of muscle sympathetic nerve discharges (Schrezenmaier et al., 2007). Indices of baroreflex sensitivity have been established to separately evaluate the vagal and adrenergic components (Vogel et al., 2005; Schrezenmaier et al., 2007). Patients with severe heart failure may show an altered BP response to the Valsalva with no fall in blood pressure during the strain phase resulting in a "square wave" blood pressure response (Felker et al., 2006). None of our patients exhibited this behaviour, all showing a substantial blood pressure drop in phase II (57.1 ± 16.8 mmHg. Mean ± *SD*).

Recent studies have shown that high frequency recordings from skin electrodes reflect stellate ganglion sympathetic nerve activity. These results have been demonstrated in animal models and humans (Jiang et al., 2015; Doytchinova et al., 2017), and have shown that episodes of ventricular tachycardia were preceded by increased skin sympathetic nerve activity within 30s of onset. Recent studies from Doytchinova et al. (Doytchinova et al., 2017) demonstrated an increased skin SNA response to the Valsalva. Whilst further studies are required to elucidate the underlying physiology and utility of skin sympathetic efferent nerve recordings, our results are nevertheless in line with theirs.

To the best of our knowledge this is the first study to demonstrate that beat-to-beat variability of APD is increased during enhanced sympathetic stimulation in humans, and in particular in humans with heart failure. This is important in view of the known association between increased beat-to-beat variability of repolarization and ventricular arrhythmia and the adverse effects of sympathetic stimulation (Shen and Zipes, 2014; Baumert et al., 2016). Several studies have examined QT interval variability (QTV) in patients with heart failure. Patients with heart failure have a higher QTV compared to age matched controls (Piccirillo et al., 2013). Heart failure patients show a marked circadian variation with an increase in QTV during the daytime (Dobson et al., 2009). Enhanced sympathetic activity by tilt table testing in heart failure patients is associated with an increased QTV although the increase may be impaired in comparison to normal subjects (Desai et al., 2004; Piccirillo et al., 2006). Heart failure patients with a history of ventricular tachycardia (VT) show higher baseline values of QTV compared to those without VT and compared to normal subjects (Nayyar et al., 2013). In the latter study Isoprenaline increased QTV in normal subjects but not in the heart failure-VT group, highlighting the limited autonomic modulation of QTV in heart failure patients (Nayyar et al., 2013).

Beta blockade has shown mixed effects on QTV. For example, no effect at rest (Piccirillo et al., 2001; Nayyar et al., 2013); a reduction during atrial pacing in normal subjects (Mine et al., 2008); no effect on head up tilt increase in QTV in heart failure patients (Nayyar et al., 2013); and reduction of QTV increase during anger recall in post MI patients (Magrí et al., 2012). Circadian variation of QTV is abolished by beta blockade (Furukawa et al., 2006).

The eight patients who were studied on a second occasion whilst on their bisoprolol showed a lessened increase in beat-to-beat variability of APD during sympathetic stimulation compared to when their bisoprolol had been discontinued, although still showing an increase. This could be due to incomplete beta-blockade or to the contribution of an additional mechanism. Such a possibility is mechano-electric feedback in response to the large pressure volume changes during the Valsalva. The effect of mechanical perturbation on APD is complex depending on multiple variables including the type of preparation, the type of stretch e.g., isotonic vs. isometric, and the timing of the stretch in relation to the timing of action potential repolarization. Cellular mechanisms involve stretch activated channels and calcium cycling (Kohl, 1998; Quinn and Kohl, 2016). Future studies might address the possibility of a role of mechano-electric feedback in beat-to-beat variability of APD.

Heart rate variability is a major component of QTV. Both APD and the QT interval are strongly cycle length dependent (Boyett and Jewell, 1978; Zaza et al., 1991). Rapid and slow processes are involved with hysteresis effects all of which vary between individuals. Since the majority of QTV studies are conducted in the presence of uncontrolled cycle length, separating rate driven QTV from actual fluctuations in QT interval is technically

challenging. Cycle length control by pacing as employed in the present studies avoids these difficulties and removes the component of QTV due to heart rate variability.

A number of factors influence beat-to-beat variability of repolarization at the cellular level including ion channel stochasticity, APD, restitution properties and calcium handling (Zaniboni et al., 2000; Heijman et al., 2013; Johnson et al., 2013; Baumert et al., 2016; Pueyo et al., 2016a). Furthermore, electrophysiological remodeling during heart failure is known to influence a wide range of electrophysiological processes and the molecular mechanisms remain somewhat controversial despite extensive investigation (Nattel et al., 2007; Cho et al., 2012; Kirk and Kass, 2013). At present it is unclear to what extent these factors may interact and be contributory to beat-to-beat variability of repolarization at the whole heart level, and how it is governed by autonomic stimulus, where electrophysiological changes in individual cells may have a limited effect due to electrotonic coupling in the syncytium. Here, we have shown that sympathetic stimulation directly changes ventricular APDs at the whole heart (coupled tissue) level in the context of heart failure. Additional measurements in future studies of ARI changes in different locations, perhaps with measurements of local strain patterns, as well as changes in conduction velocity may help to further elucidate the driving electrophysiological mechanism at the cellular level.

4.4.1 Limitations

A limitation of recording from the CRT device is that it is only possible to record from one epicardial electrode while pacing from the RV electrode. Consequently, our observations are confined to a single LV site. In view of the well-known regional variation of electrophysiological properties throughout the ventricular myocardium it is

possible that other regions may have responded differently. An unavoidable phenomenon of pacing studies is the latency period between the stimulus from the pacing lead and myocardial capture. As a result, there is a potential for variability in the cycle length. Analysis of successive activation times demonstrated a maximum variation in heart rate of ±0.3 BPM which was consistent between protocols. This would not be expected to significantly influence our results.

The SNA recordings use skin electrodes as per Doytchinova et al. (Doytchinova et al., 2017). These recordings might simultaneously detect both skin and muscle sympathetic nerve activities. In contrast, microneurography can selectively record from muscle and skin sympathetic nerves. Further studies incorporating both microneurography and SNA recordings are needed.

An additional unavoidable limitation was the inability to study a control population of normal subjects on account of the necessary requirement of an implanted biventricular pacing device. This would have been of interest on account of the known attenuation of sympathetic responses in heart failure patients. Our study findings are therefore only applicable to patients with heart failure. Finally, this study only involved the use of bisoprolol for beta-blockade. Future investigations may involve assessing how the findings from our study related to other forms of beta-blockade and the dose-response relationship in a larger clinical trial.

4.5 Conclusions

In patients with heart failure (New York Heart Association (NYHA) class I-III) beat-to-beat variability of ventricular APD was increased during an autonomic challenge associated with increased sympathetic activity. These results accord with observations on ECG QT variability and provides insight on mechanisms of known importance in the genesis of serious and fatal ventricular arrhythmias.

Chapter 5: Beat-to-beat variability of ventricular action potential duration oscillates at low frequency during sympathetic provocation in humans

This section has been adapted from *Beat-to-beat variability of ventricular action potential duration oscillates at low frequency during sympathetic provocation in humans* (Porter et al., 2018b)

5.1 Introduction

Factors which modulate or destabilize ventricular repolarization are of fundamental importance in arrhythmogenesis. Spontaneous beat-to-beat fluctuation in repolarization is an inherent property of ventricular myocardium (Baumert et al., 2016). Beat-to-beat variability of repolarization although influenced by cycle length (Boyett and Jewell, 1978) is largely due to variation in APD (Zaniboni et al., 2000) and usually measured in humans as QT interval variability (Tereshchenko et al., 2009; Hinterseer et al., 2010). Enhanced beat-to-beat variability has been shown to be associated with arrhythmia in a range of animal models (Thomsen et al., 2004; Gallacher et al., 2007; Abi-Gerges et al., 2010; Jacobson et al., 2011) and humans (Atiga et al., 1998; Haigney et al., 2004; Tereshchenko et al., 2009; Hinterseer et al., 2010; Sredniawa et al., 2012) and has been proposed as an adjunct to clinical assessment for implantable cardioverter defibrillator (ICD) implantation (Baumert et al., 2016).

Provocations which increase sympathetic activity have been shown to enhance beat-to-beat variability of repolarization (Desai et al., 2004; Piccirillo et al., 2006; Johnson et al., 2013; Porter et al., 2017). In neural signals information is coded simultaneously using two different modalities, i.e. amplitude strength or “tonic activity” and the discharge pattern, i.e. oscillation or phasic patterning (Gerstner et al., 2002; Coote, 2001; Montano et al., 2009). Recently attention has been drawn to both the physiological and clinical

importance of the phasic nature of sympathetic nerve activity in arrhythmogenesis (Rizas et al., 2014, 2016, 2017). Sympathetic nerve activity is organized in a series of low frequency bursts and it has been shown that low frequency rhythmic modulations of repolarization can be identified from the T-wave vector in the ECG which are associated with sympathetic activity (Rizas et al., 2014, 2016). When pronounced, these oscillations have been shown to be one of the strongest predictors of ventricular arrhythmia and sudden cardiac death in post myocardial infarction patients (Rizas et al., 2017). These oscillations are considered to reflect oscillations of ventricular APD. We have identified oscillations of ventricular APD in the low frequency range in humans under conditions of enhanced sympathetic activity (Hanson et al., 2014); under conditions of calcium load and reduced potassium currents common in pathological hearts, such oscillations may be arrhythmogenic by the generation of afterdepolarizations (Pueyo et al., 2016a). In addition, increased beat-to-beat variability may further contribute to destabilization of repolarization that may facilitate re-entry arrhythmias.

In this chapter, we have examined the hypothesis that in heart failure patients an acute sympathetic challenge may not only induce low frequency oscillatory behaviour of ventricular APD but that by inducing low frequency oscillations there is a resultant increase in the magnitude of APD variability. We hypothesised that these changes would be accompanied by similar changes in the low frequency behaviour of arterial blood pressure (Mayer waves (Julien, 2006)).

5.2 Methods

5.2.1 Ethical Approval

The study was approved by the West London Ethics Committee and conformed to the standards set by the Declaration of Helsinki (latest revision: 64th WMA General Assembly). Informed consent was obtained in writing from all subjects.

5.2.2 Subjects

Studies were performed in 11 ambulatory heart failure patients (all male, age 58-76) who were recipients of a CRT-D device (Quadra Assura MP™ CRT-D, St. Jude Medical). Patient characteristics are shown in **Table 5-1**. Exclusion criteria were inherited channelopathies, hypertrophic cardiomyopathy and use of Class I or III antiarrhythmics.

Table 5-1. Patient characteristics.

NYHA = New York Heart Association

Characteristics	
Ischaemic cardiomyopathy, n (%)	6 (54.5)
Ejection fraction ± SD, %	35.3 ± 13.4
NYHA class 1, n (%)	2 (18.2)
NYHA class 2, n (%)	7 (63.6)
NYHA class 3, n (%)	2 (18.2)
Diabetes mellitus, n (%)	2 (18.2)
Atrial fibrillation, n (%)	2 (18.2)
Beta-blockade, n (%)	8 (72.7)
ACE inhibitor, n (%)	9 (81.8)
Aldosterone antagonist, n (%)	7 (63.6)

5.2.3 Physiological recordings

The implanted CRT-D device was used to record unipolar electrograms from the LV epicardial lead, sampled at 512Hz (**Figure 5-1**) (Hanson et al., 2014; Chen et al., 2016). The devices used in this study allowed storage of five separate recordings of unipolar electrograms at 30 seconds duration. Synchronised simultaneous recordings of arterial blood pressure were made non-invasively using a finger cuff (Finometer pro, Finapres Medical Systems B.V., Amsterdam, The Netherlands) (Imholz et al., 1988). The signals were digitized by the MP150 System using AcqKnowledge software (Goleta, CA) and sampled at 1kHz. Simultaneous breathing activity was recorded using a respiration transducer and respiration pneumogram amplifier (TSD201 and RSP100C, Biopac Systems Inc, Goleta, CA). The respiration signal was used to confirm the end of the Valsalva and the absence of low frequency respiration which if present could potentially generate low frequency oscillation in ARI (Hanson et al., 2012).

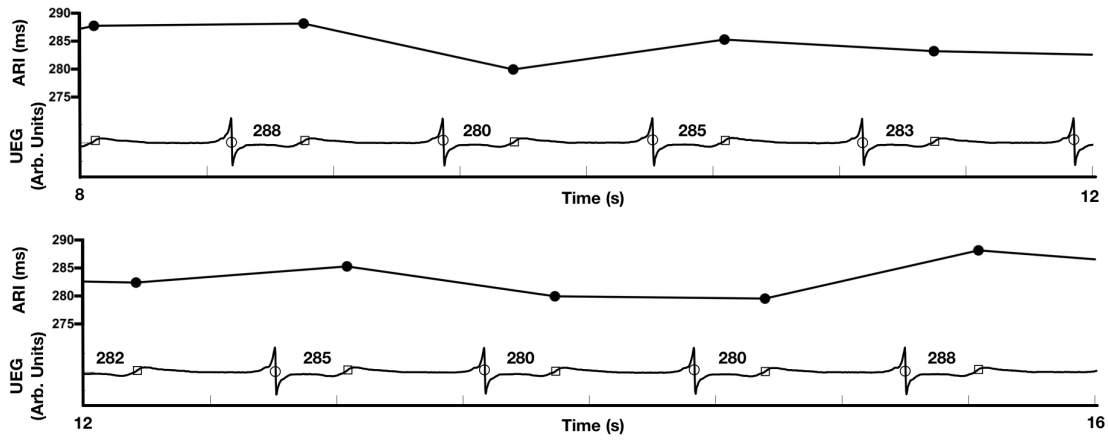


Figure 5-1. Example unipolar electrogram recorded from the left ventricular lead and the computed ARI values for the same electrogram trace.

Identification of the activation time (circle), repolarization time (square) and resultant activation-recovery interval (ARI) for each complex.

5.2.4 Protocol

Following recruitment to the study, beta-adrenergic blocking agents (bisoprolol) were discontinued for 5 days prior to the start of the protocol to allow for a sufficient wash-out period (Leopold et al., 1986). Whilst seated upright the following stages took place:

1. Fixed cycle length was achieved through RV pacing using their implanted CRT-D device. RV pacing was chosen over atrial pacing to account for a high prevalence of AF within our heart failure population and therefore standardize protocols between patients (Bueno-Orovio et al., 2014). The rate was chosen as the minimum rate required to maintain continuous capture at a fixed cycle length. A minimum adaptation period of 10 minutes took place prior to any recordings (Franz et al., 1988).
2. With the patient at rest, recordings of unipolar electrogram, blood pressure and respiration took place. Unipolar electrogram data was then extracted from the device programmer to allow further recordings in step 3.
3. Subjects were asked to perform the Valsalva maneuver (forced expiration against a fixed resistance (Doytchinova et al., 2017)) for 10 seconds. The maneuver was conducted approximately 35 seconds into the second batch of unipolar electrogram recordings. Blood pressure and respiration recordings were made continuously before, during and after the procedure.

The Valsalva maneuver is an established method of increasing sympathetic activity whereby forced expiration impedes venous return resulting in a reduction in ventricular pressure and volume, and a baroreflex increase in sympathetic activity (Booth et al., 1962; Korner et al., 1976; Smith et al., 1987). Sympathetic activity has been shown to be greatly enhanced during the strain phase of the Valsalva maneuver in healthy control subjects in studies using microneurographical recordings of muscle sympathetic nerve discharges

(Schrezenmaier et al., 2007). Indices of baroreflex sensitivity have been established to separately evaluate the vagal and adrenergic components (Vogel et al., 2005; Schrezenmaier et al., 2007).

5.2.5 Analysis of data

Raw data

Raw digital unipolar electrogram traces were analysed off-line using custom built MATLAB software (MathWorks Inc, Natick, Mass) as described previously (Chen et al., 2013, 2016). Each 30 second output from the CRT-D device was truncated to form one long data sequence of approximately 150 seconds in length. Any possible overlap between successive 30 second sequences was found by searching for matching traces at the start/end of successive traces and was removed. ARIs were measured from the time of minimum dV/dt of the electrogram QRS complex, representing local activation time (AT), to the time of maximum dV/dt of the subsequent T-wave, representing local repolarization time (RT) (Wyatt et al., 1981; Haws and Lux, 1990; Coronel et al., 2006; Potse et al., 2009; Hanson et al., 2012, 2014). Blood pressure recordings were analysed for systolic blood pressure (SBP) and the maximum rate of systolic pressure increase (dP/dt_{max}) for each beat using a script written in MATLAB (MathWorks Inc, Natick, Mass). Ectopic beats (0.6±0.8% of beats across all recordings) were removed from analysis together with the successive beat.

Beat-to-beat variability analysis

We have previously demonstrated a transient temporal increase in the short-term variability of ARI immediately following the Valsalva using a 10-beat moving window (Porter et al., 2017). Here our analysis focusses on longer time periods of recording to allow for frequency domain analysis. As such the immediate 60 seconds following

termination of the Valsalva was compared with the resting recordings taken prior to the onset of the Valsalva. The respiration recordings were used to determine the timing of the termination of the Valsalva.

Beat-to-beat variability of ARI was computed over the entire 60 second period as per established QT variability measures (Baumert et al., 2016). The standard deviation of ARI (SDARI) was computed as:

$$SDARI = \sqrt{\frac{1}{N}\sum(ARI_n - ARI_{mean})^2}$$

the ARI variance normalised to the square mean ARI (nSDARI) was computed as:

$$nSDARI = \frac{SDARI^2}{ARI_{mean}{}^2}$$

the standard deviation of SBP (SD-SBP) and dP/dt_{max} (SD-dP/dt_{max}), and the normalized variability values of SBP (nSD-SBP) and dP/dt_{max} (nSD-dP/dt_{max}) were computed with the same formula as for ARI. The same formula was applied to AT-AT intervals to assess for any evidence of a change in conduction variability generated by the Valsalva.

Spectral analysis

Spectral analysis was performed over the same 60 second recordings used to compute beat-to-beat variability measures. Before spectral analysis was performed, linear interpolation was applied to fill in missing AT-AT interval, ARI or blood pressure values (Malik, 1996). An auto-regressive model fitted to each AT-AT interval, ARI and blood

pressure segment was computed using the Yule–Walker method. Following the recommendation of Kay (Kay, 1999), model orders were tested in the range L/3 to L/2, with L the number of beats. The optimal model order was chosen as that which minimized Akaike's Information Criterion (Akaike, 1974) and the residuals were required to pass whiteness test. The low and high frequency variability in AT-AT interval, ARI, SBP and dP/dt_{max} were then calculated by integrating the band power across the bandwidth 0.04–0.15 Hz for low frequency and 0.15-0.4 Hz for high frequency. The normalized low frequency and high frequency variability were also calculated by dividing the low frequency and high frequency variability by the total power in the range above 0.04 Hz ('normalization power'), as recommended by Malik (Malik, 1996). Both markers represent the power of the low frequency and high frequency component relative to the total power of the spectrum. As sympathetic activity is organised in a series of low frequency bursts, the low frequency and normalised low frequency are used to expose changes in sympathetic activity. **Figure 5-2** shows the construction of the low frequency and high frequency band in the spectrum and the corresponding changes in low frequency and high frequency power in the ARI series of one patient following Valsalva.

