Physiological time-series investigations of cardiovascular regulation in healthy young adults during physical exercise.

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Physiological time-series investigations of cardiovascular regulation in healthy young adults during physical exercise

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Summary

Physiological parameters may be recorded non-invasively to gain information on cardiovascular function which can then characterise populations with various pathologies. Physical exercise produces specific autonomic nervous system (ANS) changes. There has been no comprehensive profiling of cardiovascular function during exercise or simultaneous characterisation of the influence of exercise on cardiac ventricular function and electrical activity. This work aims to address that, using a combination of physiological parameters.

Between-lead agreement for ambulatory electrocardiographic (ECG) depolarisation-repolarisation (QT) interval was quantified during rest and exercise. In contrast to cardiac interval (RR) data, between-lead bias and limits of agreement for QT interval data should be quantified when reporting results from an ambulatory ECG system and between-gender QT differences should also be accounted for. ECG electrode location appears to significantly affect QT-RR hysteresis, the shortening of the post-exercise QT interval relative to that at similar heart rates during exercise or pre-exercise rest, further emphasising the need for standardisation of ECG electrode placement.

Sample entropy (SampEn) measures data complexity. Few studies have compared SampEn of RR data (SampEn-RR) during exercise, whilst none have examined SampEn for the corresponding QT interval (SampEn-QT). Fractal analysis assesses data correlation and scaling structures. Detrended fluctuation analysis (DFA) provides a scaling exponent (α) which describes these properties. This has not been quantified for RR interval data during post-exercise recovery and has not been reported for QT interval data. Differences in α magnitudes for RR and QT data suggest that these quantities have different fractal properties.

Exercise perturbs the resting QT-RR relationship via hysteresis. The QT variability index (QTVI) quantifies the relative autonomic influence on the atrial and ventricular myocardium during rest and exercise. QTVI is a consistent measure of cardiac ventricular function and as such appears to be a more useful index than other parameters based on RR or QT interval alone.

Publications included in the text

Lewis MJ, Short AL. (2008). Relationship between electrocardiographic RR and QT interval variability and indices of ventricular function in healthy subjects. *Physiological Measurement,* 29(1), 1-13.

Lewis MJ, Short AL. (2007). Sample entropy of electrocardiographic RR and QT time-series data during rest and exercise. *Physiological Measurement,* 28(6), 731-744.

Lewis MJ, Short AL. (2006). Differences in QT interval determined from multi-lead ambulatory ECG during rest and physical exercise. *Biomedical Signal Processing and Control,* 1, 219-228.

Lewis MJ, Rassi D, Short AL. (2006). Analysis of the QT interval and its variability in healthy adults during rest and exercise. *Physiological Measurement,* 27(11), 1211-26.

Lewis MJ, Short AL. (2006). Hysteresis of electrocardiographic depolarisation-repolarisation intervals during dynamic physical exercise and subsequent recovery. *Physiological Measurement,* 27(2), 191-201.

DECLARATION

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STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked.

Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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Abbreviations used in the text

ACE – angiotensin converting enzyme

ACh – acetylcholine

ACI – acceleration index

ANS – autonomic nervous system

AR – autoregression

ApEn – approximate entropy

BMI – body mass index

BP – blood pressure

BPV – blood pressure variability

BRS – baroreceptor sensitivity

CAD – coronary artery disease

CAN – cardiac autonomic neuropathy

CHF – congestive heart failure

CI – cardiac output index

CNS – central nervous system

CO – cardiac output

COPD – chronic obstructive pulmonary disease

CPAP – continuous positive airway pressure

CPET – cardiopulmonary exercise testing

CSA – central sleep apnoea

DAN – diabetic autonomic neuropathy

DFA – detrended fluctuation analysis

ECG – electrocardiograph

EDV – end diastolic volume

EDVI – end diastolic volume index

EF – ejection fraction

ENS – enteric nervous system

FEV_1 – forced expiratory volume in one second

FBM – fractional Brownian motion

FFT – fast Fourier transformation

FGN – fractional Gaussian noise

FVC – forced vital capacity

HF – high frequency

HR – heart rate

HR_{max} – maximum heart rate

HRV – heart rate variability

IC – index of contractility

ICG – impedance cardiography

LF – low frequency

LQTS – long QT syndrome

LVET – left ventricular ejection time

LVWI – left ventricular work index

MI – myocardial infarction

MVV – maximum voluntary ventilation

NN50 – the number of interval differences of successive RR intervals >50ms

NYHA – New York Heart Association

OSA – obstructive sleep apnoea

PDM – principal dynamic mode

pNN50 – NN50/number of RR intervals

PNS – peripheral nervous system

PP – pulse pressure

PSD – power spectral density

QT interval – the duration of ventricular depolarisation-repolariasation

QT_a – QT interval measured from Q wave onset to T wave apex

QT_c – heart rate corrected QT interval

QT_e – QT interval measured from Q wave onset to T wave end

QT_m – mean QT interval

QT_v – QT interval variance

QTVI – QT variability index

QTVN – QT_v normalised for mean QT

REM – rapid eye movement

RMSSDNN – the root of the mean squared differences of successive RR intervals

RR interval – the duration between two adjacent R wave peaks

RR_m – RR interval variability normalised for mean heart rate

RR_v – RR interval variability

RSA – respiratory sinus arrhythmia

RT interval – ventricular repolarisation interval

SampEn – sample entropy

SampEn-QT – SampEn of QT time series data

SampEn-RR – SampEn of RR time series data

SA node – sinoatrial node

SCD – sudden cardiac death

SDANN – the standard deviation of the average of RR intervals in time segments

SDNN – the standard deviation of RR intervals in a given time period

SNS – somatic nervous system

SV – stroke volume

SVI – stroke volume index

TFC – thoracic fluid content

TFM – Task Force Haemodynamic Monitor

TPR – total peripheral resistance

$\dot{V}_E$ – minute ventilation

VLF – very low frequency

$\dot{V}O_2$ – oxygen uptake

$\dot{V}O_{2max}$ – peak oxygen uptake

WR_{max} – work capacity

Chapter 1 Thesis overview

1.1 Introduction

The main theme of this thesis is the investigation and characterisation of cardiac and haemodynamic responses to physical exercise. In a normal physiologically and neurally intact heart, successive cardiac cycles (RR intervals) are non-uniformly separated in the time domain. Investigation of this temporal variability in RR interval data is commonly referred to as heart rate variability (HRV) analysis. Frequency domain techniques, including those based on Fourier or wavelet transformation, can be used to separate the HRV signal into band-limited components. The "power" of a signal can be estimated within a finite frequency range by calculating its "power spectral density" (PSD), and the PSD may be integrated to determine the power within a defined bandwidth.

Hyndman *et al.* (1971), Sayers (1973) and Kitney & Rompelman (1980) were among the first to implement power spectral analysis for the investigation of HRV and other haemodynamic parameters. Since then, the Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology has published guidelines for the measurement, physiological explanation and clinical interpretation of HRV (Task Force ESC & NAPSE 1996). These guidelines suggest three bandwidths are of importance in the power spectra of adult HRV data, each having association with different heart rate modulation mechanisms.

Neural regulation of the heart rate takes place as a result of the interplay between sympathetic and parasympathetic modulation of the electrical activity of the sinoatrial (SA) node. These two branches of the autonomic nervous system (ANS) demonstrate a reciprocal activity relationship (Malliani *et al.* 1994). A

fundamental hypothesis suggesting the use of spectral analysis in HRV studies is that the two branches of the ANS influence the heart rate in a frequency-dependent way. Information about the contribution of each system to the main power spectral components has been obtained using neuropharmacological techniques (Randall *et al.* 1991). These studies have suggested that: (i) High frequency (HF) components of the HRV signal are mediated via the parasympathetic system, the sympathetic system being too slow to respond at these frequencies, and (ii) HRV components in the low frequency (LF) range are mediated via both sympathetic and parasympathetic systems (Akselrod *et al.* 1981,1985). Additionally, it has been suggested that the relative power of the LF and HF components (the LF/HF ratio) might provide an accurate index of sympathovagal balance (Pagani *et al.* 1986).

Equivocal findings have been reported from investigations comparing the distribution of HRV power during rest with that during dynamic exercise. Bernardi *et al.* (1990) and Rimoldi *et al.* (1992) reported an increase in LF power and a decrease in HF power during low intensity exercise hence implying an increase in the LF/HF ratio during low intensity exercise, a trend similarly described by Tulppo *et al.* (2001). However, several authors (Casadei *et al.* 1995, Perini *et al.* 1990) have described reductions in LF power at higher exercise intensities whilst at yet higher exercise intensities Arai *et al.* (1989) and Bernardi *et al.* (1990) documented increased HF power. Variations in both the mode and the relative intensity of exercise employed in different HRV studies have made it difficult to reliably compare their results.

The experimental work forming the basis of this thesis is presented as five studies which are detailed in chapters 3, 4 and 5, an overview of these chapters is provided below. Subjects were selected from two separate cohorts owing to time

restrictions and subject availability, however all subjects were broadly from the same population, that is physically active young adults. All studies discussed in chapters 3 and 4 contain data from a single cohort, the two studies which form chapter 5 however use data taken from different cohorts.

1.2 Chapter 3 Investigating the relationship between QT and RR time-series during rest and exercise

Following physical exercise, the electrocardiographic ventricular depolarisation-repolarisation (QT) interval is shortened relative to that at similar heart rates during exercise or pre-exercise rest. This lag in QT adaptation to the recovering heart rate has been described as "hysteresis" (Ahnve & Vallin 1982). Multi-lead ambulatory electrocardiogram (ECG) recording offers recognised advantages for QT interval analysis, especially during physical exercise. However, data are usually reported for a single lead and do not quantify between-lead variability, leading to possible misinterpretation. Prior to undertaking a quantification of QT-RR hysteresis, it is essential to understand whether the choice of electrode (lead) position influences the magnitude of either the RR or QT intervals (for example, owing to either technical artifact or differences in physiological information content). No previous study has quantified the influence of ECG electrode placement on the magnitude and temporal variation of QT-RR hysteresis following physical exercise. In the preliminary study, quantification of the between-lead agreement for QT and RR time-series data recorded during rest and during dynamic exercise was sought, prior to the subsequent analysis of QT-RR hysteresis.

A three-lead Holter ECG was recorded continuously during pre-exercise rest, exercise and recovery for male and female subjects undertaking a progressive sub-

maximal bicycle ergometer exercise test. Beat-to-beat cardiac cycle (RR) and QT_a (Q wave end to T wave apex) data were measured for each sinus beat following observation which indicated that the QT_a interval provided a reliable measure of ventricular depolarisation and repolarisation. Bland-Altman analysis (Bland & Altman 1999) was used to quantify between-lead agreement for RR and QT intervals. Mean between-lead difference (bias) was significantly ($p<10^{-6}$) greater for QT compared with RR and in males the between-lead limits of agreement (LOA) for QT were significantly ($p<10^{-6}$) greater than for RR. Relative to mean parameter values, the between-lead LOA for RR was low, with maximal values (% of mean) of 0.1% (male) and 0.3% (female). Paired-lead bias for RR was also relatively low (5.0% and 6.7%), but paired-lead bias for QT (8.3% and 6.9%) and paired-lead LOA for QT (13.1% and 12.8%) were notably larger. These results indicate that the between-lead bias and LOA for QT interval data should be quantified when reporting results from a multi-lead ambulatory ECG system. Interpretation of the results of such studies should take account of between-lead and between-gender QT differences, and should note the physiological conditions during recording. This type of analysis however, does not necessarily indicate that any one lead is better than another.

QT_a-RR hysteresis was subsequently calculated as the difference in QT_a magnitude at identical heart rates during the pre-exercise rest or exercise period and the post-exercise recovery period. The results of this analysis indicated some significant ($p<0.05$) between-lead and between-gender differences in the calculated hysteresis values. Hysteresis was generally greatest during the second or third minute post-exercise and in males. Hysteresis was significantly diminished during late recovery and in males, and was significantly diminished during late recovery compared with early recovery periods ($p<0.05$). QT_a-RR hysteresis is significantly affected by the locations of the electrodes used to record

the surface ECG, emphasising the need for standardisation of ECG electrode placement in future investigations.

1.3 Chapter 4 Complexity of electrocardiographic RR and QT time-series data during rest and exercise

Sample entropy (SampEn) is a measure of data complexity. Few studies have compared the SampEn of RR data (SampEn-RR) during differing physiological states and none have examined SampEn for the corresponding QT interval (SampEn-QT). Chapter 4 details the quantification of SampEn-RR and SampEn-QT during rest and for a range of exercise workloads. The utility of SampEn for discriminating between physiological states, and the relationship of SampEn-RR with traditional measures of HRV is assessed. Twelve males of similar age, mass and aerobic fitness participated. A three-lead ECG was recorded continuously during pre-exercise, progressive bicycle exercise and recovery, and beat-to-beat RR and QT intervals were quantified for sinus beats. SampEn and HRV were calculated within consecutive one-minute periods throughout. Consistent estimation of SampEn-RR and SampEn-QT was possible with an appropriate choice of SampEn parameters. SampEn-RR was sensitive to differing physiological conditions but its discriminating ability was poorer than that of linear HRV indices. SampEn-RR was also negatively correlated with normalised LF and LF/HF parameters. Changes in SampEn for RR and QT data were interpreted in terms of the altered ANS control of either the atrial or ventricular myocardium (or both) during discrete physiological states. It is speculated that the observed greater complexity in QT data might be explained by a direct ANS influence on the ventricular myocardium.

Fractal analysis assesses the correlation and scaling structure within data. Detrended fluctuation analysis (DFA) provides a scaling exponent (α) that describes these properties. Previous studies have quantified α for RR data during rest and exercise, but not during recovery. The influence of age and physical fitness on α during exercise has not been considered. Furthermore, α has not been reported for QT interval data. The further aim of this chapter was to investigate short-term (α_1) and long-term (α_2) scaling exponents for RR and QT data using DFA during rest and physical exercise. This was specifically approached by comparing α_1 and α_2, thereby examining their relationships with HRV parameters, and assessing their sensitivities to differing physiological states. A three-lead ECG was recorded continuously during rest, progressive bicycle exercise and recovery. Beat-to-beat RR and QT intervals were quantified and α_1 and α_2 were quantified using DFA during each physiological state. Short-term α_1 (for RR) was the only exponent sensitive to differing physiological conditions, but its discriminating ability was poorer than most HRV indices. α_1 and α_2 (for RR) correlated with various indices of HRV, confirming that the fractal characteristic of RR data is associated with ANS activity. Differences in scaling exponent magnitudes for RR and QT data indicates that these quantities have dissimilar fractal properties. This is possibly a reflection of differing mechanisms of temporal modulation for these two markers of cardiac electrical activity.

1.4 Chapter 5 Investigation of QT interval variability and its association with ventricular function in healthy adults during rest and exercise

1.4.1 Study 1

The QT interval is known to vary in part as a function of heart rate (HR) or RR interval, although previous studies have indicated a substantial HR-independent component in resting QT interval variability (Porta *et al.* 1998, Almeida *et al.* 2006). There have been few studies of the influence of dynamic physical exercise

on the QT interval or of the QT–RR relationship during these conditions. However it has been shown that QT shortening during exercise is in part independent of heart rate (Rickards & Norman 1981). It has since been suggested that the ANS has a direct influence on the ventricular myocardium, and this might affect cardiac repolarisation independently of heart rate modulation via the SA node (Shimizu *et al.* 1994, Magnano *et al.* 2002). To date there have been no reports of the quantification of HR-dependent and HR-independent influences on the atrial and ventricular myocardium during physical exercise. The QT variability index (QTVI) introduced by Berger *et al.* (1997) quantifies the relative magnitude of temporal variability in QT and RR intervals and can be interpreted with regard to the relative autonomic modulation of these two parameters. Previous studies have suggested that the magnitude of QTVI is a risk factor for arrhythmia (Berger *et al.* 1997, Atiga *et al.* 1998,2000). QTVI might therefore be a useful index for delineating the relative autonomic influences on the atrial and ventricular myocardium, and this might be linked to the potential risk of cardiac arrhythmia.

The aim of this study was to quantify the relationship between QT and RR interval variabilities during rest, exercise and subsequent recovery. After establishing that the data provided evidence of a HR-independent component of QT variability, quantification of the QTVI index during these conditions was sought. It was postulated that the temporal variation of QTVI during exercise reflects the differential autonomic regulation of electrical activity in the atrial and ventricular myocardium, and might reflect the risk of cardiac arrhythmia during exercise. The data collected from the different leads of a clinical Holter ECG system was also compared, and the differences in the results for males and females were examined.

A three-lead Holter ECG was recorded continuously during pre-exercise, exercise and recovery for subjects undertaking a progressive bicycle ergometer exercise test. Mean values of RR, QT, QT_c (QT interval corrected for heart rate), QTVI and mean-normalised QT variance (QTVN) were determined. At the onset of exercise QTVI increased rapidly compared with pre-exercise rest and remained significantly elevated throughout exercise and recovery. There were significant differences between QT_aVI and QT_eVI (QT interval measured from Q wave onset to T wave apex (QT_a) and T wave end (QT_e), respectively) throughout the experimental protocol, however, detection of T wave apex was considered more accurate than T wave end. QTVI was significantly reduced in males compared with females prior to exercise but was similar thereafter, possibly owing to QT intervals being longer in females than males. The results suggest a simultaneous withdrawal of ANS modulation of the atrial myocardium and an accentuation of ANS modulation of the ventricular myocardium during exercise. Hence QT variability is possibly modulated by an autonomic influence on heart rate, as the QT interval is partly heart rate dependent, along with the influence of ion (calcium) channel modification.

1.4.2 Study 2

Ultimately, HRV or QT variability only have value with regard to their clinical utility and it would be of use for clinicians to understand how these variables are related to more familiar clinical indicators (such as left ventricular function, as assessed by left ventricular ejection fraction and stroke volume). To date there has been no simultaneous characterisation of the influence of physical exercise on cardiac ventricular function and cardiac electrical conduction variability. Consequently little is known about the relationship between ventricular function and either HRV or QT variability. Subsequent to the analysis of QTVI during

exercise, an assessment of the relationship between QTVI and ventricular function was sought.

A three-lead Holter ECG was recorded continuously during pre-exercise, exercise and recovery for subjects undertaking a progressive bicycle ergometer exercise test. Mean values of RR and QT, their variabilities (RMSSD and SDNN (section 2.5.4.2.1)) and their relative variability (QTVI) were determined. Traditional indices of ventricular function: stroke volume index (SVI), cardiac output index (CI), left ventricular ejection time (LVET), end diastolic volume index (EDVI), ejection fraction (EF), index of contractility and acceleration index (IC and ACI, both of which are measures of the inotropic state of the myocardium), total peripheral resistance (TPRI), left ventricular work index (LVWI), systolic and diastolic blood pressure (BP) and pulse pressure (PP) were determined on a beat-to-beat basis via impedance cardiography. Beat-to-beat blood pressure was recorded simultaneously using a technique employing photoplethysmography and vascular unloading.

Multiple linear regression analysis using the Stepwise method resulted in significant models for each of the dependent variables (RR, QT, RR and QT variabilities, QTVI) using indices of ventricular function as predictor variables. Notably QTVI reflected both SVI and ACI (which are measures of cardiac 'output' per contraction and the force of contraction, respectively), hence indicating that QTVI was strongly related to measures of cardiac pumping efficiency. This relationship was largely unperturbed by physical exercise, in contrast with the results for all other dependent variables. This work suggests that QTVI is a consistent measure of cardiac ventricular function, and as such is a more useful index than other parameters based on RR or QT interval alone.

Chapter 2 Autonomic nervous system control of the cardiovascular system

2.1 The peripheral nervous system

The peripheral nervous system (PNS) consists of all nerves and neurons which do not lie within the central nervous system (CNS) (Appendix I), but extend outward serving the limbs and organs. It provides a nerve supply to the rest of the body and relays messages to and from the CNS. It consists of 12 pairs of cranial nerves which originate in the brain and 31 pairs of spinal nerves which originate at the spinal cord. The spinal nerve pairs are subdivided into 8 pairs of cervical nerves, 12 pairs of thoracic nerves, 5 pairs of lumbar nerves, 5 pairs of sacral nerves and 1 pair of coccygeal nerves, corresponding to the vertebrae to which they are connected.

Unlike the CNS, the PNS is not protected by bone or the blood-brain barrier (the endothelial cells which protect the brain from blood chemicals) leaving it vulnerable to toxins and injury. The PNS consists of the somatic nervous system (SNS), the enteric nervous system (ENS) and the autonomic nervous system (ANS). Each of the 43 nerve pairs of the PNS belong to one or more of these systems.

2.1.1 The somatic nervous system

The somatic nervous system is responsible for coordinating bodily movement. It is that part of the PNS associated with volitional control through the recruitment of skeletal muscle and with the reception of external stimuli, hence it regulates the body within its surroundings through the senses. The SNS therefore includes all the neurons connected with muscle, skin and the sensory organs. The SNS however also provides an automatic and involuntary reaction to a stimulus,

examples being the patellar reflex, the aversion reflex and correction in response to postural imbalance.

2.1.2 The enteric nervous system

The enteric nervous system manages all aspects of digestion, from the oesophagus to the stomach, small intestine and colon. There are more neurons in the ENS than there are in the spinal cord, the system having immense intricacy. The ENS is responsible for the complex behaviour of the bowel propulsive peristaltic movement, and other motions which result in digestive mixing. The ENS also regulates intestinal blood supply, mucosal epithelial water and electrolyte transport. The ENS is capable of autonomous behaviour including the coordination of reflexes, as it receives considerable innervation from the ANS and thus is often considered to be part of it.

2.1.3 The autonomic nervous system

It was noted by Gaskell in 1886 following demonstration of the complete structural and functional relationships of the ANS, that some nerves provide inhibitory and excitatory function. The ANS is the part of the PNS that controls homeostasis, the constancy of the content in tissues of gasses, ions and nutrients and the maintenance of heart rate and blood pressure (Cannon 1932). It does so largely by controlling cardiovascular and respiratory functions, but also salivation, perspiration, pupil diameter, genitourinary function and secretions from exocrine and endocrine glands, by supplying nerves to all internal organs and to blood vessels. It may therefore be considered predominantly an efferent system (Freeman *et al.* 2006). The ANS is controlled by the hypothalamus and so its actions are not controlled by the brain but are involuntary and reflex in nature.

Autonomic nerves constitute all efferent fibres which leave the CNS, with the exception of those which innervate skeletal muscle. There are however some afferent autonomic fibres which mediate visceral sensation and regulate vasomotor and respiratory reflexes, examples being the baroreceptors and chemoreceptors in the carotid sinus and aortic arch (section 2.6) which are concerned with the control of heart rate, blood pressure and respiratory activity. Signals from these afferent fibres are usually carried to the CNS by major autonomic nerves such as the vagus, splanchnic or pelvic nerves, although afferent pain fibres from blood vessels may be carried by somatic nerves. Motor neurons of the ANS belong to one of three categories that have specific effects on their target organs: sympathetic, parasympathetic or enteric.

2.1.3.1 The sympathetic nervous supply

The term sympathetic originated in the second century A.D. when Galen suggested that "sympathy" exists between all parts of the body. The sympathetic nervous system consists of ganglia which are anterior to the vertebral column and travel the length of the body from thoracic vertebra T1 through to lumbar vertebra L2. The system is therefore known as the thoraco-lumbar outflow (Freeman *et al.* 2006). The sympathetic nervous system responds to impending danger or stress and enables the body to be prepared for "fight or flight". Its primary function is to increase cardiac output during episodes of stress.

Other sympathetic responses include diversion of blood flow from the skin and splanchnic vessels (vessels supplying and draining the viscera) to those supplying skeletal muscle, raised heart rate and blood pressure, increased pupil dilation and lens relaxation allowing more light to enter the eyes, bronchiolar dilation, contraction of sphincters and metabolic changes such as the mobilisation of fat

and glycogen, along with the sense of excitement one feels owing to the increase of noradrenaline (Section 2.4) in the vascular system.

2.1.3.2 The parasympathetic nervous supply

Parasympathetic nervous fibres exit the brain stem and sacral segments of the spinal cord and supply the thorax, abdomen and pelvic regions via the vagus, oculomotor, facial and glossopharyngeal nerves (these are cranial nerves X, III, VII and IX respectively) and also via the spinal nerves that exit the cord from the sacral vertebrae S2 through to S4. The sacral neural fibres form pelvic plexuses which innervate the distal colon, rectum, bladder and reproductive organs; the system is therefore described as the craniosacral outflow (Freeman *et al.* 2006). Parasympathetic influence is evident when one is resting and feels relaxed and is responsible for pupil constriction, lowering of heart rate, dilation of the blood vessels, and stimulation of the digestive system (salivary gland secretion and acceleration of intestinal peristalsis) and genitourinary system. In keeping with the "rest and digest" functions, parasympathetic activity mediates digestion of food and indirectly, the absorption of nutrients and excretion of waste material.

2.1.3.3 Pharmacology: Cholinergic effects

Autonomic pre-ganglionic neurons in the CNS and all parasympathetic post-ganglionic neurons release acetylcholine (ACh). They are termed cholinergic neurons and ACh release is followed by increased parasympathetic mediation of target-organ function. The specific ACh receptors have been subdivided pharmacologically according to the actions of the alkaloids muscarine and nicotine. The actions of ACh at pre-ganglionic regions in both the parasympathetic and sympathetic systems are mimicked by nicotine and all autonomic ganglia are therefore termed nicotinic. Nicotinic transmission also

occurs at the neuromuscular junction, in the CNS, the adrenal medulla and at some sympathetic post-ganglionic sites. However, the action of ACh at the parasympathetic post-ganglionic nerve endings is mimicked by muscarine. Muscarinic transmission also occurs at certain sites in the CNS.

2.1.3.4 Pharmacology: Adrenergic effects

Norepinephrine (noradrenaline) and epinephrine (adrenaline) are hormones classed as catecholamines. The majority of sympathetic post-ganglionic neurons release norepinephrine and so are termed adrenergic; that is, they are strongly influenced by sensory inputs from somatic and visceral sources. Exceptions to this classification are limited to populations of sympathetic neurons innervating sweat glands, which are cholinergic. The adrenal medulla are the central cores of the adrenal glands, which are under the control of the hypothalamus; they are innervated by sympathetic pre-ganglionic neurons and respond to nervous impulses, thereby releasing adrenaline and noradrenaline into the blood stream, although the majority of noradrenaline is synthesised to adrenaline. In situations involving physical or psychological stress, secretion rate is markedly increased, producing a widespread increase in sympathetic activity.

The actions of catecholamines are mediated by specific postsynaptic cell surface receptors. Pharmacological subdivision of these receptors into two groups (α and β) was first suggested by Ahlquist (1948), based upon the effects of adrenaline at peripheral sympathetic sites. These have since been further subdivided on functional and anatomical grounds. Thus β_1-adrenoceptor mediated effects in the heart (increased force and rate of contraction) have been differentiated from those which produce smooth muscle relaxation in the bronchi and blood vessels (β_2-adrenoceptor effects). Similarly, α-adrenoceptor mediated effects such as

vasoconstriction have been termed α_1-effects, to differentiate them from the feedback inhibition by noradrenaline on its own release from pre-synaptic terminals, which is mediated by α_2-adrenoceptors.

However, the classification is more complex. Many organs have both β_1 and β_2 adrenoceptors; for example, the heart has one β_2-adrenoceptor for every three β_1-adrenoceptors. The receptors also show differing responses to adrenaline and noradrenaline. At β_1 adrenoceptors in the heart, adrenaline and noradrenaline appear to have an equal effect, whereas β_2 adrenoceptors in smooth muscle are more sensitive to circulating adrenaline than noradrenaline.

2.2 Electrical activity of the heart

The heart is the major organ of the cardiovascular system, causing blood to flow to all extremities of the body through the vasculature by repeated, rhythmic contractions which establish blood pressure. The sympathetic and parasympathetic nervous systems continuously moderate the inherent rhythmicity of the heart via their action on specific parts of it, with the final pace of the heart being defined by the balance between these two branches of the ANS.

The cardiac wall consists of three main layers. The epicardium is the outer layer of the wall and consists of connective tissue covered by epithelium. Beneath the epicardium lies the myocardium. The myocardium is composed of specialist cardiac muscle cells with the unique ability to carry action potentials as well as to contract, similar to neurons in nerve fibres. Some myocardial cells also have the property of automaticity, that is the ability to spontaneously generate an

electrical action potential, and these particular myocytes are described as myogenic. The myocardium is subject to two opposed electrical control mechanisms. First-order electrical control is via the sinoatrial (SA) node and arises from sympathetic discharge in the region of the SA node. Second-order electrical control comes via parasympathetic influence from the spinal vertebral ganglia and vagus nerves. The innermost layer of the cardiac wall is the endocardium which lines the chambers of the heart, covers the heart valves and is continuous with the inner lining of the blood vessels. Purkinje fibres are also located in the endocardium, which participate in the electrical conduction and contraction of the cardiac ventricles.

Under normal conditions in a healthy heart, the SA node exhibits total control of the cardiac cycle, as its action potentials are initiated most frequently, exciting other cells to generate their own synchronised action potentials. The spontaneous depolarisation of the SA node is continuously modulated by sympathetic and parasympathetic neural input, sympathetic via the superior, middle and inferior nerves and parasympathetic via the vagus nerve. Sympathetic and parasympathetic nerve fibres terminate at the SA node, the atria, the ventricles and the coronary vessels and parasympathetic nerve fibres also terminate at the AV node.

2.3 Electrocardiography and haemodynamic monitoring

An electrocardiograph (ECG) is an expression of the ionic cellular changes generated during the myocardial depolarisation-repolarisation cycle and provides information on cardiac integrity via both its morphological and temporal characteristics. Although elaborate and sophisticated imaging facilities are used for assessing heart and circulation morphology and function, the ECG remains

the first-step approach in patient evaluation (Passino & Emdin 2008) and is the single most commonly performed clinical investigation (Bayés de Luna *et al.* 2006). The spread of electrical impulses (electrical depolarisation-repolarisation wavefront) that constitutes the heart beat is externally measurable by electrodes attached to the skin. Placed either side of the heart, these measure potential differences and hence electrical activity within different regions of the heart. An ECG therefore enables measurement and diagnosis of abnormal conduction and rhythm, particularly those caused by conductive tissue damage. Techniques based on temporal analysis of the ECG have been used as markers of autonomic modulation of the heart. These have included HRV, QT interval variability and heart rate turbulence analyses.

Whereas a 12-lead ECG provides a representation of cardiac electrical activity, typically of short duration and used for morphological diagnosis, a Holter or ambulatory monitor allows the recording and subsequent temporal analysis of cardiac rhythm over 24 hours or more, which has particular value for individuals with arrhythmias. Holter monitors usually employ between 3 and 8 leads connected to a small recorder which is typically attached to the individual's clothing.

Einthoven in 1895 identified the primary topographic features of the ECG waveform now known as the PQRST waveform; these are:

- The P wave, representing atrial depolarisation which is of low amplitude and therefore at times masked by the QRS complex

- The PR interval, representing the time taken for the electrical impulse to pass from the SA node and through the AV node and the His-Purkinje system, thereby initiating ventricular contraction
- The QRS complex, representing ventricular depolarisation
- The T wave, representing the recovery of ventricular cells to pre-excitation potentials

A typical cardiac waveform is illustrated in Figure 2.1.

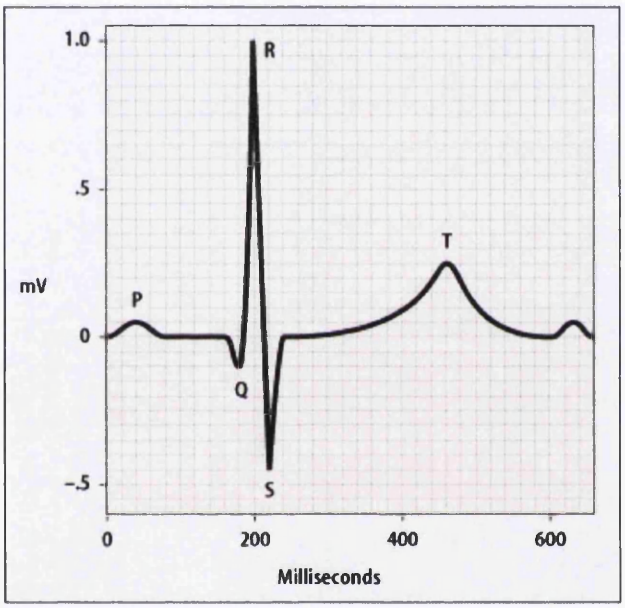


Figure 2.1 A typical cardiac waveform (http://campus-llamaquique.uniovi.es/virtual/docencia/teleco/4.aplicaciones/biomedica/bio.htm)

2.3.1 Temporal ECG parameters: RR interval

The RR interval is the time elapsed between two adjacent R waves on the ECG. Strictly, as the P wave represents atrial depolarisation and is therefore the beginning of the cardiac cycle, a full cycle is represented by the PP interval, but as the R wave is more easily identifiable on the ECG owing to difficulties in

precisely verifying P wave onset, the RR interval is used as a surrogate and more accurate index of cardiac cycle duration.