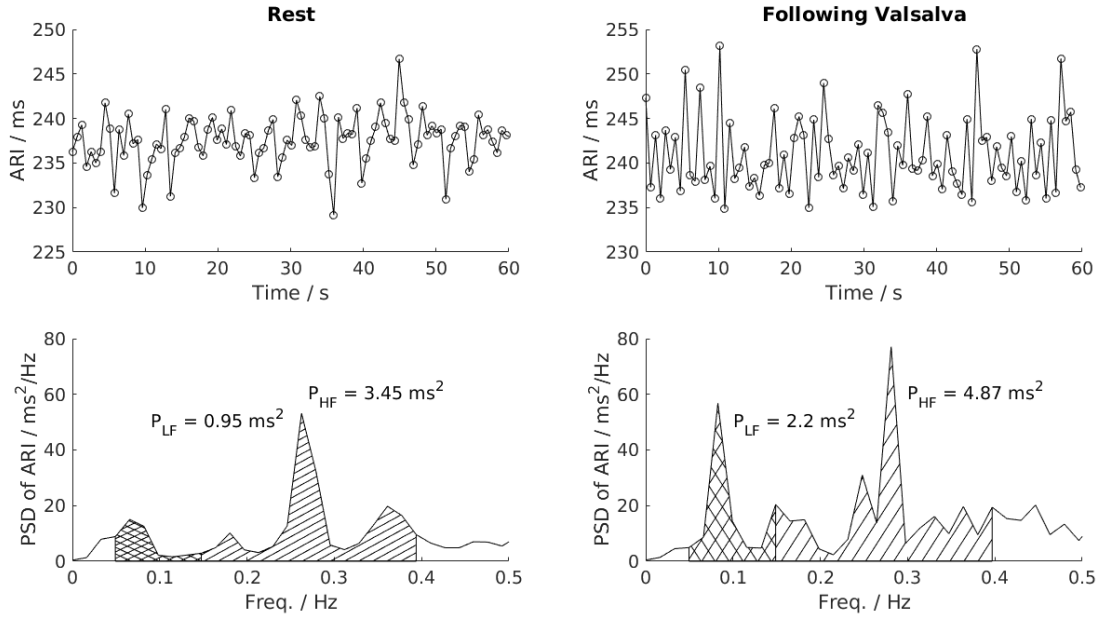


Figure 5-2. Power spectral analysis of low-frequency (0.04-0.15Hz) and high-frequency (0.15-0.4Hz) variability.

This example shows a clear increase of low frequency variability of ARI in one patient following the Valsalva. PSD = Power spectrum density, P_{LF} = low frequency power, P_{HF} = high frequency power.

5.2.6 Statistical Analysis

Results are presented as mean±standard deviation for normally distributed variables and as median and interquartile range (IQR) for nonnormally distributed variables. Continuous variables were compared using the Wilcoxon signed-rank test or Mann-Whitney U test for related or independent observations, respectively. Correlation between variables was expressed using the Spearman correlation coefficient (r_s). A *P* value of <0.05 was considered to be statistically significant for all tests.

5.3 Results

All unipolar electrogram recordings and respiratory recordings were analysable. 3 blood pressure recordings were inadequate to allow analysis throughout the entire 60 seconds (2 during the resting period and 1 in the 60 seconds following termination of the Valsalva). A total of 22 unipolar electrogram, 22 respiratory recordings, and 19 blood pressure recordings were analysed. Throughout the protocol the pacing cycle length remained constant for all patients. The mean pacing cycle length used was 703±80 ms.

5.3.1 Activation-recovery interval and blood pressure variability.

Mean ARI, SBP and dP/dt_{max} measurements during rest and following the Valsalva are reported in **Table 5-2**. Mean SDARI increased significantly following the Valsalva compared to the control period (p=0.019). Mean nSDARI also increased significantly (p=0.032). **Figure 5-3** shows individual values of SDARI and nSDARI during control and following the Valsalva. As expected, mean ARI did not change between control and following stimulus (p=0.147). There was no evidence of an increase in conduction variability following the Valsalva as assessed by AT-AT interval variability: AT-AT interval variability at rest (4.42±0.41 ms) and following the Valsalva (4.52±0.57 ms) was similar (p=0.7).

Table 5-2. Mean activation-recovery interval (ARI) and blood pressure measurements at rest and following the Valsalva.

SD = Standard deviation, nSD = Normalised SD, SBP = Systolic blood pressure, dP/dt_{max} = maximum rate of systolic pressure increase. Values are shown as means (±SD).

	Control	Post Valsalva	*P Value*
Mean ARI, ms	245.95 (±32.94)	244.75 (±33.63)	0.147
SDARI, ms	4.57 (±1.23)	5.52 (±0.99)	0.019
nSDARI, nu	$4.06x10^{-4}$ ($±3.36x10^{-4}$)	$5.59x10^{-4}$ ($±2.82x10^{-4}$)	0.032
Mean SBP, mmHg	113.42 (±19.13)	115.96 (±28.84)	0.426
SD-SBP, mmHg	4.99 (±2.80)	18.74 (±4.35)	0.004
nSD-SBP, nu	$2.31x10^{-3}$ ($±2.05x10^{-3}$)	$3.74x10^{-2}$ ($±3.05x10^{-2}$)	0.004
Mean dP/dt max, mmHg/s	959.11 (±471.54)	925.73 (±311.25)	0.652
SD-dP/dt max, mmHg/s	82.29 (±54.86)	276.96 (±58.17)	0.004
nSD-dP/dt max, nu	$8.47x10^{-3}$ ($±6.34x10^{-3}$)	$11.3\ x10^{-2}$ ($±6.34x10^{-2}$)	0.004

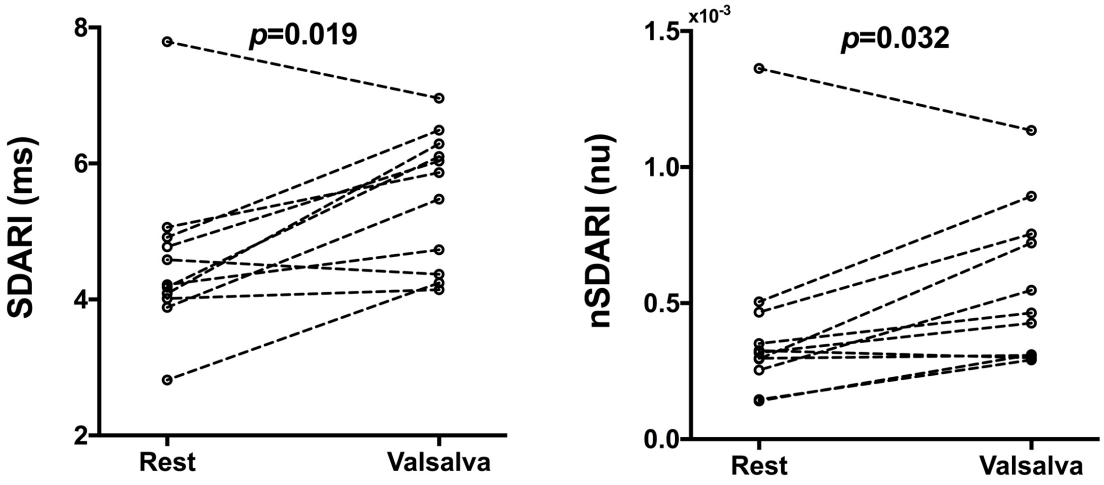


Figure 5-3. Individual beat-to-beat variability measures of activation-recovery intervals (ARIs) at rest and following the Valsalva

SD = Standard deviation, nSD = Normalised SD

Mean SD-SBP increased following the Valsalva (p=0.004). Mean nSD-SBP also significantly increased (p=0.004). **Figure 5-4A** shows individual values of SD-SBP and nSD-SBP during control and following the Valsalva. Mean SD-dP/dt_{max} increased

following the Valsalva (p=0.004). Mean nSDdP/dt_{max} also significantly increased (p=0.004). **Figure 5-4B** shows individual values of SD-dP/dt_{max} and nSD-dP/dt_{max} during control and following the Valsalva. As per the behaviour observed in mean ARI, there was no change in mean SBP (p=0.426) nor mean dP/dt_{max} (p=0.652).

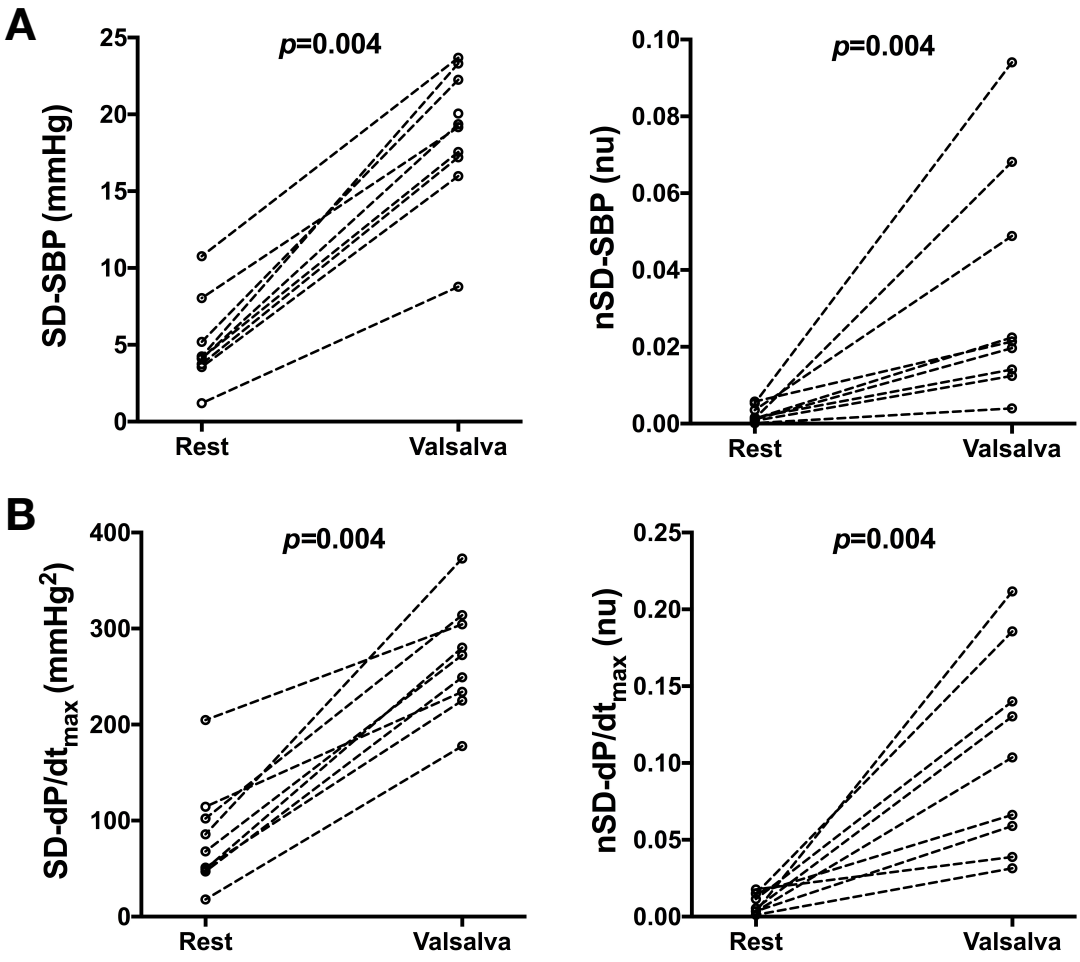


Figure 5-4. Individual beat-to-beat variability measures of (A) systolic blood pressure (SBP) and (B) the maximum rate of systolic pressure increase (dP/dt_{max}) at rest and following the Valsalva.

SD = Standard deviation, nSD = Normalised SD

Evaluation of the heart rate response for assessment of individuals autonomic response to the Valsalva was not possible due to the fixed paced cycle length. It has also been documented that heart failure patients may show an altered blood pressure response to

the Valsalva without a fall in blood pressure during the strain phase known as a 'square wave' blood pressure response (Felker et al., 2006). None of our patients exhibited this behaviour (including the 2 patients with NYHA 3 class heart failure), all showing a substantial blood pressure drop in phase II of the Valsalva (Porter et al., 2017). To determine the significance of this blood pressure drop on the beat-to-beat variability of ARI in individual patients, a correlation analysis with the individual change from rest in SDARI and nSDARI was performed. Δ SDARI and Δ nSDARI showed no correlation with the degree of mechanical blood pressure drop (p=0.958 and p=0.689 respectively).

The behaviour of SDARI in patients with ischaemic cardiomyopathy (ICM) (n=6) vs. non-ischaemic cardiomyopathy (NICM) (n=5) was similar. There was no observed difference in resting SDARI (4.15±0.78 ms vs. 5.08±1.56 ms, p=0.537), nor SDARI post Valsalva (5.41±0.76 ms vs. 5.65±1.3 ms, p=0.537) between patients with ICM vs. NICM.

5.3.2 Frequency analysis

The respiratory frequency at rest (0.28±0.08 Hz) and following termination of the Valsalva (0.26±0.11 Hz) was similar (p=0.473). This excluded the possibility of low frequency respiration producing associated low frequency changes in blood pressure and ARI at rest and post Valsalva.

Mean spectral energy measurements of ARI, SBP and dP/dt_{max} during rest and following the Valsalva are reported in **Table 5-3**. Mean LF power of ARI (LF-ARI) significantly increased following the Valsalva (p=0.002). When normalised the mean LF power of ARI continued to demonstrate a significant increase during the 60 seconds following the Valsalva when compared to control (p=0.019). **Figure 5-5** demonstrates the individual values of LF and nLF power of ARI during control and following the Valsalva. The

Valsalva did not produce significant increases in the LF power of AT-AT intervals (rest: 0.73±0.57 vs. post Valsalva: 2.76±4.57, p=0.365), nor in the normalised LF power of AT-AT intervals (rest: 0.04±0.05 vs. post Valsalva: 0.13±0.2, p=0.365).

Table 5-3. Mean spectral analysis measurements of activation-recovery interval (ARI), systolic blood pressure (SBP) and the maximum rate of systolic pressure increase (dP/dt_{max}) at rest and following the Valsalva.

HF = high frequency power, nHF = normalized HF power, LF = low frequency power, nLF = normalized LF power. Values are shown as means (±SD).

	Control	Post Valsalva	*P* Value
HF-ARI, ms^2	10.61 (±6.52)	14.15 (±8.69)	0.007
nHF-ARI, nu	0.53 (±0.16)	0.51 (±0.2)	0.765
LF-ARI, ms^2	2.65 (±1.7)	7.37 (±5.1)	0.002
nLF-ARI, nu	0.15 (±0.09)	0.28 (±0.19)	0.019
HF-SBP, $mmHg^2$	7.88 (±7.04)	36.73 (±27.17)	0.004
nHF-SBP, nu	0.46 (±0.19)	0.17 (±0.12)	0.004
LF-SBP, $mmHg^2$	9.45 (±9.15)	244.11 (±175.83)	0.004
nLF-SBP, nu	0.49 (±0.17)	0.84 (±0.11)	0.004
HF-dP/dt_{max}, $mmHg^2/s^2$	2794.07 (±3354.78)	4403.17 (±2730.79)	0.129
nHF-dP/dt_{max}, nu	0.46 (±0.2)	0.15 (±0.1)	0.004
LF-dP/dt_{max}, $mmHg^2/s^2$	2174.1 (±2626.31)	31417.85 (±19097.79)	0.004
nLF-dP/dt_{max}, nu	0.41 (±0.18)	0.82 (±0.12)	0.004

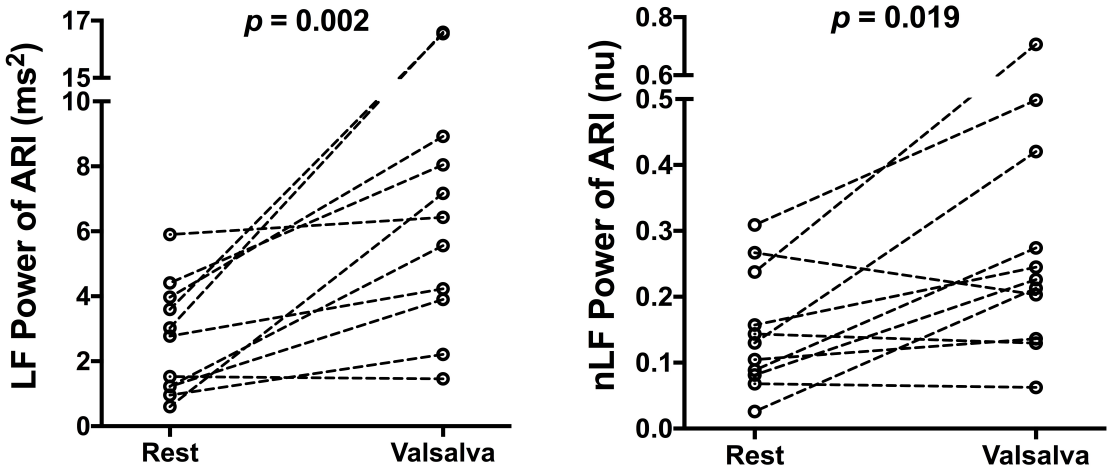


Figure 5-5. Effect of Valsalva on low frequency (LF) power and normalised LF (nLF) power of activation-recovery intervals (ARIs) at rest and following the Valsalva

The Valsalva produced the same modulation of spectral energy measurements of blood pressure with that seen in ARI. Mean LF and nLF power of SBP and dP/dt_{max} significantly increased following the Valsalva (p=0.004). **Figure 5-6A and 5-6B** demonstrate the individual values of LF and nLF power of SBP and dP/dt_{max} during control and following the Valsalva.