2.3.2 Temporal ECG measurements: QT interval

The QT interval is measured from the beginning of the QRS complex to the end of the T wave and therefore represents the time for both ventricular depolarisation and repolarisation to occur. There is no distinct atrial repolarisation wave represented in the ECG waveform as it occurs during ventricular depolarisation, and since it has small amplitude it is masked by the larger ventricular-generated QRS complex. The QT interval on the standard 12-lead ECG is influenced by a variety of physiological and pathological factors such as heart rate, autonomic balance, hormonal concentration, drugs, heart disease, ventricular dysfunction and electrolyte disturbances (Sredniawa *et al.* 2005) and also genes and gene products (Moss 2006) that can modify the function of specific cardiac ion channels.

The QT interval and its variability have diagnostic and screening relevance with regard to pro-arrhythmic potential. Recently the importance of careful assessment of all drugs for pro arrhythmic effects has been recognised (Sheridan 2000, Shah 2002). Drugs that have the potential to alter cardiac ion channel activity resulting in delayed conduction or repolarisation (hence a prolonged QT interval) are routinely screened in the preclinical and clinical settings (Berger *et al.* 2005). Additionally, congenital long QT syndrome (LQTS) is an abnormality of cardiac repolarisation that can cause syncope and sudden death (Levine *et al.* 2008). It was first described (Jervell & Lange-Nielsen 1957) in a family of four children with congenital deafness, syncope and QT interval prolongation, three of whom died suddenly. There are ten distinct genetic LQTS phenotypes, each

with a specific ion channel abnormality (Levine *et al.* 2008). Notably, individuals with the LQT1 phenotype (a potassium channel abnormality; Chiang & Roden (2000)) are most likely to experience a cardiac event during exercise, particularly and for as yet unexplained reasons, when swimming (Schwartz *et al.* 2001).

Detection of mean QT interval increases of the order of several milliseconds is subject to various difficulties such as improper lead placement, electrode noise, or an incorrect choice of leads. Notably QT prolongation is more evident in some leads than others (owing to both physiological and technical reasons) and different ECG machines can present varying values of QT interval for the same subject owing to the use of differing detection algorithms. In addition, incorrect choices for the start of the Q wave and end of the T wave can occur owing to the relatively low amplitudes of these waves. Methodologically it is easier to measure from Q onset to T_a (T_{apex}, the peak of the T wave) than to T_e (T_{end}, the end of the T wave, where it meets the ECG baseline) (Davey 1999), although it remains unclear whether QT interval variability within the T_a–T_e interval has importance. In support of the use of QT_a interval is the finding that in normal resting subjects, most inter- and intra-individual QT interval variability occurs in the R to T_a portion of the QT_e interval (Merri *et al.* 1989).

Ambulatory ECG (Holter) monitoring has allowed the assessment of dynamic QT under differing physiological states such as exercise and rest, and has facilitated beat-to-beat analysis over extended periods. Assessment of the QT interval and its relationship with the corresponding RR interval during various conditions (such as rest and exercise) is not, contrary to what might be expected, fixed or uniform. Quantification of the QT-RR relationship under different physiological conditions holds promise as a tool for identifying individuals at increased risk of

arrhythmic events and for studying the effects of drugs on ventricular repolarisation (Sredniawa *et al.* 2005).

Throughout the subsequent sections of this chapter, QT interval adaptation is discussed where appropriate, in parallel with HRV in a range of pathophysiological conditions.

2.3.2.1 Heart rate correction of the QT interval

The QT interval typically needs correction for the influence of heart rate owing to the negative correlation between heart rate and QT interval. Slower heart rates are associated with longer QT intervals and vice versa. This can be problematic when attempting to distinguish between, for example, QT interval changes that are drug-induced and those caused by natural physiological processes. Heart rate correction aims to provide a standardised value that would have been measured in the same subject had the heart rate been 60 beats per minute and allows comparison between QT intervals recorded at different heart rates. Corrected QT interval (QT_c) is commonly based around formulae proposed by Bazett (1920) and Fridericia (1920) (Equations 1 & 2) although there are many other formulae utilising logarithms, exponentials and regression.

Equation 1 (Bazett): $$QTc = \frac{QT}{\sqrt{RR}}$$

Equation 2 (Friderica): $$QTc = \frac{QT}{\sqrt[3]{RR}}$$

To assess the performance of a particular heart rate correction formula, the correlation between QT_c intervals calculated using the formula and the RR

intervals can be assessed. If it differs from zero, the correction formula is not truly successful (Sredniawa *et al.* 2005).

Previous work has suggested that a specific correction formula may work well for a specific data set but might not be universally applicable (Malik 2001) as the formulae do not account for age, gender and levels of aerobic training. The reasons for this high inter-individual variability include environmental influence and inherent genetically determined differences in the QT-RR relationship (Batchvarov *et al.* 2002). In addition, females have longer QT_c intervals than males (Genovesi *et al.* 2007) and the QT interval is prolonged during sleep (Extramiana *et al.* 2005). An ideal approach would be for each individual to have a personalised correction derived from different heart rates. *Ad hoc* correction which may be sufficient in clinical practice is too simplistic and inaccurate to be used in precise drug studies (Malik 2004), although there is no evidence that such individual rate correction would improve discrimination of diseases such as hereditary LQTS (Toivonen 2002). Bazett's formula has been questioned as it over-corrects QT at high heart rates and under-corrects at low heart rates which can hide the pro-arrhythmic toxicity of drugs slowing the heart rate (Yash & Toal 2003). The cube root correction of Friderica accounts for this but is not reliable at high heart rates. It is thought that Bazett's formula can only be applied to correct the QT interval within a range of heart rates between 50 and 90 beats per minute, the same limitation applying to Fridericia's formula although to a lesser degree (Sredniawa *et al.* 2005).

The alternative use of the so-called "bin method" that matches heart rates of on-drug and off-drug data is linked to other potential difficulties (Malik 2005). The "bin method" compares QT intervals recorded at closely matched heart rates before and after intervention (either mechanical or pharmaceutical). Pairs of RR

and QT intervals are distributed into groups (bins) according to the immediately preceding RR value. Post intervention pairs are collected at the same value of RR, but with new, altered QT interval values and compared with the baseline pairs. The bin method has particular importance in infants (as this group has higher heart rates owing to smaller ventricular size and greater metabolic needs), in individuals with slow or high heart rates and when investigating drugs which induce significant changes in heart rate (Sredniawa *et al.* 2005), as it allows the comparison of QT interval for any RR interval. The bin method has limitations in that there are not always sufficient numbers of recorded QT intervals available for analysis and owing to autonomic tone, QT interval may be different at identical heart rates, (the phenomenon of "hysteresis"). It should also be noted that heart rate correction ignores the dynamicity of QT-RR relationship (Yash & Toal 2003).

2.3.2.2 QT-RR hysteresis

It has been observed that QT changes lag several minutes behind heart rate changes, a phenomenon referred to as "QT-RR hysteresis" (Ahnve & Vallin 1982). Simply, the QT interval does not adapt to heart rate changes quickly, taking a number of beats to adjust in response to changes in heart rate. This lag has been shown in cellular and pacing studies (Franz *et al.* 1988, Lau *et al.* 1988, Lee *et al.* 1999) to have two components:

1. An immediate rapid component with a very short time-constant that acts over 1-2 cardiac cycles
2. A delayed component with a time constant of the order of 1 minute or greater

Toivonen (2002) has since noted that full adaptation of the QT interval to a change in heart rate takes between one and three minutes. New methods of QT dynamics assessment that take into account these phenomena have been proposed (Sredniawa *et al.* 2005). These methods include the computation of linear regressions between QT and RR intervals and measures of the regression gradient, this defining the percentage of QT interval change per unit of RR interval change. Using these methods, dynamic QT changes have been observed in relation to ischemic heart disease, advanced heart failure and cardiac arrest.

Pueyo *et al.* (2003) used a global optimisation algorithm to estimate the lag between RR and QT changes. They found that on average, 140 seconds of the preceding RR data have an influence on the QT interval. Benatar & Decraene (2001) quantified RR, QT and QT_c in children at rest, during high-intensity physical exercise, and during six minutes of post-exercise recovery. They observed that lengthening of the QT interval in response to decreasing heart rate began only after two minutes of recovery. Notably, Chauhan *et al.* (2002) observed that QT hysteresis values at one and two minutes post-exercise were significantly greater in women than in men, which suggests that the cardiovascular response to exercise and exercise training may be different in males and females (Genovesi *et al.* 2007). These authors suggest that females in particular may benefit from intervention aimed at increasing physical activity as a tool for prevention of cardiovascular morbidity and mortality.

2.3.2.3 The QT variability index

The QT variability index (QTVI) suggested by Berger *et al.* (1997) quantifies the relative magnitudes of temporal variability in QT and RR intervals and has been interpreted as an indirect measure of autonomic regulation of cardiac ventricular

electrophysiology, although this hypothesis has never been directly tested (Malik 2008). QTVI is the base 10 log ratio of QT variability (QT_v) normalised for mean QT interval (QT_m) squared and heart rate variability (RR_v) normalised for mean heart rate (RR_m) squared:

$$QTVI = \log_{10}\left\{\frac{(QT_v)/(QT_m)^2}{(RR_v)/(RR_m)^2}\right\}$$

The QTVI therefore compares the magnitude of temporal variability in QT and RR time-series. The ability to assess ANS influence at the ventricular level is appealing as it is there that ventricular arrhythmias originate (Malik 2008).

Several previous studies have indicated an association between changes in QTVI and altered ANS activity. QTVI is decreased after postural tilt in healthy subjects (Piccirillo *et al.* 2001) and is increased in individuals with chronic renal failure, this being associated with diabetes and coronary disease (Johansson *et al.* 2004) and in individuals with congestive heart failure (Raghunandan *et al.* 2005). Moreover increased QTVI is a risk factor for sudden cardiac death (Atiga *et al.* 1998), indicates a greater susceptibility for malignant ventricular arrhythmias (Berger *et al.* 1997, Atiga *et al.* 1998) and is associated with an independent risk for ventricular tachycardia or ventricular fibrillation (Haigney *et al.* 2004). In asymptomatic individuals with congestive heart failure and mild ventricular systolic dysfunction, the QTVI could be helpful in stratifying the risk of sudden cardiac death (Piccirillo *et al.* 2007). Furthermore, the combination of a high QTVI and low RR variability is associated with an increased risk of primary cardiac arrest among individuals without clinically recognised heart disease (Whitsel *et al.* 2001). It is also notable that Atiga *et al.* (1998) suggested a non-exercise QTVI value of 0.1 or greater to be a discriminator for higher risk of arrhythmic events. QTVI and its association with ventricular function in healthy adults during rest and exercise is detailed in chapter 5.

The first study (Baumert *et al.* 2008a) of the relationship between QT variability and cardiac norepinephrine spillover (measuring cardiac sympathetic activity, the direct "gold-standard"), conducted in individuals with depression and panic disorder, was unable to find any correlation between the variables. This resulted from two facets of QTVI being problematic. Firstly, QT interval measurements neglect hysteresis and secondly, exact QT interval duration in any selected ECG lead does not provide a consistent measure of the overall duration of ventricular repolarisation (Malik 2008).

2.3.3 Haemodynamic monitoring

Critical care patients regularly require determination of their cardiac output (CO), typically expressed as the volume of blood ejected into systemic circulation from the left ventricle per minute (De Maria & Raisinghani 2000). The primary aim of haemodynamic monitoring is the determination of the adequacy of oxygen delivery to the tissues (Summers *et al.* 2003). Whilst determination of blood pressure, heart rate, respiratory rate and oxygen saturation is clinically readily available, the application of these measurements to provide an assessment of the true haemodynamic state is difficult, as blood pressure and heart rate are not well correlated with blood flow in acute clinical conditions (Wo *et al.* 1993).

2.3.3.1 Traditional indices of cardiac output

CO is commonly determined via a thermodilution pulmonary artery balloon flotation Swan-Ganz catheter (Engoren & Barbee 2005) although the process has been associated with complications related to its invasiveness, such as pneumothorax, arterial puncture, arrhythmias and bacteremia, hence questions remain regarding the efficacy of catheter use (Levett & Replogle 1979, Stetz *et al.* 1982, Nishikawa & Dohi 1993, Connors *et al.* 1996, Kern & Shoemaker 2002,

Sandham *et al.* 2003). Clinicians do not yet have a "gold standard" for the measurement of CO (De Maria & Raisinghani 2000, Chakravarthy 2008). However, traditional standards remain: the dye dilution method which is similarly invasive and is unable to measure beat-to-beat changes (Spiering *et al.* 1998); or the Fick method (Engoren & Barbee 2005) which has limited use in individuals with lung abnormalities (Light 1988, Takala *et al.* 1989) as it relies on the analysis of inspired and expired gases. In current practice however, gas analysis is not routinely conducted, hence thermodilution has replaced the Fick method as the reference standard for CO measurement (Albert *et al.* 2004).

2.3.3.2 Impedance cardiography

Impedance cardiography (ICG) is a non-invasive technique for the evaluation of haemodynamic parameters (Strobeck & Silver 2004) and also records the electrical activity in the myocardium as a non-diagnostic ECG which is used as part of the ICG calculation process (De Maria & Raisinghani 2000). It allows the state of the circulatory system and trends in changes in haemodynamic parameters to be assessed easily, quickly and cheaply through the specific characterisation of stroke volume (SV) (the volume of blood pumped from the heart with each beat) and hence stroke volume index (SVI) (stroke volume normalised for body surface area to account for different body sizes and hence heart sizes) derived from the impedance waveform and the ECG using specific algorithms (De Maria & Raisinghani 2000). It also allows assessment of the following (adapted from Sodolski & Kutarski 2007):

- Total peripheral resistance (TPR) (the sum of the resistance of all peripheral vasculature)

- Contractility – the measurement of acceleration index (ACI) (a measure of the inotropic state of the myocardium)
- Left ventricular ejection time (LVET) (the time for ejection of blood from the left ventricle)
- End diastolic volume (EDV) (the blood volume in a ventricle at the end of diastole)
- Ejection fraction (EF) (the fraction of the end diastolic volume pumped out of a ventricle per beat)
- Left ventricular work index (LVWI) (the work the left ventricle does in pumping blood each minute)
- Thoracic fluid content (TFC) (a reflection of the total (intravascular and extravascular) fluid volume contained within the thorax)
- Systolic and diastolic blood pressures (BP)
- Pulse pressure (PP) (the change in BP over a cardiac cycle)

One new device for the non-invasive monitoring of haemodynamic function is the Task Force Haemodynamic Monitor (TFM) (CNSystems Medizintechnik GMBH, Austria), which allows the beat-to-beat analysis of SV and CO along with beat-to-beat BP analysis and the derivation of TPR (Fortin *et al.* 1997, Fortin *et al.* 1998, Gratze *et al.* 1998).

2.3.3.3 The theory of impedance cardiography

The thorax is predominantly muscle, lung, fat, skin, bone and air, all of which have a high resistivity, and blood, which has a low resistivity resulting from its electrolytic fluid base (Summers *et al.* 2003). It can be postulated therefore that the majority of any electrical current applied to the thorax would flow through the blood filled aorta and vena cava as they offer the paths of least impedance. Kubicek *et al.* (1966) considered that observed impedance alterations occurring

within this thoracic conductor would reflect changes in the volume of the great vessels. In the ICG method, low amplitude, high frequency alternating current is transmitted through the thorax and seeks the low impedance path of the aorta (Albert *et al.* 2004); each cardiac cycle alters the blood volume and velocity within the aorta which therefore alters its impedance. The ICG measures the baseline thoracic impedance and compares it with the dynamic impedance changes that occur through each cardiac cycle, providing a measure of CO. Typically, sensors are placed each side of the neck and thorax providing outer and inner "circuits". The outer sensors provide the electrical field and the inner sensors measure thoracic impedance.

From Ohm's law, the impedance (z) between two points is equal to the ratio of the potential difference (E) between the points and the size of the electrical current (I) flowing between the points:

$$Z = E/I$$

Furthermore, if impedance is dependent on the cross-sectional area (A), length (L) and resistivity (ρ) of the conductor, then:

$$Z = \rho(L/A)$$

Changes in impedance can be related to changes in volume (V) (where $V = A \times L$) leading to:

$$Z = \rho(L^2/V)$$

Hence, when applied to the body, the impedance (Z) of the thorax is inversely proportional to the content of fluid (V) within the thoracic cavity and this result is the fundamental principle behind the concept of ICG (Summers *et al.* 2003). ICG was pioneered by Nyboer *et al.* (1940) who used the technique to evaluate the flow of blood in limbs (Sodolski & Kutarski 2007). Their technique recorded a

proportional impedance response to volumetric changes in the arm and related impedance change ($\delta Z/\delta t$) to original base impedance (Z_o) and volume change ($\delta v/\delta t$) as follows:

$$\delta V/\delta t = -\rho\left(L^2/{Z_o}^2\right)\delta Z/\delta t$$

The technique was then furthered by Kubicek *et al.* (1966) by the definition of a new equation determining SV which incorporated the maximum value of the first derivative of the impedance waveform [$(\delta Z/\delta t)_{max}$] and LVET. The inclusion of $(\delta Z/\delta t)_{max}$ overcame the limitations of signal artifact produced by respiration, which prevented accurate assessment of ejection time, and magnitude of impedance change attributable to blood volume change in the aorta, hence (where SV represents δV and LVET represents δt):

$$SV = \rho\left(L^2/{Z_o}^2\right)(\delta Z/\delta t)_{max}\, LVET$$

Sramek (1982) refined the technique through the use of a truncated cone model of the thorax rather than the previous cylindrical model and proposed the length of the thorax to be 17% of the patient height. Bernstein (1986) modified it further by accounting for the mass of the individual in an attempt to more accurately define the thoracic volume.

The TFM utilises a combination of refined hardware and software based on the Kubicek and Sramek modifications detailed above and a novel set of shortband electrodes which combine ease of application and reproducibility by creating a particularly homogeneous electrical field in the thorax (Fortin *et al.* 2006).

2.3.3.4 Clinical application of impedance cardiography

ICG is used in the diagnosis and treatment of hypertensive patients (Sharman *et al.* 2004, Strobeck & Silver 2004, Ferrario 2005, Ventura *et al.* 2005) as

hypertension is associated with abnormal CO and arterial distensibility which can be calculated via the relationship between SV and PP (Sodolski & Kutarski 2007). It has also had utility in therapeutic decision making in dyspnoea (Springfield *et al.* 2004, Strobeck & Silver 2004), coronary artery bypass surgery recuperative care (Kaukinen *et al.* 2003, Van De Water *et al.* 2003, Kokkonen *et al.* 2005), in intensive care (Neath *et al.* 2005, Veale *et al.* 2005), in dialysis patients (Yoshii *et al.* 2005, Wynne *et al.* 2006), in the optimisation of cardiac pacemaker programming (Belott 1999, Tse *et al.* 2003, Gimbel 2005) and in heart failure (Yancy & Abraham 2003, Vijayaraghavan *et al.* 2004), although it should be noted that a recent study (Kieback *et al.* 2009) found poor correlation between the ICG and thermodilution methods in measuring SVI in this group of individuals. Furthermore, ICG has been used in monitoring circulatory system changes during pregnancy (Heethaar *et al.* 1995, van Oppen *et al.* 1996).

2.3.3.5 Comparison of cardiac output determination methods

Early generation ICG showed inconsistent performance (Belardinelli *et al.* 1996, Marik *et al.* 1997) as there was consistent overestimation of CO (De Maria & Raisinghani 2000). Despite the algorithmic improvements made by Sramek (1982) and Bernstein (1986), results were weakly correlated with thermodilution and the Fick method (Yakimets & Jensen 1995) and it appeared therefore that ICG would have little useful clinical application (Marik *et al.* 1997, De Maria & Raisinghani 2000). However, improvements in signal processing, software, hardware and algorithmic calculation have led to ICG becoming a valuable investigative tool (Sodolski & Kutarski 2007). Current generation devices are able to provide precise, repeatable results (Sodolski & Kutarski 2007) which are comparable with results obtained from invasive methods as confirmed in multiple comparative studies (De Maria & Raisinghani 2000, Greenberg *et al.* 2000, Strobeck *et al.* 2000, Drazner *et al.* 2002, Sageman *et al.* 2002, Van De Water *et al.* 2003, Strobeck &

Silver 2004) and the trends of ICG-derived CO changes closely match CO changes assessed by thermodilution (Shoemaker *et al.* 1998). Thermodilution is known to have a variation of up to 20% in individual measurements (De Maria & Raisinghani 2000) which is improved to 10% variation by averaging over three measurements (Fortin *et al.* 2006). However, the available data would not yet support ICG replacing invasive methods in the critical care setting (Bayram & Yancy 2009). Notably, ICG can now be used to record beat-to-beat measurements and to electronically mark events in relation to postural changes and changes in exercise workload (De Maria & Raisinghani 2000).

Specifically for the TFM however, the variation of CO is smaller and hence the reproducibility of results is better when compared with thermodilution, even in individuals with severe heart failure (Fortin *et al.* 2006). Fortin *et al.* (2006) attributed this to the modified algorithm used and the new electrode array producing a stable electrical field. They concluded that the TFM appears suitable for monitoring intensive care cardiac patients.

2.3.3.6 Limitations of impedance cardiography

ICG results are considered unreliable when there is consistent aortic insufficiency, septic shock or hypertension, in individuals of height less than 120 cm or over 230 cm and of mass less than 30 kg or over 155 kg (Sodolski & Kutarski 2007). Specifically for the TFM, inaccurate CO values have been found in the presence of cardiac shunt, in individuals with heart valve insufficiency or stenosis and in individuals with high levels of thoracic fluid accumulation (Fortin *et al.* 2006).

2.4 Chronotropic effects on the heart

Heart rate regulation is a highly intricate process controlled by components of the CNS and PNS through positive and negative feedback loops, giving rise to very complicated dynamics (Kanters *et al.* 1996). Physically, cardiac control is overseen by:

- The medulla oblongata processing sensory information from the ANS
- The higher somatomotor centres of the brain constantly updating the medulla oblongata with neural information to regulate cardiac output
- The hypothalamus which transmits physical expressions of emotion via the ANS
- Sensory receptors providing peripheral input from the viscera leading to neurochemical release
- Renal control of blood volume and the renin-angiotensin system (section 2.4.2)
- Further endocrine factors including thyroxin and reproductive hormones
- Respiratory influence including the respiratory sinus arrhythmia (RSA) (section 2.5.4.2.4)

Chronotropic effects on the heart are exerted by the following physiological parameters: blood pressure, blood composition, temperature, breathing rate and breath amplitude. These influences are achieved via the release of chemical substances which act on the heart to cause a positive or negative chronotropic effect as shown in Table 2.1.

Table 2.1 Chemical substances which chronotropically affect the heart

Chemical	Released By	Chronotropic effect
Noradrenaline (norepinephrine)	Sympathetic ANS fibres	positive
Adrenaline (epinephrine)	Adrenal medulla	positive
Acetylcholine	Parasympathetic ANS fibres	negative

2.4.1 Chronotropic influences of the ANS

Sympathetic activity increases the rate of atrial depolarisation via the release of noradrenaline at the SA node, leading to tachycardia and decreased HRV. The tendency toward sympathetic influence exists even during conditions of equilibrium. Parasympathetic stimulation favours the liberation of acetylcholine, which reduces the rate of depolarisation of the SA node leading to bradycardia and increased HRV. There also exists a tendency towards parasympathetic influence during equilibrium, hence the constantly modulating antagonistic nature of the two branches of the ANS (Lewis 2005). Additionally, this modulatory process is governed by the CNS with a closed loop control system. The CNS receives input stimuli and uses the ANS to send the appropriate signal to the heart. This signal then alters heart rate as well as other heart rate-dependent cardiovascular variables such as blood pressure and information about these changes is fed back to the CNS (García-Sánchez *et al.* 2006). Added to this is the input from other sensory feedback systems, lending immense complexity to overall heart rate modulation.

2.4.2 The renin-angiotensin-aldosterone system

The renin-angiotensin-aldosterone system is an endocrine system that influences cardiac output through the regulation of blood volume, vascular resistance and hence blood pressure. This occurs via the formation of angiotensin I in blood and tissues which is synthesised from angiotensinogen, released by the liver. This is further synthesised by the angiotensin converting enzyme (ACE) to angiotensin II, which in turn stimulates the release of aldosterone from the adrenal cortex which acts to maintain fluid and sodium levels in the kidneys. Angiotensin II also constricts blood vessels hence increasing systemic vascular resistance and arterial pressure. There is widespread involvement of angiotensin II in the pathophysiology of cardiovascular diseases as well as diabetic nephropathy (Ferrario 2006). Angiotensin II also exerts several actions on the sympathetic nervous system and also on central baroreflex modulation (section 2.6.3), accounting for its ability to increase blood pressure without a reflex bradycardia (Reid 1992).

2.5 Heart rate variability

2.5.1 History and origins

The fluctuation of haemodynamic parameters on a beat-to-beat basis was reported although not described by Hales (1733) following the initial measurement of quantitative blood pressure through the insertion of a glass capillary tube into the carotid artery of a horse to view the blood level, "it would rise and fall at and after each pulse two, three, or four inches; and sometimes it would fall twelve or fourteen inches." However, the Chinese physician Wang Shuhe wrote in the third century A.D. "If the pattern of the heart beat becomes as regular as the tapping of a woodpecker or the dripping of rain from the roof, the patient will be dead in four days" (Cheng 2000). The modern clinical

relevance of HRV was identified when it was recognised that RR interval alterations preceded foetal distress and before a critical change in heart rate became apparent following possible hypoxia (Hon & Lee 1965). This relevance was furthered during the 1980s when HRV became a strong indicator of mortality following myocardial infarction (MI). This section will provide an overview of the standards, methods and relevance of HRV analysis.

2.5.2. What is heart rate variability?

Heart rate variability is the change in duration of cardiac cycles that is observed on a beat-to-beat basis in living organisms. HRV is commonly determined non-invasively via the ECG. Theoretically this would be achieved by measuring the time interval between the initial deflections of the P waves, or the intervals between this point and the end of the T wave, for consecutive cardiac cycles. However, technical difficulties render this method unrealistic and consequently, as previously discussed, an alternative proxy measure of cardiac cycle length, the "RR interval" (the duration between two adjacent R wave peaks) is used. Measurement of the RR interval thus allows calculation of heart rate (heart rate (beats per minute) = 60/RR (seconds)), and different authors have variously used either RR interval or heart rate as the basis of HRV investigations. HRV analysis therefore quantifies the amount by which the RR interval (or heart rate) changes from one cardiac cycle to the next.

2.5.3 General interpretation of HRV

HRV analysis provides a versatile, non-invasive clinical method for assessing the integrity of the cardiovascular control system in a variety of disease states (Akselrod *et al.* 1981). HRV is generally interpreted as a marker of the balance which exists between the influence of the sympathetic and parasympathetic

influences of the ANS on the SA node of the heart (To reiterate: release of epinephrine and norepinephrine by the sympathetic nervous system and acetylcholine released by the parasympathetic nervous system (Malpas 2002)). Significantly altered HRV can be found not only in cardiac diseases but also in a wide variety of pathophysiological disorders characterised by disturbance of ANS function (Several of these disorders are detailed in Appendix II).

In the young and/or healthy individual, the physiological variability of the ANS is reflected by the pattern (variability and structural complexity) of HRV. With progressing age or disease, HRV becomes more regular with a marked loss of variability and complexity. Greater variability of heart rate indicates greater integrity of physiological control mechanisms and as a general rule, higher values of HRV are associated with more functionally efficient cardiac autonomic control mechanisms (Lewis 2005).

The relationship between the ANS and cardiac mortality has been widely investigated, with HRV acknowledged as one of the most sensitive markers of this relationship. Clinical data based on numerous studies since the early 1990s has generally considered decreased global HRV as a strong predictor of increased all-cause cardiac and/or arrhythmic mortality (Sztajzel 2004). Despite the intensive research on various aspects of HRV, it has not been definitively established why altered (reduced) HRV has been associated with early death (Huikuri *et al.* 1999). This is largely because conventional measures of HRV permit high risk group definition but without the specificity to identify individuals with elevated sudden cardiac death (SCD) risk (Kanters *et al.* 1996).

2.5.4 Technical measurement of HRV

HRV assessment is, in general, performed to provide some form of evaluation of ANS function. There are two common settings in which HRV is measured (Kleiger *et al.* 2005). The first is assessment under controlled laboratory conditions with short-term measurements designed to challenge the ANS via certain manoeuvres (section 2.5.4.3.4). The second is the determination of HRV from 24-hour ECG recordings taken whilst subjects perform their usual daily activities. This method has been particularly useful for risk stratification in various pathologies and also for quantifying autonomic dysfunction (Eckberg 1980, Billman & Hoskins 1986, Bigger *et al.* 1988).

2.5.4.1 Standards and guidelines for HRV assessment and interpretation

The establishment of the Task Force by the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Task Force ESC & NASPE 1996) led to the development and application of appropriate guidelines for the standardisation of HRV measurement and analysis. The specific goals of the Task Force were to:

- Standardise nomenclature and develop definitions of terms
- Specify standards of measurement
- Define physiological and pathophysiological correlates
- Describe currently appropriate clinical applications
- Identify areas for future research

Cerutti *et al.* (2006) suggested another Task Force initiative would be appropriate to update current thinking and understanding, to take account of results obtained over the time since the initial Task Force.

2.5.4.2 Quantification of HRV

The Task Force guidelines (Task Force ESC & NASPE 1996) detail the quantification of HRV using statistical and geometrical methods in the time and frequency domains. HRV has been assessed in numerous ways, including both linear and non-linear methods, which are detailed below.

2.5.4.2.1 Linear time-domain methods

Time domain analysis gives a measure of the magnitude of HRV but little detail about the underlying rhythms which cause the variability, as the temporal sequence of individual RR intervals is lost during averaging. However, time domain indices are simple to calculate using statistics derived from the intervals between RR complexes on an ECG. For example, the standard deviation of all the RR intervals in a given period is referred to as SDNN. "NN" refers to normal-to-normal intervals, or adjacent beats originating at the SA node. SDNN is the square root of variance and as variance mathematically equates to the total power of spectral analysis, SDNN reflects all cyclic components responsible for variability over the recording period (Task Force ESC & NASPE 1996). SDNN is considered an estimate of overall HRV, encompassing sympathetic and parasympathetic influences (Piestrzeniewicz *et al.* 2008).

A further interval based measurement, SDANN, expresses the standard deviation of the average of RR intervals over segments (usually 5 minutes) of the total

monitoring period and is considered to represent variation resulting from circadian rhythms (Freeman *et al.* 2006). SDANN provides a "smoothed out" version of SDNN by measuring long term fluctuations (Bigger *et al.* 1988) which result from mainly sympathetic output (Piestrzeniewicz *et al.* 2008). SDANN is less subject to errors caused by artifact than SDNN as the averaging process minimises the effects of missed beats and ectopic complexity, hence it is much less affected by abnormal rhythm and may even permit risk stratification in atrial fibrillation (Kleiger *et al.* 2005). Further time domain measures include RMSSD, the square root of the mean squared differences of successive RR intervals, NN50, the number of interval differences of successive RR intervals >50ms and pNN50, the proportion derived by dividing NN50 by the total number of RR intervals (Task Force ESC & NASPE 1996). The latter three indices are generally thought to reflect vagal modulation of the SA node (Freeman *et al.* 2006).

2.5.4.2.2 Geometric methods

The Task Force guidelines (Task Force ESC & NASPE 1996) suggested several time domain geometric measures of HRV such as the HRV triangular index, the differential index and the Poincaré plot which is a method to assess complex non-linear behaviour in physiological signals. In HRV analysis it presents a graphical representation of temporal correlations within the RR intervals (Woo *et al.* 1992). It is a scatter plot of an RR interval time-series plotted against the same series delayed by one cardiac interval. In the Poincaré plot, the dispersion of points perpendicular to the line of identity (the "width" of the scatterplot) reflects the level of short-term variability (Brennan *et al.* 2002). This dispersion can be quantified by the standard deviation of the distances of the points from the line of identity and is equivalent to RMSSDNN. The standard deviation of points along the line of identity (the "length" of the scatterplot) reflects the standard deviation of the RR intervals (SDNN). A major advantage of Poincaré plots is their ability to

identify beat-to-beat cycles and patterns in data that are difficult to identify with spectral analysis (Brennan *et al.* 2002).