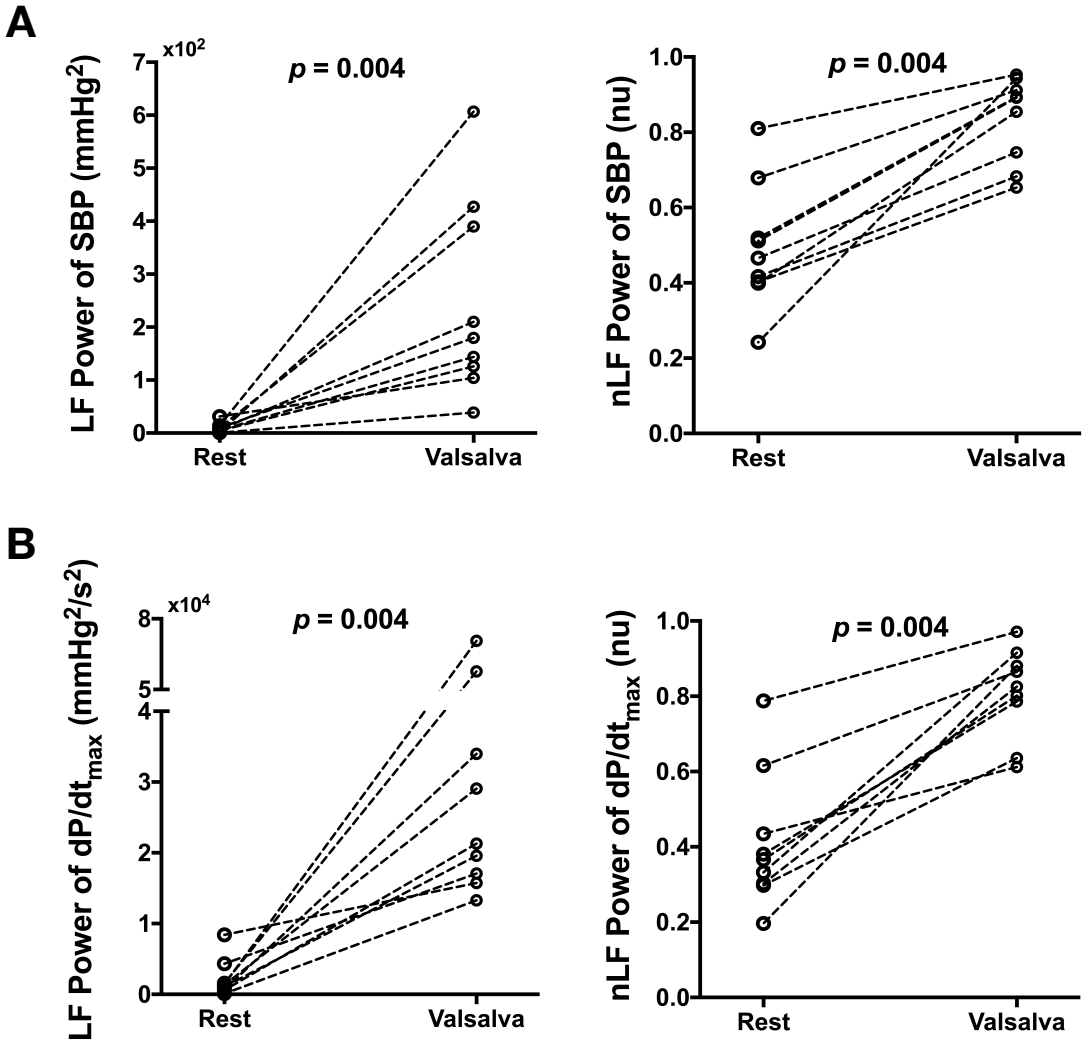


Figure 5-6. Effect of Valsalva on low frequency (LF) power and normalised LF (nLF) power of (A) systolic blood pressure (SBP) and (B) the maximum rate of systolic pressure increase (dP/dt_{max}) at rest and following the Valsalva

The behaviour of LF-ARI in patients with ICM vs. NICM was similar. There was no observed difference in resting LF-ARI (1.98±1.44 ms^2 vs. 3.47±1.77 ms^2, p=0.247), nor LF-ARI post Valsalva (6.98±5.48 ms^2 vs. 7.84±5.18 ms^2, p=0.792) between patients with ICM vs. NICM.

A very strong positive correlation was found between LF-SBP and LF-dP/dt_{max} (r_s= .933, n=19, p<0.001) (**Figure 5-7**). A positive correlation was also found between LF-ARI and

LF-SBP (r_s= .681, n=19, p=0.001) and between LF-ARI and LF-dP/dt_{max} (r_s= .623, n=19, p=0.004).

There was a strong positive correlation between SDARI and LF-ARI (r_s= .679, n=22, p<0.001) (**Figure 5-8**). Importantly there was no apparent association between the haemodynamic response to the Valsalva (assessed by the blood pressure drop in phase II) and the increases in the individual oscillatory behaviour of APD: Δ LF-ARI showed no correlation with the degree of mechanical blood pressure drop (p=0.235).

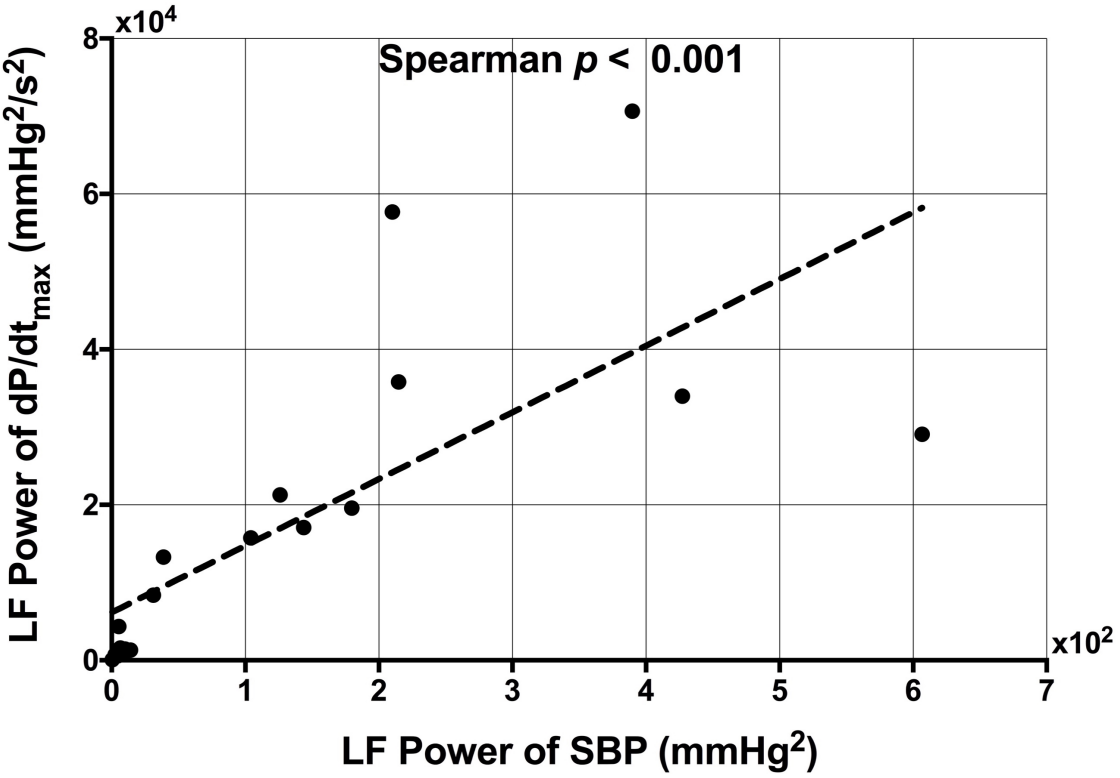


Figure 5-7. Scatterplot demonstrating the significant correlation between low frequency (LF) power of systolic blood pressure (SBP) and the LF power of the maximum rate of systolic pressure increase (dP/dt_{max})

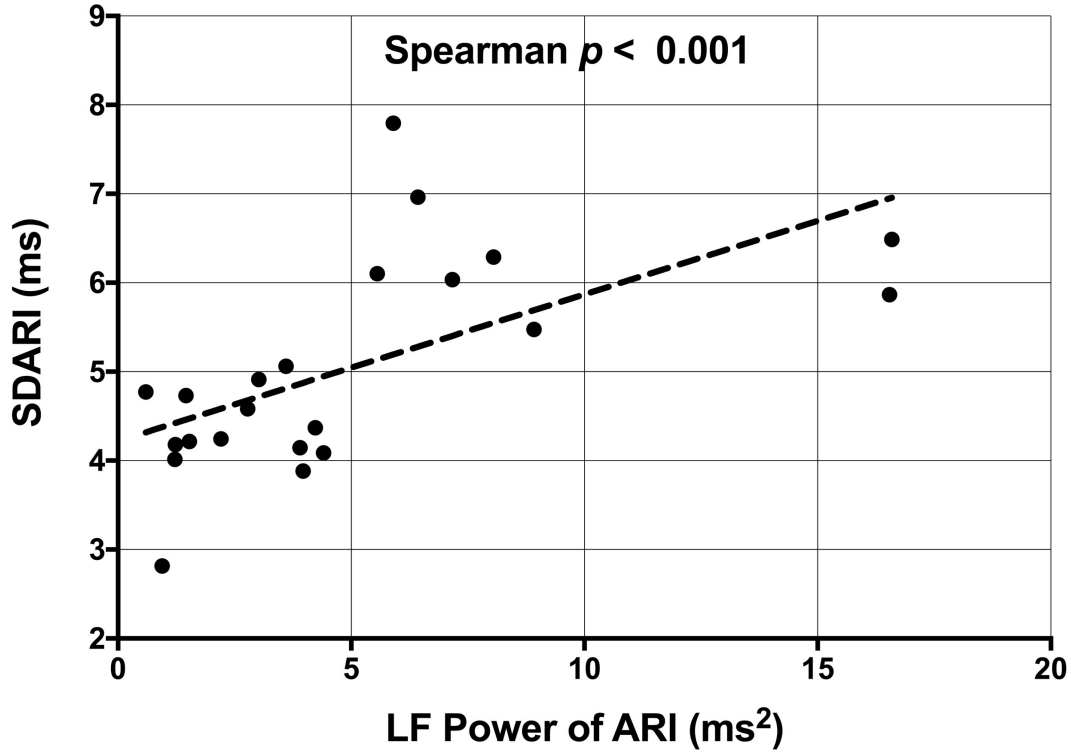


Figure 5-8. Scatterplot demonstrating the significant correlation between the low frequency (LF) power of activation-recovery intervals (ARIs) and the beat-to-beat variability of ARI (SDARI)

5.4 Discussion

The work in this chapter has investigated the oscillatory behaviour of ventricular repolarization in the low frequency range (0.04-0.15Hz) in response to a standard autonomic challenge (Valsalva maneuver). ARIs as a conventional surrogate for APD were recorded from the LV epicardial lead of an implanted biventricular pacing device while pacing from the RV lead. The main findings were: (1) beat-to-beat variability of APD (ARI) increased associated with an increase in beat-to-beat variability of systolic blood pressure and dP/dt_{max} of systolic pressure, (2) the low frequency power of APD increased also associated with an increase in the low frequency power of systolic blood pressure and dP/dt_{max}, (3) the increase in beat-to-beat APD variability correlated with increasing low frequency power of APD.

Sympathetic nerve activity is organized in bursts in a range of frequencies including the so called low frequency range in the region of 0.04-0.15 Hz, i.e. approximately one every 10 seconds in humans (Pagani et al., 1986; Malliani et al., 1991; Pagani et al., 1997; Furlan et al., 2000; Coote, 2001; Montano et al., 2009). Spectral analysis of blood pressure usually reveals a low frequency component. Fluctuations in blood pressure at this frequency (Mayer waves (Julien, 2006)) are generally attributed to the response of peripheral vascular resistance to phasic sympathetic nerve input. Beat-to-beat variability of heart rate also exhibits a low frequency component although the contribution of sympathetic activity remains a subject of discussion. Recently attention has been drawn to the presence of oscillations in the morphology of the T wave on the ECG in humans at approximately the 0.1 Hz frequency which are enhanced by sympathetic provocation (orthostatic challenge, exercise) and reduced by beta-adrenergic blockade (Rizas et al., 2014, 2016). These T wave oscillations have been attributed to oscillations in ventricular

repolarization in response to low frequency sympathetic nerve input and assumed to reflect corresponding oscillations in ventricular APD. We have recently demonstrated the presence of low frequency oscillations of ventricular APD in humans (Hanson et al., 2014). In the present study we confirm the presence of these low frequency APD oscillations and demonstrate an increase following a conventional sympathetic provocation maneuver consistent with the findings of Rizas and colleagues on the ECG T wave (Rizas et al., 2014, 2016).

Extrapolation from our findings on beat-to-beat variability of APD with cycle length maintained constant to studies of QT variability is not straightforward. In physiological conditions QT variability is substantially influenced by heart rate variability due to the rapid and slow components of the cycle length dependence of APD (Franz et al., 1988; Zaza et al., 1991; Cabasson et al., 2012). QT variability when cycle length is held constant is considered to be due to both variation in APD and variation in activation pattern and hence conduction time (Baumert et al., 2016). In our studies, the Valsalva did not induce changes in the variability of conduction time nor in the low frequency oscillatory behaviour of conduction.

Beat-to-beat variability of repolarization has been demonstrated in a range of experimental models and humans (Zaniboni et al., 2000; Tereshchenko et al., 2010; Hinterseer et al., 2010). Exaggerated beat-to-beat variability of repolarization is known to be associated with arrhythmogenesis in animal models (Thomsen et al., 2004; Gallacher et al., 2007; Abi-Gerges et al., 2010; Jacobson et al., 2011) and in humans (Atiga et al., 1998; Haigney et al., 2004; Tereshchenko et al., 2009; Hinterseer et al., 2010; Średniawa et al., 2012) and increase in the normal beat-to-beat variability in the timing of repolarization is associated with pro-arrhythmia (Baumert et al., 2016).

Enhanced sympathetic activity has been shown to increase beat-to-beat variability of repolarization in canine ventricular myocytes (Johnson et al., 2013), the QT interval (Desai et al., 2004; Piccirillo et al., 2006) and recently of ventricular APD in humans (Porter et al., 2017). A previous study in normal subjects using spectral analysis of QT interval variability showed an increase in low frequency oscillation during both mental stress and exercise. This was attributed to a combined effect of increased sympathetic activity on RR interval variability and a direct effect on ventricular myocardium (Negoescu et al., 1993). A subsequent study using atrial pacing at a constant rate to eliminate RR interval variability confirmed that mental stress increased the low frequency power of QT variability. These results suggested a direct rate independent effect of enhanced sympathetic activity (Negoescu et al., 1997). The present study during constant steady state pacing extends these observations to the level of the ventricular action potential. We show that not only does a sympathetic challenge increase beat-to-beat variability of APD but also imparts a low frequency oscillation such that the beat-to-beat variability of APD waxes and wanes over a roughly 10 second cycle.

5.4.1 Mechanisms

Oscillations of ventricular repolarization in the low frequency range independent of RR interval have been demonstrated in human endocardium (Hanson et al., 2014), body surface ECG recordings of the T-wave vector (Rizas et al., 2014, 2016, 2017) and in in-silico modelling studies (Pueyo et al., 2016a). These rhythmic fluctuations are increased by enhanced sympathetic activity and reduced by beta-adrenergic blockade and occur in the spectral range of oscillations characteristic of sympathetic nerve activity (0.04-0.15 Hz). Modelling studies of the dynamics of the ventricular APD (Pueyo et al., 2016a) suggest that the phasic nature of amplification with sympathetic stimulation is related to the different phosphorylation kinetics in response to beta-adrenergic stimulation of

inward depolarizing calcium current (ICaL) and outward repolarizing potassium current (IKs) (Liu et al., 2012; Xie et al., 2013; Ruzsnavszky et al., 2014). The oscillatory behaviour was markedly enhanced by incorporating interaction with beta adrenergic effects on myocardial stretch by calcium mechanisms and stretch activated channels, and also by incorporating calcium overload and downregulation of potassium channels, both of which are characteristic features of pathological hearts and remodelling and both known to promote the development of after-depolarizations and arrhythmias (Weiss et al., 2015).

Our study showing a correlation between the increase in low frequency patterning of APD and the increase in beat-to-beat variability of APD suggests a possible interaction between the underlying mechanisms. While a number of mechanism have been proposed as the cellular basis for beat-to-beat variability of repolarization, it is likely that spontaneous sarcoplasmic calcium release plays a fundamental role (Li et al., 2012; Kim et al., 2013; Johnson et al., 2013; Baumert et al., 2016). Calcium release from the sarcoplasmic reticulum varies on a beat-to-beat basis and in calcium overloaded cells may generate beat-to-beat variability of APD (Johnson et al., 2013). The apparently random nature of beat-to-beat variability favours a stochastic process, and stochastic variation in gating of a wide range of ion channels has been shown to influence beat-to-beat variability (Tanskanen et al., 2005; Johnson et al., 2013; Heijman et al., 2013). It is at present uncertain to what extent these effects observed in isolated cells may be operative in the whole heart due to electrotonic interaction between cells (Zaniboni et al., 2000; Baumert et al., 2016). However, in pathological hearts where cell coupling is reduced and in the presence of calcium overload and reduced repolarization reserve beat-to-beat variability may be arrhythmogenic by inducing early or late after-depolarizations or initiating re-entry. Modelling studies exploring the mechanisms of sympathetic enhancement of low

frequency oscillation of APD showed an important contribution of accompanying mechanical changes mediated by stretch activated channels (Pueyo et al., 2016a). Recent work in a canine model of chronic atrioventricular block and reduced repolarization reserve has shown that beat-to-beat variability in preload enhanced beat-to-beat variability of repolarization and was proarrhythmic. This effect was blocked by streptomycin therefore strongly suggesting a role of stretch activated channels in the modulation of beat-to-beat variability of APD (Stams et al., 2016).

5.4.2 Clinical perspective

Risk stratification for the identification of patients at high risk of sudden cardiac death, particularly post MI, remains a major challenge. In view of the multiple mechanisms involved it is unlikely that a single test would prove sufficient and a combination of clinical characteristics with a selection of stratification tools may be more appropriate (Dagres and Hindricks, 2013). Oscillation of ventricular repolarization related to sympathetic activity, referred to as periodic repolarization dynamics (PRD) has been identified as a strong predictor of sudden cardiac death (Rizas et al., 2014, 2017) and is currently involved in a randomized prospective multicentre trial in 17 centres in Germany (Hamm et al., 2017). However, the mechanisms underlying PRD remain poorly understood. PRD provides incremental prognostic information to exercise induced T wave alternans (TWA) and is capable of detecting patients not identified by TWA (Rizas et al., 2014). The two may be complementary acting through different mechanism, PRD probably relates to low frequency sympathetic activity and TWA to high frequency oscillations related to calcium handling (Narayan et al., 2008). PRD may be obtained at rest whereas TWA requires exercise or invasive procedures to induce a heart rate increase to the region of 2 Hz. We have previously demonstrated the presence of oscillation of the ventricular APD at the sympathetic nerve frequency in humans (Hanson et al., 2014) and

modelling studies identified a cellular mechanism related to the phosphorylation kinetics of ion channels (Pueyo et al., 2016a). The present study extends our knowledge on mechanisms underlying the oscillatory behaviour of repolarization at the level of the ventricular action potential oscillation in humans and a possible interaction with beat-to-beat variability of APD, an important proarrhythmic mechanism. Further studies investigating the oscillatory behaviour of electrophysiological properties at the intact heart, tissue and cellular level may help refine its potential use in risk stratification as well as pointing toward novel therapeutic modalities.

5.4.3 Limitations

An implanted biventricular pacing device was a basic requirement for the study which negated the possibility of incorporating a control group of subjects. Consequently, our findings are only directly applicable to patients with heart failure as we have not been able to study a population of patients with normal hearts. Similar studies of normal hearts would be of high importance to fully determine the significance of the observed changes in beat-to-beat variability of APD. Our study includes both patients with ischaemic and non-ischaemic cardiomyopathy. Although we observed changes in beat-to-beat variability of APD and also the low frequency behaviour of APD in both groups, our study is underpowered to determine whether subtle differences in behaviour exist between these populations. Although all of our patients had a typical haemodynamic response to the Valsalva, this may not be representative of the spectrum of NYHA classes of heart failure. Our study is underpowered to observe differences in behaviour between various functional classes of heart failure. Our observations are confined to a single LV epicardial site as it was only possible to record from one epicardial electrode whilst maintaining a constant cycle length through RV pacing. In view of the regional variation in electrophysiological properties throughout the myocardium other regions may have

yielded different results. Recordings were made during free breathing and respiration is known to affect repolarization lability. Due to a high prevalence of atrial fibrillation amongst our population of heart failure patients we chose RV pacing over atrial pacing to achieve fixed cycle length. Future studies would assess ARI variability during atrial pacing for further validation. Due to the fixed paced cycle length that is established throughout the protocol it is not possible to evaluate the heart rate response to the Valsalva and we are therefore not able to determine the Valsalva ratio which is a well-established measurement of the autonomic response.

5.5 Conclusions

In patients with heart failure and implanted CRT-D devices physiological provocation to increase sympathetic activity induced oscillations of LV APD in the low frequency range (0.04-0.15 Hz). Coherence analysis suggested an interaction between the low frequency oscillatory behaviour of APD and the beat-to-beat variability. These observations provide insight at the level of the ventricular action potential into mechanisms underlying low frequency oscillation of repolarization derived from the ECG T-wave which have been shown to be exaggerated by sympathetic stimulation and are strongly predictive of sudden cardiac death in post MI patients. Further work aimed at unravelling mechanisms at the cellular level may help to elucidate the link to arrhythmogenesis and possibly point to therapeutic targets.

Chapter 6: Complex interaction between low-frequency APD oscillations and beat-to-beat APD variability in humans is governed by the sympathetic nervous system

6.1 Introduction

Factors which influence the stability of ventricular repolarization are important in arrhythmogenesis. Enhanced oscillation of ventricular repolarization in the low frequency range and increased beat-to-beat variability of ventricular repolarization are two of the strongest predictors of arrhythmia and sudden cardiac death (Atiga et al., 1998; Thomsen et al., 2004; Haigney et al., 2004; Gallacher et al., 2007; Larisa G Tereshchenko et al., 2009; Hinterseer et al., 2010; Abi-Gerges et al., 2010; Jacobson et al., 2011; Średniawa et al., 2012; Baumert et al., 2016; Rizas et al., 2014, 2016, 2017, 2019). Both are enhanced by sympathetic stimulation and recent studies suggest a possible interactive mechanism (Porter et al., 2018b). However, the mechanisms underlying the effect of beta-adrenergic stimulation on LF oscillations of repolarization and beat-to-beat variability of repolarization remain unclear.

Oscillations of ventricular repolarization measured from the ECG T-wave vector referred to as periodic repolarization dynamics (PRD) have been attributed to oscillations in APD at the frequency of the sympathetic nerves (approx. 0.05-0.1 Hz). Ventricular APD measured as ARIs has recently been shown to oscillate in this frequency range (Hanson et al., 2014). The LF power of APD has been shown to be increased by sympathetic provocation (Porter et al., 2018b). The recent finding of LF oscillations in short term variability of ventricular APD (Porter et al., 2018b) raises the possibility of an association between LF oscillations of APD and beat-to-beat variability.

Computational modelling has provided early insight into the mechanisms underlying these oscillations of APD, the effect of beta-adrenergic stimulation and their relationship to the initiation of ventricular arrhythmias (Pueyo et al., 2016a, 2016b). More recent

studies on the effect of beta-adrenergic blockade suggest that the cellular mechanisms underlying modulation of LF APD and beat-to-beat variability of APD are strongly influenced by the initial conditions on APD (Sampedro-Puente et al., 2019). The purpose of the present study was to examine this hypothesis in humans in vivo.

We have studied 12 patients during cardiac catheterisation allowing us to measure ARIs as an approximation for APD at 10 RV and 10 LV endocardial sites in order to investigate the effect of acute beta-adrenergic blockade on LF oscillations of ventricular APD and on beat-to-beat variability of APD, and the possible interaction between the two. Cycle length was held constant with RV pacing to avoid confounding effects due to the cycle length dependency of APD.

6.2 Methods

6.2.1 Ethical approval

The study was approved by the ethics committee of Guy's and Thomas' Hospitals and conformed to the standards set by the Declaration of Helsinki (latest revision: 59th World Medical Association General Assembly). All patients gave written, informed consent.

6.2.2 Subjects

Studies were performed in 12 patients (10 males, 2 females, aged 41–69, median 61) during the course of routine clinical radiofrequency ablation procedures for atrial fibrillation. Four patients had paroxysmal atrial fibrillation, and eight patients had persistent atrial fibrillation. All subjects had normal biventricular function and none of them were known to have ventricular scar or disordered conduction due to bundle branch abnormalities. Studies were performed in the un-sedated state and cardio-active medications were discontinued for 5 days before the study.

6.2.3 Protocol

Subjects were paced from the right ventricular apex using a Biotronik (Berlin, Germany) stimulator (model UHS 3000) at 2x diastolic threshold and 2 ms pulse width, at a cycle length >20 beats/min faster than the intrinsic AF rate (median, 500 ms) to avoid breakthrough intrinsic beats. **Figure 6-1** shows the set-up of both recording decapolar catheters and the pacing catheter. A 2-min period of adaptation to the paced cycle length was applied before starting a controlled breathing protocol. Breathing was controlled

throughout the protocol at 0.25Hz and 0.5Hz. Recordings took place for 90s during each controlled breathing cycle. First, a control period was established with the breathing protocol performed in absence of any autonomic blocking agents. Pacing was then stopped and the subject received metoprolol at a dose sufficient to reduce the intrinsic heart rate by 10 beats/min (iv; dose range, 2–10 mg), and after a further 10 minutes for equilibration the pacing (at the same paced cycle length as the control) and breathing protocol was repeated as above.

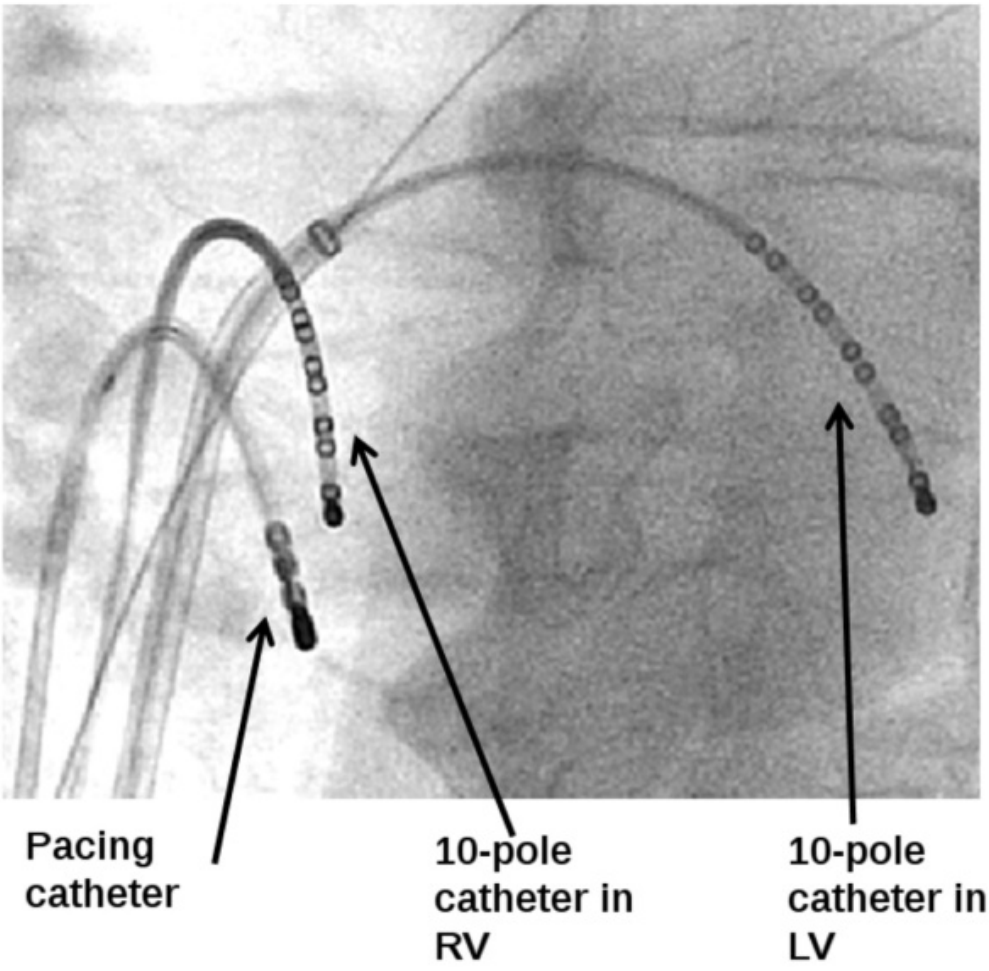


Figure 6-1. Fluoroscopic image of the right ventricular (RV) and left ventricular (LV) decapolar catheter electrodes and the RV pacing catheter.

6.2.4 Measurements

Continuous synchronous recordings of femoral arterial blood pressure and unipolar electrograms at 10 endocardial RV and 10 endocardial LV sites were obtained before routine clinical radiofrequency ablation procedures for atrial fibrillation in the cardiac catheterization lab at St Thomas' Hospital in London, as described previously (Hanson et al., 2012; van Duijvenboden et al., 2015). Unipolar electrograms and blood pressure recordings were digitized at 1,200 Hz (Ensite 3000; Endocardial Solutions) and analyzed offline.

6.2.5 Data analysis

Unipolar electrograms were analyzed for ventricular APDs at each recording site by measuring ARIs using the Wyatt method (Wyatt et al., 1981). This method has been validated in theoretical, computational, and experimental studies (Wyatt et al., 1981; Haws and Lux, 1990; Coronel et al., 2006; Potse et al., 2009). According to this method, activation is measured at the moment of minimum dV/dt of the QRS complex of the unipolar electrogram and repolarization at the moment of maximum dV/dt of the T-wave. ARIs were measured automatically using in house developed algorithms. Heuristic-based screening was used to identify and discount any cases where the T-wave was indistinct or corrupt. Blood pressure recordings were analyzed for SBP and dP/dt_{max} as a measure of myocardial contractility.

To establish evenly-sampled series, any beats for which ARI, SBP or dP/dt_{max} measurements could not be determined were replaced by linear interpolation between the surrounding beats. Recordings were rejected from the analysis if these surrogate beats constituted more than 10% of any series.

The low frequency (LF) power in each ARI series was estimated by calculating the bandpower in the low-frequency band (0.04-0.15Hz) using the Thomson's multitaper method with three Slepian tapers, which is known to be robust against noise (Thomson, 1982). The same was applied to the calculate the high frequency (HF) power in the high-frequency band (0.25-0.5 Hz). The LF and HF powers were then averaged for RV and LV poles. **Figure 6-2** shows the construction of the LF and HF band in the spectrum and the corresponding changes in LF and HF power in the ARI series of one patient following introduction of beta-blockade.

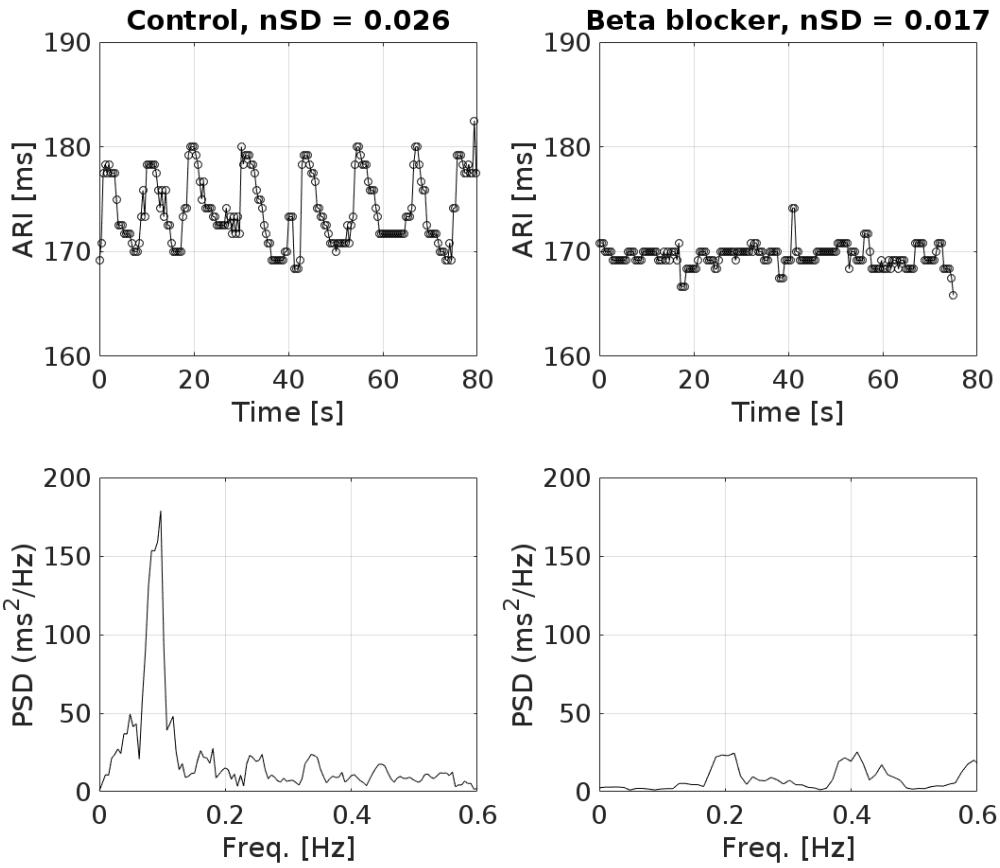


Figure 6-2. Power spectral analysis of low-frequency (0.04-0.15Hz) and high-frequency (0.25-0.5Hz) variability during control and following beta-blockade.

This example shows a clear decrease of low frequency power of ARI in one patient following the introduction of beta-blockade. This is coupled with a reduction in the normalised standard deviation (nSD) of activation-recovery intervals (ARIs).

PSD = Power spectrum density.

Beat-to-beat variability of ARI was computed for each endocardial recording site over the entire recording as per established QT variability measures (Baumert et al., 2016). The standard deviation of ARI (SDARI) was computed as:

$$SDARI = \sqrt{\frac{1}{N}\sum(ARI_n - ARI_{mean})^2}$$

the ARI variance normalised to the square mean ARI (nSDARI) was computed as:

$$nSDARI = \frac{SDARI^2}{ARI_{mean}{}^2}$$

Similar to the LF power, we computed the average SDARI across LV and RV poles. The normalized variability values of SBP (nSD-SBP) and dP/dt_{max} (nSD-dP/dt_{max}) were computed with the same formula as for ARI.

6.2.6 Statistical analysis

Results were averaged across the two separate breathing cycles for both control recordings and following introduction of beta-blockade. Results are presented as mean±standard deviation for normally distributed variables and as median and interquartile range (IQR) for nonnormally distributed variables. The effect of beta-blockade on LF power for ARI, SBP and dP/dt_{max} was tested for statistical significance using the two-tailed paired Wilcoxon signed-rank test. Results were considered significant at $p < 0.05$.

6.3 Results

6.3.1 Effect of beta blockade on group data

Beta-blockade resulted in a significant reduction of LF power of ARI (8.6 ± 4.5 ms^2 vs. 5.5 ± 3.5 ms^2, $p = 0.027$) (**Figure 6-3A**) and the LF power of SBP (1.4 $x10^{-3}$ ± 1.2 $x10^{-3}$ $mmHg^2$ vs. 0.4 $x10^{-3}$ ± 0.5 $x10^{-3}$ $mmHg^2$, $p = 0.027$) (**Figure 6-3B**). A trend to reduction was observed for the LF power of dP/dt_{max} (0.7 $x10^{-6}$ ± 1 $x10^{-6}$ vs. 0.1 $x10^{-6}$ ± 0.2 $x10^{-6}$ $mmHg^2/s^2$, $p = 0.129$) (**Figure 6-3C**).

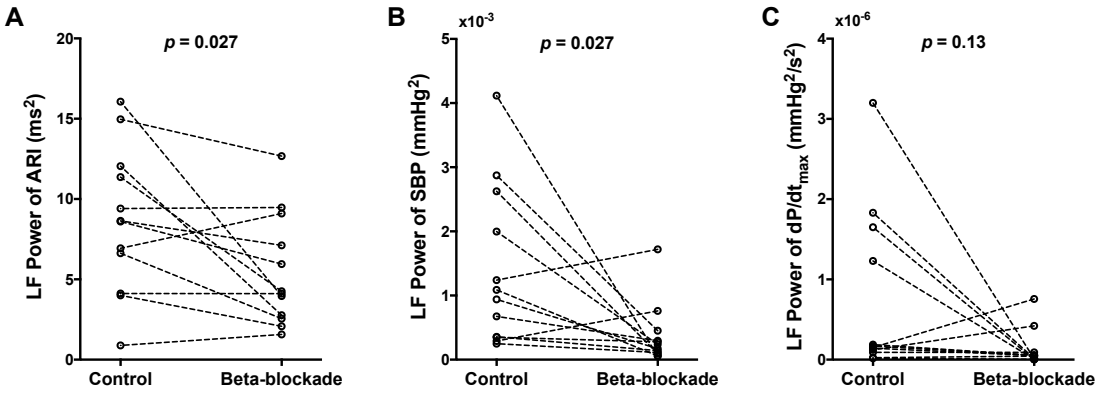


Figure 6-3. Effect of beta-blockade on the low frequency (LF) power of (A) activation-recovery intervals (ARIs), (B) systolic blood pressure (SBP) and (C) the maximum rate of systolic pressure increase (dP/dt_{max}).

No effect of beta-blockade was seen on the HF power of ARI (6.5 $x10^{-3}$ ± 3 $x10^{-3}$ ms^2 vs. 6.1 $x10^{-3}$ ± 3.4 $x10^{-3}$ ms^2, $p = 0.91$), SBP (1.9 $x10^{-3}$ ± 1 $x10^{-3}$ $mmHg^2$ vs. 1.7 $x10^{-3}$ ± 1 $x10^{-3}$ $mmHg^2$, $p = 0.424$), nor dP/dt_{max} (7.6 $x10^{-7}$ ± 7.4 $x10^{-7}$ $mmHg^2/s^2$ vs. 3.2 ± 3.9 $mmHg^2/s^2$, $p = 0.052$).

No immediate effect of beta-blockade was seen on the beat-to-beat variability of ARI (nSD-ARI: 0.04 ± 0.01 nu vs. 0.03 ± 0.01 nu, $p = 0.733$), SBP (nSD-SBP: 0.07 ± 0.03 nu vs. 0.05 ± 0.02 nu, $p = 0.151$), nor dP/dt_{max} (nSD-dP/dt_{max}: 0.23 ± 0.15 nu vs. 0.16 ± 0.08 nu, $p = 0.266$).