2.5.4.2.3 Further non-linear methods

Non-linear properties of RR time series have been investigated as linear statistical HRV measures do not describe complex dynamic RR interval time series (Meyer & Stiedl 2003). Non-linear measures make no assumption of uniformity (as required by linear descriptive statistical methods) and quantify dynamic aspects of heart rate control whilst maintaining the information on cardiac function (Komatsu *et al.* 1997). These non-linear methods differ from linear methods in that they measure the correlation properties of time series data, rather than their variability (Galaska *et al.* 2008). Such techniques have included measures of both the complexity or predictability of data (such as entropy, which indicates the logarithmic likelihood that the patterns of the data that are close to each other will remain close for the next comparison within a longer pattern (Khandoker *et al.* 2009)) and of the structure of data (such as self-similarity or scaling descriptions). For example, RR time series possess a strong $1/f^{\beta}$ type scaling component (Kobayashi & Musha 1982, Peng *et al.* 1995), suggesting that RR time-series have the property of self-similarity, which can be determined from fractal analysis (Mandelbrot 1982, Meyer *et al.* 1998a,1998b). A mono-fractal process is characterised by a single scaling exponent throughout its duration. Several methods exist for the estimation of fractal properties in data, including power spectral density analysis, detrended fluctuation analysis (DFA), rescaled range analysis, dispersional analysis, maximum likelihood estimation and scaled windowed variance methods.

Prior to selecting a fractal analysis method, an informed choice for the most appropriate descriptive model of the data under investigation is necessary, since each technique may perform differently for different model assumptions. The DFA technique introduced by Peng *et al.* (1995) yields a scaling exponent (α) that can be used to describe the correlation structure of data. In this way it is possible to distinguish between data that possess long-range power-law correlations ($0.5< \alpha \leq 1.0$) and those that have correlation structure but not in the form of a power-law ($\alpha > 1.0$). The specific cases of $\alpha = 0.5$, $\alpha = 1.0$ and $\alpha = 1.5$ indicate, respectively, data that are uncorrelated (white noise), data that have the correlation characteristics of 1/f noise, and data that have the correlation characteristics of Brown noise (the integral of white noise). Previous studies have shown that DFA can be used to estimate fractal properties in data assumed to follow either fractional Brownian motion (FBM) or fractional Gaussian noise (FGN) models (Eke *et al.* 2000,2002). DFA has an important advantage in that it avoids spurious detection of long-range correlations resulting from non-stationary conditions (Peng *et al.* 1995, Eke *et al.* 2000, Ivanov *et al.* 1999). It has also previously been asserted that the accuracy of estimating fractal properties depends on the length of the analysed data set, and that reliable determination requires at least 2^{12} data points (Eke *et al.* 2000,2002). However, Delignieres *et al.* (2006) showed that DFA (and most other techniques) provide acceptable estimates of the scaling exponent for both FBM and FGN data sets consisting of 128 or more points.

Several studies have associated the fractal characteristic of RR data with cardiac integrity, a lack of fractal structure being associated with pathology (Peng *et al.* 1995, Iyengar *et al.* 1996, Pikkujamsa *et al.* 1999, Perkiomaki *et al.* 2003). Investigations of the effects of physical exercise on the fractal properties of RR data have also shown that the mono-fractal scaling exponent is influenced by

physical stress (Tulppo *et al.* 2001, Hautala *et al.* 2003). The DFA α scaling exponents for RR data have been shown to have clinical utility. For example, Makikallio *et al.* (1998) found that, of all the HRV indices assessed in that study, α_1 (short-term scaling exponent) was the strongest independent predictor in differentiating between patients with coronary artery disease (CAD) and healthy subjects (α_1 being significantly greater in patients). Jokinen *et al.* (2001) noted that α_1 was more sensitive than traditional HRV measures in detecting age-related changes in HRV in patients with CAD. Recently Baumert *et al.* (2007) observed an increase in α prior to the onset of ventricular fibrillation in patients with severe congestive heart failure (CHF).

However, there is some evidence that RR time-series are too complex to be adequately described by a single scaling exponent. Consequently the "multi-fractal" analysis approach, introduced by Frisch & Parisi (1985) and Halsey *et al.* (1986), has been used to describe RR time-series in terms of multiple local scaling indices, with several authors associating changes in RR multi-fractality with cardiac pathology (Ivanov *et al.* 1999,2001).

To date there has been:

- *No determination of the influence of age and physical fitness on the sensitivity of the scaling factor to different exercise work loads*
- *No quantification of the temporal behaviour of the scaling factor during the post-exercise period*
- *No investigation of the fractal nature of the QT interval in the ECG*

Two common indices of entropy, namely approximate entropy (ApEn; Pincus 1991) and sample entropy (SampEn; Richman & Moorman 2000), can be used to

quantify signal complexity within short time segments (Fusheng *et al.* 1998). ApEn and SampEn both quantify the probability that sequences of patterns in a data set that are initially closely related remain close on the next incremental comparison within a specified tolerance, with lower entropy values indicating a more predictable, hence less complex time series. ApEn differs from SampEn in that its calculation involves counting a self-match for each sequence of patterns, and this leads to bias in ApEn (Pincus & Goldberger 1994, Pincus 1995). Consequently ApEn has two drawbacks: (1) it is a function of the length of the data set being analysed, yielding values lower than expected for short data sets (it suggests greater similarity within the data than actually exists); (2) it lacks relative consistency across differing testing conditions (i.e. for different parameters of the entropy index). Conversely, SampEn does not count self-matches and therefore has reduced bias. SampEn is also largely independent of record length and displays better relative consistency than ApEn (Richman & Moorman 2000).

Both ApEn and SampEn have been used to examine non-linear aspects of physiological time series data, including the RR interval derived from the ECG. Although the physiological interpretation of non-linear indices such as entropy is not completely understood, a reduction in non-linear characteristics has been associated with both cardiac and autonomic neural pathology. Previous studies have investigated the influence of various factors on the entropy (complexity) of RR time-series data, including the effects of age (Pikkujamsa *et al.* 1999, Platisa & Gal 2006b), gender (Ryan *et al.* 1994, Platisa & Gal 2006a), posture (Javorka *et al.* 2002, Vuksanovic & Gal 2005, Merati *et al.* 2006) and both cardiac and non-cardiac pathology (Jokinen *et al.* 2001, Merati *et al.* 2006, Platisa & Gal 2006b). There is therefore some evidence to suggest the utility of quantifying the entropy of cardiac interval time-series data to characterise and discriminate between different physiological and pathological states.

- *To date there have been very few investigations of the effects of exercise on RR entropy, and these have involved either single low-intensity workloads (Tulppo et al. 2001) or have quantified entropy only during the pre- and post-exercise periods (Javorka et al. 2002)*
- *The influence of exercise work load on RR time-series complexity is unknown, and estimation of the sensitivity of entropy to changes in work load would further the full characterisation of the mediators of HRV*
- *There has been no investigation of the entropy of the QT interval of the ECG*

2.5.4.2.4 Linear frequency domain methods

It is generally accepted that frequency domain processing of HRV signals may be of more use than time domain methods as information is provided on both the magnitude of variability and its specific distribution as a function of frequency. The frequency dependent manner in which the branches of the ANS influence heart rate fundamentally suggests the utility of power spectral density (PSD) analysis. It has been used extensively in the investigation of HRV and its underlying rhythms, along with other haemodynamic parameters since the 1970s (Hyndman *et al.* 1971, Sayers *et al.* 1973).

The PSD approach provides information on what the underlying HRV rhythms are, the physiological processes which they represent and the relative contribution of each underlying rhythm to heart rate. It is based on the spectral estimation methods of autoregression (AR) (parametric) or fast Fourier transformation (FFT) (non-parametric) which uses the notion that any periodic signal may be expressed as a sum of an infinite set of sine and cosine functions with different characteristic periods of oscillation and different weighting coefficients (Freeman *et al.* 2006). The advantage of the FFT-based methods is

their computational efficiency compared with AR methods, although the latter provides smoother and more easily interpretable spectral shapes (Task Force ESC & NASPE 1996).

Three major bandwidths of activity have generally been identified in the power spectrum of adult beat-to-beat heart rate data. The power of the signal over a frequency bandwidth is determined from integration of the PSD over that frequency band. The three major bandwidths are:

- Very low-frequency (VLF) < 0.04 Hz
- Low-frequency (LF) 0.04 - 0.15 Hz (long term variability)
- High-frequency (HF) 0.15 - 0.4 Hz (short term variability)

These have been described physiologically by Pagani *et al.* (1986) and Akselrod *et al.* (1981,1985). In addition, Yamamoto *et al.* (1991) identified HF components at frequencies above the Task Force's recommended HF bandwidth limit. These parasympathetically mediated HF components are probably of respiratory origin, possibly reflecting tidal volume modulation of heart rate, and could be either neurally or mechanically mediated (Lewis 2005).

VLF activity, which may only adequately be assessed by longer term measurement with large streams of uninterrupted data (Malliani 1999), is associated with thermoregulation (Sayers 1973), vasomotor activity (Kamath & Fallen 1993) and the renin-angiotensin system (Akselrod *et al.* 1981) and results from sympathetic discharge. For example, VLF might reflect the direct effect of temperature on pacemaker activity at the SA node and indirect effects mediated by the ANS altering heart rate and HRV. Raising the core body temperature from 36.0°C to 36.6°C can cause an increase in heart rate of the order of 40 beats/min in

males, whereas cooling the heart initiates bradycardia, a technique used in cardiac surgery (Stauss 2003) with the likely effect of a reduction of HRV with increasing heart rate and vice versa.

The origin of the variability exhibited in the LF band has two opposing viewpoints and has been the most contentious with regard to physiological interpretation (Malpas 2002): 1) that it reflects sympathetic and parasympathetic tone and 2) that it is generated by baroreceptor modulation of sympathetic and parasympathetic tone (section 2.6.3) and is therefore largely an index of baroreceptor gain (Malliani *et al.* 1991), although others (Houle & Billman 1999) have suggested that this is dependent on the experimental means used to augment the sympathetic activity. Both viewpoints accept that LF oscillation involve the action of the SNS on the vasculature. Workers believing LF oscillations reflect sympathetic tone have proposed a central oscillator model which details an apparent ability of the CNS to generate LF oscillations. Work supporting this central oscillator theory has not been widely endorsed. The dominant and accepted physiological theory is that involving the baroreflex feedback loop which results from baroreceptor action. However, Malpas (2002) stated that baroreflex activity alone cannot fully explain LF oscillations and suggested that other reflex pathways or CNS components involved with modulating sympathetic nervous activity may alter the LF component. Contentiously, Malpas (2002) concluded that the Task Force's (Task Force ESC & NASPE 1996) statement that a measure of autonomic balance (section 2.5.4.2.5) is obtainable from the LF/HF ratio appears incorrect.

HF activity is associated with respiratory modulation of HRV via both respiratory frequency and tidal volume influences (Hirsch & Bishop 1981, Bernadi *et al.* 1989, Yamamoto *et al.* 1991). The closed loop coupling between heart rate and

blood pressure has an input into HRV through respiratory sinus arrhythmia (RSA). RSA is an autonomic phenomenon, which may have a positive influence on gas exchange in the lung via efficient ventilation/perfusion matching, although the mechanisms responsible are not totally clear (Yasuma & Hayano 2004). RSA is pronounced in children and young adults, declines with age and can be absent in the elderly and those with disease (Hughson *et al.* 1995). Heart rate decreases during expiration (expirational bradycardia) and increases during inspiration (inspirational tachycardia) through influences on the flow of sympathetic and parasympathetic impulses to the SA node. Inspiration impedes vagus nerve activity leading to sympathetic dominance and this pattern is reversed on expiration (Jordan & Spyer 1987). The difference in RR interval duration between inspiration and expiration can be regarded as an indication of the magnitude of RSA (Eckberg 1983, Task Force ESC & NASPE 1996).

Reduced RSA has been demonstrated in pathophysiology including CHF (Saul *et al.* 1988), hypertension (Maver *et al.* 2004), weight gain (Arrone *et al.* 1995) and diabetes (Lindmark *et al.* 2003) although the causal relationship is unclear, with a similar situation existing in the RSA and hypertension relationship (Masi *et al.* 2007). The consistent associations found between autonomic dysregulation, indicated by reduced RSA, and a variety of cardiovascular risk factors and disease processes make RSA an important target for further investigation. However despite it being a commonly used variable in physiological research, the methods for its quantification are not standardised (Denver *et al.* 2007).

Inter-individual variations in the distribution of HRV power in resting humans have been partly explained by such factors as age, gender and exercise training status. Power in each of the standard frequency bandwidths has been found to be negatively correlated with age in both males and females (Ryan *et al.* 1994,

Jensen-Urstad *et al.* 1997). It has also been reported that the LF and total power components of HRV are greater in males (Jensen-Urstad *et al.* 1997, Ramaekers *et al.* 1998), but that the HF component is greater in females (Ryan *et al.* 1994).

In addition to the frequency bands detailed above, the Task Force (Task Force ESC & NASPE 1996) classified an ultra low frequency band (<0.00335Hz), the most prominent oscillation within this band of the PSD being the circadian rhythm, with the ANS contributing significantly to circadian HRV via the paraventricular nucleus of the hypothalamus (Stauss 2003).

To fully appreciate the temporal dynamics of the band limited frequency components of the PSD, combined time and frequency domain analysis and presentation is required. In its simplest form, this can be achieved by splitting the acquired physiological signal into multiple periods of short duration (for example of the order of one minute each). The PSD is then calculated for each section in order to construct a graphical representation of the temporal dynamics of the power distribution (Lewis 2005). Assessment of the regions in which this time-frequency representation shows high levels of power in each of the frequency bands provides information on the dominant mechanisms of heart rate control by the ANS. Analyses of this kind have indicated that there is a marked difference in the cardiac control methods apparent during periods of rest compared with during exercise. Figure 2.2 (a) shows an example of an RR and time-frequency plot and Figure 2.2 (b) shows the equivalent data as a PSD plot.

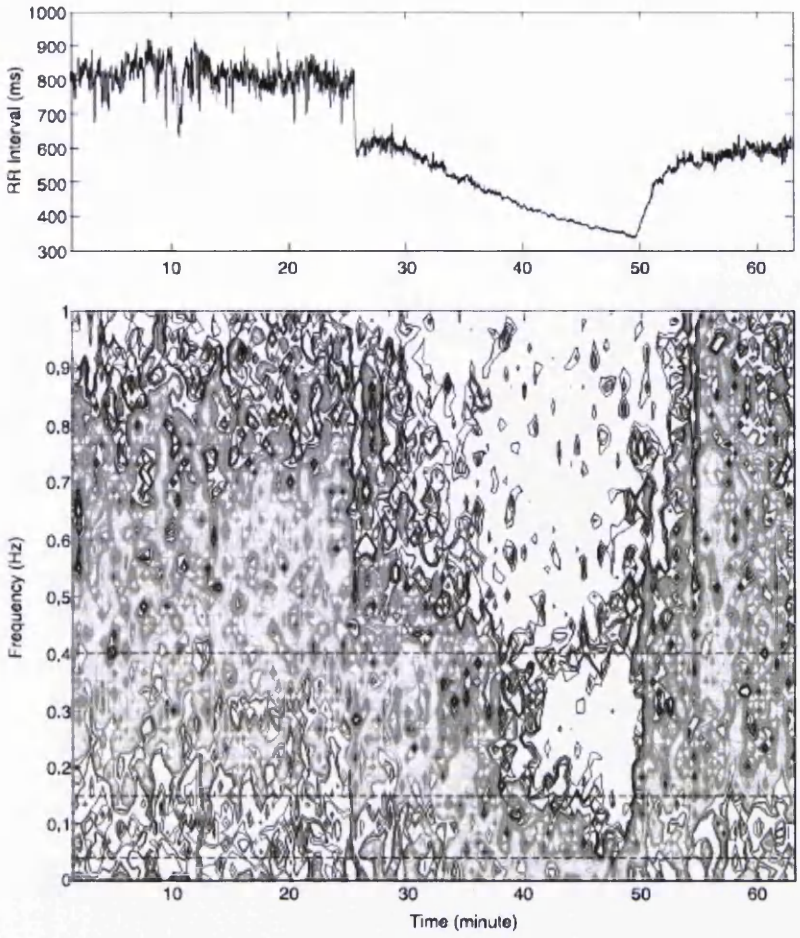


Figure 2.2 (a) Reproduced with permission from Lewis (2005)

The upper figure shows the RR interval for a healthy 21-year-old subject during rest (0-24 minutes), progressive cycling exercise to 85% age-predicted maximal heart rate (24-48 minutes) and post exercise recovery (48-63 minutes). The lower figure is a time-frequency spectrogram plot for the same subject over the same period as above. Signal power is indicated by greyscale, high power to low power dark grey to light grey. The horizontal lines delineate the three frequency bandwidths recommended for HRV analysis (Task Force ESC & NASPE 1996). During rest there is high power in each bandwidth with the LF component dominating. During exercise, LF and HF powers are progressively reduced and VLF becomes dominant. During recovery, power is restored to all three bandwidths but HF power remains below the resting value. An alternative to this, displaying averaged power distributions calculated during specified periods of

rest, high intensity exercise and recovery for the same subject is given in figure 2.2 (b).

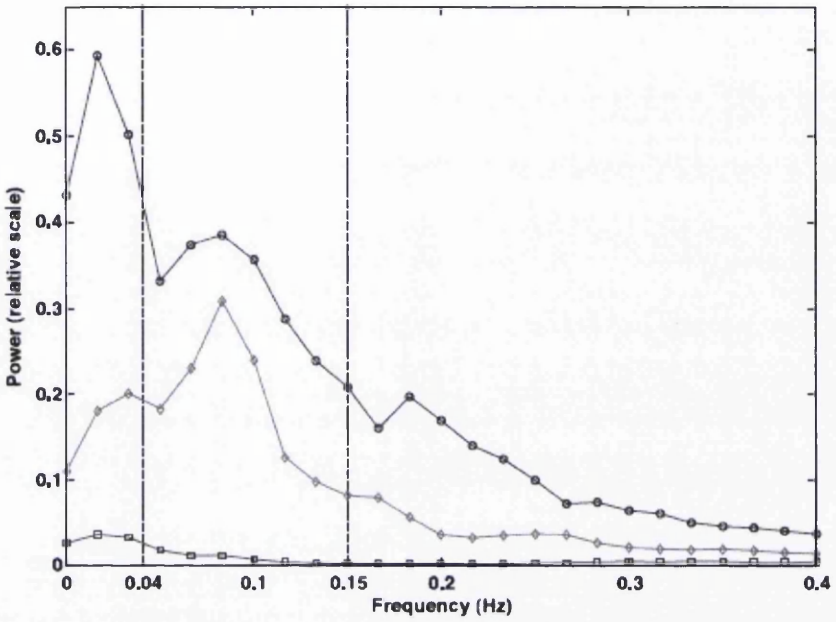


Figure 2.2 (b) reproduced with permission from Lewis (2005)

Figure 2.2 (b) is an alternative representation of the data presented in Figure 2.2 (a) for selected periods of rest (upper plot, circles), moderate to high intensity exercise (lower plot, squares) and subsequent recovery (middle plot, diamonds). Each plot represents the averaged power spectrum calculated for the following ten minute periods: Rest (1-10 minutes), exercise (30-40 minutes), recovery (50-60 minutes). A substantial reduction in power across the full range of frequencies during exercise may be seen and power continues to diminish during the recovery period. The VLF, LF and HF bands are indicated by the vertical lines.

2.5.4.2.5 The concept of sympathovagal balance

The complex interactions between the sympathetic and parasympathetic inputs to the SA node has led to the concept of "sympathovagal balance" reflecting the state which results from the dynamic interplay between the two ANS branches (Goldberger 1999). The dominant ANS branch at a particular time is largely determined by the physical and emotional states of an individual owing to the different physiological control processes evident at that time (Lewis 2005). Changes in LF band power are generally understood to reflect a combination of sympathetic and parasympathetic ANS outflow variations, whilst changes in the HF band power reflect vagal nerve modulation of cardiac output. The relative power of the LF and HF components of the power spectrum (i.e. the LF/HF power ratio) provides an accurate index of sympathovagal balance (Kitney 1984, Malliani *et al.* 1991, Ori *et al.* 1992).

Investigators in the field have opposing views regarding sympathovagal balance (Goldberger 1999). The concept of sympathovagal balance has been challenged by Eckberg (1997) as the reciprocity of the opposing branches of the ANS is not always present (for example, the case of the mammalian diving reflex whereby facial immersion in cold water initiates bradycardia and peripheral vasoconstriction) although it is largely felt that this is inapplicable to humans (Malliani 1999). Eckberg (1997) stated that calculations of sympathovagal balance may obscure rather than illuminate human physiology and pathophysiology.

A major deficiency of the LF/HF power ratio approach in the assessment of sympathovagal balance is that the method is linear and therefore does not account for the non-linearity of the ANS (Zhong *et al.* 2006). These workers introduced principal dynamic mode (PDM) analysis as a method of separating the

dynamic characteristics of the two branches of the ANS and reported that use of the traditional PSD method is less accurate for diagnosing dysautonomia compared with the PDM method.

As previously stated, altered levels of autonomic balance from normal values are associated with pathological conditions as well as aerobic training. For example, enhanced sympathetic and reduced vagal activities have been associated with hypertension (Pagani *et al.* 1986). Stressful conditions such as orthostatic tilt, Valsalva manoeuvre, mental stress or moderate physical exercise have been shown to alter the sympathovagal balance in favour of elevated sympathetic activity (increased LF activity, decreased HF activity), together with a reduction in total ANS activity (reduced overall power in the HRV spectra (Malliani *et al.* 1991)). However, several studies have, in fact, shown HF to gradually increase during exercise, whereas LF power decreases (Pichon *et al.* 2004). The HF and LF components provide measurement of the degree of autonomic modulation within these bands rather than absolute magnitude of autonomic tone (Task Force ESC & NAPSE 1996) which could account for the paradoxical reported increase in the HF component. Findings such as these have suggested that the interplay of multiple inputs to the heart need to be considered rather than autonomic balance in isolation. The physiological controversy surrounding sympathovagal balance is an indication of the extremely complex continuous modulation from numerous physiological processes acting at the SA node. Sympathovagal balance is a concept that currently has no definition and the most precise way to actually characterise it is unknown (Goldberger 1999).

The majority of frequency domain HRV investigations have focused on the detection of spectral peaks, in the belief that they reflect single regulatory cardiovascular control mechanisms. However, non-periodic variations appear on

the PSD as powers spread over a broadband frequency with "noise-like variability" (Braun *et al.* 1998). Therefore both periodic and non-periodic behaviour should be analysed, a methodological bridge possibly being Poincaré scatter plots (Japundžić-Žigon 2001). However, the circumstances under which non-linear HRV is applicable have not been fully elucidated (Stein & Reddy 2005) although these authors acknowledged that a combination of traditional and non-linear HRV may be optimal for risk stratification. They further suggested that future Holter systems should have more sensitive beat detection and classification algorithms to allow routine calculation of non-linear HRV for risk stratification in the clinical setting.

2.5.4.3 Technical procedures for HRV assessment

Assessment of HRV is based on the analysis of consecutive sinus rhythm RR intervals derived from either short term (2 to 5 minutes) or long term ECG recording (24 to 48 hours) (Gang & Malik 2003). In clinical applications long term recording methodology has been predominant whereas short term recordings can provide a practical and flexible assessment of HRV during non-steady-state conditions such as those occuring during normal ambulatory activity and postural manoeuvre or during physical exercise. Practically all modern Holter ECG recording systems have dedicated modules for the calculation of long and short-term HRV (Cerutti *et al.* 2006).

2.5.4.3.1 Influence and standardisation of postural manoeuvre

Postural change from supine to standing is used in clinical practice to elicit an autonomic shift from parasympathetic to sympathetic dominance in an attempt to examine pathophysiological influences on ANS function. For example, the head-up tilt test compares favourably with other diagnostic methods for the evaluation

of syncope (Mukherjee *et al.* 2006). The HRV response to changing posture is a more sensitive measure of ANS cardiac modulation than supine resting HRV in individuals with cardiac pathology as damage in this case results primarily from sympathetic impairment which can only be estimated with a stimulus (Carnethon *et al.* 2002). Orthostatic stress by passive graded head-up tilt reduces stroke volume through pooling of 500-1000 ml of blood in the lower extremities (Jacob *et al.* 1998) and activates the baroreflex (section 2.6.3) to maintain systemic blood pressure and cardiac output (Shamsuzzaman *et al.* 1998). Absence of this reflex may cause orthostatic hypotension and syncope (Freeman 2008) particularly in the elderly and in individuals with cardiovascular disease and is a classic manifestation of sympathetic vasoconstrictor failure.

HRV analysis for different body positions has been compared in supine and left and right recumbent positions (Ryan *et al.* 2003), supine and sitting positions (Sipinkova *et al.* 1997), supine and standing positions (Chen *et al.* 1999, Siebert *et al.* 2004) and supine, standing and head-up and head-down tilt positions (Lida *et al.* 1999). In healthy individuals, autonomic balance does not change significantly between different recumbent positions but is clearly different between supine and vertical postures (Watanabe *et al.* 2007). Also, compared with the supine position, the right lateral decubitus position can increase vagal modulation in the healthy and also in those with CAD and acute myocardial infarction (MI) (Yang *et al.* 2008) and this position induces the greatest increase in HRV complexity compared with supine and left lateral decubitus position (Kim *et al.* 2005). This has clear relevance for the need of standardisation of experimental protocols to avoid the confounding influences of postural variations between subjects.

2.5.4.3.2 Influence and standardisation of respiration

Several respiratory influences such as breathing frequency or tidal volume affect HRV indices (Badra *et al.* 2001, Brown *et al.* 1993, Sanderson *et al.* 1996) possibly confounding results unless controlled. Although HRV analysis conducted with spontaneous breathing could remain unaffected in qualitative terms, it is of interest to control all possible confounders (Tripathi 2004).

Spectral HRV analysis (section 2.5.4.2.4) assumes that HF oscillations result from RSA whereas LF oscillations are purely cardiovascular in origin (Malliani *et al.* 1991). This implies that lung-volume oscillation frequencies are within the HF band (Pinna *et al.* 2006) a condition which is not always apparent. For example, in some trained individuals, the breathing frequency drops into the LF band during rest (Strano *et al.* 1998). Also, in individuals with a regular HF band breathing pattern, the breathing frequency displays variability between and within individuals (Hirsch & Bishop 1981). This variability then would likely translate as a variability of HF spectral parameters (Pinna *et al.* 2006).

Voluntary respiratory control at frequencies ≥ 0.2 Hz has been advocated in an attempt to standardise short-term HRV analysis by avoiding spectral leakage of respiratory components into the LF band; this should result in attributing LF and HF spectral components to the correct physiological processes (Brown *et al.* 1993, Sanderson *et al.* 1996, Cooke *et al.* 1998, Strano *et al.* 1998). However, consensus on the use of controlled breathing protocols in the absence of definitive standardised guidelines has not been reached (Pinna *et al.* 2006).

2.5.4.3.3 Standardisation of water ingestion

Water ingestion has several effects on cardiovascular autonomic regulation (Brown *et al.* 2005). Drinking 500 ml of water augments sympathetic vasoconstrictor tone measured through muscle sympathetic nerve activity and plasma norepinephrine levels (Scott *et al.* 2001). In the healthy young adult, this sympathetic activation causes little or no change in blood pressure but results in a pressor response of around 11 mmHg in the healthy elderly (Jordan *et al.* 2000). In individuals with autonomic failure, drinking water can increase blood pressure by more than 30 mmHg for an hour or more (Jordan *et al.* 2000, Cariga & Mathias 2001). The exact mechanism of this pressor effect has caused controversy (Brown *et al.* 2005). Cariga & Mathias (2001) argued that it is not ANS reflex based owing to the time course involved, but Jordan *et al.* (2000) refuted this as autonomic blockade virtually eliminates the pressor effect. As well as sympathetic activation, water drinking apparently enhances parasympathetic tone in the healthy young, demonstrated by heart rate reduction and an increase in HRV (Routledge *et al.* 2002). These authors considered that an increase in parasympathetic tone buffers the pressor effect, a central mechanism absent in older subjects and those with autonomic failure.

Clinically, excess water ingestion may result in dangerous blood pressure elevation in individuals with supine hypertension or poor autonomic regulation (Jordan 2002). However, drinking water significantly improves orthostatic tolerance (Lu *et al.* 2003) and benefits individuals with orthostatic syncope (Shannon *et al.* 2002) within 15 minutes of drinking 500 ml of water (Schroeder *et al.* 2002). Dehydration however produces decreased orthostatic tolerance and resting tachycardia (Charkoudian *et al.* 2003). These authors also reported that mild exercise-induced dehydration causes an increase in resting heart rate and a decrease in baroreceptor sensitivity (section 2.6.3.1) which are potential

mechanisms for post-exercise orthostatic intolerance and tachycardia in healthy individuals. This has clear implications for the need for standardisation of water ingestion prior to the commencement of ANS functional testing.

2.5.4.3.4 Standard clinical assessment of cardiovascular autonomic function

Autonomic dysfunction may result from primary neurological disease such as pure autonomic failure or multiple system atrophy secondary to a wide range of diseases (Ducla-Soares *et al.* 2007). It is commonly assessed using tests for abnormalities in cardiovascular reflexes, although ANS integrity can also be tested in other systems including the gastrointestinal, urogenital, sudomotor and neuroendocrine systems (Ewing & Clarke 1986). However, tests in systems other than the cardiovascular are more complex (Ryder & Hardisty 1990). Cardiovascular tests involve measurement of heart rate or RR interval during the Valsalva manoeuvre (Levin 1966), deep breathing (Wheeler & Watkins 1973), normal breathing (Murray *et al.* 1975), standing up (Ewing *et al.* 1978), tilting from supine to upright (Sundkvist *et al.* 1980) and variation over 24-hours (Ewing *et al.* 1985). ANS assessment has also been described using the blood pressure response to standing up (Clarke *et al.* 1979), tilting from supine to upright (Sundkvist *et al.* 1980) and sustained hand grip (Ewing *et al.* 1974). Ewing *et al.* (1981) proposed a battery of five autonomic tests, the "Ewing battery". It consists of:

- Mean maximum/minimum ratio during three Valsalva manoeuvres (a test of parasympathetic function)
- Mean maximum-minimum heart rate difference during six deep breaths (a test of parasympathetic function)

- The 30/15 ratio after standing (ratio of the 30th RR interval to the 15th, upon standing there is a reflex tachycardia, maximal around the 15th beat which diminishes thereafter) (a test of parasympathetic function)
- The systolic blood pressure fall after standing (a test of sympathetic function)
- The diastolic blood pressure rise during sustained handgrip (a test of sympathetic function)

The use of this battery has allowed classification of autonomic dysfunction according to severity, patients being characterised according to the number of abnormal test results (Ryder & Hardisty 1990).

Following the work of Ewing *et al.* (1981), O'Brien *et al.* (1986) described the "O'Brien battery". This uses only one Valsalva manoeuvre and only one deep breath, reducing testing time and patient discomfort. Additionally, the O'Brien battery "normal" ranges are age-related, unlike the Ewing battery which was developed before the influence of aging on autonomic function was considered (Ryder & Hardisty 1990). Wieling (1987) stated however that the O'Brien battery provides much numerical information which is not supported by physiological interpretation. This view was supported by Ewing (1990) with the suggestion that the O'Brien battery contains physiological misconception. Whichever battery is used, the tests are commonly time consuming, require standardisation and patient compliance and are not always performable owing to an individual's clinical status (Khandoker *et al.* 2009).

2.6 Blood pressure and the cardiac cycle

Blood pressure is the pressure exerted by the circulating blood on the walls of blood vessels and is a consequence of cardiac contraction which forces the stroke volume (the ejection of blood from one ventricle in a single beat) into the aorta. This initial flow causes blood pressure which results from the inherent resistance of the vasculature. The pumping action of the heart together with total peripheral resistance of the blood vessels, maintains a continuous pressure difference between the arterial and venous sides of the vascular system, so allowing tissue perfusion. The relationship between mean blood pressure (BP), stroke volume (SV) and total peripheral resistance (TPR) is given by:

$$BP = SV \times HR \times TPR$$

TPR is an indicator of the existing state of vasoconstriction and vasodilation, predominantly controlled by sympathetic tone. Arterioles present the main resistance to the flow of blood. Sympathetic nerves, which control the action of arterial wall muscle fibre, maintain resistance at a level sufficient for high arterial blood pressure by constricting the channel of the arteriole. Arterial pressure is also affected by the chemical composition of the blood. Decreased oxygen or increased carbon dioxide partial pressures cause a reflex elevation of blood pressure. Respiratory activity is therefore an important regulator of arterial pressure. At each stage of the cardiac cycle, there is a synchronous fluctuation in blood pressure.

Variation in blood pressure is dependent on several inter-linked regulating mechanisms. Generally, positive chronotropic influences result in increased blood pressure, whilst negative chronotropic influences result in decreased blood pressure owing to the relationship between heart rate (HR) and cardiac output (CO).

$$CO = HR \times SV$$

Blood pressure is regulated via the vasomotor centre of the medulla oblongata. Output from the vasomotor centre changes the heart rate to return blood pressure to acceptable levels. The renin-angiotensin-aldosterone system also provides hormonal control of blood pressure through renin production causing increased circulating levels of angiotensin II. This leads to vasoconstriction and increased blood pressure and also to increased sodium, hence water retention in the blood.