6.3.2 Influence of initial values on the response to beta-blockade

The effect of beta-adrenergic blockade on the group data was small. However, a wide range of control values was evident and when our results were expressed in relation to control values a highly significant effect of beta blockade was apparent. Subjects in whom the initial control values of LF of ARI were large showed a greater change in the magnitude of the oscillations following beta-blockade compared to subjects in whom the initial values were low. When control oscillations of ARI were large beta-blockade reduced their magnitude. When control oscillations were small the response to beta-blockade was minimal or variable ($r_s = 0.62$, $p = 0.037$) **(Figure 6-4A)**. A similar relationship was observed for beat-to-beat variability of ARI ($r_s = 0.79$, $p = 0.003$) **(Figure 6-4B)**, and the LF power of SBP and dP/dt_{max} ($r_s = 0.78$, $p = 0.004$ and $r_s = 0.84$, $p = 0.001$ respectively) **(Figures 6-4C, 6-4D).**

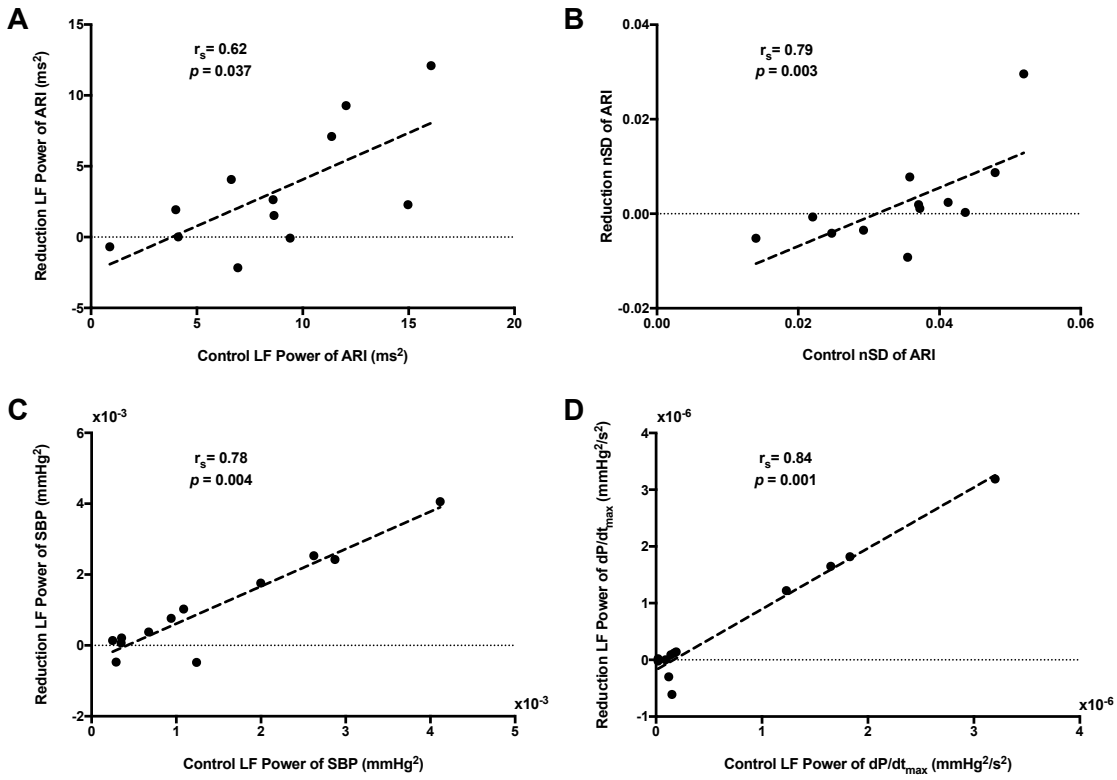


Figure 6-4. Scatterplots demonstrating the significant relationship between baseline values and the reduction seen following beta-blockade in: (A) LF power of ARI, (B) beat-to-beat variability of ARI (nSDARI), (C) LF power of systolic blood pressure (SBP), and (D) the LF power of the maximum rate of systolic pressure increase (dP/dt_{max}).

6.3.3 Relationship between low frequency power and beat-to-beat variability of activation-recovery intervals

There was a strong relationship between the reduction in LF power of ARI and the reduction of beat-to-beat variability of ARI in response to beta-blockade ($r_s = 0.9$, $p < 0.001$) **(Figure 6-5)**. There was no significant relationship between the change in HF power of ARI and the reduction of beat-to-beat variability of ARI ($r_s = 0.55$, $p < 0.07$). Similar relationships were observed between the reduction in LF power and the reduction

in beat-to-beat variability of both SBP ($r_s = 0.727$, $p < 0.007$) and dP/dt_{max} ($r_s = 0.72$, $p < 0.008$).

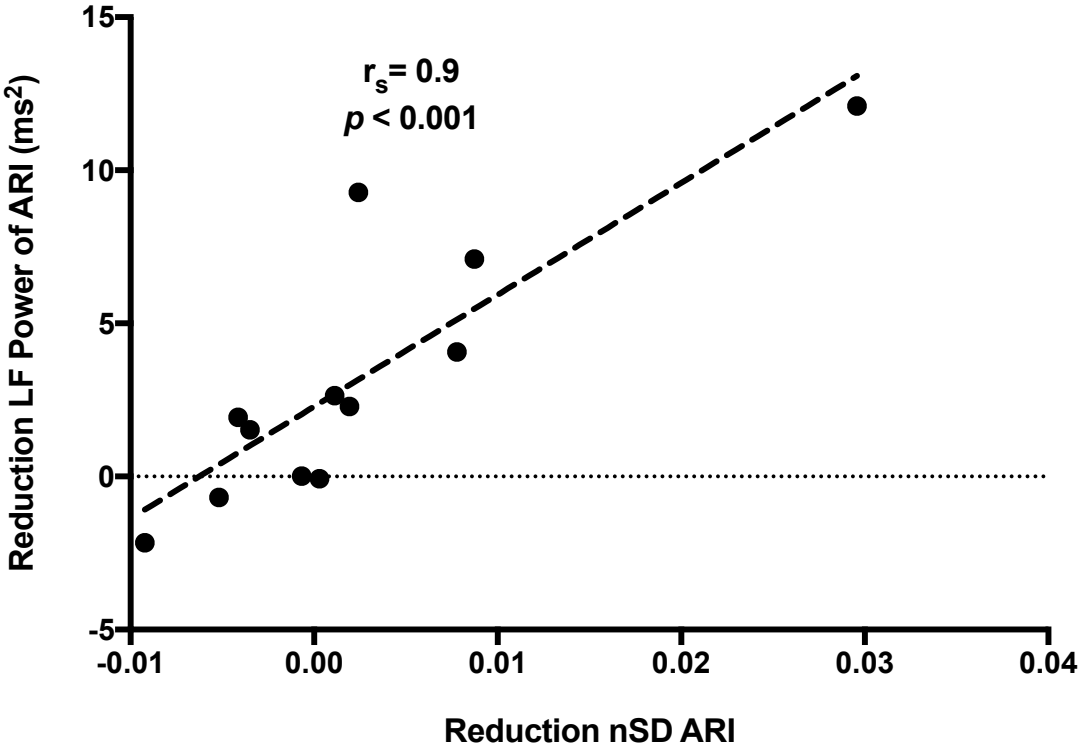


Figure 6-5. Scatterplot demonstrating the significant relationship between the beta-blockade induced reduction in the LF power of ARI and the witnessed reduction in the beat-to-beat variability of ARI (nSDARI).

6.4 Discussion

This chapter presents studies exploring the effect of acute beta-adrenergic blockade on low frequency oscillations of ventricular APD (approximated by ARI) and on beat-to-beat APD variability at 10 RV and 10 LV endocardial sites in patients with normal ventricles. Cycle length was maintained constant with right ventricular pacing to eliminate confounding effects of cycle length dependency and breathing was controlled throughout the protocol at 0.25Hz and 0.5Hz. Our main findings were: (1) we observed a wide variation of control values of LF power and beat-to-beat variability of ARI, SBP and dP/dt_{max}; (2) beta-adrenergic blockade was associated with a significant reduction of LF power of ARI and SBP, (3) individually no clear impact of beta-blockade on the beat-to-beat variability of ARI, SBP and dP/dt_{max} was demonstrated, however (4) there was a strong correlation between the reduction seen in the LF power of ARI, SBP and dP/dt_{max} following beta-blockade, and the reduction in beat-to-beat variability.

Whereas oscillations in heart rate variability have long been recognised and the underlying mechanisms the subject of much debate (Parati et al., 2006), oscillations of ventricular APD at the low frequency have only relatively recently been identified (Hanson et al., 2014). These LF APD oscillations identified in humans using ARI recordings from the ventricular myocardium, are independent of variation in R-R interval and independent of respiration (Hanson et al., 2014). They frequently occur in association with LF oscillations in blood pressure (Mayer waves) (Julien, 2006). Oscillation of ventricular repolarization at the low frequency has recently been identified from the body surface ECG T-wave vectors and these are also independent of R-R interval variability and respiration and are attributed to LF oscillation of ventricular APD (Rizas et al., 2014). When enhanced these oscillations are strongly predictive of arrhythmia and sudden

cardiac death (Rizas et al., 2014, 2016, 2017, 2019; Hamm et al., 2017). The magnitude of both ARI and T-wave vector oscillations are increased during sympathetic stimulation (Porter et al., 2017, 2018b; Rizas et al., 2014) and it has been suggested they may be related to the intrinsic low frequency oscillation of sympathetic nerve activity.

Recently it has been observed that the intrinsic beat-to-beat variation in APD also exhibits phasic variation at the low frequency, which is enhanced during increased sympathetic stimulation (Porter et al., 2018b). This raises the question of a possible interaction between LF ARI and intrinsic beat-to-beat variation in ARI. Enhanced beat-to-beat variability of repolarization, measured clinically as QT variability or experimentally as APD variability, is well known to predispose to malignant ventricular arrhythmias (Atiga et al., 1998; Haigney et al., 2004; Thomsen et al., 2004; Gallacher et al., 2007; Tereshchenko et al., 2009; Hinterseer et al., 2010; Abi-Gerges et al., 2010; Jacobson et al., 2011; Średniawa et al., 2012; Baumert et al., 2016) and a possible interaction may have important mechanistic implications in this context.

It has been shown that sympathetic provocation increases LF power of the QT interval (Baumert et al., 2016). Paradoxically studies using beta-adrenergic blockade have shown a mixed response of beat-to-beat variability of the QT interval with either no change or an increase or decrease (Baumert et al., 2016). It is generally agreed that QT variability is influenced by R-R variability which may be relevant. Our observations that the response of APD variability to beta-blockade during clamped heart rate is strongly influenced by initial conditions may in part provide an explanation.

Recent computational research has shown that the major ionic contributors to inter-individual differences in LF oscillations of APD and beat-to-beat APD variability are I_{Kr},

I_{CaL} and I_{K1} (Sampedro-Puente et al., 2019). In this study, a set of stochastic human ventricular action potential models was developed by individually varying the ionic conductance of I_{Kr}, I_{CaL} and I_{K1} from their nominal values in the O'Hara-Virág-Varró-Rudy (ORd) action potential model (O'Hara et al., 2011). Beta-adrenergic and mechanical stretch effects were included in the models to simulate sympathetic modulation of ventricular electrophysiology at the cell level (Pueyo et al., 2016b; Sampedro-Puente et al., 2019). For each of the simulated models, normalized measures of LF oscillation magnitude of APD (nmLF) and beat-to-beat APD variability (nSD-APD) were computed before and after beta-blockade. In accordance with the clinical observations of this study, beta-blockade in these simulated cells led to a remarkable reduction in nmLF and also in nSD-APD. Importantly, these simulations showed a wide range of nmLF and nSD-APD initial values as well as of their changes in response to beta-blockade. In line with the presented clinical data, higher nmLF and nSD-APD initial values were associated with larger beta-blockade-induced decreases in the magnitudes of both markers. A strong correlation was observed between the effects of beta-blockade on nmLF and nSD-APD.

The reduction in nmLF in response to beta-blockade, which could be observed to a greater or lesser extent in all the virtual cells, can be explained on the basis of beta-adrenergic stimulation enhancing LF oscillations of APD via differential phosphorylation and dephosphorylation kinetics of cellular PKA targets (mainly I_{CaL} and I_{Ks}) (Pueyo et al., 2016b; Sampedro-Puente et al., 2019). For nSD-APD, the reduction induced by beta-blockade is justified by the fact that beta-adrenergic stimulation modulates, on the one hand, the LF oscillations of APD and, on the other hand, the stochastic gating of ionic currents active during the repolarization phase (Sampedro-Puente et al., 2019).

Mechanoelectric feedback (MEF) has been suggested to contribute to the development of LF oscillations and beat-to-beat variability of APD in humans in vivo [Hanson et al, 2014] and by computational simulation, these adrenergic and mechanical actions have been shown to synergistically potentiate the oscillatory behavior and temporal variability of cellular ventricular repolarization (Pueyo et al., 2016b; Sampedro-Puente et al., 2019), in accord with the well-known potentiation of MEF effects by beta-adrenergic stimulation (Horner et al., 1996; Puglisi et al., 2013). The role of MEF, possibly through stretch-activated channels, in contributing to beat-to-beat variability of ventricular repolarization is supported by experimental evidence in the chronic atrioventricular-block dog model, where beat-to-beat preload changes have been shown to increase short-term variability of monophasic APD (Stams et al., 2016).

6.4.1 Limitations

The study population were patients with ostensibly normal ventricles undergoing routine ablation procedures for supraventricular arrhythmias. 8 of the 12 patients had persistent atrial fibrillation and therefore the possibility of some ventricular remodelling cannot be excluded. Recordings were made from 20 localised right and left ventricular endocardial sites and it is possible that other regions may have yielded different results.

6.4.2 Clinical implications

Understanding the mechanisms underlying the interaction between beta-adrenergic stimulation, the LF oscillatory behaviour of APD and beat-to-beat APD variability is important for the development of therapeutic strategies for the prevention of arrhythmia and sudden cardiac death. Enhanced oscillations of ventricular repolarization in the LF range measured from the ECG T-wave vector and referred to as periodic repolarization

dynamics (PRD) have emerged as one of the strongest predictors of arrhythmia and sudden cardiac death in cardiac patients and are the subject of ongoing clinical trials (Rizas et al., 2014, 2016, 2017, 2019; Hamm et al., 2017). The present work identifies several specific features of the interaction between beta-adrenergic stimulation, the LF oscillatory behaviour of APD and beat-to-beat APD variability that are reproducible by computational modelling which enables mechanistic insight to be gained at the cellular level.

6.5 Conclusions

In patients with normal ventricles acute beta-adrenergic blockade modulated LF oscillatory behaviour of ventricular APD (measured as ARIs) and beat-to-beat variability of APD in a manner that was dependent on initial APD values. A strong correlation was present between the effect of beta-adrenergic stimulation on LF oscillation of APD and beat-to-beat variability of APD. These findings are discussed in relation to computational modelling which reproduced the clinical findings and investigated cellular mechanisms. These observations provide valuable insight into the strong association of LF oscillations of ventricular repolarization and arrhythmic and sudden cardiac death. Further work is warranted to improve our understanding in order to develop therapeutic strategies.

Chapter 7: Conclusion

The overall aim of this thesis was to explore in vivo human repolarization at the level of the ventricular APD. The main objectives were:

1) To explore the association between increased repolarization instability and increased risk of ventricular tachyarrhythmia at the level of the ventricular APD.

2) To compare the behaviour of the ventricular APD with body surface repolarization and in doing so explore the limitations of repolarization instability as a marker of arrhythmic risk.

3) To demonstrate the influence of an autonomic stimulus on the beat-to-beat variability of ventricular APD.

4) To assess the potential beneficial action of beta-adrenergic blockade in the stabilization of beat-to-beat variability of repolarization at the level of ventricular APD.

7.1 Original contributions

1) Whilst numerous studies exist looking at various metrics of repolarization, Chapter 2 represents the first demonstration of the association between increased LV beat-to-beat variability of APD and increased incidence of ventricular tachyarrhythmia in patients with heart failure. These findings are supportive of the possible utility of beat-to-beat variability of repolarization as an adjunct to risk stratification.

2) In Chapter 3 the temporal behaviour of body surface ECG QT intervals is shown to correlate significantly with that of the temporal behaviour of RV and LV APD. Whilst correlations were significant they were not perfect. These results highlight that the QT interval is a strong marker of both LV and RV repolarization. However, as enhanced dispersion of repolarization is mechanistic in arrhythmogenesis these results likely highlight the accepted flaws in the body surface QT interval. The body surface QT interval is representative of global repolarization and subtle regional inhomogeneity of repolarization would be missed by QT interval assessment alone.

3) In Chapter 4 an autonomic stimulus (Valsalva) produced increases in beat-to-beat variability of ventricular APD in patients with heart failure. This was associated with increases in haemodynamic indices supportive of increased sympathetic activity. These findings provide insight into the mechanisms behind the interplay of the autonomic nervous system with the genesis of ventricular tachyarrhythmia.

4) In Chapter 5 the oscillatory behaviour of ventricular APD following an autonomic stimulus was investigated. These findings show for the first time how the low frequency oscillations of APD increase in response to an autonomic challenge. Furthermore, the individual increase in low frequency oscillation of APD following the stimulus correlated significantly with the witnessed increase in beat-to-beat variability of APD. These observations provide insight at the level of the ventricular APD into mechanisms underlying low frequency oscillation of repolarization which is known to be strongly predictive of sudden cardiac death.

5) Chapters 4 and 6 explore the impact of beta-adrenergic blockade on APD variability. Chapter 4 shows for the first time the ability of beta-adrenergic blockade to suppress

the resultant increase in beat-to-beat variability of APD following an autonomic challenge. Chapter 6 presents the impact of beta-adrenergic blockade on low frequency oscillatory behaviour of APD during resting conditions. It demonstrates for the first time the ability of beta-adrenergic blockade to suppress the low frequency oscillatory behaviour of APD and again how this reduction in low frequency oscillation correlates with the reduction in beat-to-beat variability of APD.

7.2 Future work

Risk stratification of patients at high risk of sudden cardiac death remains a major challenge and it is unlikely that one single test will ever prove 100% sensitive. Chapter 2 demonstrates a novel risk marker in the prediction of spontaneous ventricular tachyarrhythmia, however, Chapter 3 highlights inhomogeneity of behaviour between regional local APD and also between local APD and a global measure of repolarization. Prospective studies incorporating global and regional repolarization variability may help improve the sensitivity and specificity of these markers by determining an individual's propensity to increased dispersion of repolarization.