The blood pressure regulatory mechanisms described above involves three physiological sensors as detailed below.

2.6.1 Mechanoreceptors

Mechanoreceptors are located in the left ventricle, right atrium and large veins and respond to force changes in cardiovascular walls. They act as physiological transducers, converting a physical variable into an electrochemical signal which is portrayed via changes in electrolyte and hormone concentrations.

2.6.2 Chemoreceptors

Chemoreceptors are located centrally in the medulla oblongata and in the periphery and respond to changes in the concentration of blood gases. Chemoreceptors, found in the carotid bodies, sense decreases in the oxygen content of the blood. Those found in the aortic bodies sense increases of carbon dioxide and hydrogen ion concentrations in the blood. Stimulation of these chemoreceptors causes an increase in respiratory rate which stimulates the vasomotor centre such that heart rate, cardiac output, and blood pressure are modified.

2.6.3 Baroreceptors

Baroreceptors are a specific type of mechanoreceptor located in the auricles of the heart and venae cavae, although those with highest sensitivity are found in the carotid sinuses and aortic arch. The carotid sinus baroreceptors are innervated by the glossopharyngeal nerve (cranial nerve IX); the aortic arch baroreceptors are innervated by the vagus nerve (cranial nerve X). Baroreceptor activity (neural response to stimulation) travels along these nerves to the medulla oblongata. Baroreceptors detect the pressure of blood flowing through them and regulate blood flow by signalling the need for an increase or decrease in TPR and CO hence providing an important short term neural control system for maintaining cardiovascular stability (Sato *et al.* 2005). This occurs via a negative feedback mechanism whereby blood pressure increases are detected and responded to through the inhibition of sympathetic and activation of parasympathetic signals from the cardiac control centre. Rising blood pressure initiates vascular stretching and baroreceptors are activated. Heart rate is reduced, peripheral vasculature is dilated and blood pressure is therefore decreased towards normal levels. Conversely, a drop in blood pressure causes a decrease in the number of signals received at the baroreceptors and thus a balancing increase in blood pressure and cardiac output occurs. The baroreceptors are also responsible for rapid adjustments in blood pressure as seen when one moves from a sitting to a standing position (section 2.5.4.3.1). During dynamic exercise, there exists the paradox of the requirement of elevated blood pressure and the blood pressure lowering function of the baroreceptor.

2.6.3.1 Baroreflex sensitivity

Cardiovascular baroreflex sensitivity (BRS) is the arterial baroreflex-mediated change in the RR interval per unit change in systolic blood pressure (Sagawa 1983). Reduced sensitivity of baroreflex control of heart rate has been used

widely as a marker for cardiovascular disease, being associated with an adverse prognosis. Intervention aimed at improving BRS including physical training may reduce the risk of onset of cardiac events (Parati 2005). Influences such as respiration and central neural mechanisms and the action of the renin-angiotensin system continually influence BRS (Lanfranchi & Somers 2002).

BRS has been shown to decrease with age and in the presence of hypertension, CAD, physical deconditioning and heart failure (Kardos *et al.* 2001). Diminished BRS has also been shown to commonly occur in conjunction with diabetes and provides a marker of poor prognosis following MI (Ryan *et al.* 2007). In hypertensive individuals, diminished BRS is associated with increased blood pressure variability (BPV) as the baroreflex is unable to induce the necessary cardiac and vascular adjustments in response to short term blood pressure fluctuations. Lenard *et al.* (2005) explored BRS in young healthy sedentary and endurance exercise trained subjects with and without a family history of hypertension. They found that reduced BRS characterises not only individuals with hypertension but also normotensive offspring of hypertensive individuals.

Dynamic exercise simultaneously increases arterial pressure and heart rate (Sala-Mercado *et al.* 2007) through a combination of sympathoexcitation and vagal withdrawal. The arterial baroreflex operating point is reset in relation to workload although the specific contribution of each branch of the ANS is unclear (Ogoh *et al.* 2005). These authors demonstrated that in the transition from rest to mild, moderate and heavy exercise workloads, the operating point of the baroreflex is progressively relocated to operate around the prevailing arterial pressure. This work verified previous observations (Bevegård & Shepherd 1966, Melcher & Donald 1981, Walgenbach & Donald 1983, Walgenbach & Shepherd 1984) that baroreceptor control of heart rate is maintained around an exercise

induced increase in heart rate. However, until the early 1990s, it was generally accepted that baroreflex control of blood pressure was lost during exercise to enable the parallel increase in heart rate (Raven *et al.* 2006). Many studies confirming baroreflex resetting have indicated that central command activation (a "feed-forward" mechanism resulting from the brain assessing future sympathetic activity requirements (Williamson *et al.* 2006)) or the exercise pressor reflex (generated by activation of mechanical and chemical skeletal muscle receptors) or both, facilitate the resetting (Iellamo *et al.* 1997, Norton *et al.* 1999, Ogoh *et al.* 2002, Gallagher *et al.* 2006).

Baroreceptor resetting in exercise mimics that in hypertension (Joyner 2006) and exercise therapy in the hypertensive can restore baroreflex control of heart rate and blood pressure to more normal values (Timmers *et al.* 2004).

2.6.3.2 Quantifying baroreceptor sensitivity

There are three main indices of BRS (Lanfranchi & Somers 2002). The first gives the degree of change in heart rate or sympathetic signal for a given change in blood pressure, quantified by the response of the cardiovascular system to the application of an external mechanical or pharmacological stimulus. The second is an alternative in-vivo approach which evaluates spontaneous baroreflex modulation of heart rate by identifying sequences of successive beats from the ECG which show progressive increases in systolic pressure being followed by a progressive lengthening of RR interval (or vice versa). The gradient of the regression line between systolic pressure and RR interval gives the magnitude of baroreflex gain. The third method is provided by cross-spectral analysis of systolic pressure and RR interval or of sympathetic nerve signal to muscle. This method relies on the fact that the low frequency band of the power spectrum (section

2.5.4.2.4) is modulated by the baroreflex and that RR interval and systolic pressure fluctuate at the same frequency.

The immense complexity of neural control mechanisms at times stops the baroreflex from overcoming non-baroreflex influences on blood pressure, hence occasionally systolic changes are not coupled with the reflex change in RR interval (Di Rienzo *et al.* 2001). These authors suggested a new approach to the quantification of baroreceptor function: the baroreflex effective index which quantifies the number of times the baroreflex has clearly driven the SA node, rather than BRS, which quantifies the power of the reflex when SA node drive has taken place. They reported that beat-to-beat RR interval changes are only induced by the baroreflex in response to 21% of systolic blood pressure changes owing to central inhibitory influences or to interference at SA node level by non-baroreflex mechanisms.

2.7 Applications of HRV analysis during physical activity

2.7.1 Introduction

Cardiovascular alterations to exercise constitute well coordinated responses throughout the body, with virtually every organ system doing more work than when at rest, whilst at times receiving impaired blood flow (Sheriff *et al.* 2006). Exercise therapy is now widely utilised owing to its effectiveness in the rehabilitation of individuals with cardiac disease and also lifestyle-related conditions such as diabetes mellitus and obesity (Ino-Oka *et al.* 2009). That aside, the risk of sudden death is dramatically increased during and immediately after exercise compared with sedentary periods (Mittleman & Siscovick 1996, Albert *et al.* 2000). Although heart rate alone may be a powerful tool in the assessment of autonomic tone (Lahiri *et al.* 2008), it provides no direct detailed information

regarding either the sympathetic or parasympathetic inputs. The profile of heart rate change during exercise and in the post-exercise recovery period however is recognised as having more prognostic significance than during rest (Jouven *et al.* 2005).

A lower resting heart rate is achieved largely through parasympathetic input from vagal tone in healthy individuals. The major physiological means of enhancing basal vagal tone and lowering resting heart rate is habitual dynamic exercise (Katona *et al.* 1982, Smith *et al.* 1989, Sugawara *et al.* 2001) resulting in increased cardiac electrical stability and protection against ventricular fibrillation (Hull *et al.* 1994). Conversely, impairment of vagal tone and elevated resting heart rate occurs with physical deconditioning (Wichi *et al.* 2009).

2.7.2 Cardiopulmonary exercise testing

Cardiopulmonary exercise testing (CPET) has become the most widely used method for initial evaluation of suspected coronary disease and its severity (Kligfield & Lauer 2006), being used diagnostically but more so prognostically. It provides assessment of the integrative exercise responses involving the pulmonary, cardiovascular and skeletal muscle systems which are not adequately reflected through the measurement of individual organ system function. Exercise capacity is a powerful predictor of mortality, being a superior prognostic marker compared with smoking history, hypertension or prior infarction (Maeder *et al.* 2005). An individual's exercise capacity however depends not only on age, gender and level of fitness, but also on the mode of exercise employed and the exercise protocol applied (Myers 1996). In North America, treadmill use is more popular, whereas in Europe, bicycle ergometer testing is more commonly used. Studies comparing the two modalities have generally revealed a higher peak oxygen

uptake ($\dot{V}O_{2max}$), higher maximal heart rate and better sensitivity in detecting coronary ischemia using the treadmill compared with the bicycle (Maeder *et al.* 2005). However, bicycle ergometry testing is often the preferred method owing to its reduced upper body motion which simplifies ECG and blood pressure recording (Myers 1996).

CPET use in patient management has increased with the understanding that resting pulmonary and cardiac function testing does not reliably predict exercise performance or functional capacity and that overall health status correlates better with exercise tolerance than with resting measurements (Albouaini *et al.* 2007). The clinical variables elicited during exercise testing are markers of general cardiovascular fitness and ANS function (an individual is considered to have a positive diagnosis if signs or symptoms of ischemia are displayed such as ST segment depression (Miller 2008)) these include (adapted from Miller 2008):

- Exercise duration
- Exercise hypotension (a fall in systolic blood pressure occurring at maximal workload)
- Exercise hypertension (an increase in blood pressure occurring at maximal workload)
- Chronotropic incompetence (failure to use 80% of heart rate reserve (De Sutter *et al.* 2006)
- Heart rate variability including the HF and LF components

Notably, the utility of CPET to evaluate ANS function and its modulation of the cardiovascular system has recently become recognised owing to the increasing evidence of its prognostic value (Freeman *et al.* 2006). Data regarding ANS

balance can be obtained through the evaluation of several discrete aspects of the cardiopulmonary exercise test including: the pre-test period, the heart rate response to dynamic exercise and heart rate recovery from the exercise test.

2.7.3 Heart rate increase at the onset of exercise

Generally it takes around one second to induce a 25% heart rate increase and in the following seconds, the acceleration increases further until it reaches a steady level within one-three minutes (Coote & Bothams 2001). Nobrega *et al.* (1990) investigated the heart rate response during the first 4 seconds of exercise onset and found that a relative tachycardia within this period was exclusively a result of vagal withdrawal independent of sympathetic activation, hence an exaggerated initial heart rate response to exercise could signal autonomic (specifically parasympathetic) dysfunction (Chaitman 2007). Leeper *et al.* (2007) demonstrated for the general population and for a subset of those subjects with documented CAD that a greater rise in heart rate early in treadmill exercise is associated with increased survival. Interestingly, Falcone *et al.* (2005) reported the converse in a similar population using a semi-supine bicycle ergometer protocol, as faster vagal withdrawal in response to dynamic exercise heightens the deleterious effect of sympathetic activation unopposed by vagal activity. Although the two studies arrived at different conclusions, both have value as the major difference between them was body position (Chaitman 2007). The increase in heart rate with exercise in the supine position may be more rapid than in the upright position as stroke volume contributes less to cardiac output compared with the upright position in which the increase is the result of augmented stroke volume and heart rate. The two studies had fundamental design differences in that the patient population considered by Leeper *et al.* (2007) were "considerably sicker" than those considered by Falcone *et al.* (2005), specifically in terms of autonomic dysfunction, smoking history and β-adrenergic blocker use. Chaitman (2007)

suggested however that in the clinical setting, rapid acceleration of heart rate in the early phases of exercise should not be used to predict cardiovascular outcomes until there is a clearer understanding of the autonomic mechanisms governing heart rate response during these periods in individuals both in the upright and supine positions and in medicated populations.

2.7.4 Heart rate during progressive exercise

During exercise, cardio-acceleration results from parasympathetic inhibition at low exercise intensities and from both parasympathetic inhibition and sympathetic activation at moderate intensities (Shepard 1987). The evaluation of maximal heart rate during CPET has been conducted in a variety of subjects, with and without cardiovascular disease. Vagal withdrawal with the onset of exercise can result in an initial increase in heart rate of 30 to 50 beats per minute, but further increases are considered to result from sympathetic activation. Clinically, the general "rule of thumb" is that the higher the heart rate reached during testing, the better the prognosis (Freeman *et al.* 2006).

Many factors affect maximal heart rate during dynamic exercise, including age, although a consistently poor correlation has been found in this relationship (Freeman *et al.* 2006). Stepwise multiple regression analysis performed by Londeree & Moeschberger (1984) on more than 23000 subjects from the literature revealed that age alone accounted for 75% of the variability in maximal heart rate and other factors including mode of exercise, level of aerobic fitness and country of origin added only an additional 5%. The authors found that heart rates at maximum work rate were lower when using bicycle ergometry as the exercise method compared with treadmill use and lower still with swimming. They also

confirmed that trained individuals generally have lower maximal heart rates than untrained subjects.

Jouven *et al.* (2005) tested the hypothesis that among apparently healthy individuals, SCD is likely to be associated with abnormal heart rate profiles during exercise and subsequent recovery. They used a bicycle ergometer protocol and found that the prognostic value of the absolute increase in heart rate was greater than that of either the resting heart rate or heart rate recovery, suggesting that altered neural control of cardiac function contributes to SCD. The authors suggested that autonomic imbalance may precede the onset of arrhythmia, aiding in the early identification of at-risk individuals.

2.7.5 Heart rate recovery

In general, initial heart rate recovery post-exercise is linked to inhibition of sympathetic activity and parasympathetic reactivation (Arai *et al.* 1989, Imai *et al.* 1994, Pierpont *et al.* 2000) although the specific timing of these events has not been fully examined (Suzuki *et al.* 2008). Slower changes in the stimuli to chemoreceptors and baroreceptors accompanying clearance of metabolites, along with delayed elimination of excess body heat and catecholamines are thought to be other contributory factors, although parasympathetic activation is considered the main mechanism of cardio-deceleration post exercise (Javorka *et al.* 2002). Kannankeril *et al.* (2004) analysed heart rate in healthy individuals during maximal exercise and subsequent recovery under normal physiological conditions as well as during selective parasympathetic blockade with atropine. They demonstrated that even at maximal exercise, the parasympathetic system has a small but significant effect on heart rate, with rapid reactivation upon cessation of exercise within the first minute post-exercise and a steady increase until 4

minutes into recovery at which time parasympathetic input to heart rate remains constant. Sundaram *et al.* (2004) considered how sympathetic withdrawal contributes to heart rate recovery following sub-maximal exercise. Subjects were analysed during normal physiological conditions along with selective β-adrenergic blockade with propranolol, selective parasympathetic blockade with atropine, or double blockade with both agents. These workers reported that early heart rate recovery following exercise is complex but predominantly a result of parasympathetic reactivation with sympathetic and non-autonomic components playing lesser roles.

Heart rate recovery post-exercise is becoming an increasingly important prognostic index (Cole *et al.* 1999, Shetler *et al.* 2001, Watanabe *et al.* 2001). Cole *et al.* (2000) showed that a delayed decrease in heart rate during the first minute post-exercise of less than 12 beats per minute following graded exercise is a powerful predictor of overall mortality regardless of achieved workload or changes in heart rate during exercise. The majority of studies (and all those discussed above) revealing an association between heart rate recovery in the first minute post exercise and clinical outcome have used treadmill exercise. The two studies assessing the prognostic value of heart rate recovery in the first minute after bicycle ergometer testing have provided conflicting conclusions. Notably, slower heart rate recovery in the first minute provides a strong predictor of SCD among apparently healthy men (Jouven *et al.* 2005), although Gaibazzi *et al.* (2004) failed to find a significant correlation between heart rate recovery and SCD. Further investigation into the physiological basis and prognostic significance of autonomic function during recovery may delineate the relationship between autonomic dysfunction, all-cause mortality and SCD (Lahiri *et al.* 2008).

2.7.6 Heart rate variability and exercise

The Task Force guidelines (Task Force ESC & NAPSE 1996) did not assess the impact of physical exercise on the physiological variables that directly affect HRV. However a number of studies have investigated the influence on HRV of exercise and subsequent recovery in the time and frequency domains and have also involved the use of pharmacological blockade, measurement of plasma norepinephrine concentration and muscle sympathetic activity (Sone *et al.* 2004). Improved physical training status has also been associated with increased HF power and, paradoxically, with either an increased or decreased LF power (Furlan *et al.* 1993, Janssen *et al.* 1993, Aubert *et al.* 2001, Verlinde *et al.* 2001). In addition, the application of non-linear dynamical analysis methods, such as quantification of sample entropy, can provide information about cardiovascular system control in exercise which is undetectable by conventional linear HRV (Javorka *et al.* 2002).

Equivocal findings have been reported from investigations comparing the distribution of HRV power during rest with that during dynamic exercise. Bernardi *et al.* (1990) and Rimoldi *et al.* (1992) reported an increase in LF power and a decrease in HF power during low intensity exercise, both studies therefore implying an increase in the LF/HF ratio during low intensity exercise, a trend similarly described by Tulppo *et al.* (2001) and Pichon *et al.* (2004). Casadei *et al.* (1995) and Perini *et al.* (1990) however described reductions in LF power at higher exercise intensities, whilst at yet higher exercise intensities, Arai *et al.* (1989) and Bernardi *et al.* (1989) documented increased HF power. Variations in both the mode and the relative intensity of exercise employed in these studies have made it difficult to reliably compare their results and to re-iterate, the LF and HF components indicate rates of sympathetic and parasympathetic modulation rather than reflecting the magnitude of their tone. Additionally there

exists the potential confounder of non-normalisation. Measurement of VLF, LF and HF power components is usually made in absolute values of power (ms^2), but LF and HF may be measured in normalised units (Pagani *et al.* 1986, Malliani *et al.* 1991) which represent the relative value of each power component in proportion to the total power minus the VLF component (Task Force ESC & NASPE 1996).

There is no universally accepted pattern of change of HRV during exercise. This is largely owing to between-study differences in subject characteristics such as age and physical fitness, data analysis methods and data accuracy resulting from the use of different experimental apparatus. However, the general consensus is that values for the LF, HF and total power components of HRV at high work rates are diminished, compared with during rest and low work rates (Yamamoto *et al.* 1991, Polanczyk *et al.* 1998, Hautala *et al.* 2003, Cottin *et al.* 2004). Some authors have also reported gradual reductions in LF and HF powers during progressive maximal exercise, these trends appearing to be exponential in nature (Rimoldi *et al.* 1992, Tulppo *et al.* 1996, Perini *et al.* 2000, Lewis & Kingsley 2003) and interpreted as representations of progressive and substantial withdrawal of ANS control.

2.7.7 Influence of respiratory sinus arrhythmia on HRV during dynamic exercise

Modulation of SA node activity via respiratory frequency and respiratory tidal volume is the main mechanism influencing the HF component of HRV. However, accurate data regarding RSA action during progressive exercise are scarce owing to the lack of stationarity and the difficulty in controlling breathing patterns encountered during exercise (Blain *et al.* 2005). The central frequency of RSA in HRV analysis has been shown to correlate strongly with respiratory frequency

(Bernardi *et al.* 1989) and increases linearly with metabolic demand (Perini *et al.* 1998). Unsurprisingly, several authors have identified HRV components at frequencies exceeding the Task Force's recommended HF bandwidth (0.15 to 0.4 Hz) during exercise (Cottin *et al.* 2004, Nakamura *et al.* 1993, Yamamoto *et al.* 1991, Warren *et al.* 1997). Consequently these authors chose to reclassify the HF bandwidth used in their studies, using one of several alternative bandwidths (0.15-0.8 Hz, 0.1-1.0 Hz, 0.15-1.0 Hz or 0.15-1.5 Hz). Moreover, Blain *et al.* (2005) found that RSA occurred throughout exercise, and was greatest during the highest work rates. These authors also observed that the amplitude of RSA did not differ when ventilation was kept constant for differing respiratory frequency and tidal volume combinations, and suggested that this implied a mechanical process for RSA. These results provide a clear rationale for extending the HF bandwidth during exercise to include the underlying respiratory frequency: failing to do this would exclude at least part of the influence of RSA on the HF component of HRV. Nevertheless, and in the absence of specific guidelines, most studies continue to report HRV parameters (including the commonly compared LF/HF ratio) using the Task Force's restricted HF bandwidth.

2.7.8 QT interval during and after exercise

The physiology of autonomic effects on QT interval dynamics following exercise is not well understood owing to the changing autonomic profile that occurs during exercise (Sundaram *et al.* 2008). However, it is generally accepted that the QT interval shortens with progressive exercise (Sarma *et al.* 1988, Roukema *et al.* 1998, Rajappan *et al.* 2003) this being observed throughout a wide range of heart rate and involving heart rate dependent and independent QT modulation (Porta *et al.* 1998, Almeida *et al.* 2006). However, rate correction imprecision, particularly at high exercise intensities has precluded a fundamental understanding of the autonomic effects of exercise and recovery on the QT

interval (Sundaram *et al.* 2008). It is notable that QT interval evaluation during exercise is subject to several methodological problems: noise and baseline drift make the determination of the end of the T wave difficult and fusion of the end of the T wave with the subsequent P wave further obscures the end of cardiac repolarisation (Kligfield & Lauer 2006). Limitations aside, several studies have suggested that lengthening of the rate-corrected QT interval with exercise identifies myocardial ischemia (Elgoff *et al.* 1987, Arab *et al.* 2000, Watanabe *et al.* 2000) particularly in individuals with exercise related ventricular arrhythmias (Cuomo *et al.* 1989).

To date, there has been:

1. *No comparison of QT and its variability between different leads of the ECG system*
2. *No simultaneous analysis of QT and RR variability during exercise*
3. *No assessment of the relationship between these parameters and ventricular function*
4. *No previous study which has quantified the influence of ECG electrode placement on QT-RR hysteresis*
5. *No investigation of the entropy of the QT interval during different physiological conditions (and different exercise workloads)*
6. *No examination of the relationships between sample entropy and traditional HRV parameters and no comparison of the ability of these parameters to distinguish between physiological states*

2.8 Current research trends and priorities

Whilst the initial focus of HRV application was the prediction of mortality in post-MI patients, or those with valvular or congestive heart disease, the major clinical focus of HRV research now is the prediction of the onset of fatal ventricular arrhythmias (Kleiger *et al.* 2005). Recent advances in HRV analysis have come about through the use of more sophisticated signal processing of beat-to-beat cardiovascular variability. A variety of data analysis algorithms and tools, such as wavelet analysis and methods of non-linear analysis, together with improved processing power, have substantially improved both ECG signal acquisition and its temporal and morphological analysis in recent years. Emphasis now needs to be given to bridging the two areas of HRV signal processing and physiological modelling (Cerutti *et al.* 2006) in order to simplify aspects of multi-system dynamics to provide new avenues for innovative interpretations and explorations. A major continuing challenge is also to demonstrate the utility and clinical implications of specific measures of HRV in diagnosis and monitoring so that such measures become part of routine patient care. For example, HRV may be clinically useful in the functional assessment of cardiac autonomic regulation in critical care where HRV can aid diagnosis and prognosis and inform treatment strategy (Lewis 2005). HRV, used as a clinical tool, is likely to become increasingly common in future as a result of the more widespread implementation of HRV analysis facilities into clinical monitoring and informatics systems.

Although there is a significant body of evidence demonstrating an association between endurance exercise training and HRV (Al-Ani *et al.* 1996, Pichot *et al.* 2000, Iellamo *et al.* 2002, Iwaski *et al.* 2003, Tulppo *et al.* 2003, Hautala *et al.* 2004, Kiviniemi *et al.* 2006) the use of HRV measurement as a tool for adjusting endurance training programmes has not been widely assessed. Kiviniemi *et al.*

(2007) however, through HF power assessment, suggested that improved cardiorespiratory fitness can be achieved by using daily HRV measurement for exercise prescription when compared with a standardised training regime. The challenge remains to individually define the training stimulus that produces optimum metabolic and structural changes in the cardiovascular and neuromuscular systems without compromising recovery (Hautala *et al.* 2009).

The recent understanding that HRV may in fact be representative of complex phenomena which reflects the non-linear fluctuations of cardiac-autonomic outflow in a fractal or entropic or chaotic manner requires further investigation. Wu *et al.* (2009) suggested that definitive testing of these divergent characterisations is the key to unravelling the physiological mechanisms of HRV, critical for its effective use as a non-invasive marker for cardiac risk assessment and stratification.

Several challenges remain in the development of a more complete understanding of the mechanisms of cardiovascular control during physical activity, and the influences thereon of physical training. In this regard it has become apparent that a comprehensive understanding of ANS changes during exercise and recovery requires a multimodal approach. Only in recent years has this been achievable through the introduction of more advanced signal processing and numerical analysis techniques and the introduction of advanced haemodynamic measurement systems such as the TFM. To reiterate section 2.3.3.2, the TFM allows non-invasive continuous measurement of blood pressure via the vascular unloading technique and beat-to-beat stroke volume measurement with ICG (Fortin *et al.* 1998). These beat-to-beat data also enable real-time calculation of HRV, BPV, BRS, systemic vascular resistance and measures of cardiac ventricular performance such as ejection fraction. Measurement of these parameters

synchronously with indices HRV during rest, quantified exercise and subsequent recovery, is likely to be the best strategy in the quest to understand the intricacies of physiological control. Such information will be of value in assessing the influences of acute exercise and of physical training on cardiovascular regulation. Furthermore it would provide a comparative model to help elucidate pathophysiological changes in a variety of cardiac and systemic disorders, thereby integrating our understanding of autonomically-mediated physiological control mechanisms, and the most appropriate quantitative methods for their assessment. This will be an important translational step towards establishing cardiovascular autonomic function testing as a clinically useful tool.

It is reasonable to postulate that progress towards this goal will be expedited through quantification of:

- *The QT-RR (hysteresis) relationship during exercise*
- *The influence of ECG electrode placement on RR and QT, their variabilities and the QT-RR hysteresis relationship*
- *An analysis of the ability of sample entropy to discriminate between different physiological states (and different exercise workloads)*
- *The relationships between sample entropy and traditional HRV parameters*
- *A determination of the influence of age and physical fitness on the sensitivity of the DFA scaling factor to different exercise work loads*
- *A quantification of the temporal behaviour of the DFA scaling factor during the post-exercise period*
- *An investigation of the fractal nature of the QT interval in the ECG*
- *The relationship between indices of HRV and QTV and clinical measures of cardiac function (in particular ventricular function)*
- *The effects of gender and individual exercise work capacity on the above*

Chapter 3 Investigating the relationship between QT and RR time-series during rest and exercise

3.1 Rationale

Multi-lead ambulatory ECG recording offers recognised advantages for ventricular QT interval analysis, especially during physical exercise. However, data are usually reported for a single lead and do not quantify between-lead variability, leading to possible misinterpretation. There may then be significant between-lead and between-gender QT interval differences when reporting results from a multi-lead ambulatory ECG under conditions of physical stress. Therefore, the magnitude of the lag in QT interval adaptation to recovering heart rate following exercise (which has been described as QT-RR hysteresis (Ahnve & Vallin 1982)) may also be dependent on accurate ECG electrode placement. No previous studies have quantified the influence of ECG electrode placement on hysteresis following physical exercise.

3.2 Aims and objectives

The aims of this chapter were:

1. To assess the between-lead agreement for QT and RR intervals obtained from a commercial three-lead ambulatory ECG system
2. To characterise QT-RR hysteresis using this ECG system during physical exercise within a range of low to high intensities and during subsequent recovery

The objectives of the studies were:

1. To quantify between-lead agreement for QT and RR intervals during conditions of rest, physical exercise and subsequent recovery using a Bland-Altman analysis
2. To determine the influence of gender on between-lead agreement for these parameters
3. To quantify the magnitude and temporal variation of QT-RR hysteresis using standardised electrode locations
4. To quantify between-lead differences in QT-RR hysteresis
5. To quantify the influence of gender on QT-RR hysteresis

3.3 Methodology

All physiological testing and measurements were performed in the Exercise Physiology Laboratory at the Sport and Exercise Science Research Centre, Swansea University and the investigation was approved by the departmental ethics committee. Subject health screening was undertaken using the American Heart Association/American College of Sports Medicine pre-participation screening questionnaire (Balady *et al.* 1998). All subjects were apparently healthy non-smokers and were physically active to a similar level. Subjects were free of cardiovascular and chronic respiratory problems (including asthma), had no history of sleep apnoea, central or peripheral nervous system disorder, and were not taking any medication at the time of investigation. All subjects were informed in writing about the demands of the study, and subsequently gave their written informed consent for participation prior to commencing the study. Subjects were not permitted to consume tea, coffee, alcohol or a heavy meal within two hours prior to physiological assessment, and no physical exercise was permitted within the 24 hour period prior to assessment.

The preliminary and main study populations used for the work of this chapter are both subsets of a main subject group which underwent an identical testing protocol. The subjects were investigated continuously during a progressive exercise test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport; Lode, Netherlands). The bicycle test was chosen in order to allow simple quantification of exercise workload, to diminish movement artifact through improved upper body stability compared with treadmill use and to maximise subject comfort and safety in line with the American Thoracic Society Statement on Cardiopulmonary Exercise Testing (ATS 2003). Following the recommendations of this statement, a sub-maximal incremental protocol was employed. The work rate began at 60 W for 5 min in order to achieve standardised baseline conditions and thereafter increased in 30 W increments every 3 minutes until the subjects reached 85% of their age-predicted (220-age (years)) maximal heart rate. This endpoint was chosen in an attempt to keep subjects below the anaerobic threshold.

Subjects were instructed to pedal at a constant cadence of 60 rpm, although a further advantage of using an electromagnetically braked ergometer was that fluctuations in cadence were automatically adjusted for workload by the ergometer, thereby providing power output control, this having particular importance when subjects became fatigued. RR interval (Reynolds Holter recorder; Del Mar Reynolds Medical Ltd., UK), breath-by-breath respiratory frequency (f_B), minute ventilation (the volume of gas inhaled (or exhaled) in one minute) ($\dot{V}_E$) and oxygen consumption ($\dot{V}O_2$) (Oxycon Pro; Jaeger, Germany) were recorded simultaneously. Prior to testing, subjects underwent baseline spirometric assessment of the form:

- Forced expiratory volume in one second (FEV_1), the largest volume of gas forcibly exhaled in one second
- Forced vital capacity (FVC), the largest amount of gas forcibly exhaled following maximal inspiration
- Maximum voluntary ventilation (MVV), the maximum amount of gas which could be exhaled and inhaled in one minute

During testing the Category Ratio (CR)-10 scale adapted from Borg (1982) was used at regular intervals in order to assess subjects' perceived exertion. Prior to and immediately post exercise, pulse oximetry was conducted (Nonin 8500 digital handheld pulse oximeter; Nonin Inc., USA) in order to screen for possible hypoventilation. Following the cessation of exercise subjects stood for 10 minutes recovery. The following is a step-by-step description of the experimental process undertaken for the assessment of each subject:

- Calibration of the Oxycon Pro gas analysis system
- Mass and height of the subject measured
- Lode ergometer set correctly (saddle height and handlebar reach)
- 85% of age predicted heart rate calculated
- Baseline spirometry characterisation undertaken
- Oxycon Pro and Lode ergometer control systems programmed for a continuous ramped protocol with recovery period
- Reynolds Holter recorder attached to the subject and recording started
- Subject settled comfortably on the Lode ergometer to sit for 15 minutes of rest investigation prior to exercise
- Pulse oximetry reading taken
- Oxycon Pro gas analysis started

- Oxycon Pro and Reynolds systems synchronised by the manual insertion of "markers" at the beginning of exercise
- Following 5 minutes of exercise "markers" inserted to the Oxycon Pro and Reynolds systems to label the transition to the higher work rate
- The previous step repeated at every 3 minute stage of the protocol until the subject reached 85% of age predicted maximum heart rate
- The subject shown the CR-10 perception scale at each exercise work rate
- At 85% of age predicted maximum heart rate the subject is removed from the Lode ergometer and the Oxycon Pro system is manually "forwarded" to signify the recovery period
- The subject stands for 10 minutes post exercise recovery

A subject undergoing testing is illustrated in Figure 3.1.

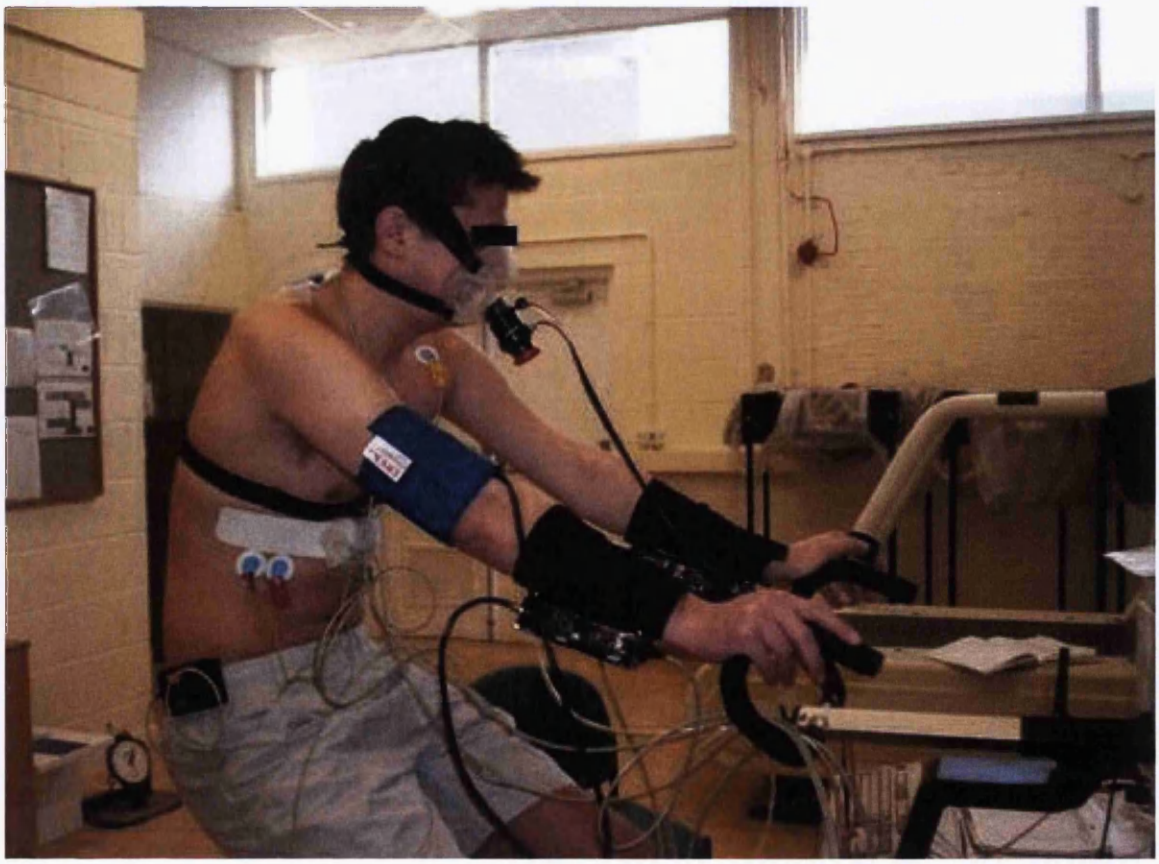


Figure 3.1 A subject undergoing testing

3.3.1 Preliminary study subject characteristics

Nine males (age 23.6 ± 4.7 years, mass 85.7 ± 8.5 kg (mean ± SD)) and eight females (age 21.9 ± 4.8 years, mass 64.0 ± 4.2 kg) volunteered to take part in the investigation. Individual maximal oxygen uptake ($\dot{V}O_{2max}$) values confirmed the similarity of aerobic fitness within both groups: males (46.7 ± 5.2 $ml \cdot kg^{-1} \cdot min^{-1}$) and females (40.1 ± 8.8 $ml \cdot kg^{-1} \cdot min^{-1}$). Work capacity ($WR_{max}$), estimated from the relationship between work rate and heart rate and extrapolating to maximum heart rate (HR_{max}), were: 289 ± 46 W (males) and 195 ± 45 W (females) (mean ± SD).

3.3.2 Main study subject characteristics

Six males (age 23.5 ± 4.3 years, mass 87.5 ± 8.8 kg (mean ± SD)) and six females (age 22.2 ± 5.3 years, mass 64.8 ± 4.7 kg) volunteered to take part in the investigation. Individual maximal oxygen uptake ($\dot{V}O_{2max}$) values confirmed the similarity of aerobic fitness within both groups: 46.4 ± 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$ (males) and 39.4 ± 7.9 $ml \cdot kg^{-1} \cdot min^{-1}$ (females). WR_{max}, estimated from the relationship between work rate and heart rate and extrapolating to HR_{max}, were: 295 ± 52 W (males) and 202 ± 43 W (females) (mean ± SD).

3.4 Physiological measurement

3.4.1 Apparatus

A Reynolds Lifecard CF digital Holter recorder (Del Mar Reynolds Medical Ltd., UK) was used to record a three-lead ECG throughout exercise and for 10 minute periods pre- and post-exercise. The ECG leads were positioned in the modified V5, CC5, modified V5R electrode configuration (Figure 3.2).

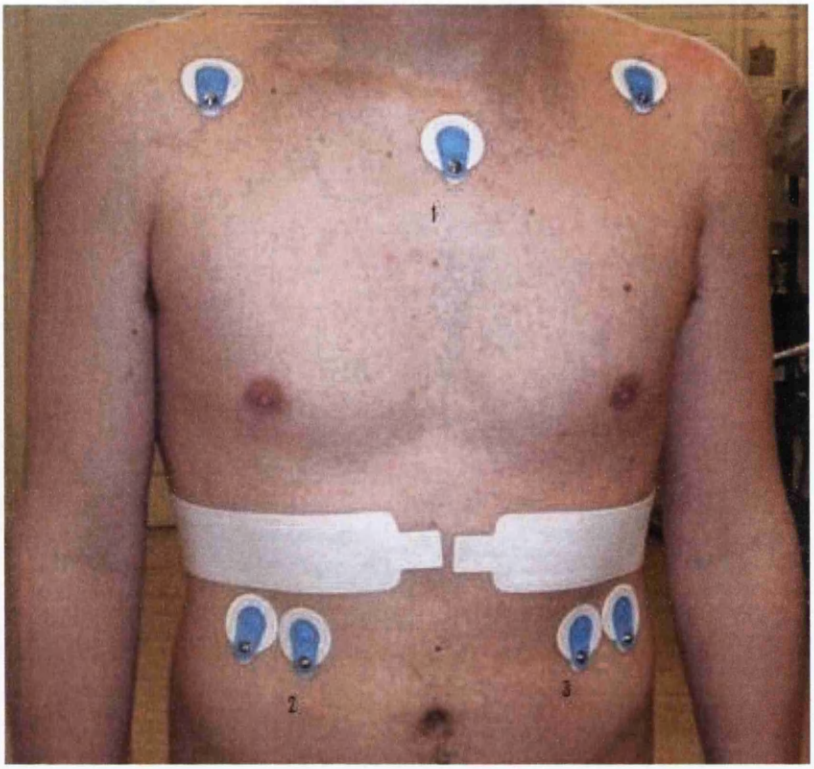


Figure 3.2 A subject with electrodes 1-3 positioned in the modified V5, CC5, modified V5R configuration

This system provided ECG data with a sample accuracy of 2.5 μV (magnitude of least significant bit; 12-bit resolution) and 128 Hz sampling frequency. Subsequently, each lead was separately analysed using a Reynolds Pathfinder digital analyser with Reynolds Research Tools software (Del Mar Reynolds Medical Ltd., UK). All ECG recordings used for subsequent analysis in these studies were free of any form of morphologically abnormal beat, and this was verified by both the Holter system and by human observation prior to subsequent analysis. When either the RR or QT intervals were considered to be anomalous, both the RR and QT data points were removed from the data set. This occurred infrequently (and mainly during exercise), resulting in fewer than 1% of the data being removed.

All QT analysis in this chapter was based on the QT_a interval (Q wave onset to T wave apex). Preliminary observations prior to beginning the study showed that the apex of the T wave (T_a) was more reliably measurable by the Holter system's detection algorithm compared with the end point of the T wave (T_e). This was evident as exercise progressed to higher intensities, where the elevated heart rates made it particularly problematic to detect T_e, but T_a could still be reliably detected. (Subsequent studies assessing the reliability of T_a as an estimate of the ventricular depolarisation-repolarisation phase are presented in chapter 5). Only sinus rhythm beats were considered in these analyses.

3.5 Data processing

3.5.1 Preliminary study

Mean values within consecutive one-minute windows were calculated for both QT and RR data sets for all three ECG leads. Subsets of the averaged QT and RR data sets were defined that represented these data within specified intervals during exercise and during the pre- and post-exercise phases:

1. Consecutive one-minute intervals during the pre-exercise resting phase, which lasted for 10-minutes
2. The final one-minute period (in order to represent physiologically steady-state conditions) of each exercise stage
3. Consecutive one-minute intervals during the post-exercise resting phase, which lasted for 10-minutes

Single pre-exercise values for RR and QT were then calculated for each of the three leads, using the mean of the ten one-minute averages during this period in

order to obtain representative values for pre-exercise rest. These were considered appropriate values as there was no substantial physiological variation during this time. The method described by Bland & Altman (1999) was used to quantify the between-lead bias and associated limits of agreement (LOA) for the RR and QT data recorded for each pair of ECG leads. Mean values of between-lead difference (bias) and the corresponding LOA (bias $\pm 2 \times$ SD of between-lead differences) were calculated for the whole pre-exercise interval, for the final one-minute interval of each exercise stage, and for consecutive one-minute intervals during recovery. Composite paired-lead values that represented the mean bias and associated LOA for the whole experimental period were also calculated.

The Lilliefors test for goodness of fit to a Normal distribution confirmed that the data samples were Normally distributed and therefore the suitability of the selected parametric statistical tests. Student's two-sample t-tests for unequal variance (heteroscedastic data) were used to assess between-lead and between-gender differences in the data at each stage of the experimental protocol. All data quoted in the text represent mean ± SD. Error bars in the figures represent the SEM (standard error in the mean) for the plotted parameters.

3.5.2 Main study

The non-uniformly sampled QT and RR data sets were linearly interpolated with a sampling frequency of 2 Hz to provide data that were uniformly sampled in the time-domain (sampling interval 0.5 seconds, this made it computationally easier to accurately measure time intervals in the analysis). Mean values were then calculated for consecutive non-overlapping one-minute windows as described in section 3.5.1, for both QT and RR data sets, separately for all three ECG leads.

The one-minute mean RR data were next re-sampled to provide a reference RR data set with sampling intervals of 10 ms (to improve RR resolution and thus the accuracy of the subsequent algorithm used in this analysis). This procedure was performed separately for the rest/exercise and the post-exercise recovery periods to assist in computational efficiency. The corresponding QT data were then linearly interpolated as a function of the uniformly sampled RR data. This allowed the determination of RR and QT within consecutive one-minute post-exercise time intervals, as well as the QT values at the same matched RR values during the rest/exercise periods. This enabled the calculation of QT-RR hysteresis, defined as the difference in magnitude of QT for the same value of RR during the rest/exercise and post-exercise recovery periods. A positive value of hysteresis was defined as a shortened post-exercise QT relative to that during the exercise or pre-exercise periods (i.e. a positive hysteresis value indicated a lagging QT recovery towards pre-exercise values).

The Lilliefors test for goodness of fit to a Normal distribution confirmed that the data samples were Normally distributed and therefore confirmed the suitability of the selected parametric statistical tests. One way analysis of variance (ANOVA) was used to compare the magnitudes of hysteresis calculated at each stage of the post-exercise recovery period. Multiple pairwise comparisons were performed for these data using Tukey's honestly significant difference criterion, and differences were considered significant for $p<0.05$. Student's two-sample t-tests for unequal variance (heteroscedastic data) were used to assess between-lead and between-gender differences in the data at each stage. All data quoted in the text represent mean ± SD.

3.6 Results

3.6.1 Preliminary study

Owing to the difficulty of measuring QT interval during the elevated heart rates associated with moderate- to high-intensity exercise, QT data were available only for exercise intensities up to 68 ± 8% of WR_{max} in males and up to 75 ± 10% of WR_{max} in females (equivalent to mean heart rates of 152 ± 9 bpm in males and 157 ± 4 bpm in females).

3.6.1.1 Between-lead agreement for RR and QT data

There were no significant differences in the RR values obtained from the three leads at any stage of the protocol for either males or females. In contrast, there were significant ($p<0.05$) differences in the QT interval values obtained from the different leads during exercise and recovery periods in both males and females (Figures 3.3 (a) & 3.3 (b) respectively).

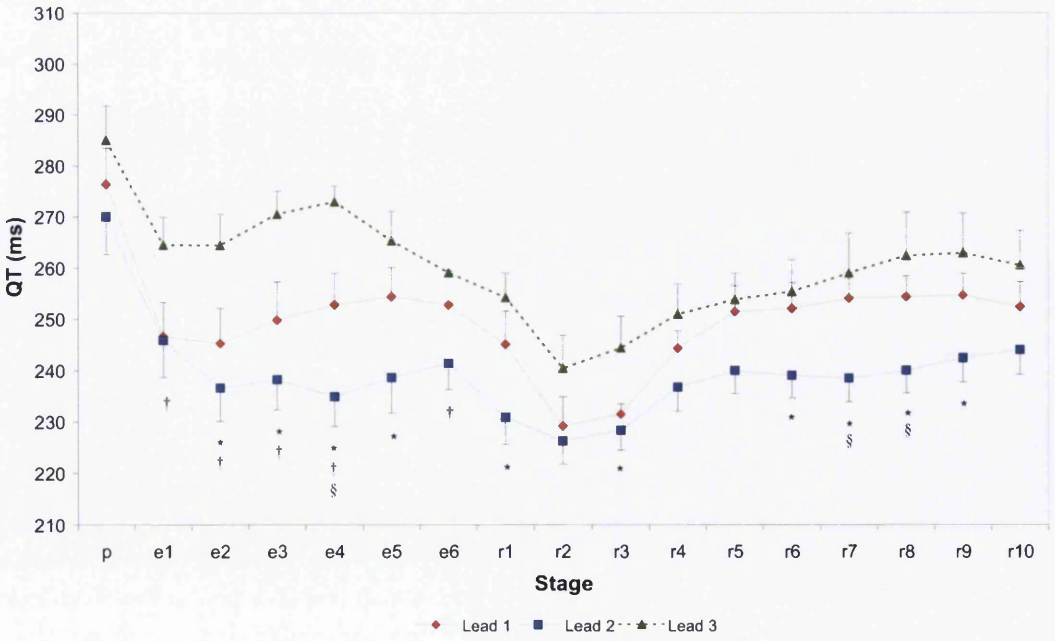


Figure 3.3 (a) Group mean values of QT interval for each ECG lead (male group) (lead 1, lead 2, lead 3 = ECG leads 1, 2 & 3; § = significant difference between lead 1 and lead 2; † = significant difference between lead 1 and lead 3; * = significant difference between lead 2 and lead 3) (p = pre-exercise stages, e = exercise stages, r = recovery stages)

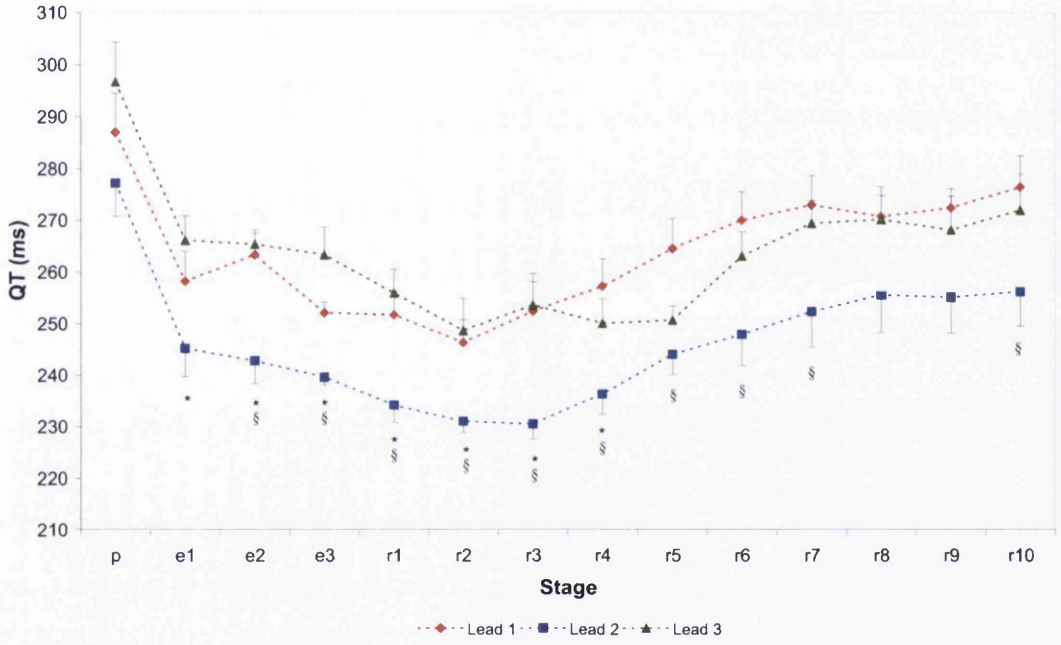


Figure 3.3 (b) Group mean values of QT interval for each ECG lead (female group) (lead 1, lead 2, lead 3 = ECG leads 1, 2 & 3; § = significant difference between lead 1 and lead 2; † = significant difference between lead 1 and lead 3; * = significant difference between lead 2 and lead 3) (p = pre-exercise stages, e = exercise stages, r = recovery stages)

Figures 3.3 (c) & 3.3 (d) present example Bland-Altman plots (between-lead differences plotted against mean values calculated for those leads) for beat-to-beat RR and QT intervals respectively from a typical male subject. The plots show the mean between-lead bias for these ECG parameters together with the associated

limits of agreement (LOA), plotted separately for the rest, exercise and recovery periods. Bias and LOA are indicated separately for each defined one-minute stage during exercise and recovery. The plots show that the range of LOA for QT is much larger than for RR, particularly during pre-exercise rest. Figure 3.3 (d) (iii) also shows a non-linear relationship between bias and mean QT, with an inflection point in this example for QT values of around 260 ms. There is no such trend in RR for the equivalent lead pair (Figure 3.3 (c) (iii)). Similar results were obtained for the other paired-lead analyses in each subject.

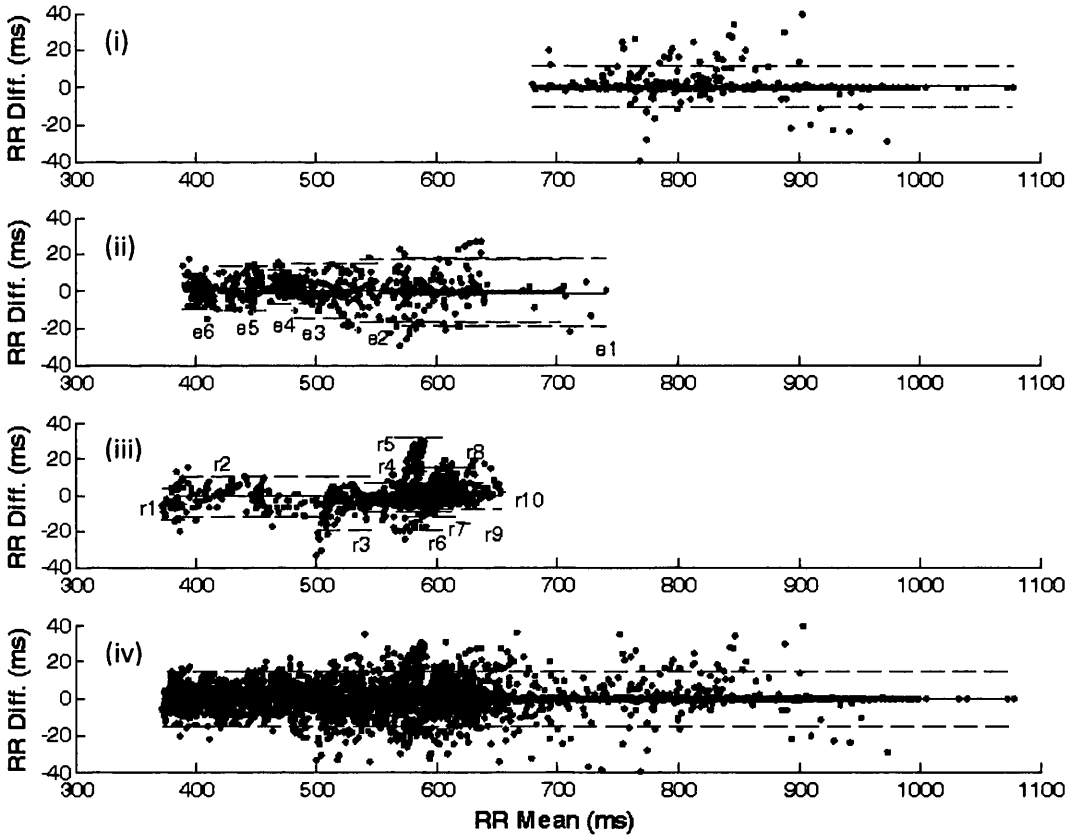


Figure 3.3 (c) Bland-Altman analysis of between-lead agreement for RR data (lead 1-lead 3, for a typical male subject) (Horizontal bars show ranges of measured intervals: Solid = mean bias; dashed = upper and lower limits of agreement. Values are indicated separately for each defined one-minute stage during exercise (six stages, e1-e6 [plot (ii)]) and recovery (ten stages, r1-r10) [plot (iii)]). Plot (i) illustrates pre-exercise rest and plot (iv) illustrates the complete testing period

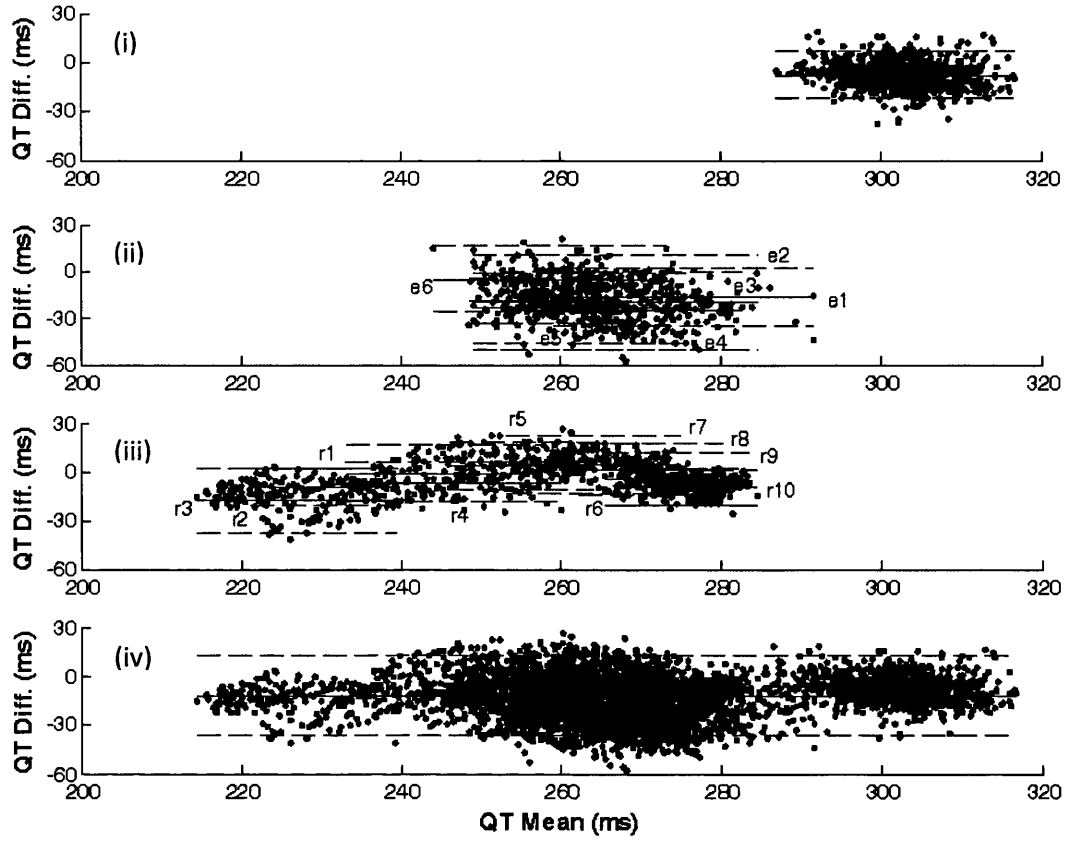


Figure 3.3 (d) Bland-Altman analysis of between-lead agreement for QT data (lead 1-lead 3, for a typical male subject) (Horizontal bars show ranges of measured intervals: Solid = mean bias; dashed = upper and lower limits of agreement. Values are indicated separately for each defined one-minute stage during exercise (six stages, e1-e6 [plot (ii)]) and recovery (ten stages, r1-r10) [plot (iii)]). Plot (i) illustrates pre-exercise rest and plot (iv) illustrates the complete testing period

3.6.1.2 Group mean values of bias and LOA for RR and QT data

Bland-Altman analysis was also used to calculate the group mean values of bias and the associated LOA for RR and QT, using each lead-pair. These data are shown in Figures 3.4 (a) and 3.4 (b), respectively (male group only). There were no significant between-stage differences in mean bias or LOA for either RR or QT

for any lead-pair. The LOA for RR tended to be largest during pre-exercise and during late recovery and smallest during exercise and early recovery. There was no discernible trend for QT LOA during the experimental protocol. There were occasional significant differences in mean bias of QT for males and females.

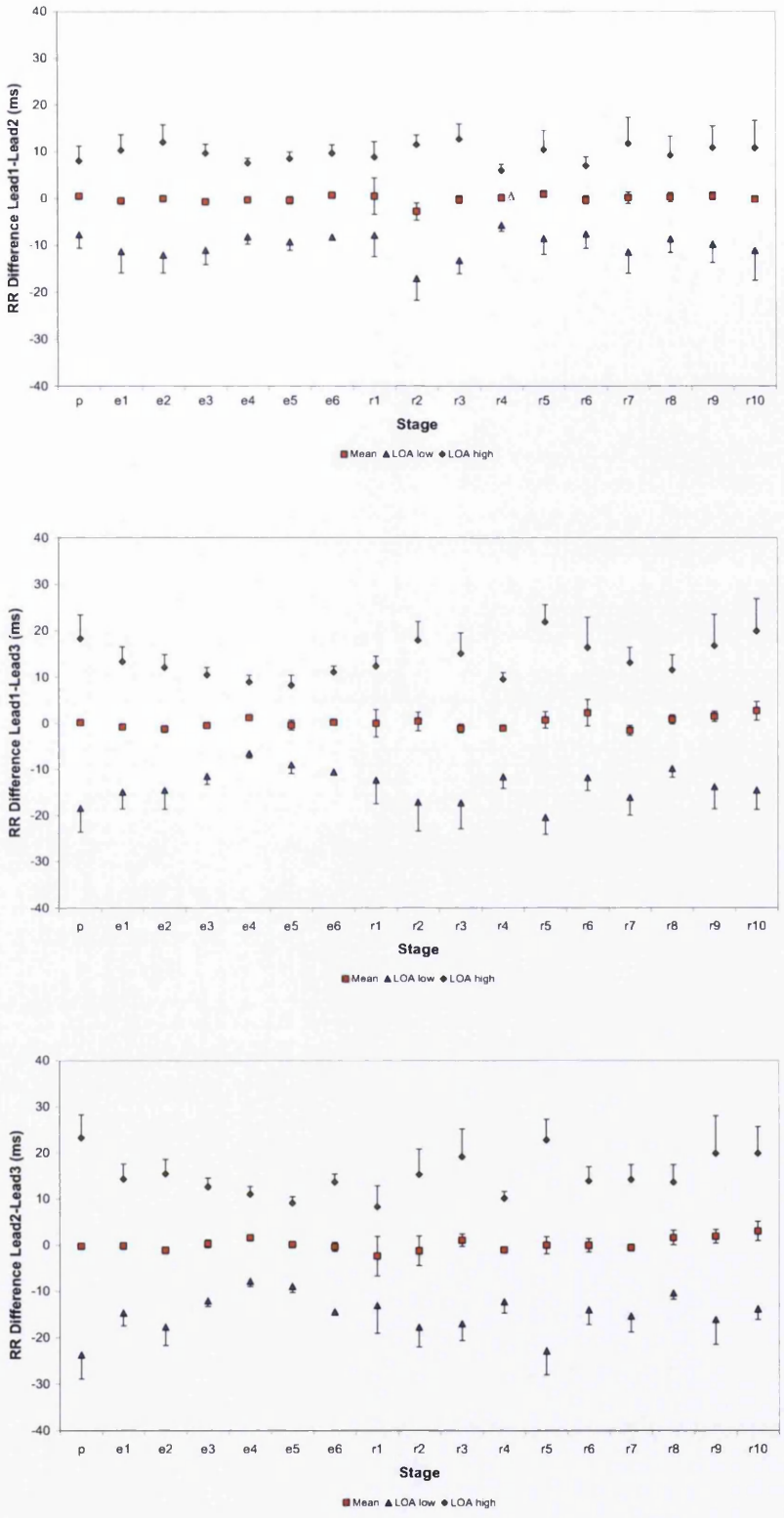
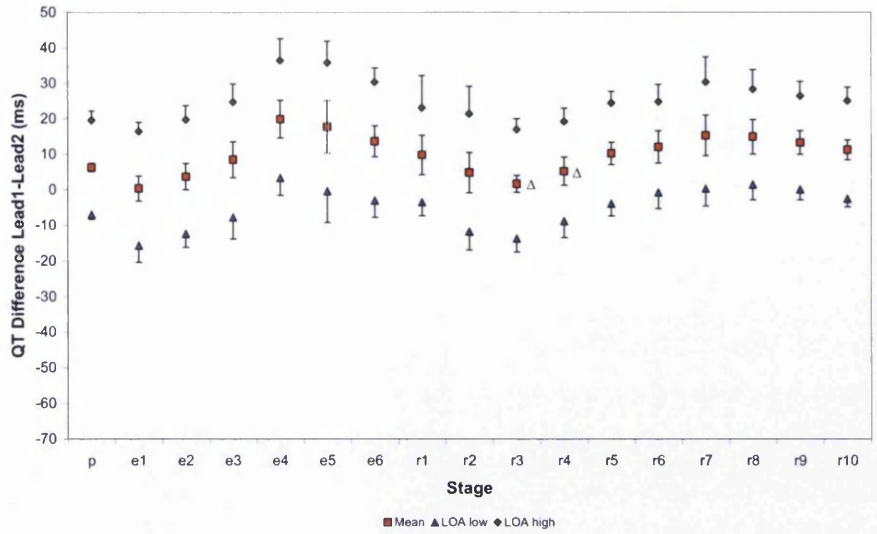
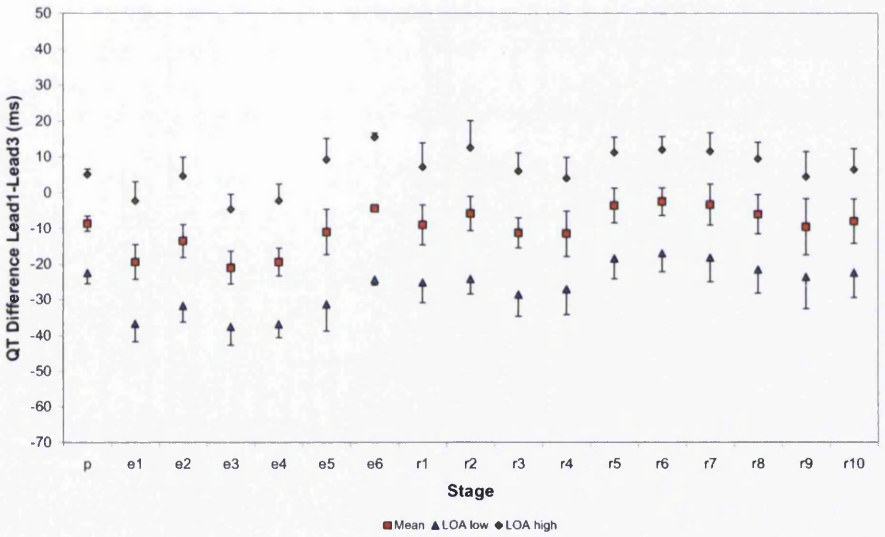


Figure 3.4 (a) Group mean values of between-lead bias and associated limits of agreement (LOA low, LOA high) determined from Bland-Altman analysis of RR interval for each protocol stage (male group) (Top: leads 1&2; Middle: leads 1&3; Bottom: leads 2&3. Δ = significant difference between male and female groups)


QT Difference Lead1-Lead2 (ms)
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
p
e1
e2
e3
e4
e5
e6
r1
r2
r3
r4
r5
r6
r7
r8
r9
r10
Stage
Mean ▲LOA low ◆LOA high


QT Difference Lead1-Lead3 (ms)
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
p
e1
e2
e3
e4
e5
e6
r1
r2
r3
r4
r5
r6
r7
r8
r9
r10
Stage
Mean ▲LOA low ◆LOA high

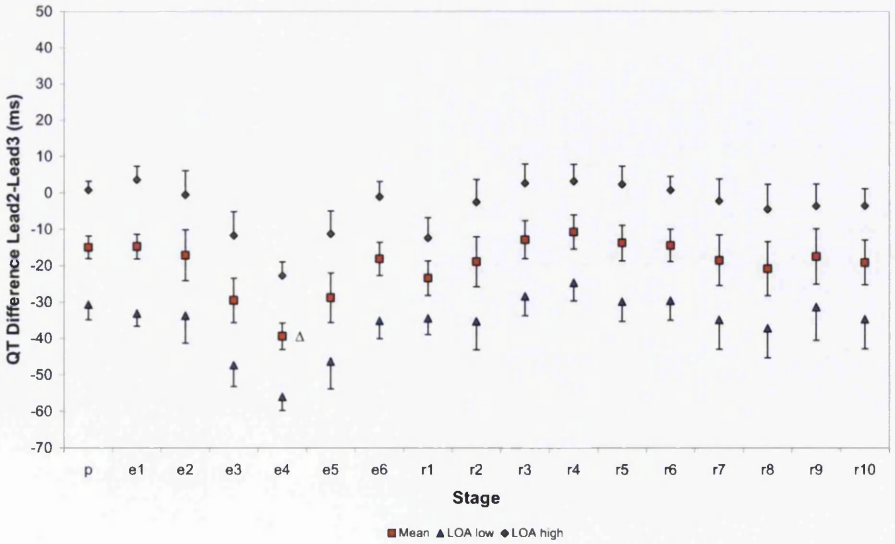

QT Difference Lead2-Lead3 (ms)
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
p
e1
e2
e3
e4
e5
e6
r1
r2
r3
r4
r5
r6
r7
r8
r9
r10
Stage
Mean ▲LOA low ◆LOA high

Figure 3.4 (b) Group mean values of between-lead bias and associated limits of agreement (LOA low, LOA high) determined from Bland-Altman analysis of QT interval for each protocol stage (Male Group) (Top: leads 1&2; Middle: leads 1&3; Bottom: leads 2&3. Δ = significant difference between male and female groups)

Tables 3.1 (a) & 3.1 (b) present mean values of the bias and LOA for RR and QT intervals, respectively, taken over the whole recording period for each of the lead pairs. The ranges of LOA for RR were similar for each lead pair, and were slightly larger in females ($p<0.05$). The results were similar with regard to LOA for QT, with larger values for females in two of the lead pairs ($p<0.01$). The mean bias was significantly larger for QT compared with RR in two or more lead pairs for both males and females ($p<10^{-6}$). There were also isolated between-gender differences in mean bias of RR and QT ($p<0.05$). The results shown in Tables 3.1 (a) and 3.1 (b) were also quantified relative to the mean parameter values (% of the mean absolute RR or QT value during the whole recording period): paired-lead bias for RR was 0.1% (males) and 0.3% (females); range of paired-lead LOA for RR was 5.0% (males) and 6.7% (females); paired-lead bias for QT was 8.3% (males) and 6.9% (females); range of paired-lead LOA for QT was 13.1% (males) and 12.8% (females).