Increases of beat-to-beat variability of APD prior to the onset of ventricular tachyarrhythmia have been demonstrated in animals (Wijers et al., 2018). This work highlights the potential for real-time analysis of beat-to-beat variability of ARI using implanted cardiac devices with pre-emptive delivery of overdrive pacing to stabilize the electrophysiology during times of increased repolarization instability. However, these findings remain to be demonstrated in humans. Further work should investigate the temporal behavior of APD variability prior to the onset of ventricular tachyarrhythmia in

man. Given the findings of Chapter 3, this work should incorporate regional and global measures of repolarization instability.

Chapters 4 and 6 demonstrate the benefit of beta-adrenergic blockade during times of rest and times of autonomic stress. However, these studies were unable to assess various forms of beta-blockade or investigate for a dose-response relationship which may help in the direction of therapeutic targets in clinical practice. A larger clinical trial should be conducted to study this.

Studies have previously highlighted shortening of basic APD in response to favorable reverse remodeling in patients with CRT. The impact of ventricular remodeling and reverse remodeling on beat-to-beat variability of APD requires further investigation. A prospective study incorporating recordings of APD variability before CRT implant and again during assessment of response to CRT should be conducted. These findings could have many implications including an understanding of possible further advantages or disadvantages to CRT.

1st Author publications, arising from thesis work

1) **Porter, B.** et al. Autonomic modulation in patients with heart failure increases beat-to-beat variability of ventricular action potential duration. Front. Physiol. 8, (2017).

2) **Porter, B.** et al. Beat-to-Beat Variability of Ventricular Action Potential Duration Oscillates at Low Frequency During Sympathetic Provocation in Humans. Front. Physiol. 9, 12–77 (2018).

3) **Porter, B.** et al. Left ventricular activation-recovery interval variability predicts spontaneous ventricular tachyarrhythmia in heart failure patients. Heart Rhythm (2018).

1st Author conference presentations, arising from thesis work

1) **Porter, B.** et al. Ventricular action potential duration variability is enhanced in heart failure patients with spontaneous ventricular tachycardia or fibrillation. EHRA-Congress, Barcelona, Spain. 2018

2) **Porter, B.** et al. Prediction Of Ventricular Tachyarrhythmias By Intracardiac Activation Recovery Interval Variability. Heart Rhythm Sessions, Boston, USA. 2018

3) **Porter, B.** et al. Myocardial fibrosis in non-ischaemic dilated cardiomyopathy is associated with increased activation recovery interval variability. EHRA-Congress, Lisbon, Portugal. 2019

4) **Porter, B.** et al. Presence of myocardial fibrosis is associated with increased activation-recovery interval variability. Heart Rhythm Sessions, San Francisco, USA. 2019

Bibliography

Abi-Gerges, N., Valentin, J.P., and Pollard, C.E. 2010. "Dog Left Ventricular Midmyocardial Myocytes for Assessment of Drug-Induced Delayed Repolarization: Short-Term Variability and Proarrhythmic Potential." *British Journal of Pharmacology* 159 (1): 77–92.

Abisse, S.S., Lampert, R., Burg, M., Soufer, R., and Shusterman, V. 2011. "Cardiac Repolarization Instability during Psychological Stress in Patients with Ventricular Arrhythmias." *Journal of Electrocardiology* 44 (6): 678–83.

Akaike, H. 1974. "A New Look at the Statistical Model Identification." *IEEE Transactions on Automatic Control* 19 (6): 716–23.

Amos, G.J., Wettwer, E., Metzger, F., Li, Q., Himmel, H.M., and Ravens, U. 1996. "Differences between Outward Currents of Human Atrial and Subepicardial Ventricular Myocytes." *The Journal of Physiology* 491 (Pt 1 (Pt 1): 31–50.

Andrews, C.M., Srinivasan, N.T., Rosmini, S., Bulluck, H., Orini, M., Jenkins, S., Pantazis, A., et al. 2017. "Electrical and Structural Substrate of Arrhythmogenic Right Ventricular Cardiomyopathy Determined Using Noninvasive Electrocardiographic Imaging and Late Gadolinium Magnetic Resonance Imaging." *Circulation: Arrhythmia and Electrophysiology* 10 (7).

Atiga, W.L., Calkins, H., Lawrence, J.H., Tomaselli, G.F., Smith, J.M., and Berger, R.D. 1998. "Beat-to-Beat Repolarization Lability Identifies Patients at Risk for Sudden Cardiac Death." *Journal of Cardiovascular Electrophysiology* 9 (9): 899–908.

Bardy, G.H., Lee, K.L., Mark, D.B., Poole, J.E., Packer, D.L., Boineau, R., Domanski, M., et al. 2005. "Amiodarone or an Implantable Cardioverter-Defibrillator for Congestive Heart Failure." *The New England Journal of Medicine* 352 (3): 225–37.

Baumert, M., Porta, A., Vos, M.A., Malik, M., Couderc, J.P., Laguna, P., Piccirillo, G., Smith, G.L., Tereshchenko, L.G., and Volders, P.G.A. 2016. "QT Interval Variability in Body Surface ECG: Measurement, Physiological Basis, and Clinical Value: Position Statement and Consensus Guidance Endorsed by the European Heart Rhythm Association Jointly with the ESC Working Group on Cardiac Cellular Electroph." *Europace* 18 (6): 925–44.

Baumert, M., Starc, V., and Porta, A. 2012. "Conventional QT Variability Measurement vs. Template Matching Techniques: Comparison of Performance Using Simulated and Real ECG." Edited by Natasha M Maurits. *PLoS ONE* 7 (7): e41920.

Bezzina, C.R., Jong, J.S.S.G. de, Tanck, M.W.T., Scicluna, B.P., Roden, D.M., Dekker, L.R.C., Bardai, A., et al. 2010. "Genome-Wide Association Study Identifies a Susceptibility Locus at 21q21 for Ventricular Fibrillation in Acute Myocardial Infarction." *Nature Genetics* 42 (8): 688–91.

Booth, R.W., Ryan, J.M., Mellett, H.C., Swiss, E., and Neth, E. 1962. "Hemodynamic Changes Associated with the Valsalva Maneuver in Normal Men and Women." *The Journal of Laboratory and Clinical Medicine* 59 (2): 275–85.

Boyett, M.R., and Jewell, B.R. 1978. "A Study of the Factors Responsible for Rate-Dependent Shortening of the Action Potential in Mammalian Ventricular Muscle." *The Journal of Physiology* 285 (December): 359–80.

Bueno-Orovio, A., Hanson, B.M., Gill, J.S., Taggart, P., and Rodriguez, B. 2012. "In Vivo Human Left-to-Right Ventricular Differences in Rate Adaptation Transiently Increase Pro-Arrhythmic Risk Following Rate Acceleration." Edited by Rajesh Gopalrao Katare. *PLoS ONE* 7 (12): e52234-12.

Bueno-Orovio, A., Hanson, B.M., Gill, J.S., Taggart, P., and Rodriguez, B. 2014. "Slow Adaptation of Ventricular Repolarization as a Cause of Arrhythmia?" *Methods of Information in Medicine* 53 (04): 320–23.

Burdon-Sanderson, J., and Page, F.J.M. 1882. "On the Time-Relations of the Excitatory Process in the Ventricle of the Heart of the Frog." *Journal of Physiology* 2 (5–6): 385–412.

Buxton, A E, Lee, K.L., Fisher, J.D., Josephson, M.E., Prystowsky, E.N., and Hafley, G. 1999. "A Randomized Study of the Prevention of Sudden Death in Patients with Coronary Artery Disease." *New England Journal of Medicine* 341 (25): 1882–90.

Buxton, Alfred E, Lee, K.L., DiCarlo, L., Gold, M.R., Greer, G.S., Prystowsky, E.N., O'Toole, M.F., et al. 2000. "Electrophysiologic Testing to Identify Patients with Coronary Artery Disease Who Are at Risk for Sudden Death." *The New England Journal of Medicine* 342 (26): 1937–45.

Cabasson, A., Meste, O., and Vesin, J.M. 2012. "Estimation and Modeling of QT-Interval Adaptation to Heart Rate Changes." *IEEE Transactions on Biomedical Engineering* 59 (4): 956–65.

Carmeliet, E. 2017. "Cardiac Ionic Currents and Acute Ischemia: From Channels to Arrhythmias." *Physiological Reviews* 79 (3): 917–1017.

Chen, Z., Hanson, B., Sohal, M., Sammut, E., Child, N., Shetty, A., Boucher, R., et al. 2013. “Left Ventricular Epicardial Electrograms Show Divergent Changes in Action Potential Duration in Responders and Nonresponders to Cardiac Resynchronization Therapy.” *Circulation: Arrhythmia and Electrophysiology* 6 (2): 265–71.

Chen, Z., Hanson, B., Sohal, M., Sammut, E., Jackson, T., Child, N., Claridge, S., et al. 2016. “Coupling of Ventricular Action Potential Duration and Local Strain Patterns during Reverse Remodeling in Responders and Nonresponders to Cardiac Resynchronization Therapy.” *Heart Rhythm* 13 (9): 1898–1904.

Chien Suu, K., and Surawicz, B. 1976. “Ventricular Monophasic Action Potential Changes Associated with Neurogenic T Wave Abnormalities and Isoproterenol Administration in Dogs.” *The American Journal of Cardiology* 38 (2): 170–77.

Cho, H., Barth, A.S., and Tomaselli, G.F. 2012. “Basic Science of Cardiac Resynchronization Therapy: Molecular and Electrophysiological Mechanisms.” *Circulation: Arrhythmia and Electrophysiology* 5 (3): 594–603.

Coote, J.H. 2001. “Multiple Oscillators in Autonomic Control.” *Journal of Physiology* 533 (2): 313.

Coronel, R., Bakker, J.M.T. de, Wilms-Schopman, F.J.G., Opthof, T., Linnenbank, A.C., Belterman, C.N., and Janse, M.J. 2006. “Monophasic Action Potentials and Activation Recovery Intervals as Measures of Ventricular Action Potential Duration: Experimental Evidence to Resolve Some Controversies.” *Heart Rhythm* 3 (9): 1043–50.

Cranefield, P.F. 1977. “Action Potentials, Afterpotentials, and Arrhythmias.” *Circulation*

Research.

Cupples, L.A., Hofman, A., Chugh, S.S., Ehret, G., Witteman, J.C.M., Kao, W.H.L., Bardai, A., et al. 2011. "Identification of a Sudden Cardiac Death Susceptibility Locus at 2q24.2 through Genome-Wide Association in European Ancestry Individuals." Edited by Mark I McCarthy. *PLoS Genetics* 7 (6): e1002158.

Dagres, N., and Hindricks, G. 2013. "Risk Stratification after Myocardial Infarction: Is Left Ventricular Ejection Fraction Enough to Prevent Sudden Cardiac Death?" *European Heart Journal* 34 (26): 1964–71.

Dekker, L.R.C., Bezzina, C.R., Henriques, J.P.S., Tanck, M.W., Koch, K.T., Alings, M.W., Arnold, A.E.R., et al. 2006. "Familial Sudden Death Is an Important Risk Factor for Primary Ventricular Fibrillation." *Circulation* 114 (11): 1140–45.

Desai, N., Raghunandan, D.S., Mallavarapu, M., Berger, R.D., and Yeragani, V.K. 2004. "Beat-to-Beat Heart Rate and QT Variability in Patients with Congestive Cardiac Failure: Blunted Response to Orthostatic Challenge." *Annals of Noninvasive Electrocardiology : The Official Journal of the International Society for Holter and Noninvasive Electrocardiology, Inc* 9 (4): 323–29.

Diego, J.M. Di, and Antzelevitch, C. 1993. "Pinacidil-Induced Electrical Heterogeneity and Extrasystolic Activity in Canine Ventricular Tissues Does Activation of Atp-Regulated Potassium Current Promote Phase 2 Reentry?" *Circulation* 88 (3): 1177–89.

Dobson, C.P., Rovere, M.T. La, Olsen, C., Berardinangeli, M., Veniani, M., Midi, P., Tavazzi, L., and Haigney, M. 2009. "24-Hour QT Variability in Heart Failure."

Journal of Electrocardiology 42 (6): 500–504.

Doytchinova, A., Hassel, J.L., Yuan, Y., Lin, H., Yin, D., Adams, D., Straka, S., et al. 2017. "Simultaneous Noninvasive Recording of Skin Sympathetic Nerve Activity and Electrocardiogram." *Heart Rhythm* 14 (1): 25–33.

Duijvenboden, S. van, Hanson, B., Child, N., Orini, M., Rinaldi, C.A., Gill, J.S., and Taggart, P. 2015. "Effect of Autonomic Blocking Agents on the Respiratory-Related Oscillations of Ventricular Action Potential Duration in Humans." *American Journal of Physiology - Heart and Circulatory Physiology* 309 (12): H2108–17.

Duncan, E., Thomas, G., Johns, N., Pfeffer, C., Appanna, G., Shah, N., Hunter, R., Finlay, M., Schilling, R.J., and Sporton, S. 2010. "Do Traditional VT Zones Improve Outcome in Primary Prevention ICD Patients?" *Pacing and Clinical Electrophysiology : PACE* 33 (11): 1353–58.

Ellis, W.S., Eisenberg, S.J., Auslander, D.M., Dae, M.W., Zakhor, A., and Lesh, M.D. 1996. "Deconvolution: A Novel Signal Processing Approach for Determining Activation Time from Fractionated Electrograms and Detecting Infarcted Tissue." *Circulation* 94 (10): 2633–40.

Ellison, K.E., Hafley, G.E., Hickey, K., Kellen, J., Coromilas, J., Stein, K.M., Lee, K.L., and Buxton, A.E. 2002. "Effect of β-Blocking Therapy on Outcome in the Multicenter Unsustained Tachycardia Trial (MUSTT)." *Circulation* 106 (21): 2694–99.

Felker, G.M., Cuculich, P.S., and Gheorghiade, M. 2006. "The Valsalva Maneuver: A Bedside 'Biomarker' for Heart Failure." *American Journal of Medicine* 119 (2):

117–22.

Franz, M.R., Swerdlow, C.D., Liem, L.B., and Schaefer, J. 1988. “Cycle Length Dependence of Human Action Potential Duration In Vivo and Different Steady-State Frequencies.” *J. Clin. Invest,* 82 (September): 972–79.

Friedlander, Y., Siscovick, D.S., Weinmann, S., Austin, M.A., Psaty, B.M., Lemaitre, R.N., Arbogast, P., Raghunathan, T.E., and Cobb, L.A. 1998. “Family History as a Risk Factor for Primary Cardiac Arrest.” *Circulation* 97 (2): 155–60.

Furlan, R., Porta, A., Costa, F., Tank, J., Baker, L., Schiavi, R., Robertson, D., Malliani, A., and Mosqueda-Garcia, R. 2000. “Oscillatory Patterns in Sympathetic Neural Discharge and Cardiovascular Variables during Orthostatic Stimulus.” *Circulation* 101 (8): 886–92.

Furukawa, Y., Shimizu, H., Hiromoto, K., Kanemori, T., Masuyama, T., and Ohyanagi, M. 2006. “Circadian Variation of Beat-to-Beat QT Interval Variability in Patients with Prior Myocardial Infarction and the Effect of β-Blocker Therapy.” *PACE - Pacing and Clinical Electrophysiology* 29 (5): 479–86.

Gallacher, D.J., Water, A. Van de, Linde, H. van der, Hermans, A.N., Lu, H.R., Towart, R., and Volders, P.G.A. 2007. “In Vivo Mechanisms Precipitating Torsades de Pointes in a Canine Model of Drug-Induced Long-QT1 Syndrome.” *Cardiovascular Research* 76 (2): 247–56.

Gerstner, W., Kreiter, A.K., Markram, H., and Herz, A.V.M. 2002. “Neural Codes: Firing Rates and Beyond.” *Proceedings of the National Academy of Sciences* 94 (24): 12740–41.

Goldberger, J., Basu, A., Boineau, R., Buxton, A.E., Cain, M.E., Canty, J.M., Chen, P.-S., et al. 2014. "Risk Stratification for Sudden Cardiac Death: A Plan for the Future." *Circulation* 129 (4): 516–26.

Graham, I., Ata, D., Borch-Johnsen, K., Boysen, G., Burell, G., Cifkova, R., Dallongeville, J., et al. 2017. "European Guidelines on Cardiovascular Disease Prevention in Clinical Practice (Ending). Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice." *Rational Pharmacotherapy in Cardiology*. School of Health and Caring Sciences, Linnaeus University, Stagneliusgatan 14, Kalmar, Sweden. joep.perk@lnu.se.

Grant, A.O. 2009. "Cardiac Ion Channels." *Circulation. Arrhythmia and Electrophysiology* 2 (2): 185–94.

Guduru, A., Lansdown, J., Chernichenko, D., Berger, R.D., and Tereshchenko, L.G. 2013. "Longitudinal Changes in Intracardiac Repolarization Lability in Patients with Implantable Cardioverter-Defibrillator." *Frontiers in Physiology* 4 AUG (August): 1–10.

Guinamard, R., Chatelier, A., Demion, M., Potreau, D., Patri, S., Rahmati, M., and Bois, P. 2004. "Functional Characterization of a Ca2+-Activated Non-Selective Cation Channel in Human Atrial Cardiomyocytes." *Journal of Physiology* 558 (1): 75–83.

Haigney, M.C., Zareba, W., Gentlesk, P.J., Goldstein, R.E., Illovsky, M., McNitt, S., Andrews, M.L., and Moss, A.J. 2004. "QT Interval Variability and Spontaneous Ventricular Tachycardia or Fibrillation in the Multicenter Automatic Defibrillator Implantation Trial (MADIT) II Patients." *Journal of the American College of*

Cardiology 44 (7): 1481–87.

Hamm, W., Rizas, K.D., Stülpnagel, L. von, Vdovin, N., Massberg, S., Kääb, S., and Bauer, A. 2017. "Implantable Cardiac Monitors in High-Risk Post-Infarction Patients with Cardiac Autonomic Dysfunction and Moderately Reduced Left Ventricular Ejection Fraction: Design and Rationale of the SMART-MI Trial." *American Heart Journal* 190 (August): 34–39.

Hanson, B., Child, N., Duijvenboden, S. Van, Orini, M., Chen, Z., Coronel, R., Rinaldi, C.A., Gill, J.S., Gill, J.S., and Taggart, P. 2014. "Oscillatory Behavior of Ventricular Action Potential Duration in Heart Failure Patients at Respiratory Rate and Low Frequency." *Frontiers in Physiology* 5 (379): 191.

Hanson, B., Gill, J., Western, D., Gilbey, M.P., Bostock, J., Boyett, M.R., Zhang, H., Coronel, R., and Taggart, P. 2012. "Cyclical Modulation of Human Ventricular Repolarization by Respiration." *Frontiers in Physiology* 3 SEP (January): 379.

Hanson, B., Sutton, P., Elameri, N., Gray, M., Critchley, H., Gill, J.S., and Taggart, P. 2009. "Interaction of Activation-Repolarization Coupling and Restitution Properties in Humans." *Circulation. Arrhythmia and Electrophysiology* 2 (2): 162–70.

Haws, C.W., and Lux, R.L. 1990. "Correlation between in Vivo Transmembrane Action Potential Durations and Activation-Recovery Intervals from Electrograms. Effects of Interventions That Alter Repolarization Time." *Circulation* 81 (1): 281–88.

Heijman, J., Zaza, A., Johnson, D.M., Rudy, Y., Peeters, R.L.M., Volders, P.G.A., and Westra, R.L. 2013. "Determinants of Beat-to-Beat Variability of Repolarization Duration in the Canine Ventricular Myocyte: A Computational Analysis." Edited by

Andrew D McCulloch. *PLoS Computational Biology* 9 (8): e1003202.

Hinterseer, M., Beckmann, B.M., Thomsen, M.B., Pfeufer, A., Ulbrich, M., Sinner, M.F., Perz, S., et al. 2010. "Usefulness of Short-Term Variability of QT Intervals as a Predictor for Electrical Remodeling and Proarrhythmia in Patients with Nonischemic Heart Failure." *American Journal of Cardiology*.

Hintsa, T., Puttonen, S., Toivonen, L., Kontula, K., Swan, H., and Keltikangas-Järvinen, L. 2010. "A History of Stressful Life Events, Prolonged Mental Stress and Arrhythmic Events in Inherited Long QT Syndrome." *Heart* 96 (16): 1281–86.

Hjalmarson, Å., Goldstein, S., Fagerberg, B., Wedel, H., Waagstein, F., Kjekshus, J., Wikstrand, J., et al. 2000. "Effects of Controlled-Release Metoprolol on Total Mortality, Hospitalizations, and Well-Being in Patients with Heart Failure: The Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure (MERIT-HF)." *Journal of the American Medical Association* 283 (10): 1295–1302.

Hodgkin, A.L., and Huxley, A.F. 1990. "A Quantitative Description of Membrane Current and Its Application to Conduction and Excitation in Nerve." *Bulletin of Mathematical Biology* 52 (1–2): 25–71.

Horner, S.M., Murphy, C.F., Coen, B., Dick, D.J., Harrison, F.G., Vespalcova, Z., and Lab, M.J. 1996. "Contribution to Heart Rate Variability by Mechanoelectric Feedback: Stretch of the Sinoatrial Node Reduces Heart Rate Variability." *Circulation*.

Huang, J.L., Chiou, C.W., Ting, C.T., Chen, Y.T., and Chen, S.A. 2001. "Sudden Changes in Heart Rate Variability during the 1999 Taiwan Earthquake." *American*

Journal of Cardiology 87 (2): 245–48.

Imholz, B.P.M., Montfrans, G.A.V., Settels, J.J., Hoeven, G.M.A.V. Der, Karemaker, J.M., and Wieling, W. 1988. "Continuous Non-Invasive Blood Pressure Monitoring: Reliability of Finapres Device during the Valsalva Manoeuvre." *Cardiovascular Research* 22 (6): 390–97.

Investigators, A. versus I.D. (AVID). 1997. "A Comparison of Antiarrhythmic-Drug Therapy with Implantable Defibrillators in Patients Resuscitated from near-Fatal Ventricular Arrhythmias." *The New England Journal of Medicine* 337 (22): 1576–83.

Investigators, C.A.S.T. (CAST). 1989. "Preliminary Report: Effect of Encainide and Flecainide on Mortality in a Randomized Trial of Arrhythmia Suppression after Myocardial Infarction." *The New England Journal of Medicine* 321 (6): 406–12.

Jacobson, I., Carlsson, L., and Duker, G. 2011. "Beat-by-Beat QT Interval Variability, but Not QT Prolongation per Se, Predicts Drug-Induced Torsades de Pointes in the Anaesthetised Methoxamine-Sensitized Rabbit." *Journal of Pharmacological and Toxicological Methods* 63 (1): 40–46.

Janse, M.J., and Wit, A.L. 1989. "Electrophysiological Mechanisms of Ventricular Arrhythmias Resulting from Myocardial Ischemia and Infarction." *Physiological Reviews* 69 (4): 1049–1169.

January, C.T., Chau, V., and Makielski, J.C. 2012. "Triggered Activity in the Heart: Cellular Mechanisms of Early after-Depolarizations." *European Heart Journal* 12 (suppl F): 4–9.

January, C.T., and Riddle, J.M. 1989. “Early Afterdepolarizations: Mechanism of Induction and Block. A Role for L-Type Ca2+ Current.” *Circulation Research* 64 (5): 977–90.

Jiang, Z., Zhao, Y., Doytchinova, A., Kamp, N.J., Tsai, W.C., Yuan, Y., Adams, D., et al. 2015. “Using Skin Sympathetic Nerve Activity to Estimate Stellate Ganglion Nerve Activity in Dogs.” *Heart Rhythm* 12 (6): 1324–32.

Johnson, D.M., Heijman, J., Bode, E.F., Greensmith, D.J., Linde, H. van der, Abi-Gerges, N., Eisner, D.A., Trafford, A.W., and Volders, P.G.A. 2013. “Diastolic Spontaneous Calcium Release From the Sarcoplasmic Reticulum Increases Beat-to-Beat Variability of Repolarization in Canine Ventricular Myocytes After -Adrenergic Stimulation.” *Circulation Research* 112 (2): 246–56.

Jouven, X., Desnos, M., Guerot, C., and Ducimetière, P. 1999. “Predicting Sudden Death in the Population: The Paris Prospective Study I.” *Circulation* 99 (15): 1978–83.

Julien, C. 2006. “The Enigma of Mayer Waves: Facts and Models.” *Cardiovascular Research* 70 (1): 12–21.

Kaikkonen, K.S., Kortelainen, M.L., Linna, E., and Huikuri, H. V. 2006. “Family History and the Risk of Sudden Cardiac Death as a Manifestation of an Acute Coronary Event.” *Circulation* 114 (14): 1462–67.

Kay, S.M. 1999. *No Title*. *Theory and Application*. Prentice-Hall PTR.

Kim, J.J., Němec, J., Papp, R., Strongin, R., Abramson, J.J., and Salama, G. 2013. “ Bradycardia Alters Ca 2+ Dynamics Enhancing Dispersion of Repolarization and Arrhythmia Risk .” *American Journal of Physiology-Heart and Circulatory*

Physiology 304 (6): H848–60.

Kirk, J.A., and Kass, D.A. 2013. "Electromechanical Dyssynchrony and Resynchronization of the Failing Heart." *Circulation Research* 113 (6): 765–76.

Køber, L., Thune, J.J., Nielsen, J.C., Haarbo, J., Videbæk, L., Korup, E., Jensen, G., et al. 2016. "Defibrillator Implantation in Patients with Nonischemic Systolic Heart Failure." *New England Journal of Medicine* 375 (13): 1221–30.

Kohl, P. 1998. "Cellular Mechanisms of Cardiac Mechano-Electric Feedback." *The Canadian Journal of Cardiology* 14 (1): 1–10.

Korner, P.I., Tonkin, A.M., and Uther, J.B. 1976. "Reflex and Mechanical Circulatory Effects of Graded Valsalva Maneuvers in Normal Man." *Journal of Applied Physiology* 40 (3): 434–40.

Krishnan, S.C., and Antzelevitch, C. 1993. "Flecainide-Induced Arrhythmia in Canine Ventricular Epicardium: Phase 2 Reentry?" *Circulation* 87 (2): 562–72.

Kunze, D.L. 2004. "Cardiac Na Currents and the Inactivating, Reopening, and Waiting Properties of Single Cardiac Na Channels." *The Journal of General Physiology* 86 (5): 691–719.

Lampert, R., Jain, D., Burg, M.M., Batsford, W.P., and McPherson, C.A. 2000. "Destabilizing Effects of Mental Stress on Ventricular Arrhythmias in Patients with Implantable Cardioverter-Defibrillators." *Circulation* 101 (2): 158–64.

Lampert, R., Joska, T., Burg, M.M., Batsford, W.P., McPherson, C.A., and Jain, D. 2002. "Emotional and Physical Precipitants of Ventricular Arrhythmia." *Circulation* 106

(14): 1800–1805.

Lampert, R., Shusterman, V., Burg, M., McPherson, C., Batsford, W., Goldberg, A., and Soufer, R. 2009. "Anger-Induced T-Wave Alternans Predicts Future Ventricular Arrhythmias in Patients With Implantable Cardioverter-Defibrillators." *Journal of the American College of Cardiology* 53 (9): 774–78.

Lampert, R., Shusterman, V., Burg, M.M., Lee, F.A., Earley, C., Goldberg, A., Mcpherson, C.A., Batsford, W.P., and Soufer, R. 2005. "Effects of Psychologic Stress on Repolarization and Relationship to Autonomic and Hemodynamic Factors." *Journal of Cardiovascular Electrophysiology* 16 (4): 372–77.

Leopold, G., Ungethum, W., Pabst, J., Simane, Z., Buhring, K., and Wiemann, H. 1986. "Pharmacodynamic Profile of Bisoprolol, a New Beta 1-selective Adrenoceptor Antagonist." *British Journal of Clinical Pharmacology* 22 (3): 293–300.

Leor, J., Poole, W.K., and Kloner, R.A. 2002. "Sudden Cardiac Death Triggered by an Earthquake." *New England Journal of Medicine* 334 (7): 413–19.

Li, Q., O'Neill, S.C., Tao, T., Li, Y., Eisner, D., and Zhang, H. 2012. "Mechanisms by Which Cytoplasmic Calcium Wave Propagation and Alternans Are Generated in Cardiac Atrial Myocytes Lacking T-Tubules—Insights from a Simulation Study." *Biophysical Journal* 102 (7): 1471–82.

Liu, G.X., Choi, B.R., Ziv, O., Li, W., Lange, E. de, Qu, Z., and Koren, G. 2012. "Differential Conditions for Early After-Depolarizations and Triggered Activity in Cardiomyocytes Derived from Transgenic LQT1 and LQT2 Rabbits." *Journal of Physiology* 590 (5): 1171–80.

Lloyd-Jones, D.M., Wilson, P.W.F., Larson, M.G., Beiser, A., Leip, E.P., D'Agostino, R.B., and Levy, D. 2004. "Framingham Risk Score and Prediction of Lifetime Risk for Coronary Heart Disease." *The American Journal of Cardiology* 94 (1): 20–24.

Lukas, A., and Antzelevitch, C. 1996. "Phase 2 Reentry as a Mechanism of Initiation of Circus Movement Reentry in Canine Epicardium Exposed to Simulated Ischemia." *Cardiovascular Research* 32 (3): 593–603.

Magrí, D., Piccirillo, G., Quaglione, R., Dell'armi, A., Mitra, M., Velitti, S., Barba, D. Di, Lizio, A., Maisto, D., and Barillà, F. 2012. "Effect of Acute Mental Stress on Heart Rate and QT Variability in Postmyocardial Infarction Patients." *ISRN Cardiology* 2012 (5): 1–9.

Malik, M. 1996. "Heart Rate Variability. Standards of Measurement, Physiological Interpretation, and Clinical Use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology." *European Heart Journal*. American Heart Association, Inc.

Malik, M. 2008. "Beat-to-Beat QT Variability and Cardiac Autonomic Regulation." *American Journal of Physiology - Heart and Circulatory Physiology* 295 (3): H923–25.

Malliani, A., Pagani, M., Lombardi, F., and Cerutti, S. 1991. "Cardiovascular Neural Regulation Explored in the Frequency Domain." *Circulation* 84 (2): 482–92.

Mantravadi, R., Gabris, B., Liu, T., Choi, B.R., Groat, W.C. De, Ng, G.A., and Salama, G. 2007. "Autonomic Nerve Stimulation Reverses Ventricular Repolarization Sequence in Rabbit Hearts." *Circulation Research* 100 (7).

Maruyama, M., Lin, S.F., Xie, Y., Chua, S.K., Joung, B., Han, S., Shinohara, T., et al. 2011. "Genesis of Phase 3 Early Afterdepolarizations and Triggered Activity in Acquired Long-QT Syndrome." *Circulation: Arrhythmia and Electrophysiology* 4 (1): 103–11.

Meisel, S.R., Dayan, K.I., Pauzner, H., Chetboun, I., Arbel, Y., David, D., and Kutz, I. 1991. "Effect of Iraqi Missile War on Incidence of Acute Myocardial Infarction and Sudden Death in Israeli Civilians." *The Lancet* 338 (8768): 660–61.

Millar, C.K., Kralios, F.A., and Lux, R.L. 1985. "Correlation between Refractory Periods and Activation-Recovery Intervals from Electrograms: Effects of Rate and Adrenergic Interventions." *Circulation* 72 (6): 1372–79.

Mine, T., Shimizu, H., Hiromoto, K., Furukawa, Y., Kanemori, T., Nakamura, H., Masuyama, T., and Ohyanagi, M. 2008. "Beat-to-Beat QT Interval Variability Is Primarily Affected by the Autonomic Nervous System." *Annals of Noninvasive Electrocardiology* 13 (3): 228–33.

Mines, G.R. 1914. "On Circulating Excitations in Heart Muscles and Their Possible Relation to Tachycardia and Fibrillation." *Transactions of the Royal Society of Canada*.

Montano, N., Furlan, R., Guzzetti, S., Mcallen, R.M., and Julien, C. 2009. "Analysis of Sympathetic Neural Discharge in Rats and Humans." *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 367 (1892): 1265–82.

Moss, A.J, Hall, W.J., Cannom, D.S., Daubert, J.P., Higgins, S.L., Klein, H., Levine, J.H.,

et al. 1996. “Improved Survival with an Implanted Defibrillator in Patients with Coronary Disease at High Risk for Ventricular Arrhythmia. Multicenter Automatic Defibrillator Implantation Trial Investigators.” *The New England Journal of Medicine* 335 (26): 1933–40.

Moss, A.J, Zareba, W., Hall, W.J., Klein, H., Wilber, D.J., Cannom, D.S., Daubert, J.P., et al. 2002. “Prophylactic Implantation of a Defibrillator in Patients with Myocardial Infarction and Reduced Ejection Fraction.” *The New England Journal of Medicine* 346 (12): 877–83.

Myerburg, R.J., Kessler, K.M., and Castellanos, A. 1992. “Sudden Cardiac Death. Structure, Function, and Time-Dependence of Risk.” *Circulation* 85 (1 Suppl): I2-10.

Myers, R.W., Pearlman, A.S., Hyman, R.M., Goldstein, R.A., Kent, K.M., Goldstein, R.E., and Epstein, S.E. 1974. “Beneficial Effects of Vagal Stimulation and Bradycardia during Experimental Acute Myocardial Ischemia.” *Circulation* 49 (5): 943–47.

Nánási, P.P., Magyar, J., Varró, A., and Ördög, B. 2017. “Beat-to-Beat Variability of Cardiac Action Potential Duration: Underlying Mechanism and Clinical Implications.” *Canadian Journal of Physiology and Pharmacology* 95 (10): 1230–35.

Narayan, S.M., Bayer, J.D., Lalani, G., and Trayanova, N.A. 2008. “Action Potential Dynamics Explain Arrhythmic Vulnerability in Human Heart Failure: A Clinical and Modeling Study Implicating Abnormal Calcium Handling.” *Journal of the American College of Cardiology* 52 (22): 1782–92.

Nattel, S., Maguy, A., Bouter, S. Le, and Yeh, Y.-H. 2007. "Arrhythmogenic Ion-Channel Remodeling in the Heart: Heart Failure, Myocardial Infarction, and Atrial Fibrillation." *Physiological Reviews* 87 (2): 425–56.

Nayyar, S., Roberts-Thomson, K.C., Hasan, M.A., Sullivan, T., Harrington, J., Sanders, P., and Baumert, M. 2013. "Autonomic Modulation of Repolarization Instability in Patients with Heart Failure Prone to Ventricular Tachycardia." *American Journal of Physiology-Heart and Circulatory Physiology* 305 (8): H1181–88.

Negoescu, R., Dinca-Panattescu, S., Filcescu, V., Ionescu, D., and Wolf, S. 1997. "Mental Stress Enhances the Sympathetic Fraction of QT Variability in an RR-Independent Way." *Integrative Physiological and Behavioral Science* 32 (3): 220–27.

Negoescu, Radu, Skinner, J.E., and Wolf, S. 1993. "Forebrain Regulation of Cardiac Function Spectral and Dimensional Analysis of RR and QT Intervals." *Integrative Physiological and Behavioral Science* 28 (4): 331–42.

Ng, G.A., Brack, K.E., Patel, V.H., and Coote, J.H. 2007. "Autonomic Modulation of Electrical Restitution, Alternans and Ventricular Fibrillation Initiation in the Isolated Heart." *Cardiovascular Research* 73 (4): 750–60.

Noble, D. 1962. "A Modification of the Hodgkin--Huxley Equations Applicable to Purkinje Fibre Action and Pace-Maker Potentials." *The Journal of Physiology* 160 (2): 317–52.

Noble, Denis. 2007. "From the Hodgkin-Huxley Axon to the Virtual Heart." *Journal of Physiology*. Department of Physiology, Anatomy and Genetics, Parks Road, Oxford, OX1 3PT, UK. denis.noble@physiol.ox.ac.uk: John Wiley & Sons, Ltd (10.1111).

O'Hara, T., Virág, L., Varró, A., and Rudy, Y. 2011. "Simulation of the Undiseased Human Cardiac Ventricular Action Potential: Model Formulation and Experimental Validation." *PLoS Computational Biology*.

Oosterhoff, P., Tereshchenko, L.G., Heyden, M.A.G. Van Der, Ghanem, R.N., Fetics, B.J., Berger, R.D., and Vos, M.A. 2011. "Short-Term Variability of Repolarization Predicts Ventricular Tachycardia and Sudden Cardiac Death in Patients with Structural Heart Disease: A Comparison with QT Variability Index." *Heart Rhythm* 8 (10): 1584–90.

Pagani, M., Lombardi, F., Guzzetti, S., Rimoldi, O., Furlan, R., Pizzinelli, P., Sandrone, G., Malfatto, G., Dell'Orto, S., and Piccaluga, E. 1986. "Power Spectral Analysis of Heart Rate and Arterial Pressure Variabilities as a Marker of Sympatho-Vagal Interaction in Man and Conscious Dog." *Circulation Research* 59 (2): 178–93.

Pagani, M., Montano, N., Porta, A., Malliani, A., Abboud, F.M., Birkett, C., and Somers, V.K. 1997. "Relationship between Spectral Components of Cardiovascular Variabilities and Direct Measures of Muscle Sympathetic Nerve Activity in Humans." *Circulation* 95 (6): 1441–48.

Palamarchuk, I., Baker, J., and Kimpinski, K. 2016. "Non-Invasive Measurement of Adrenergic Baroreflex during Valsalva Maneuver Reveals Three Distinct Patterns in Healthy Subjects." *Clinical Neurophysiology* 127 (1): 858–63.

Papadakis, M., Sharma, S., Cox, S., Sheppard, M.N., Panoulas, V.F., and Behr, E.R. 2009. "The Magnitude of Sudden Cardiac Death in the Young: A Death Certificate-Based Review in England and Wales." *Europace*.