Table 3.1 (a) Mean bias and associated limits of agreement (± SEM) of the RR interval for paired leads, calculated for the entire recording period

Lead Pair	Mean Bias (ms)	Lower Mean LOA (ms)	Upper Mean LOA (ms)	Range Mean LOA (ms)
Male Group				
1,2	-0.2 ± 0.2 †	-10.0 ± 0.7	9.6 ± 0.5	19.6 ± 1.0 †$^{\Delta}$ *(1,3)(2,3)
1,3	0.2 ± 0.3 †$^{\Delta}$	-13.5 ± 0.9	13.9 ± 1.0	27.4 ± 1.8 † *(1,2)
2,3	0.1 ± 0.3 †$^{\Delta}$	-14.8 ± 1.0	15.0 ± 1.1	29.9 ± 2.0 *(1,2)
Female Group				
1,2	0.1 ± 0.5 † *(2,3)	-14.0 ± 1.3	14.3 ± 1.8	28.3 ± 2.9 $^{\Delta}$
1,3	-1.3 ± 0.6 $^{\Delta}$	-18.9 ± 2.2	16.2 ± 2.2	35.0 ± 4.2
2,3	-1.5 ± 0.5 †$^{\Delta}$ *(1,2)	-19.9 ± 2.2	17.0 ± 1.7	36.9 ± 3.7

($^{\Delta}$ = significant between-gender difference ($p<0.05$); *(x,y) = significant difference between lead-pair x,y; † = significant difference between values for QT and RR ($p<10^{-6}$))

Table 3.1 (b) Mean bias and associated limits of agreement (± SEM) of the QT interval for paired leads, calculated for the entire recording period

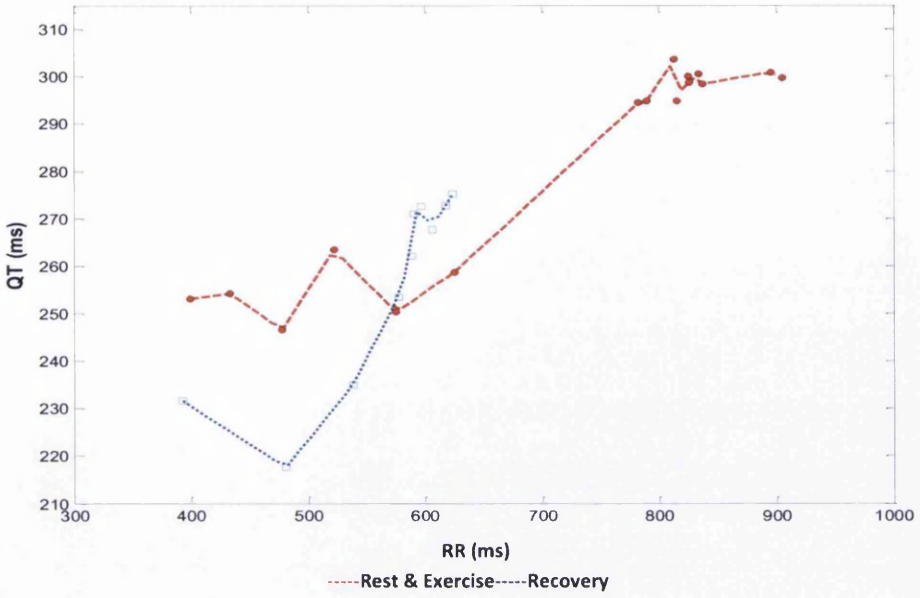
Lead Pair	Mean Bias (ms)	Lower Mean LOA (ms)	Upper Mean LOA (ms)	Range Mean LOA (ms)
Male Group				
1,2	9.9 ± 1.4 †$^{\Delta}$ *(1,3)(2,3)	-5.1 ± 1.4	24.9 ± 1.4	30.0 ± 0.8 † *(1,3)
1,3	-9.9 ± 1.4 †$^{\Delta}$ *(1,2)(2,3)	-26.2 ± 1.6	6.5 ± 1.4	32.8 ± 1.0 † *(1,2)
2,3	-19.6 ± 1.8 † *(1,2)(1,3)	-35.5 ± 1.9	-3.7 ± 1.7	31.7 ± 0.8
Female Group				
1,2	17.5 ± 1.0 †$^{\Delta}$ *(1,3)(2,3)	1.3 ± 1.3	33.8 ± 1.4	32.5 ± 1.7
1,3	0.0 ± 1.9 $^{\Delta}$ *(1,2)(2.3)	-16.3 ± 1.9	16.3 ± 2.2	32.7 ± 1.6
2,3	-17.5 ± 1.3 † *(1,2)(1,3)	34.1 ± 1.2	-0.9 ± 1.8	33.2 ± 1.7

($^{\Delta}$ = significant between-gender difference ($p<0.01$); *(x,y) = significant difference between lead-pair x,y; † = significant difference between values for QT and RR ($p<10^{-6}$))

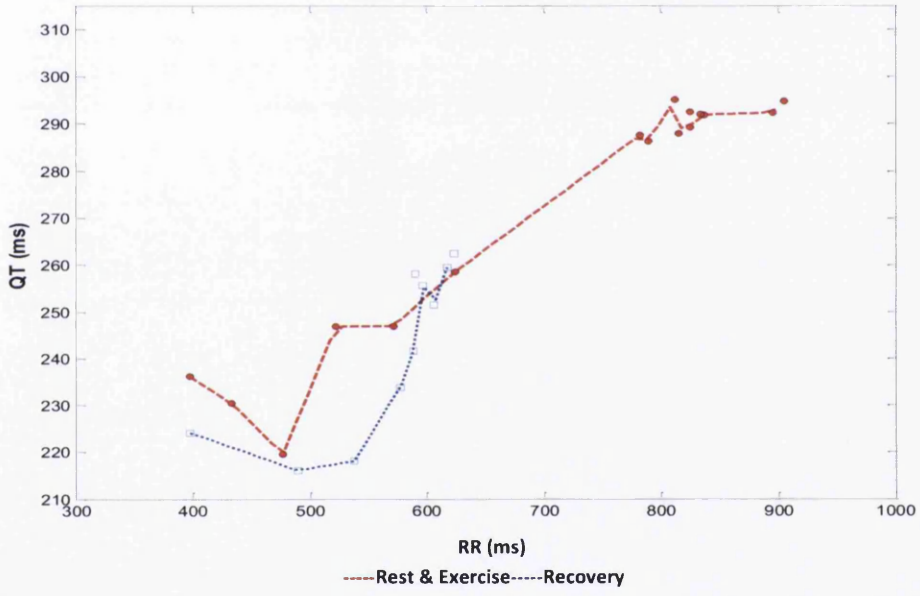
3.6.2 Main study: analysis of QT-RR hysteresis

Figure 3.5 (a)-(c) shows the magnitude of the QT interval as a function of the corresponding RR interval for a male subject, determined from each of the three ECG leads throughout the exercise and recovery periods. These plots demonstrate the magnitude of QT_a-RR hysteresis and the typical shapes of the QT_a-RR "loops" that were observed for individual subjects. It is notable that the shapes of these loops often varied substantially between the different ECG leads recorded for the same subject.

(a)


QT (ms)
RR (ms)
----Rest & Exercise-----Recovery

(b)


QT (ms)
RR (ms)
----Rest & Exercise-----Recovery

(c)

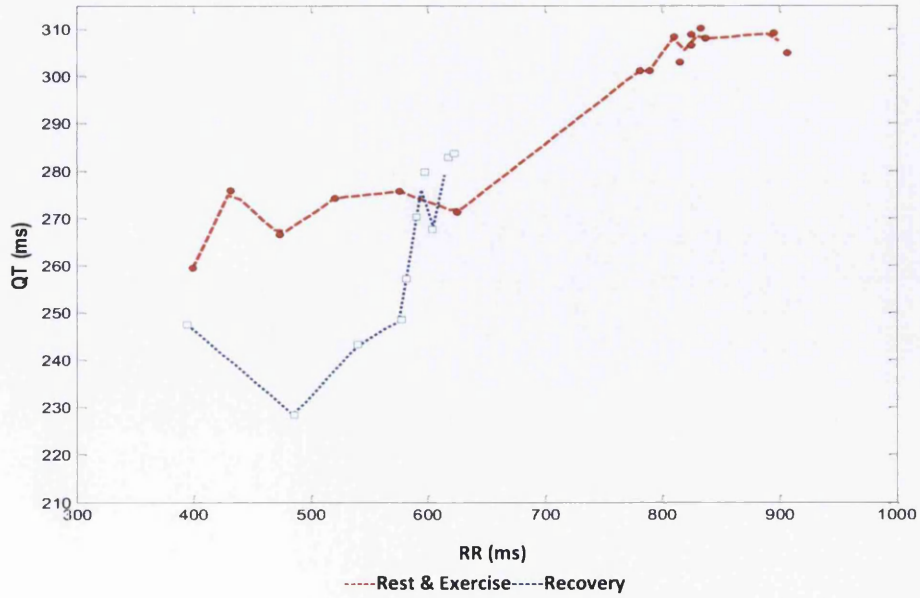
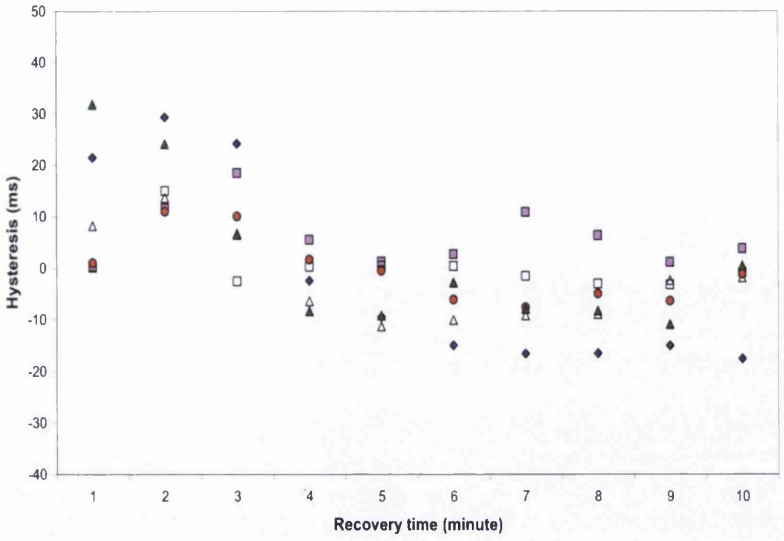

QT (ms)
RR (ms)
----Rest & Exercise-----Recovery

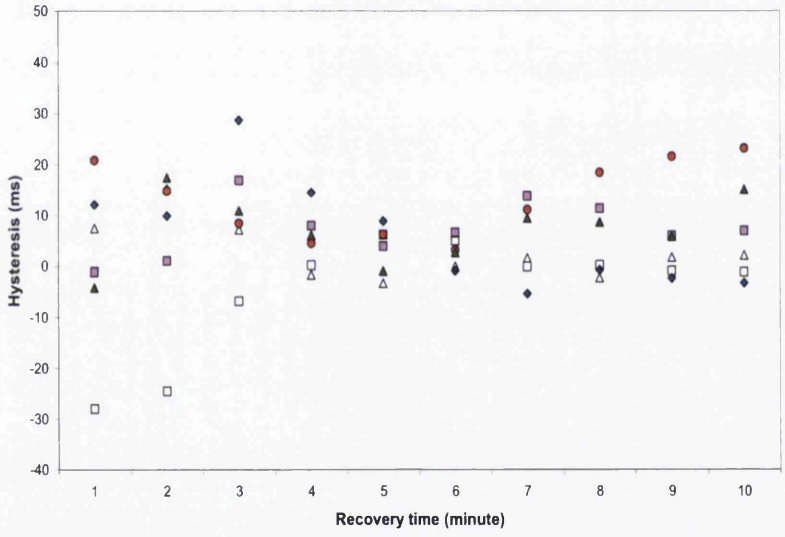
Figure 3.5 QT interval as a function of the corresponding RR interval for a single typical male subject: (a) ECG lead 1, (b) ECG lead 2, (c) ECG lead 3

The magnitude of the QT_a-RR hysteresis calculated for each consecutive one-minute interval of the post-exercise recovery period is shown separately for each subject in Figure 3.5 (d)-(f) (males) and Figure 3.5 (g)-(i) (females). The magnitudes and temporal trends in hysteresis were similar for the majority of male individuals, with females demonstrating a greater inter-individual variability in hysteresis.

(d)



(e)



(f)

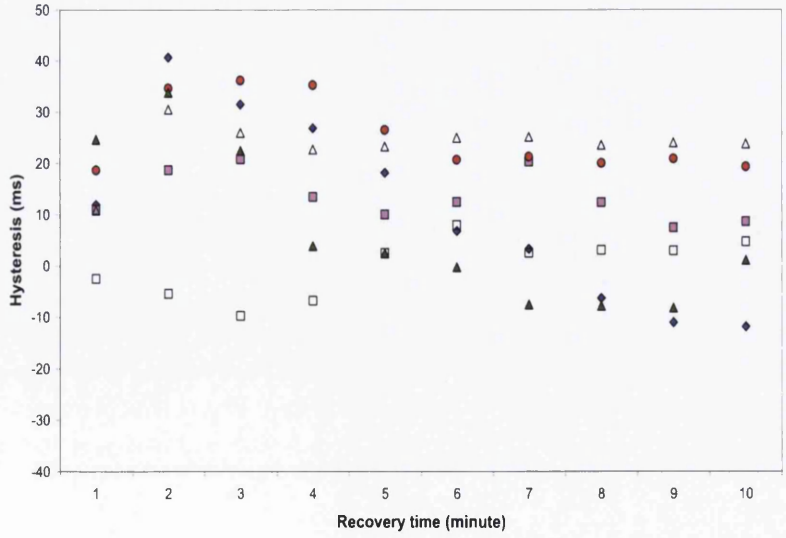
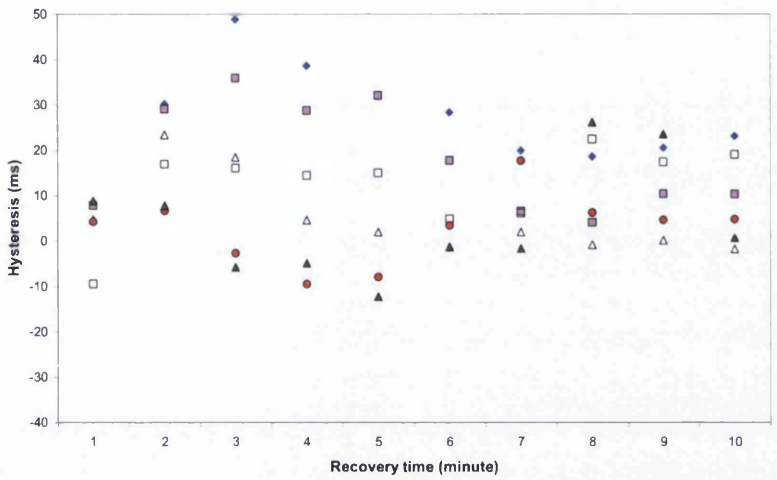
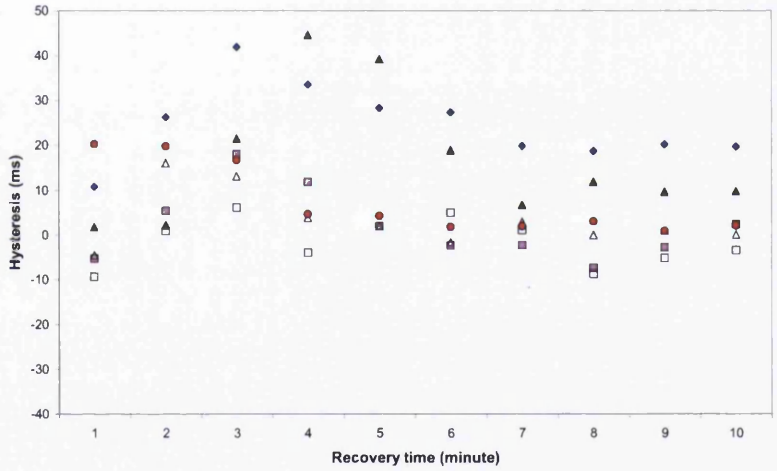


Figure 3.5 (d-f) QT-RR hysteresis as a function of recovery time (minutes post-exercise); male group. Symbols represent different individuals (n=6): (d) ECG lead 1, (e) ECG lead 2, (f) ECG lead 3

(g)



(h)



(i)

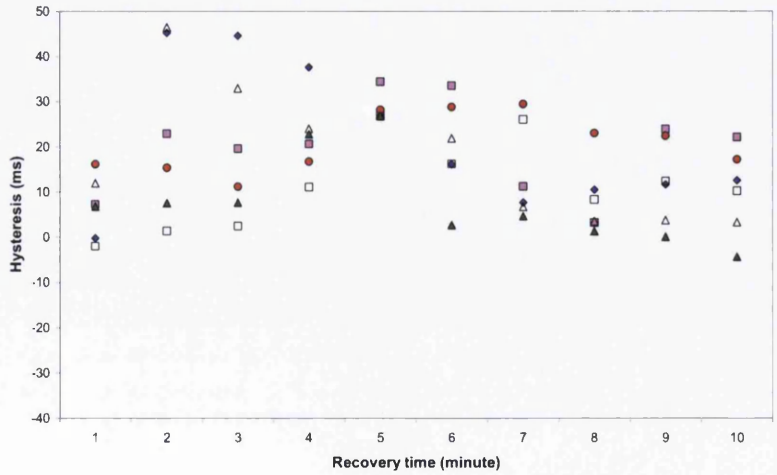


Figure 3.5 (g-i) QT-RR hysteresis as a function of recovery time (minutes post-exercise); female group. Symbols represent different individuals (n=6): (g) ECG lead 1, (h) ECG lead 2, (i) ECG lead 2

Mean hysteresis values for the subject groups are presented in Figures 3.5 (j) and 3.5 (k) for males and females respectively, demonstrating the existence of some between-lead and between-gender differences in both magnitudes and temporal trends of QT_a-RR hysteresis.

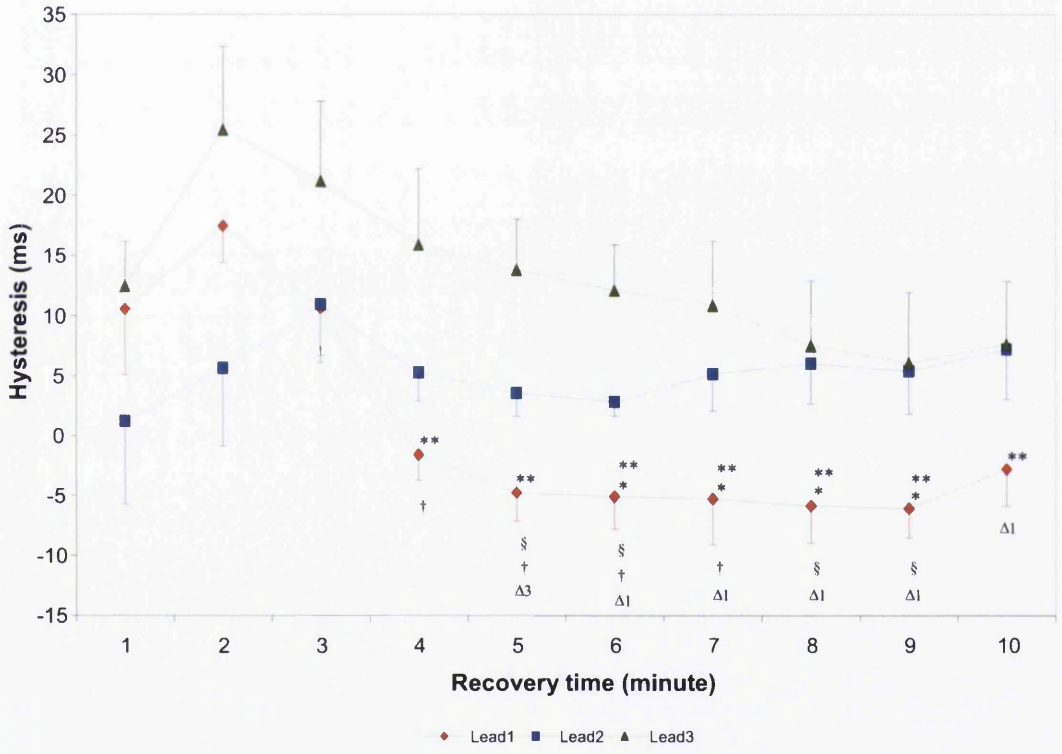


Figure 3.5 (j) Mean hysteresis as a function of recovery time (minutes post-exercise); male group. (§ = significant difference between ECG lead 1 and ECG lead 2; † = significant difference between ECG lead 1 and ECG lead 3; Δ1 = significant difference in ECG lead 1 compared with Female group; Δ3 = significant difference in ECG lead 3 compared with Female group. * = significant difference in ECG lead 1 compared with value at 1 and 3 minutes; ** = significant difference in ECG lead 1 compared with value at 2 minutes)

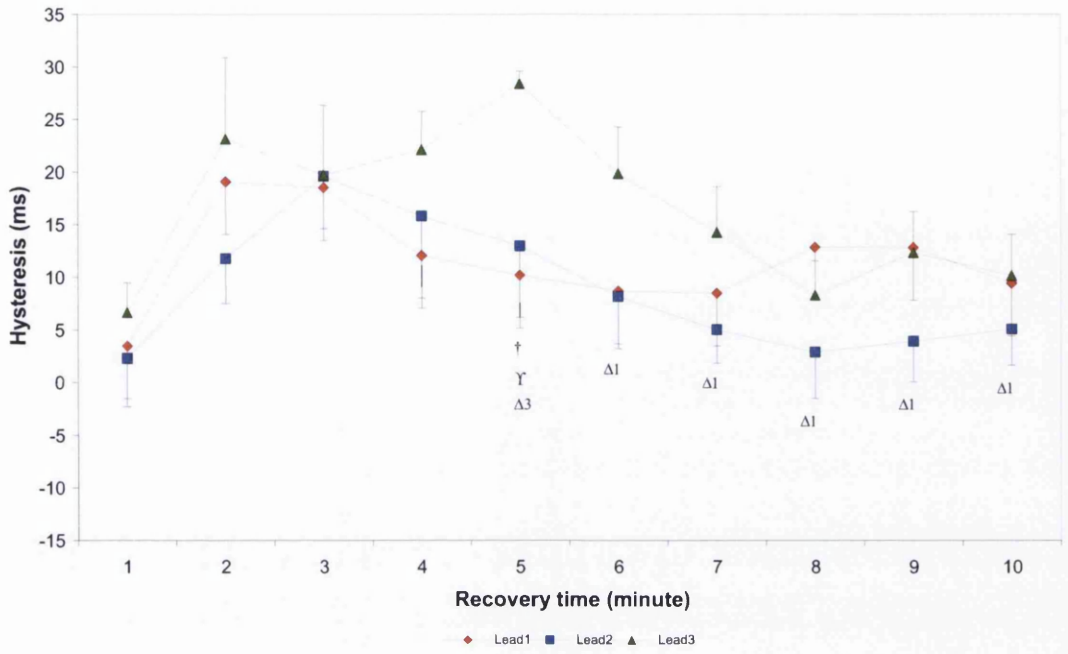


Figure 3.5 (k) Mean hysteresis as a function of recovery time (minutes post-exercise); female group. (γ = significant difference between ECG lead 1 and ECG lead 2; † = significant difference between ECG lead 1 and ECG lead 3; Δ1 = significant difference in ECG lead 1 compared with male group; Δ3 = significant difference in ECG lead 3 compared with male group)

3.6.2.1 Temporal variation of hysteresis

The magnitude of hysteresis for the male group (Figure 3.5 (j)) was greatest during either the second minute (leads 1 and 3) or the third minute (lead 2) post-exercise, with the following maximal values: 17.5 ± 7.5 ms (lead 1), 10.9 ± 11.7 ms (lead 2), 25.5 ± 16.8 ms (lead 3). After this time, the magnitude of hysteresis showed a trend towards smaller and more temporally stable values towards the end of the recovery period for all leads. The situation was similar for females in leads 1 and 2, but hysteresis calculated from lead 3 displayed some anomalous characteristics (Figure 3.5 (k)). The magnitude of hysteresis for the female group

was greatest during either the second minute (lead 1), the third minute (lead 2) or the fifth minute (lead 3) post-exercise, with the following maximal values: 19.1 ± 10.3 ms (lead 1), 19.6 ± 12.1 ms (lead 2), 28.4 ± 3.0 ms (lead 3). Again, the magnitude of hysteresis showed a trend towards smaller and more temporally stable values towards the end of the recovery period for all leads. It is notable that the hysteresis values in males were negative between the fourth and tenth minutes of recovery in lead 1 only, and during the first and second minutes of recovery in lead 2 for some subjects. Hysteresis values in females were negative in some subjects during the first minutes of recovery in leads 1 and 2, and during the eighth minute of recovery in lead 2 only.

For the male group (Figure 3.5 (j)) there were some significant differences between the hysteresis values calculated using lead 1 at different times during the post-exercise period. Hysteresis values at 1 and 3 minutes post-exercise were significantly different from each of the four hysteresis values between 6 and 9 minutes post-exercise. Hysteresis at two minutes post-exercise was significantly different from each of the hysteresis values for the period between 4 and 10 minutes post-exercise. There were no differences amongst any of the seven hysteresis values for the period between 4 and 10 minutes post-exercise, or amongst the hysteresis values for the first three minutes post-exercise. There were no differences between the hysteresis values at any stage for either lead 2 or lead 3, although there were trends towards reduced values after the second minute. For the female group (Figure 3.5 (k)) the only significant difference in hysteresis between stages was between the values calculated for lead 3 at 1 and 5 minutes post-exercise. There were no differences between the hysteresis values at any stage for either lead 1 or lead 2.

3.6.2.2 Between-lead differences in hysteresis

In males there were significant differences between the magnitudes of hysteresis calculated using leads 1 and 2 during the fifth, sixth, eighth and ninth minutes post-exercise. There were also significant differences between the magnitudes of hysteresis calculated using leads 1 and 3 for the fourth and seventh minutes of recovery. In females the only significant between-lead difference was associated with leads 1 and 3 during the fifth minute of recovery.

3.6.2.3 Between-gender differences in hysteresis

There were significant differences between the magnitudes of hysteresis calculated for males and females using lead 1 for the period between 6 and 10 minutes post-exercise. The magnitudes of hysteresis calculated using lead 3 were significantly different for males and females only during the fifth minute post-exercise. There were no gender differences in the magnitude of hysteresis calculated using lead 2.

3.7 Discussion

3.7.1 Between-lead agreement for QT and RR data

The preliminary analysis of between-lead agreement reported indicated that the range of paired-lead LOA for RR was low, with maximal values (% of mean) of 5.0% for the male group and 6.7% for the female group. However whereas the paired-lead bias for RR was also low (0.1% and 0.3% of mean respectively), the paired-lead bias (8.3% and 6.9% of mean) and LOA (13.1% and 12.8% of mean) for QT were substantially larger. It is notable that even these relatively small between-lead differences in RR (mainly attributable to the exercise and recovery periods) were observed, suggesting differences in the sensitivity of the leads to

the measured fiducial point during these conditions. This might be the result of a more pronounced influence of the signal quality in individual leads on the accuracy of the detection algorithm during these periods. The observed between-lead differences in QT might have implications for the clinical interpretation of ambulatory studies examining either the absolute magnitude or variability of the QT interval.

The electrocardiographic RR and QT intervals cannot be quantified with equivalent accuracy or precision owing to the nature of the ECG characteristics that are used in their measurement. The RR interval can be measured with a high degree of accuracy and repeatability owing to the ease of locating the R wave peak as a fiducial point. However it is more difficult to measure the QT interval, in large part owing to the ambiguity in locating either the peak or the end-point of the T wave. Importantly, the accuracy and precision of RR and QT measurement are not comparable and are likely on physiological grounds to be related to lead position. A logical and necessary first step prior to further consideration of QT and RR parameters in this thesis was therefore to determine whether QT and RR durations are independent functions of lead placement.

Kingsley *et al.* (2005) demonstrated good agreement between single-lead RR interval data from the Reynolds ambulatory ECG system and a Polar 810s heart rate monitor. Similarly, Gamelin *et al.* (2006) and Vanderlei *et al.* (2008) demonstrated good agreement between a Polar 810s heart rate monitor and twelve lead ECG systems. Whilst these studies can be interpreted as providing "implied validity" of the RR data there has been no similar assessment for QT data. Furthermore there has been no evaluation of the between-lead agreement for either RR or QT interval data recorded from this or any similar ambulatory ECG system. Specifically, between-lead differences in RR and QT data need to be

quantified throughout the physiological range of heart rates in order to determine whether they are affected by chronotropic factors. Such differences would need to be considered for example in the interpretation of: studies reporting data from only a single ECG lead, studies presenting data recorded under different physiological conditions, such as rest and exercise and data from different studies and different investigators.

It is particularly notable that between-lead variability of the QT interval, measured as the difference between maximum and minimum QT duration in a particular set of ECG leads, has generally been proposed as an index of myocardial repolarisation dispersion (Day *et al.* 1990, Barr *et al.* 1994, Macfarlane *et al.* 1998). However it has been suggested that, rather than reflecting the presence of pathology, QT dispersion might instead simply reflect the projection of cardiac electrical activity on to the different ECG lead axes (Cowan *et al.* 1988, Macfarlane 1998, Macfarlane *et al.* 1998). Quantification of the between-lead agreement for QT data is therefore important in distinguishing between "physiological" (e.g. QT interval changes during exercise or cardiac arrhythmia) and "methodological" or "technical" variation in QT intervals measured using multiple ECG leads. The preliminary study of this chapter has therefore provided useful information in this regard by quantifying the methodological (between-lead) variation in QT interval during the distinct physiological conditions of rest, physical exercise and post-exercise recovery.

This new information is also likely to be very important in the clinical interpretation of QT interval duration, especially if this involves examination of QT under different physiological conditions and/or heart rates. For example, there is impairment of QT shortening during exercise in patients with congenital LQTS (Schwartz *et al.* 1975, Chiang & Roden 2000). More recent studies have

indicated that gene mutations affecting myocardial repolarisation currents cause different patterns of adaptation of QT interval to heart rate during exercise and other autonomic stimuli (Paavonen *et al.* 2001). Analysis of the dynamic behaviour of the QT interval during these conditions might therefore facilitate clinical diagnosis of LQTS in subjects with borderline QT prolongation (Dillenburg *et al.* 2002, Toivonen 2002) and might be used to screen for individuals with a genetically determined susceptibility to repolarisation abnormalities. The results of the studies presented here suggest that any such analysis of the QT-RR relationship must take adequate account of the lead locations used in its determination. The between-lead agreement, and the influence of heart rate thereon, should be quantified for the particular ECG system used to record these data.

3.7.2 QT-RR hysteresis

3.7.2.1 Magnitude of QT_a-RR hysteresis

The magnitudes of the QT_a-RR hysteresis calculated using each of the three ECG leads were comparable with the results of previous investigations (Sundqvist & Sylven 1989, Krahn *et al.* 1997). There was also reasonably good between-subject agreement with regard to the magnitudes and temporal trends of hysteresis, particularly for the male subjects. Several characteristics of the observed data are also worthy of note, and these are discussed below.

3.7.2.2 Influence of ECG lead and gender on QT_a-RR hysteresis

It is possible that differences in hysteresis calculated using specific ECG leads are related to the relative orientations of the instantaneous atrial and ventricular depolarisation-repolarisation vectors. Projections of these myocardial electrical

vectors onto the thoracic surface are likely to result in position-dependent alterations in the temporal features of the surface ECG. It is possible that between-gender differences in hysteresis are associated with anatomical differences and their consequent influence on the temporal characteristics of the surface ECG.

3.7.2.3 Time of maximal hysteresis

As previously described (section 2.3.2.2), the lag in the adaptation of QT interval to changes in RR interval has two components: an immediate rapid component with a short time constant acting over 1-2 cardiac cycles and a delayed component with a time constant of the order of 1-3 minutes (Toivonen 2002). Furthermore, the lengthening of the QT interval in response to decreasing heart rate has previously been shown to begin after two minutes of recovery (Benatar & Decraene 2001) with post-exercise hysteresis values being significantly greater in females than males (Chauhan *et al.* 2002). The results of the present studies are in good agreement with the studies mentioned above, as maximal hysteresis values were observed during either the second or third minute post-exercise (males and females), depending on the ECG lead used to calculate this parameter and female hysteresis values were larger during the fifth minute (lead 3) post-exercise.

3.7.2.4 Possible influence of the autonomic nervous system on hysteresis

The phenomenon of hysteresis demonstrates that the relationship between the durations of QT and RR intervals throughout the physiological range of heart rate is not straightforward, as the QT-RR relationship is altered during the period of recovering heart rate following physiological stress. Previous studies have offered some insight into the possible mechanisms of this anomalous QT-RR relationship and its implications of differential regulation of cardiac depolarisation and

repolarisation. For example, there is a direct autonomic influence on the ventricular myocardium, and this might affect ventricular depolarisation-repolarisation independently of heart rate (Browne *et al.* 1982, Belardinelli & Isenberg 1983, Charpentier & Rosen 1994). It is therefore possible that QT-RR hysteresis might be associated with independent ANS modulation of the atrial and ventricular myocardia. Exercise-induced QT shortening has also been related to the level of catecholamine activity which is increased following sympathetic stimulation and this effect is independent of heart rate (Ahnve & Vallin 1982, Arrowood *et al.* 1993). It is notable that plasma norepinephrine level rises during exercise and also during the early recovery period, with a half-life of 2.8 minutes (Hagberg *et al.* 1979, Dimsdale *et al.* 1984). This suggests a temporal pattern of catecholamine influence that is consistent with maximal hysteresis values between the second and third minutes of post-exercise recovery, as observed in the present work. Plasma catecholamine level might therefore be a substantial factor in the mechanism of QT-RR hysteresis following exercise.

3.8 Limitations

For both the preliminary and main studies, the apex of the T wave rather than the end of the T wave was chosen to examine the period of ventricular depolarisation-repolarisation (QT interval), owing mainly to the improved reliability with which this parameter was measured by the Holter system's detection algorithm. However, QT_a represents only a part of the complete depolarisation-repolarisation period, although previous studies have indicated that this period contains the majority of the inter- and intra-individual variability of the QT interval (Merri *et al.* 1989, Bidoggia *et al.* 2000). In agreement with Chauhan *et al.* (2002), it was observed that the T wave apex is a more reliable fiducial point for the measurement of QT when the heart rate is very high. It has also previously been noted that the shape of the T wave becomes more

symmetrical when heart rate is elevated during exercise and recovery (Langley *et al.* 2002). However, the relative influence of this symmetry change on the location of the end point and apex of the T wave requires further investigation. The view was therefore taken that the QT_a interval provides the most reliable determination of the ventricular depolarisation-repolarisation phase during high intensity physical exercise and subsequent recovery.

3.9 Conclusion

The results of the preliminary study showed that the magnitude of paired-lead bias and the associated LOA of the bias are substantially greater for QT compared with RR, and this is true in both males and females. The difference in paired-lead bias for RR and QT is understandably related to differences in the orientation of the cardiac vector during these two phases of the cardiac cycle. The difference in LOA for RR and QT is likely related to the differential accuracy and precision with which these two parameters can be measured from the ECG. Although these findings are specific to the Reynolds Lifecard Holter system, the methodology employed here could similarly be used in other similar systems. This work suggests that the between-lead bias and associated LOA for electrocardiographic RR and QT data should be quantified in future investigations using multi-lead ambulatory ECG. Interpretation of the results of such studies should take adequate account of the between-lead variation in these parameters, and should specify each subject's physiological state.

The relationship between QT and RR intervals for the three different electrode lead configurations of an ambulatory ECG system following dynamic physical exercise was characterised and compared. The calculated magnitudes of QT_a-RR hysteresis were significantly affected by the locations of the paired ECG

electrodes used to record the surface ECG. These results emphasise the need for standardisation of ECG electrode placement in future investigations of QT_a-RR hysteresis. There is also evidence of differences in hysteresis between genders, and so a standard set of ECG electrode locations should be established separately for males and females. Further investigation of the physiological mechanisms responsible for QT_a-RR hysteresis will then be required. Standardised ECG recording methodologies should help to elucidate the hypothesised association between QT_a-RR hysteresis and differential autonomic control of atrial and ventricular myocardia.

Chapter 4 Complexity of electrocardiographic RR and QT time-series data during rest and exercise

4.1 Rationale

Variability analysis does not utilise the information available from time-dependent changes in data series. Correlation analysis however does, providing a measure of the complexity, hence predictability of data. More complex data has less predictability and could be associated with healthier physiology. Complexity estimation methods include detrended fluctuation analysis (DFA) and entropy. The influence of exercise work load on RR time-series complexity is unknown, and estimation of the sensitivity of entropy to changes in workload could further aid the full characterisation of the mediators of HRV. Also, physical exercise causes marked changes in the duration of the QT interval (Sarma *et al.* 1987, Sundqvist *et al.* 1989), observed throughout a wide range of heart rates and apparently involving both heart rate-dependent and heart rate-independent QT modulation (Porta *et al.* 1998, Almeida *et al.* 2006). It could therefore be of potential clinical significance to also examine the complexity characteristics of the QT interval during conditions of rest and exercise. Entropy and DFA may be of use in this, as although they are commonly used investigative methods, they have not been extensively assessed during exercise and recovery.

4.2 Aims and objectives

The aims of this chapter were:

1. To investigate the sample entropy (SampEn) of RR and QT interval data during different physiological conditions (rest, dynamic physical exercise and post-exercise recovery)

2. To investigate the fractal (scaling) properties of RR and QT interval data during these physiological conditions

The objectives were:

1. To estimate the optimal choice of the parameters "r" and "M" for calculating SampEn of RR and QT time-series data (SampEn-RR and SampEn-QT) during different physiological conditions (rest, dynamic physical exercise and post-exercise recovery)
2. To quantify SampEn-RR and SampEn-QT during each of these conditions
3. To assess the influence of the parameters (r,M) on SampEn-RR and SampEn-QT for a selection of "best-choice" combinations of r and M
4. To assess the ability of SampEn-RR and SampEn-QT to discriminate between different physiological states (and different exercise work loads)
5. To examine the relationships between traditional HRV parameters and each of SampEn-RR and DFA α, and to compare the ability of each of these parameters to distinguish between different physiological states
6. To quantify the fractal scaling exponents for RR and QT data using the DFA method during each of these physiological conditions
7. To examine the relationships between the scaling exponents and traditional HRV parameters

4.3 Methodology

4.3.1 Subject characteristics

Twelve males (age 20.8 ± 0.4 years, mass 78.6 ± 7.7 kg [mean ± SD]) volunteered to take part in the investigation. Subject health screening was undertaken using the American Heart Association/American College of Sports Medicine pre-

participation screening questionnaire (Balady *et al.* 1998). Further subject selection criteria were as described in section 3.3.

4.3.2 Exercise testing protocol

All measurements were carried out between 9.00AM and 10.00AM in the Exercise Physiology Laboratory at the Sport and Exercise Science Research Centre, Swansea University and the investigation was approved by the departmental ethics committee. Subjects were initially at rest and were asked to breathe in accordance with a metronome set to a rate of 15 breaths per minute (0.25 Hz) for six minutes. At the end of this period they were instructed to breathe spontaneously for a further six minutes at rest. Subjects then immediately performed a progressive exercise test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport; Lode, Netherlands). The exercise protocol was similar to that described in section 3.3 with the addition that exercise continued until volitional fatigue. Breath-by-breath oxygen uptake ($\dot{V}O_2$) data were recorded throughout using an Oxycon Pro respiratory gas analysis system (Jaeger, Germany).

4.3.3 Physiological measurement

A Reynolds Lifecard CF digital Holter recorder (Del Mar Reynolds Medical Ltd., UK) was used to record a three-lead ECG continuously throughout the pre-exercise, exercise and post-exercise periods. The ECG leads were positioned in the modified V5, CC5, modified V5R electrode configuration as shown in Figure 3.1. This system provided ECG data with a sample accuracy of 2.5 μV (magnitude of least significant bit; 12-bit resolution) and 128 Hz sampling frequency. The previously quantified between-lead differences in RR and QT data using this ECG system (preliminary study, chapter 3) led to the consideration that there would

be no benefit from quantifying SampEn or the fractal scaling exponents in each lead. All analyses in this investigation therefore were performed using data from a single ECG lead (lead 1 for each subject). The ECG recordings were analysed using a Reynolds Pathfinder digital analyser (Del Mar Reynolds Medical Ltd., UK). All ECG data used for subsequent analysis in this study were free of any form of morphologically abnormal beat, and this was verified by both the Holter system and by human observation. Beat-to-beat cardiac interval (RR) and QT interval data were automatically measured for each sinus beat and exported for further analysis using the Reynolds Research Tools software (Del Mar Reynolds Medical Ltd., UK). The QT and RR data also underwent human visual examination in order to verify the accuracy of the data prior to subsequent analysis. When either the RR or QT intervals were considered to be anomalous both the RR and QT data points were removed from the data set. This occurred infrequently (and mainly during exercise), resulting overall in fewer than 1% of the data being removed. All QT analyses in this chapter were based on the QT_e interval (Q wave onset to T wave end) as the detection performance for T_a and T_e of the Holter system used were considered broadly equivalent. Fingertip capillary blood samples were analysed for whole blood lactate and glucose concentration using a YSI 2300 Lactate and Glucose Analyser (YSI Inc., USA).

4.3.4 Data analysis

Subsets of the QT and RR data sets were defined that represented these data within specified intervals during exercise and during the pre- and post-exercise phases:

1. Consecutive one-minute intervals during the six minute period of metronomic breathing

2. Consecutive one-minute intervals during the six minute period of spontaneous breathing
3. The final one-minute period of each exercise stage (in order to represent physiologically steady-state conditions)
4. Consecutive one-minute intervals during the post-exercise resting phase, which lasted for 15-minutes

Prior to further data processing the QT and RR data were linearly detrended within consecutive one-minute segments (corresponding to the subsets defined above) in order to remove any time-dependent trends in the data.

4.3.4.1 SampEn analysis

To reiterate section 2.5.4.2.3, SampEn is defined as the negative natural logarithm of the conditional probability that two sequences of data values that are similar for "M" points will remain similar at the next point in the data set, within a tolerance "r" that is expressed as a fraction of the standard deviation of the data. This is represented mathematically as SampEn(r,M) = -ln(A/B), where A and B are the total number of forward matches of length m+1 and m, respectively (Richman & Moorman 2002). As noted by Lake *et al.* (2002) no guidelines exist for the optimal selection of the parameters r and M. Therefore in order to evaluate the theoretically optimal values of r and M for entropy analysis, an approach similar to that of Lake *et al.* (2002) was used in this analysis to find r,M such that SampEn was maximal and the associated standard error (SE-SampEn) was minimal. SampEn and its standard error were calculated using a 20 × 15 matrix of combinations of r=0.05, 0.10,…,1.00 and M=1,2,…,15. The resulting SampEn and associated SE-SampEn matrices were then normalised (by dividing each value within the matrix by the maximum value for that matrix). The product of the matrices [(normalised SampEn) × (1-normalised SE-SampEn)] was

then calculated separately for the RR and QT data sets, and individually for the pre-exercise, exercise and post-exercise states. Four r,M combinations were selected as optimal choices on the basis of this estimation procedure. SampEn was then calculated for the defined one-minute periods of the detrended RR and QT time series using each r,M parameter pair: r=0.1,M=2; r=0.15,M=2; r=0.2,M=2; r=0.25,M=2.

HRV parameters were quantified in the time domain (RMSSDNN: square root of the mean of the sum of the squares of differences between adjacent RR intervals, and SDNN: standard deviation of all RR intervals) and the frequency domain (Total power: 0.017 to 0.4 Hz, low frequency (LF: 0.04-0.15 Hz), high frequency ($HF_{0.4}$: 0.15-0.4 Hz), $LF/HF_{0.4}$, normalised LF (LF_n) and normalised $HF_{0.4}$ ($HF_{0.4n}$)) according to the Task Force guidelines on HRV (Task Force ESC & NASPE 1996). The extended high frequency component ($HF_{1.0}$: 0.15 to 1.0 Hz) was also calculated along with the corresponding $LF/HF_{1.0}$ ratio, since it has previously been shown that the Task Force's recommended $HF_{0.4}$ bandwidth does not adequately account for the influence of RSA during moderate-to-high exercise work loads (Lewis *et al.* 2007). Frequency domain data analysis procedures were as described in this previous work (Lewis *et al.* 2007): RR interval data were re-sampled using a sampling frequency of 2 Hz and then linearly de-trended and Hanning windowed in consecutive one-minute segments; the power spectral density of each segment was then calculated using the Welch periodogram method, using short-term Fourier transformation and a 50% overlap between adjacent segments. In addition, the QTVI according to the equation described by Berger *et al.* (1997) was calculated.

Each of the SampEn and HRV parameters was averaged to provide a single representative value for discrete physiological states during pre-exercise

(metronomic breathing and spontaneous breathing states) and during post-exercise (0-5, 5-10 and 10-15 minute post-exercise recovery periods). (Exercise values for each parameter were already defined for representative one-minute stages.)

4.3.4.2 Detrended fluctuation analysis

The DFA α exponents were calculated separately for RR and QT time series data using the method suggested by Little *et al.* (2006). The data were first integrated over a specified number of data points (window length n) and fitted with the local linear trend, which was then subtracted from the locally integrated data set. This was repeated for all consecutive windows of length n within the original data set. The average root-mean-squared (RMS) fluctuation of the data was then calculated over the entire length of the data set. This procedure was repeated for multiple window lengths, to give the RMS fluctuation as a function of n (F(n)). A log-log plot of F(n) against n yielded the scaling exponent (α) via the gradient of the plot. In accordance with previous work (Peng *et al.* 1995, Hautala *et al.* 2003, Penttilä *et al.* 2003) two α parameters were quantified: short-term α_1 was determined using n between 4 and 11 samples, and long-term α_2 was calculated for all greater values of n, up to the maximum permitted by the data sample. The DFA α parameters were then individually averaged to provide a single representative value for discrete physiological states during pre-exercise (metronomic breathing and spontaneous breathing states) and during post-exercise (0-5, 5-10 and 10-15 minute post-exercise recovery periods). (Exercise values for the scaling exponents were already defined for representative one-minute stages.) The relative between-state discriminating ability of the HRV parameters described in section 4.3.4.1 was compared with that of the DFA α exponents.

4.3.5 Statistical analysis

The Lilliefors test for goodness of fit to a Normal distribution was used to assess whether individual parameters in the data were Normally distributed, and thereby informed the selection of appropriate parametric or non-parametric methods of statistical analysis.

4.3.5.1 Statistical analysis-SampEn

SampEn values for the four r,M combinations were compared for each physiological state using separate mixed between-within repeated measures ANOVA for the RR and QT data. Bartlett's test was consulted to confirm the assumption of sphericity for the data in the different physiological states. A significant P value for the two-way interaction effect (physiological state × r,M combination) was deemed to represent differences in the patterns of SampEn responses to physiological state. Where simple effect analysis identified a significant difference between the SampEn values (either for different r,M in a given physiological state, or for a single r,M between different physiological states) multiple pairwise comparisons with Bonferonni confidence interval adjustment were used to identify differences between individual SampEn values.

Differences between SampEn-RR and SampEn-QT for individual physiological states were assessed using paired t-tests. Correlations between individual HRV parameters and SampEn-RR were assessed using Spearman's rank order correlation coefficient (rho) since the assumptions of Normality (Lilliefors test) and homoscedasticity (Levene's test) were violated for the HRV parameters. Friedman's non-parametric one-way repeated measures ANOVA was used to compare mean HRV parameter values between physiological states, separately for

each parameter. To enable direct comparison, this procedure was repeated for the SampEn-RR values calculated for each of the four r,M parameter pairs.

4.3.5.2 Statistical analysis-fractal characteristics

The DFA α values were compared for each physiological state using separate one-way repeated measures ANOVAs for the RR and QT data. Bartlett's test was consulted to confirm the assumption of sphericity for the data in the different physiological states. Paired t-tests were used to examine differences between α for RR and α for QT, and to examine differences between the short-term (α_1) and long-term (α_2) values for both RR and QT data, in each physiological state.

Correlations between individual HRV indices and DFA α values were assessed using Spearman's rank order correlation coefficient (rho) since the assumptions of Normality (Lilliefors test) and homoscedasticity (Levene's test) were violated for the HRV parameters. In addition to the ANOVA procedures noted above, Friedman's non-parametric one-way repeated measures ANOVA was used to compare the mean DFA α exponent values between different physiological states, separately for both RR and QT data. This was instructive as it was the statistical procedure as employed in the SampEn analysis (section 4.3.5.1), and thereby enabled direct comparison of the between-state discriminating ability of the DFA α values and HRV parameters.

All data quoted in the text represent mean ± SD. Error bars in the figures represent the SEM (standard error in the mean) for the plotted parameters. Statistical differences were considered significant at the level $P<0.05$.

4.4 Results

Individual maximal oxygen uptake ($\dot{V}O_{2max}$) values obtained during the study confirmed the homogeneity of aerobic fitness within the subject group (49.6 ± 4.8 $ml \cdot kg^{-1} \cdot min^{-1}$). Owing to the difficulty of reliably measuring QT interval during the elevated heart rates associated with exercise, QT data were available only for exercise intensities up to 70.1 ± 6.3 % $\dot{V}O_{2max}$ (equivalent to a heart rate of 151 ± 9 bpm). Table 4.1 provides a characterisation of group mean responses to the different physiological states used in the experimental protocol with regard to heart rate, relative work load, blood lactate and blood glucose.

Table 4.1 Physiological responses to different physiological states in the study (group mean ± SEM)

State	MB	SB	e1	e2	e3	e4	e5	r5	r10	r15
Heart rate (bpm)	80 ± 4	81 ± 4	103 ± 4	116 ± 4	132 ± 4	144 ± 5	151 ± 9	136 ± 4	122 ± 6	117 ± 7
Relative work rate (%VO_2 max)	20.3 ± 3.4	18.6 ± 3.1	37.7 ± 1.0	44.0 ± 1.4	54.1 ± 1.3	62.3 ± 1.5	70.1 ± 1.8	55.5 ± 2.8	46.8 ± 2.3	45.6 ± 2.9
Lactate ($mmol \cdot L^{-1}$)	-	0.9 ± 0.0	1.2 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	2.8 ± 0.2	5.0 ± 0.5	8.8 ± 0.5	-	-
Glucose ($mmol \cdot L^{-1}$)	-	4.7 ± 0.2	4.7 ± 0.2	4.6 ± 0.2	4.5 ± 0.2	4.5 ± 0.2	4.4 ± 0.3	5.2 ± 0.4	-	-

MB = metronomic breathing stage; SB = spontaneous breathing stage; e1-e5 = exercise stages 1-5; r5,r10,r15 = recovery stages (r5 = 0-5 minutes, r10 = 5-10 minutes, r15 = 10-15 minutes post-exercise)

4.4.1 SampEn results

Figure 4.1 shows a truncated version of the distributions of the product [(normalised SampEn) × (1-normalised SE-SampEn)] for differing r,M paired parameters during the resting, exercise and recovery conditions, separately for the RR and QT data sets. The plots show distinct regions in which this array

product yielded the highest values, and thereby suggests combinations of r and M that are optimal for SampEn calculation. Group mean values for SampEn-RR are presented in Figure 4.2 (a) as functions of heart rate and physiological state. Group mean values for SampEn-QT are presented in Figure 4.2 (b) as functions of heart rate and physiological state.

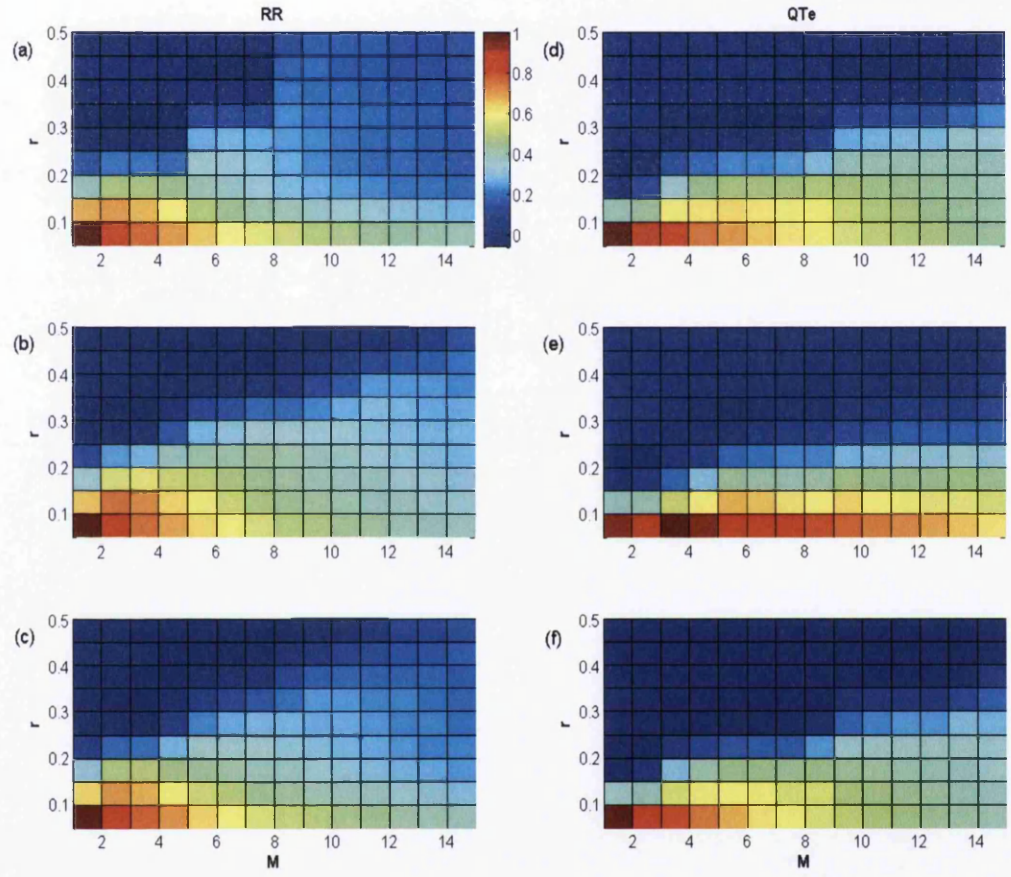


Figure 4.1 Distributions of the array product [(normalised SampEn) × (1-normalised SE-SampEn)] as a function of r and M for RR (a-c) and QT (d-f) data sets during pre-exercise (a & d), exercise (b & e) and recovery (c & f). Colour scale: Red represents larger values of the array product, blue represents smaller values as indicated in (a)

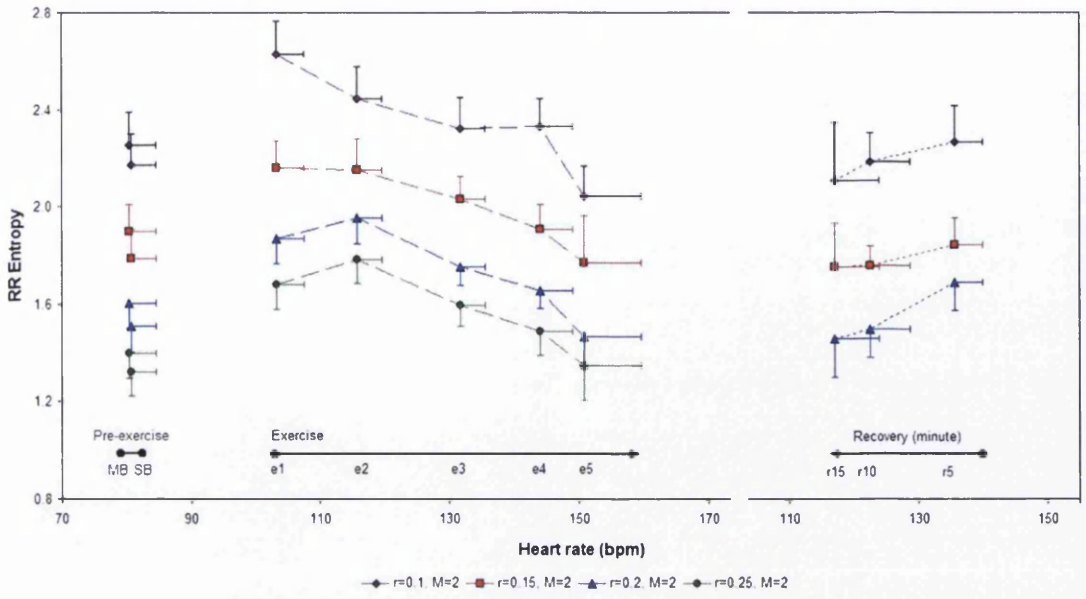


Figure 4.2 (a) SampEn-RR as a function of heart rate and physiological state. (MB = metronomic breathing stage; SB = spontaneous breathing stage; e1-e5 = exercise stages 1-5; r5,r10,r15 = recovery stages (r5 = 0-5 minutes, r10 = 5-10 minutes, r15 = 10-15 minutes post-exercise)

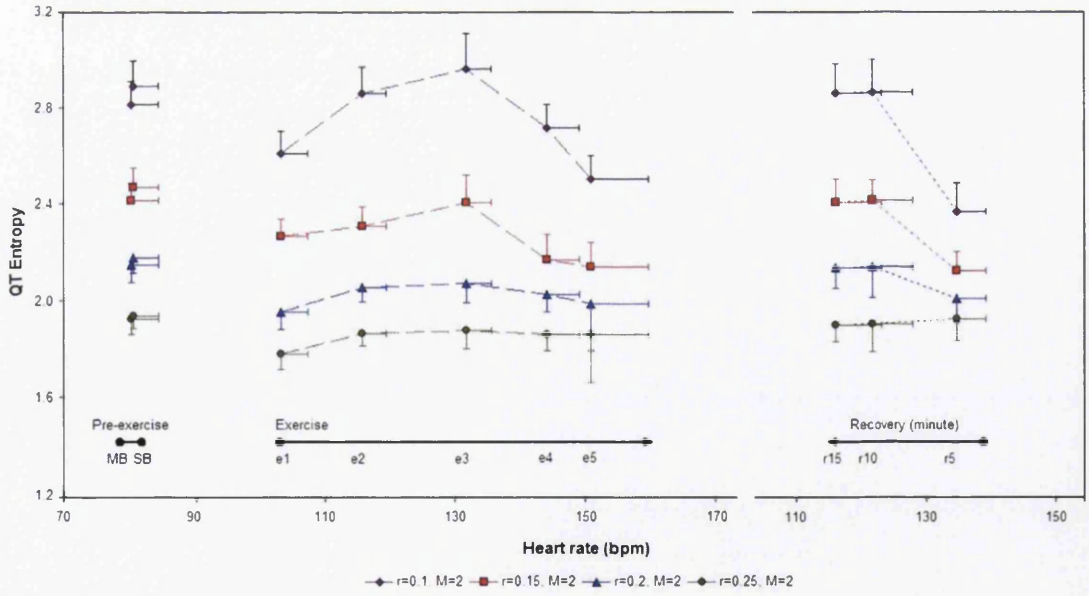


Figure 4.2 (b) SampEn-QT as a function of heart rate and physiological state. (MB = metronomic breathing stage; SB = spontaneous breathing stage; e1-e5 = exercise stages 1-5; r5,r10,r15 = recovery stages (r5 = 0-5 minutes, r10 = 5-10 minutes, r15 = 10-15 minutes post-exercise)

For the group mean values for SampEn-RR, there was a statistically significant main effect for physiological state, with a large effect size ($F_{9,345}$=9.75; p<0.0001; partial eta squared=0.203). *Post-hoc* comparisons using the Bonferroni test indicated that mean SampEn-RR during the first and second stages of exercise was significantly greater than that during metronomic breathing and spontaneous breathing, and significantly greater than that during minutes 6 to 15 of recovery. SampEn-RR during the third stage of exercise was also greater than that during minutes 11 to 15 of recovery. There were no other differences between physiological states. There was a statistically significant main effect for r,M paired parameters, with a large effect size ($F_{3,345}$=83.3; p<0.0001; partial eta squared=0.420). *Post-hoc* comparisons using the Bonferroni test indicated that SampEn-RR was significantly different for every comparison of r,M pair. The interaction effect (physiological state × r,M pair) was not statistically significant ($F_{27,345}$=0.24; p=1.0; partial eta squared=0.019), indicating that the trend of SampEn across physiological states was not influenced by the choice of r,M (i.e. different r,M for this subset of optimal combinations resulted in a consistent magnitude difference (bias) only).

For the group mean values for SampEn-QT, there was a statistically significant main effect for physiological state, with a moderately large effect size ($F_{9,360}$=3.94; p=0.0001; partial eta squared=0.090). *Post-hoc* comparisons using the Bonferroni test indicated that mean SampEn(QT_e) during the first stage of exercise was significantly less than that during both metronomic and spontaneous breathing and significantly less than that during minutes 6 to 15 of recovery. There were no other differences between physiological states. There was also a statistically significant main effect for r,M paired parameters, with a large effect size ($F_{3,360}$=180.4; p<0.0001; partial eta squared=0.600). *Post-hoc* comparisons using the Bonferroni test indicated that SampEn-QT_e was significantly different for every

comparison of r,M pair. The interaction effect (physiological state × r,M pair) was not statistically significant ($F_{27,360}$=0.30; p=0.9998; partial eta squared=0.022).

SampEn-RR was significantly less than SampEn-QT for all equivalent physiological states except during the first two stages of exercise (p=0.0001 to 0.039), when SampEn-RR was substantially elevated. SampEn-RR demonstrated a low-to-moderate negative correlation with LF_n (r=-0.24 to -0.32, p<0.021), $LF/HF_{0.4}$ (r=-0.22 to -0.27, p<0.039) and $LF/HF_{1.0}$ (r=-0.22 to -0.30, p<0.039) for each of the four r,M pairs. SampEn-RR with r=0.1 and M=2 was additionally negatively correlated with $HF_{1.0}$ and $HF_{0.4n}$ (r=-0.22, p<0.041 for both). There were no other statistically significant correlations between SampEn-RR and HRV parameters. Table 4.2 shows the HRV and SampEn parameters ranked according to their ability to discriminate between physiological states, based on the results of Friedman's one-way repeated measures ANOVA. Only SDNN and QTVI were sensitive to differences between exercise states.