Parati, G., Mancia, G., Rienzo, M. Di, Castiglioni, P., Taylor, J., and Studinger, P. 2006. "Point : Counterpoint Point : Counterpoint : Cardiovascular Variability Is / Is Not an Index of Autonomic Control of Circulation." *J. Appl. Physiol.*

Parker, G. W., Michael, L.H., Hartley, C.J., Skinner, J.E., and Entman, M.L. 1990. "Central β-Adrenergic Mechanisms May Modulate Ischemic Ventricular Fibrillation in Pigs." *Circulation Research* 66 (2): 259–70.

Parker, Gerald W., Michael, L.H., and Entman, M.L. 1987. "An Animal Model to Examine the Response to Environmental Stress as a Factor in Sudden Cardiac Death." *The American Journal of Cardiology* 60 (18): J9.

Patterson, E., Lazzara, R., Szabo, B., Liu, H., Tang, D., Li, Y.H., Scherlag, B.J., and Po, S.S. 2006. "Sodium-Calcium Exchange Initiated by the Ca2+ Transient: An Arrhythmia Trigger within Pulmonary Veins." *Journal of the American College of Cardiology* 47 (6): 1196–1206.

Paz, O., Zhou, X., Gillberg, J., Tseng, H.J., Gang, E., and Swerdlow, C. 2006. "Detection of T-Wave Alternans Using an Implantable Cardioverter-Defibrillator." *Heart Rhythm* 3 (7): 791–97.

Piccirillo, G., Cacciafesta, M., Lionetti, M., Nocco, M., Giuseppe, V. Di, Moisè, A., Naso, C., and Marigliano, V. 2001. "Influence of Age, the Autonomic Nervous System and Anxiety on QT-Interval Variability." *Clinical Science (London, England : 1979)* 101 (4): 429–38.

Piccirillo, G., Magnanti, M., Matera, S., Carlo, S. Di, Laurentis, T. De, Torrini, A., Marchitto, N., Ricci, R., and Magrí, D. 2006. "Age and QT Variability Index during

Free Breathing, Controlled Breathing and Tilt in Patients with Chronic Heart Failure and Healthy Control Subjects." *Translational Research : The Journal of Laboratory and Clinical Medicine* 148 (2): 72–78.

Piccirillo, G., Moscucci, F., Pascucci, M., Antonella Pappadà, M., D'Alessandro, G., Rossi, P., Quaglione, R., Barba, D. Di, Barillà, F., and Magrì, D. 2013. "Influence of Aging and Chronic Heart Failure on Temporal Dispersion of Myocardial Repolarization." *Clinical Interventions in Aging* 8: 293–300.

Pond, A.L., and Nerbonne, J.M. 2001. "ERG Proteins and Functional Cardiac IKr Channels in Rat, Mouse, and Human Heart." *Trends in Cardiovascular Medicine*.

Porta, A., Bari, V., Badilini, F., Tobaldini, E., Gnecchi-Ruscone, T., and Montano, N. 2011. "Frequency Domain Assessment of the Coupling Strength between Ventricular Repolarization Duration and Heart Period during Graded Head-up Tilt." *Journal of Electrocardiology* 44 (6): 662–68.

Porta, A., Girardengo, G., Bari, V., George, A.L., Brink, P.A., Goosen, A., Crotti, L., and Schwartz, P.J. 2015. "Autonomic Control of Heart Rate and QT Interval Variability Influences Arrhythmic Risk in Long QT Syndrome Type 1." *Journal of the American College of Cardiology* 65 (4): 367–74.

Porter, B., Bishop, M.J., Claridge, S., Behar, J., Sieniewicz, B.J., Webb, J., Gould, J., et al. 2017. "Autonomic Modulation in Patients with Heart Failure Increases Beat-to-Beat Variability of Ventricular Action Potential Duration." *Frontiers in Physiology* 8 (MAY).

Porter, Bradley, Bishop, M.J., Claridge, S., Child, N., Duijvenboden, S. Van, Bostock, J.,

Sieniewicz, B.J., et al. 2018a. “Left Ventricular Activation-Recovery Interval Variability Predicts Spontaneous Ventricular Tachyarrhythmia in Heart Failure Patients.” *Heart Rhythm*, December.

Porter, Bradley, Duijvenboden, S. Van, Bishop, M.J., Orini, M., Claridge, S., Gould, J., Sieniewicz, B.J., et al. 2018b. “Beat-to-Beat Variability of Ventricular Action Potential Duration Oscillates at Low Frequency During Sympathetic Provocation in Humans.” *Frontiers in Physiology* 9 (April): 12–77.

Potse, M., Vinet, A., Opthof, T., and Coronel, R. 2009. “Validation of a Simple Model for the Morphology of the T Wave in Unipolar Electrograms.” *American Journal of Physiology. Heart and Circulatory Physiology* 297 (2): H792–801.

Priori, S.G., Blomström-Lundqvist, C., Mazzanti, A., Blom, N., Borggrefe, M., Camm, J., Elliott, P.M., et al. 2015. “2015 ESC Guidelines for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the Europe.” *European Heart Journal* 36 (41): 2793–2867.

Priori, S.G., Napolitano, C., Tiso, N., Memmi, M., Vignati, G., Bloise, R., Sorrentino, V., and Danieli, G.A. 2001. “Mutataions in the Cardiac Ryanodine Receptor Gene (HRyR2) Underlie Catecholaminergic Polymorphic Ventricular Tachycardia.” *Circulation* 103 (2): 196–200.

Pueyo, E., Dangerfield, C.E., Britton, O.J., Virág, L., Kistamás, K., Szentandrássy, N., Jost, N., et al. 2016a. “Experimentally-Based Computational Investigation into Beat-to-Beat Variability in Ventricular Repolarization and Its Response to Ionic Current

Inhibition." *PLoS ONE* 11 (3): 1–20.

Pueyo, Esther, Orini, M., Rodríguez, J.F., and Taggart, P. 2016b. "Interactive Effect of Beta-Adrenergic Stimulation and Mechanical Stretch on Low-Frequency Oscillations of Ventricular Action Potential Duration in Humans." *Journal of Molecular and Cellular Cardiology* 97 (August): 93–105.

Puglisi, J.L., Negroni, J.A., Chen-Izu, Y., and Bers, D.M. 2013. "The Force-Frequency Relationship: Insights from Mathematical Modeling." *Advances in Physiology Education*.

Quinn, T.A., and Kohl, P. 2016. "Rabbit Models of Cardiac Mechano-Electric and Mechano-Mechanical Coupling." *Progress in Biophysics and Molecular Biology* 121 (2): 110–22.

Rizas, K.D., Doller, A.J., Hamm, W., Vdovin, N., Stuelpnagel, L. von, Zuern, C.S., and Bauer, A. 2019. "Periodic Repolarization Dynamics as Risk Predictor after Myocardial Infarction: Prospective Validation Study." *Heart Rhythm*.

Rizas, K.D., Hamm, W., Kääb, S., Schmidt, G., and Bauer, A. 2016. "Periodic Repolarization Dynamics: A Natural Probe of the Ventricular Response to Sympathetic Activation." *Arrhythmia & Electrophysiology Review* 5 (1): 31.

Rizas, K.D., McNitt, S., Hamm, W., Massberg, S., Kääb, S., Zareba, W., Couderc, J.P., and Bauer, A. 2017. "Prediction of Sudden and Non-Sudden Cardiac Death in Post-Infarction Patients with Reduced Left Ventricular Ejection Fraction by Periodic Repolarization Dynamics: MADIT-II Substudy." *European Heart Journal* 38 (27): 2110–18.

Rizas, K.D., Nieminen, T., Barthel, P., Zürn, C.S., Kähönen, M., Viik, J., Lehtimäki, T., et al. 2014. "Sympathetic Activity – Associated Periodic Repolarization Dynamics Predict Mortality Following Myocardial Infarction." *Journal of Clinical Investigation* 124 (4): 1770–80.

Ruzsnavszky, F., Hegyi, B., Kistamás, K., Váczi, K., Horváth, B., Szentandrássy, N., Bányász, T., Nánási, P.P., and Magyar, J. 2014. "Asynchronous Activation of Calcium and Potassium Currents by Isoproterenol in Canine Ventricular Myocytes." *Naunyn-Schmiedeberg's Archives of Pharmacology* 387 (5): 457–67.

Sakhuja, R., Shah, A.J., Keebler, M., and Thakur, R.K. 2009. "Atrial Fibrillation in Patients with Implantable Defibrillators." *Cardiology Clinics* 27 (1): 151-61-ix–x.

Sampedro-Puente, D.A., Fernandez-Bes, J., Porter, B., Duijvenboden, S. van, Taggart, P., and Pueyo, E. 2019. "Mechanisms Underlying Interactions Between Low-Frequency Oscillations and Beat-to-Beat Variability of Celullar Ventricular Repolarization in Response to Sympathetic Stimulation: Implications for Arrhythmogenesis." *Frontiers in Physiology*.

Samuels, M.A. 1997. "'Voodoo' Death Revisited: The Modern Lessons of Neurocardiology." *Neurologist* 3 (5): 293–304.

Sandhu, R.K., Costantini, O., Cummings, J.E., Poelzing, S., Rosenbaum, D.S., and Quan, K.J. 2008. "Intracardiac Alternans Compared to Surface T-Wave Alternans as a Predictor of Ventricular Arrhythmias in Humans." *Heart Rhythm* 5 (7): 1003–8.

Schrezenmaier, C., Singer, W., Swift, N.M., Sletten, D., Tanabe, J., and Low, P.A. 2007. "Adrenergic and Vagal Baroreflex Sensitivity in Autonomic Failure." *Archives of*

Neurology 64 (3): 381–86.

Shen, M.J., and Zipes, D.P. 2014. "Role of the Autonomic Nervous System in Modulating Cardiac Arrhythmias." *Circulation Research* 114 (6): 1004–21.

Smith, S.A., Salih, M.M., and Littler, W.A. 1987. "Assessment of Beat to Beat Changes in Cardiac Output during the Valsalva Manoeuvre Using Electrical Bioimpedance Cardiography." *Clinical Science (London, England : 1979)* 72 (4): 423–28.

Średniawa, B., Kowalczyk, J., Lenarczyk, R., Kowalski, O., Sędkowska, A., Cebula, S., Musialik-Łydka, A., and Kalarus, Z. 2012. "Microvolt T-Wave Alternans and Other Noninvasive Predictors of Serious Arrhythmic Events in Patients with an Implanted Cardioverter-Defibrillator." *Kardiologia Polska* 70 (5): 447–55.

Stams, T.R.G., Oosterhoff, P., Heijdel, A., Dunnink, A., Beekman, J.D.M., Nagel, R. van der, Rijen, H.V.M. van, Heyden, M.A.G. van der, and Vos, M.A. 2016. "Beat-to-Beat Variability in Preload Unmasks Latent Risk of Torsade de Pointes in Anesthetized Chronic Atrioventricular Block Dogs." *Circulation Journal* 80 (6): 1336–45.

Steinberg, J.S., Arshad, A., Kowalski, M., Kukar, A., Suma, V., Vloka, M., Ehlert, F., et al. 2004. "Increased Incidence of Life-Threatening Ventricular Arrhythmias in Implantable Defibrillator Patients after the World Trade Center Attack." *Journal of the American College of Cardiology* 44 (6): 1261–64.

Szabo, B., Sweidan, R., Rajagopalan, C. V., and Lazzara, R. 1994. "Role of Na+:Ca2+ Exchange Current in Cs+-Induced Early Afterdepolarizations in Purkinje Fibers." *Journal of Cardiovascular Electrophysiology* 5 (11): 933–44.

Taggart, P., Sutton, P., Chalabi, Z., Boyett, M.R., Simon, R., Elliott, D., and Gill, J.S. 2003. “Effect of Adrenergic Stimulation on Action Potential Duration Restitution in Humans.” *Circulation* 107 (2): 285–89.

Tanskanen, A.J., Greenstein, J.L., O’Rourke, B., and Winslow, R.L. 2005. “The Role of Stochastic and Modal Gating of Cardiac L-Type Ca2+ Channels on Early after-Depolarizations.” *Biophysical Journal* 88 (1): 85–95.

Tereshchenko, L G, Fetics, B.J., Domitrovich, P.P., Lindsay, B.D., and Berger, R.D. 2009. “Prediction of Ventricular Tachyarrhythmias by Intracardiac Repolarization Variability Analysis.” *Circulation. Arrhythmia and Electrophysiology* 2 (3): 276–84.

Tereshchenko, Larisa G., Ghanem, R.N., Abeyratne, A., and Swerdlow, C.D. 2011. “Intracardiac QT Integral on Far-Field ICD Electrogram Predicts Sustained Ventricular Tachyarrhythmias in ICD Patients.” *Heart Rhythm* 8 (12): 1889–94.

Tereshchenko, Larisa G, Fetics, B.J., and Berger, R.D. 2009. “Intracardiac QT Variability in Patients with Structural Heart Disease on Class III Antiarrhythmic Drugs.” *Journal of Electrocardiology* 42 (6): 505–10.

Tereshchenko, Larisa G, Han, L., Cheng, A., Marine, J.E., Spragg, D.D., Sinha, S., Dalal, D., Calkins, H., Tomaselli, G.F., and Berger, R.D. 2010. “Beat-to-Beat Three-Dimensional ECG Variability Predicts Ventricular Arrhythmia in ICD Recipients.” *Heart Rhythm* 7 (11): 1606–13.

Thomsen, M.B., Verduyn, S.C., Stengl, M., Beekman, J.D.M., Pater, G. De, Opstal, J. Van, Volders, P.G.A., and Vos, M.A. 2004. “Increased Short-Term Variability of

Repolarization Predicts d-Sotalol-Induced Torsades de Pointes in Dogs." *Circulation* 110 (16): 2453–59.

Thomson, D.J. 1982. "Spectrum Estimation and Harmonic Analysis." *Proceedings of the IEEE*.

Tolosana, J.M., Berne, P., Mont, L., Heras, M., Berruezo, A., Monteagudo, J., Tamborero, D., Benito, B., and Brugada, J. 2009. "Preparation for Pacemaker or Implantable Cardiac Defibrillator Implants in Patients with High Risk of Thrombo-Embolic Events: Oral Anticoagulation or Bridging with Intravenous Heparin? A Prospective Randomized Trial." *European Heart Journal*.

Trichopoulos, D., Zavitsanos, X., Katsouyanni, K., Tzonou, A., and Dalla-Vorgia, P. 1983. "Psychological Stress and Fatal Heart Attack: The Athens (1981) Earthquake Natural Experiment." *The Lancet* 321 (8322): 441–44.

Vogel, E.R., Sandroni, P., and Low, P.A. 2005. "Blood Pressure Recovery from Valsalva Maneuver in Patients with Autonomic Failure." *Neurology* 65 (10): 1533–37.

Voridis, E.M., Mallios, K.D., and Papantonis, T.M. 1983. "Holter Monitoring During 1981 Athens Earthquakes." *The Lancet*.

Waldo, A.L., Camm, A.J., deRuyter, H., Friedman, P.L., MacNeil, D.J., Pauls, J.F., Pitt, B., Pratt, C.M., Schwartz, P.J., and Veltri, E.P. 1996. "Effect of D-Sotalol on Mortality in Patients with Left Ventricular Dysfunction after Recent and Remote Myocardial Infarction. The SWORD Investigators. Survival With Oral d-Sotalol." *Lancet (London, England)* 348 (9019): 7–12.

Weiss, J.N., Garfinkel, A., Karagueuzian, H.S., Nguyen, T.P., Olcese, R., Chen, P.-S.,

and Qu, Z. 2015. “Perspective: A Dynamics-Based Classification of Ventricular Arrhythmias.” *Journal of Molecular and Cellular Cardiology* 82 (May): 136–52.

Western, D., Hanson, B., and Taggart, P. 2015. “Measurement Bias in Activation-Recovery Intervals from Unipolar Electrograms.” *American Journal of Physiology - Heart and Circulatory Physiology* 308 (4): H331–38.

Wijers, S.C., Sprenkeler, D.J., Bossu, A., Dunnink, A., Beekman, J.D.M., Varkevisser, R., Hernández, A.A., Meine, M., and Vos, M.A. 2018. “Beat-to-Beat Variations in Activation-Recovery Interval Derived from the Right Ventricular Electrogram Can Monitor Arrhythmic Risk under Anesthetic and Awake Conditions in the Canine Chronic Atrioventricular Block Model.” *Heart Rhythm* 15 (3): 442–48.

Wilbert-Lampen, U., Leistner, D., Greven, S., Pohl, T., Sper, S., Völker, C., Güthlin, D., et al. 2008. “Cardiovascular Events during World Cup Soccer.” *New England Journal of Medicine* 358 (5): 475–83.

Wyatt, R.F., Burgess, M.J., Evans, A.K., Lux, R.L., Abildskov, J.A., and Tsutsumi, T. 1981. “Estimation of Ventricular Transmembrane Action Potential Durations and Repolarization Times from Unipolar Electrograms.” *The American Journal of Cardiology* 47 (February): 488.

Xie, Y., Grandi, E., Puglisi, J.L., Sato, D., and Bers, D.M. 2013. “β-Adrenergic Stimulation Activates Early Afterdepolarizations Transiently via Kinetic Mismatch of PKA Targets.” *Journal of Molecular and Cellular Cardiology* 58 (May): 153–61.

Xue, Q., and Reddy, S. 1998. “Algorithms for Computerized QT Analysis.” *Journal of Electrocardiology* 30 (SUPPL.): 181–86.

Zaniboni, M., Pollard, A.E., Yang, L., and Spitzer, K.W. 2000. "Beat-to-Beat Repolarization Variability in Ventricular Myocytes and Its Suppression by Electrical Coupling." *American Journal of Physiology-Heart and Circulatory Physiology* 278 (3): H677–87.

Zaza, A., Malfatto, G., and Schwartz, P.J. 1991. "Sympathetic Modulation of the Relation between Ventricular Repolarization and Cycle Length." *Circulation Research* 68 (5): 1191–1203.

Zheng, Z.J., Croft, J.B., Giles, W.H., and Mensah, G.A. 2001. "Sudden Cardiac Death in the United States, 1989 to 1998." *Circulation* 104 (18): 2158–63.

Appendix

Table 8-1. Demographics and protocol involvement for patients taking part in the invasive catheter lab protocols described in chapters 3 and 6.

Protocol 1 = protocol described in chapter 3

Protocol 2 = protocol described in chapter 6

Patient	Protocol 1	Protocol 2	Gender	Age	AF classification
1	X	X	F	65	Persistent
2	X	X	M	64	Persistent
3	X	X	M	60	Persistent
4	X	X	M	60	Paroxysmal
5	X	X	M	63	Persistent
6	X	X	M	61	Persistent
7	X	X	M	62	Paroxysmal
8	X	X	M	59	Paroxysmal
9	X	X	M	41	Persistent
10	X	X	M	48	Persistent
11		X	M	69	Persistent
12		X	F	55	Paroxysmal
13	X		M	70	Persistent
14	X		F	68	Persistent