Table 4.2 Sample entropy and indices of heart rate variability for RR time-series data, in rank order according to between-state discriminating ability

Rank	Parameter	Chi-sq	P>Chi-sq	Partial eta squared
1	SDNN *	70.65	3.64×10^{-12}	0.736
2	RMSSDNN	66.13	2.88×10^{-11}	0.689
3	$HF_{0.4}$	52.63	1.27×10^{-8}	0.548
4	Total	46.86	1.63×10^{-7}	0.488
5	$HF_{1.0}$	41.80	1.48×10^{-6}	0.435
6	QTVI *	41.24	1.88×10^{-6}	0.430
7	$LF/HF_{1.0}$	32.63	7.18×10^{-5}	0.340
8	$LF/HF_{0.4}$	28.20	4.38×10^{-4}	0.294
9	LF_n	28.14	4.48×10^{-4}	0.293
10	$HF_{0.4n}$	27.05	6.93×10^{-4}	0.282
11	SampEn-RR (r=0.25,M=2)	23.72	2.60×10^{-3}	0.247
12	LF	21.37	6.20×10^{-3}	0.223
13	SampEn-RR (r=0.2,M=2)	21.35	6.30×10^{-3}	0.222
14	SampEn-RR (r=0.15,M=2)	14.62	6.71×10^{-2}	0.152
15	SampEn-RR (r=0.1,M=2)	14.33	7.37×10^{-2}	0.149

Chi-sq = Chi-squared statistic from Friedman's one-way repeated measures ANOVA; *Parameter distinguished between different exercise work loads

4.4.2 Fractal characteristics results

4.4.2.1 Results for the short-term DFA α_1 exponent

Group mean values for the short-term α_1 exponents for RR and QT data are presented in Figure 4.3 (a) as functions of heart rate and physiological state.

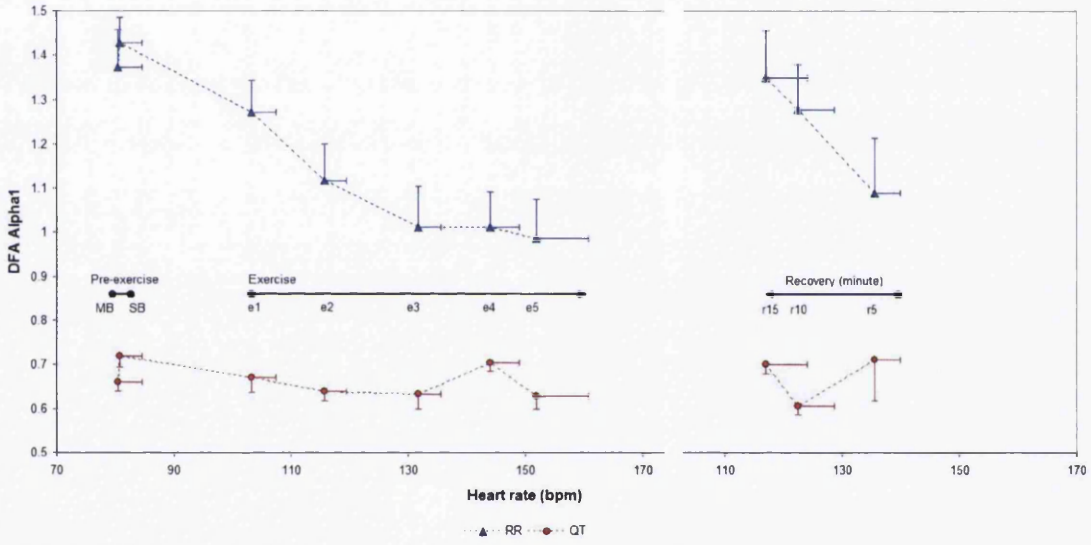


Figure 4.3 (a) DFA α_1 as a function of heart rate and physiological state. (MB = metronomic breathing stage; SB = spontaneous breathing stage; e1-e5 = exercise stages 1-5; r5,r10,r15 = recovery stages (r5 = 0-5 minutes, r10 = 5-10 minutes, r15 = 10-15 minutes post-exercise)

There was a statistically significant main effect of physiological state on both these parameters, the effect size being moderately large (α_1 (RR): $F_{9,94} = 3.11$, $p = 0.003$, partial eta squared = 0.230; α_1 (QT): $F_{9,88} = 2.53$, $p = 0.013$, partial eta squared = 0.205). However, *post-hoc* tests revealed that the only differences in α_1 (RR) were between states e5 and SB. No between-stage differences were

identified for α_1 (QT). α_1 (RR) was significantly greater than α_1 (QT) for each individual physiological state ($p<0.00001$ during rest; $0.0001<p<0.032$ during exercise; $0.001<p<0.016$ during recovery).

For the purposes of comparison with the parameters in section 4.4.1, Friedman's one-way repeated measures ANOVA was carried out to compare α_1 for the different physiological states. Friedman's ANOVA confirmed that the DFA α parameters were moderate discriminators of differing physiological states (α_1 (RR): Chi-sq = 32.94, P>Chi-sq = 1.4×10^{-4}, partial eta squared = 0.305; α_1 (QT): Chi-sq = 17.68, P>Chi-sq = 0.039, partial eta squared = 0.164). α_1 (RR) was a better state discriminator than α_1 (QT), with α_1 (RR) values during the final two stages of exercise being significantly lower compared with those at rest. However, α_1 (RR) was a relatively poor discriminator of physiological state when compared with the majority of other tested parameters. (Chi-sq = Chi-squared statistic from Friedman's one-way repeated measures ANOVA.)

It was observed that α_1 (RR) demonstrated significant low-to-moderate correlations with the following HRV parameters: RMSSDNN ($r=0.22$, $p=0.030$), SDNN ($r=0.46$, $p = 5 \times 10^{-6}$), LF ($r=0.28$, $p=0.005$), LF_n ($r=0.41$, $p = 3 \times 10^{-5}$), $LF/HF_{0.4}$ ($r=0.27$; $p=0.009$), $LF/HF_{1.0}$ ($r=0.36$; $p = 4 \times 10^{-4}$), QTVI ($r=-0.31$, $p=0.005$) and SampEn(RR) (-0.49, $p = 5 \times 10^{-6}$). There were no other statistically significant correlations between α_1 (RR) and HRV parameters. As expected, there were no statistically significant correlations between α_1 (QT) and any of the HRV parameters.

4.4.2.2 Results for the long-term DFA α_2 exponent

Group mean values for the long-term α_2 exponents for RR and QT data are presented in Figure 4.3 (b).

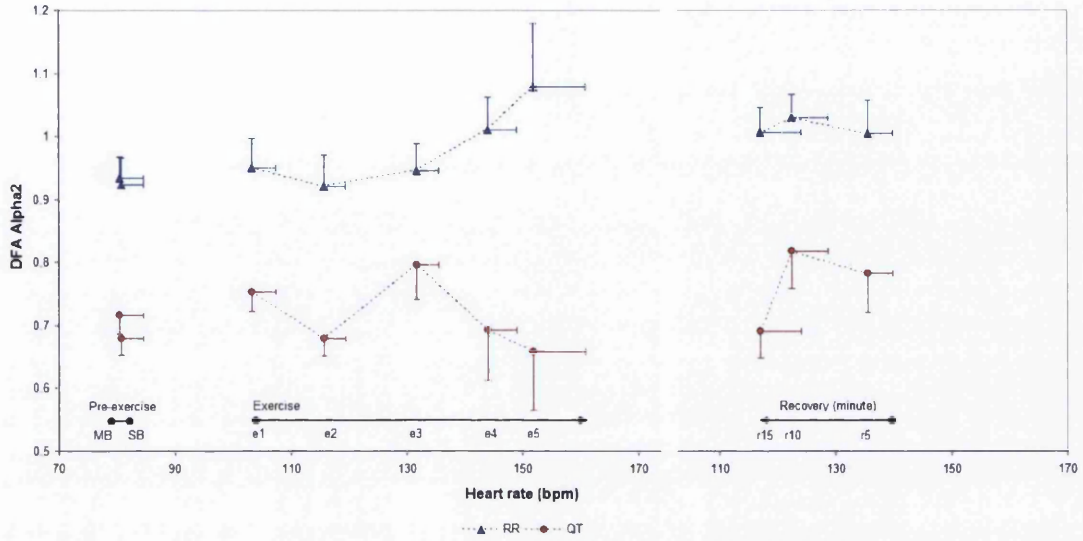


Figure 4.3 (b) DFA α_2 as a function of heart rate and physiological state. (MB = metronomic breathing stage; SB = spontaneous breathing stage; e1-e5 = exercise stages 1-5; r5,r10,r15 = recovery stages (r5 = 0-5 minutes, r10 = 5-10 minutes, r15 = 10-15 minutes post-exercise)

There was no statistically significant main effect of physiological state on either of these parameters, the effect size being small in each case (α_2 (RR): $F_{9,96} = 0.61$, $p = 0.78$, partial eta squared = 0.06; α_2 (QT): $F_{9,94} = 1.13$, $p = 0.35$, partial eta squared = 0.10). Therefore there were no differences in α_2 between physiological states for either RR or QT time series data. α_2 (RR) was again significantly greater than α_2 (QT) for each individual physiological state ($p<0.00001$ during rest; $0.001<p<0.034$ during exercise; $0.0001<p<0.002$ during recovery).

Friedman's ANOVA confirmed that the α_2 parameters were relatively poor discriminators of differing physiological states (α_2 (RR): Chi-sq = 8.40, P>Chi-sq = 0.4944, partial eta squared = 0.078; α_2 (QT): Chi-sq = 17.16, P>Chi-sq = 0.0462, partial eta squared = 0.159). Thus, although α_2 (QT) was better than α_2 (RR) in this regard, both were poorer discriminators of physiological state than the majority of other tested HRV parameters.

It was observed that α_2 (RR) demonstrated significant low-to-moderate negative correlations with the following HRV parameters: RMSSDNN (r=-0.29, p=0.003), SDNN (r=-0.23, p=0.018), Total Power (r=-0.22, p=0.028), $HF_{1.0}$ and $HF_{0.4}$ (r=-0.25, p<0.012 for both). There were no other statistically significant correlations between α_2 (RR) and HRV parameters (including SampEn and QTVI). As expected, there were no statistically significant correlations between α_2 (QT) and any of the HRV parameters.

4.5 Discussion

4.5.1 Selection of optimal values of r and M for SampEn calculation

The distributions of the array product [(normalised SampEn) × (1-normalised SE-SampEn)] suggested that there were some differences in the optimal r,M combinations for different physiological states, and between the RR and QT data sets. As noted by Pincus & Goldberger (1994), the utility of entropy (ApEn) is as a relative measure of data complexity, using a fixed r,M pair. The selection of a single r,M pair from the preliminary analysis that could be used consistently for both RR and QT data sets and for each of the different physiological states was therefore sought. The results of this procedure indicated that optimal values were within the range r=0.1-0.2 and M=2-4. The choice of the four r,M pairs for subsequent analysis was based on these results. However, M=2 for each of the

four cases was used, in order to conform to the guideline that a reasonable estimate of entropy (ApEn) requires at least 10^M data points (Pincus & Goldberger 1994). There were between 80 and 150 data points in each analysed one-minute period, and so the most appropriate choice of M was 2. One value of r outside the indicated optimal range (r=0.25) was also used in order to examine the effect of reduced tolerance.

4.5.2 Influence of physiological state on the magnitude of SampEn

The magnitudes of both SampEn-RR and SampEn-QT were strongly dependent on the selection of r and M parameters. As stated previously, SampEn-RR and SampEn-QT responded similarly to different physiological conditions for each of the four r,M pairs, demonstrating their consistency in response to differing physiological conditions. SampEn-RR showed a general trend towards increased values during the initial stages of exercise, declining as exercise intensity increased, and then initially increasing prior to a continuing further decline during recovery. SampEn-RR was significantly greater during light-to-moderate exercise work loads compared with during pre-exercise and the final 10 minutes of recovery. SampEn-QT showed a general trend towards decreased values during the initial stage of exercise, maintained a fairly uniform magnitude as exercise intensity increased (apart from when r=0.1 and M=2), and then either increased or maintained a uniform magnitude during recovery. SampEn-QT was significantly reduced during the lowest exercise work load compared with during pre-exercise and the final ten minutes of recovery. Neither SampEn-RR nor SampEn-QT distinguished between any of the other physiological states, including respiratory mode (spontaneous or metronomic breathing).

There have been several investigations of the relative influence of spontaneous and controlled (metronomic) breathing on traditional indices of HRV. Pagani *et al.* (1986) observed that controlled respiration produces a marked increase in HF power, whilst later studies showed that the influence of metronomic breathing on HRV is rate-dependent (Patwardhan *et al.* 1995, Sanderson *et al.* 1996). Strano *et al.* (1998) further suggested that standardising the respiratory rate in athletes to 15 breaths per minute (0.25 Hz) facilitates optimal estimation of the respiratory-related (HF) component of HRV. The assessment of SampEn during standardised breathing conditions was therefore sought, hence the inclusion of spontaneous and metronomic breathing in the testing protocol. A slightly elevated SampEn-RR and a slightly reduced SampEn-QT during metronomic breathing at 0.25 Hz compared with spontaneous breathing was observed, but the difference was not statistically significant. There was therefore some indication that SampEn parameters are sensitive to respiratory mode (possibly via altered parasympathetic influence or mechanical effects, or both) although this requires further investigation.

No previous study has quantified SampEn for QT time-series data. SampEn-RR was significantly lower than SampEn-QT for equivalent r,M pairs during all physiological conditions except during the initial stages of exercise, when SampEn-RR was generally at its highest values. This observation, interpreted as evidence of greater complexity in the modulation of QT interval compared with the RR interval, might be explained with regard to differential ANS modulation of the atrial and ventricular myocardium. The ANS has a direct parasympathetic influence on the ventricular myocardium, and this might affect cardiac repolarisation independently of chronotropic (heart rate) modulation of the SA node, especially at elevated heart rates (Ahnve & Vallin 1982, Shimizu *et al.* 1994, Magnano *et al.* 2002). Moreover, QT shortening during exercise has a substantial

component that is not controlled by heart rate (Rickards & Norman 1981, Milne *et al.* 1988). This therefore leads to the tentative suggestion that SampEn-QT reflects, to a degree, the combined influence of ANS modulation of both the atrial and ventricular myocardium. Moreover, during moderate-to-high exercise work loads, SampEn-QT might dominantly represent the complexity of ANS modulation of ventricular repolarisation.

Few previous studies have attempted to characterise the influence of exercise on measures of RR interval complexity. In agreement with the present work, Tulppo *et al.* (2001) observed that ApEn increased during low-intensity steady state exercise (a walking test) compared with baseline. Javorka *et al.* (2002) quantified SampEn in the standing and supine positions following sub-maximal exercise (a step test at an intensity equivalent to 70% of maximal work rate). SampEn in the supine position was significantly lower during post-exercise recovery compared with pre-exercise (and re-attained the pre-exercise value within 30 minutes), and was greater than during pre-exercise standing. However, these authors did not assess SampEn during exercise itself. In the present study, subjects were in the seated position during pre- and post-exercise, and post-exercise SampEn tended to initially increase and then decrease as recovery proceeded.

4.5.3 Relationship between SampEn-RR, heart rate and linear indices of HRV

Tulppo *et al.* (2001) noted that ApEn (and the change in ApEn from baseline during low-intensity steady state exercise) was either uncorrelated or was only weakly correlated with heart rate or spectral measures of HRV. However, Platisa & Gal (2006b) reported significant ($p<0.01$) correlations between SampEn and the natural logarithm of each of the parameters Total Power ($r=0.62$), LF ($r=0.56$), HF ($r=0.83$), and LF/HF ($r=-0.78$). Platisa & Gal (2006b) reported a generally linear

relationship between SampEn and RR and noted that at the highest heart rates the trend of reduced SampEn with increasing heart rate (observed in young subjects) was reversed in elderly subjects (SampEn increased with increasing HR). In comparison, significant low-to-moderate negative correlations between SampEn(RR) and LF_n for each of the four r,M pairs were observed in this work. Similar to Platisa & Gal (2006b), reductions in SampEn were observed when heart rate was substantially elevated in our healthy young subjects. When linear and non-linear cardiac indices were ranked according to their ability to discriminate between different physiological states, the time-domain HRV parameters (SDNN and RMSSDNN) performed best and SampEn-RR performed worst. Notably, lower values of tolerance (r) were associated with poorer discriminating ability.

4.5.4 Magnitude of α scaling exponents for RR data

Peng *et al.* (1995) noted values of $\alpha_1 = 1.201 \pm 0.178$ and $\alpha_2 = 0.998 \pm 0.124$ for RR data in healthy young adults. Pikkujamsa *et al.* (1999) noted similar values of $\alpha_1 = 1.15 \pm 0.16$ and $\alpha_2 = 1.00 \pm 0.08$. These values are consistent with the resting values observed in the present study: $\alpha_1 = 1.37 \pm 0.08$ (spontaneous breathing) and 1.43 ± 0.06 (metronomic breathing); $\alpha_2 = 0.93 \pm 0.03$ (spontaneous breathing) and 0.92 ± 0.04 (metronomic breathing). The post-exercise recovery values were similar to those at rest, ranging between 1.09 ± 0.12 and 1.35 ± 0.11 (α_1) and between 1.00 ± 0.05 and 1.03 ± 0.04 (α_2).

Hautala *et al.* (2003) noted that, compared with rest, α_1 increased from 1.13 ± 0.18 to 1.36 ± 0.11 during uniform low-intensity exercise and decreased from 1.19 ± 0.26 to 0.58 ± 0.26 during uniform high-intensity exercise. During a progressive maximal exercise test these authors observed that α_1 increased initially (from 1.07 ± 0.24 at rest to 1.50 ± 0.25 at ~40% $\dot{V}O_{2max}$) and then decreased linearly until the

end of exercise (to a value of 0.38 ± 0.10). This bidirectional change was interpreted with regard to the influence of an initial withdrawal of parasympathetic (vagal) modulation during the initial stages of exercise, since the effect was not observed when the exercise test was repeated following vagal blockade with atropine (during which α_1 reduced linearly). The authors also noted that atropine administration during rest caused α_1 to increase significantly (from 0.91 ± 0.23 to 1.37 ± 0.31), confirming a similar effect observed by Penttilä *et al.* (2003). In the present work, resting values for α_1 of between 1.37 ± 0.08 and 1.43 ± 0.06 were observed, which were comparable to the values noted by Hautala *et al.* (2003) following parasympathetic blockade at rest. A linear reduction in α_1 as a function of increasing exercise work load (to a minimum value of 0.99 ± 0.09 at ~70% $\dot{V}O_2$) was also observed, with no bidirectional change (Figure 4.3 (a)). This difference in results may be interpreted as the consequence of a greater initial reduction in cardiac parasympathetic modulation at the onset of exercise for our subjects. The subjects in the present study were physically active and reasonably well trained, and they were considerably younger than those examined by Hautala *et al.* (2003). A rapid and substantial reduction in parasympathetic activity at the onset of even low-to-moderate intensity exercise for such subjects has been previously noted (Lewis *et al.* 2007).

4.5.5 Magnitude of α scaling exponents for QT data

The α_1 values for QT were significantly lower than for RR during all physiological states: 0.66 ± 0.02 (rest, spontaneous breathing) and 0.72 ± 0.02 (rest, metronomic breathing); 0.63 ± 0.09 to 0.71 ± 0.05 (range, exercise); 0.69 ± 0.03 to 0.82 ± 0.02 (range, post-exercise recovery). The α_2 values for QT were also significantly lower than for RR during all physiological states: 0.68 ± 0.03 (rest, spontaneous breathing) and 0.72 ± 0.03 (rest, metronomic breathing); 0.66 ± 0.09 to 0.80 ± 0.05 (range, exercise); 0.69 ± 0.04 to 0.82 ± 0.06 (range, post-exercise

recovery). The α_1 and α_2 values for QT were significantly different only during parts of the rest and recovery periods.

4.5.6 Influence of physiological state on α exponents

In section 4.4.1, when linear and non-linear cardiac indices were ranked according to their ability to discriminate between different physiological states (Table 4.2), the linear time-domain HRV parameters (SDNN and RMSSDNN) performed best and the non-linear parameter SampEn-RR performed worst. Moreover, only the linear indices SDNN and QTVI were sensitive to differences between exercise states. For the detrended fluctuation analysis, the α_1 parameter for RR data displayed a moderate ability to discriminate between physiological states. However, it was a poor discriminator of physiological state when compared with the majority of the other quantified parameters, at least for the short time periods considered here. Conversely neither α_2 (for RR) nor either α_1 or α_2 (for QT) were able to differentiate between the physiological states in the study protocol in any statistically meaningful way. It is also worthy of note that Penttilä *et al.* (2003) suggested that breathing rate can significantly affect the α scaling exponent and these authors suggested that the respiratory rate should be controlled at 15 breaths per minute (0.25 Hz) to standardise protocols. However, no significant differences in α value between metronomic breathing and spontaneous breathing states were observed in the present study.

4.5.7 Relationship between α exponents and other indices of heart rate variability

It was observed that the α_1 and α_2 scaling exponents for RR demonstrated differences in their correlations with the other calculated HRV characteristics, although both exponents correlated with RMSSD and SDNN. Makikallio *et al.* (1999) previously suggested that α_1 was not correlated with any single measures

of HRV, although it was related to the LF/HF ratio. Jokinen *et al.* (2001) observed significant correlations between α_1 and SDNN, VLF, HF and ApEn and between α_2 and each of LF, HF and ApEn. Francis *et al.* (2002) also later concluded that the α_1 and α_2 indices are frequency-weighted versions of the spectral ratios LF/(HF + LF) and VLF/(LF + VLF), respectively. Hautala *et al.* (2003) observed a strong resting correlation between α_1 and LF and a strong inverse correlation between α_1 and HF_n, noting that the correlations were weaker during exercise and following parasympathetic blockade. The results of the present study are only partly consistent with these previous findings, possibly owing to differences in the measurement periods used for HRV parameter determination in the different studies.

4.6 Limitations

The electrocardiographic RR and QT intervals cannot be quantified with equivalent accuracy or precision owing to the nature of the ECG characteristics that are used in their measurement (chapter 3). Here, the detection performance for T_a and T_e of the Holter system used were considered broadly equivalent. The T_a-T_e interval contains an independent variability component (Davey 1999). These findings suggest that QT_e provides the most reliable and accurate assessment of QT interval dynamics. Nevertheless, it is possible that the measurement accuracy and precision of the QT time-series data might have influenced the calculation of SampEn-QT, and further investigation is needed to assess this. It is also recognised that it is possible that the SampEn-QT results detailed in this chapter are specific to the Holter measurement system used, and that it might be useful to quantify this parameter for other systems.

The QT interval is also known to vary in part as a function of heart rate, however, it was decided not to adjust QT interval for heart rate in this study. The limitations of universal formulae for adjusting the QT interval in this way are well recognised (Aytemir *et al.* 1999, Desai *et al.* 2003). Moreover, the results detailed in chapter 3 indicated that physical exercise appears to perturb the QT–RR relationship via a substantially altered ANS influence and so a single QT adjustment could not reliably be applied across the different physiological states. Investigation of any potential influence on SampEn-QT of heart rate adjustment of the QT interval might perhaps be the topic of further investigation.

A uniform time interval of one minute for the analysis of SampEn and fractal charecteristic α during each of the physiological states was used, rather than a uniform number of time-series data points. It could be argued that this might have influenced the results since the uniform time interval contained increasing numbers of data points as heart rate increased. However, Merati *et al.* (2006) noted that the use of either a fixed number of beats or a fixed time period for entropy calculation provides largely equivalent results and suggested that these methods are "interchangeable". Hautala *et al.* (2003) observed that there were was no difference between α_1 values calculated for either a three-minute period of RR data during exercise or a 200 beat subset of data chosen from that period. Recently, Delignieres *et al.* (2006) showed that DFA provides acceptable estimates of the scaling exponent using data sets consisting of 128 or more points. It is considered therefore that the methodology used in this chapter was appropriate given the physiological restraints, and moreover was a logical choice for enabling the direct comparison of SampEn and α and indices of HRV (also calculated for fixed one minute periods).

4.7 Conclusion

The sample entropy of RR time-series data is sensitive to differing physiological conditions when an appropriate choice of parameters is used for its calculation. The results of the study presented in this chapter suggest that values of r=0.1-0.15 and M=2 represent an optimal choice for the consistent estimation of SampEn for RR and QT data. However the ability of SampEn to discriminate between resting and exercise conditions is poorer than that of the common linear measures of HRV, and it was unable to differentiate between the work loads used in this study.

SampEn of RR data is negatively correlated with normalised LF and LF/HF parameters in cardiac control, confirming that the complexity of cardiac interval time-series is associated with ANS functional status. Pincus & Goldberger (1994) suggested that the reduction in entropy (ApEn) during pathology represents system decoupling from external inputs, or a reduction in the influence of these inputs. Changes in SampEn for RR or QT data in terms of the altered ANS control of either the atrial or ventricular myocardium (or both) during discrete physiological states are similarly interpreted here. Further investigations might usefully examine the utility of a combined analysis of SampEn for RR and QT data during specific types of pathology.

The DFA scaling exponent values for RR were in general agreement with those observed previously during rest and exercise. Post-exercise recovery values of the scaling exponents have not previously been documented; it was observed that recovery values reverted to pre-exercise values within the observation period. During rest and post-exercise recovery stages, the RR data displayed strong scaling characteristics tending towards those of brown noise (α=2). However, as

exercise work loads increased the RR data tended to demonstrate a 1/f scaling behaviour (α=1).

Scaling exponent values for QT data had not previously been reported. It was observed that α values for QT were significantly lower than those for RR during all physiological states. Moreover, the α values for QT approached 0.5 during the heaviest exercise work loads, suggesting that the QT data had the characteristics of uncorrelated white noise during these periods.

Short-term (α_1) and long-term (α_2) values for RR differed significantly during rest and during the initial stages of exercise, but were otherwise comparable, whilst α_1 and α_2 values for QT differed only for parts of the rest and recovery stages. However, α_1 for RR was found to be the better discriminator between different physiological states. These differences suggest that it is preferable to report both α_1 and α_2 exponents for RR and QT data. Nevertheless it should be borne in mind that the ability of α to discriminate between resting and exercise conditions is poorer than that of the majority of common linear and non-linear measures of HRV. The α_1 exponent for RR was found to be negatively correlated with various linear and non-linear indices of HRV, confirming that the fractal characteristic of cardiac interval time-series data is associated with ANS functional status.

These results suggest that RR and QT have dissimilar fractal structures, possibly reflecting differences in the mechanisms of temporal modulation of these two aspects of cardiac electrical activity. Further investigations might usefully compare the fractal characteristics of both RR and QT data in cases of cardiac or neural pathology.

Chapter 5 Investigation of QT interval variability and its association with ventricular function in healthy adults during rest and exercise

5.1 Rationale

Perturbation of the resting QT-RR relationship during physical exercise is possibly caused by the onset of differential parasympathetic modulation of the atrial and ventricular myocardium. One method of investigating this non-invasively could be through the quantification of the QTVI which reflects the ratio of variabilities of QT and RR time-series and might be related to the heart rate-dependent and heart rate-independent influences on the QT interval. Simultaneous characterisation of the influence of physical exercise on cardiac ventricular function and cardiac electrical variability has not been previously conducted, hence little is known about the relationship between ventricular function and either heart rate (RR) or repolarisation (QT) variability. The relationship between QTVI and ventricular function could be of clinical interest and it is postulated that QTVI might have use as a non-invasive surrogate marker of ventricular function.

5.2 Aims and objectives

The aims of this chapter were:

1. To quantify electrocardiographic QT interval and RR interval variability during rest and dynamic physical exercise
2. To quantify indices of cardiac ventricular function, as assessed indirectly by ICG and non-invasively by ECG measurement, through the determination of RR and QT intervals and their variabilities in the time-domain and their relative variabilities via the QTVI

The objectives were:

1. To interpret QT and RR variabilities in terms of relative autonomic modulation of the atrial and ventricular myocardium
2. To characterise the relationships between QT, QT_c and RR intervals and to consider their associations with differential autonomic regulation
3. To quantify the above in terms of the data collected from the different leads of a clinical Holter ECG system
4. To examine whether the above are influenced by gender
5. To characterise measures of cardiac ventricular function during rest, progressive (maximal) physical exercise and post-exercise recovery
6. To examine and compare the relationships between ventricular function indices and standard measures of RR and QT interval variability (including QTVI) using multiple regression analysis
7. To examine whether the relationship between these parameters is altered following high-intensity physical exercise

5.3 Introduction

5.3.1 Study 1: Analysis of QT interval and its variability

There have been relatively few studies of the influence of dynamic physical exercise on the QT interval or of the QT-RR relationship during these conditions. Several authors have reported a linear relationship between uncorrected QT and both heart rate and RR interval during light exercise (Kligfield *et al.* 1996, Mayuga 2001, Chauhan 2002). However, Mayuga *et al.* (2001) compared several heart rate correction models for a range of heart rates during exercise, concluding that a natural logarithmic model was the best description of the QT-RR relationship. The dynamic QT-RR relationship is further complicated by the fact that the magnitude of QT shortening in response to increased heart rate depends on the mechanism causing the increase. Increased heart rate during atrial pacing

causes a smaller reduction in QT interval compared with that during exercise at similar heart rates (Rickards & Norman 1981, Milne *et al.* 1982). In addition, QT interval shortening has been observed during exercise at uniformly-paced heart rates in patients with heart block (Rickards & Norman 1981). Porta *et al.* (1998) used spectral analysis to model the relationship between the RR and ventricular repolarisation (RT_a) intervals. These authors observed that RT_a was heart rate-dependent within the standard low frequency and high frequency bandwidths, but there was a substantial heart rate-independent contribution to this parameter at very low frequencies. Almeida *et al.* (2006) used a similar approach and found that the heart rate-independent contribution to QT variability was greater than 40% in the majority of healthy subjects in their study. These studies demonstrated that QT interval shortening during exercise has a substantial component that is not controlled by heart rate. It has since been shown that the ANS has a direct influence on the ventricular myocardium, and this might affect cardiac repolarisation independently of chronotropic modulation via the SA node (Shimizu *et al.* 1994, Magnano *et al.* 2002).

To date there have been no reports of the quantification of heart rate-dependent and heart rate-independent influences on the atrial and ventricular myocardium during physical exercise. To reiterate section 2.3.2.3, QTVI quantifies the relative magnitude of temporal variability in QT and RR intervals and can be interpreted with regard to the relative autonomic modulation of these two parameters. QTVI might therefore be a useful index for delineating the relative autonomic influences on the atrial and ventricular myocardium.

5.3.2 Study 2: Relationship between ventricular function, RR variability and QT variability

Several previous studies have indicated an association between changes in QTVI and altered ANS activity. For example, QTVI is decreased after postural tilt in healthy subjects (Piccirillo *et al.* 2001) and is increased in patients with chronic renal failure (Johansson *et al.* 2004). Moreover, increased QTVI is a risk factor for sudden cardiac death (Atiga *et al.* 1998,2000), indicates a greater susceptibility for malignant ventricular arrhythmias (Berger *et al.* 1997, Atiga *et al.* 1998,2000) and is associated with an independent risk for ventricular tachycardia or ventricular fibrillation (Haigney *et al.* 2004). Recently, Piccirillo *et al.* (2007) found that an elevated QTVI was a strong predictor of SCD in chronic heart failure patients who were asymptomatic and had only a slightly depressed ejection fraction.

However, despite its increasing use in clinical research, QTVI during physical exercise, a physiological condition during which there are marked changes in both ANS activity and cardiac functional performance, has not been extensively assessed. To date, there has been no investigation of the relationship between QT or RR variability parameters or QTVI and traditional indices of cardiac functional performance. In particular it would be of great clinical interest to establish which aspects of cardiac performance have the greatest association with QTVI. Moreover there appears to have been no previous study in which the range of cardiac ventricular performance indices have been characterised and compared during conditions of rest, exercise and subsequent recovery.

5.4 Methodology

Although this chapter is presented as two studies, each study population is a subset of a main group which underwent an identical testing protocol. All recordings were performed in the Exercise Physiology Laboratory at the Sport and Exercise Science Research Centre, Swansea University and the investigation was approved by the departmental ethics committee. Subject health screening was undertaken using the American Heart Association/American College of Sports Medicine pre-participation screening questionnaire (Balady *et al.* 1998). Further subject selection criteria were as described in section 3.3.

5.4.1 Study 1

5.4.1.1 Subjects

Nine males (age 23.6 ± 4.7 years, mass 85.7 ± 8.5 kg [mean ± SD]) and eight females (age 21.9 ± 4.8 years, mass 64.0 ± 4.2 kg) volunteered to take part in the investigation. Individual maximal oxygen uptake ($\dot{V}O_{2max}$) values confirmed the similarity of aerobic fitness within both groups: males (46.7 ± 5.2 $ml \cdot kg^{-1} \cdot min^{-1}$) and females (40.1 ± 8.8 $ml \cdot kg^{-1} \cdot min^{-1}$). All subjects performed a progressive sub-maximal exercise test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport; Lode, Netherlands). The testing protocol used is described in section 3.3. Maximal work rates (WR_{max}, used to calculate sub-maximal workloads) were estimated from the relationship between work rate and heart rate, extrapolated to the age-predicted maximal heart rate (220-age (years)): 289 ± 46 W (males) and 195 ± 45 W (females).

5.4.1.2 Physiological measurement

A Reynolds Lifecard CF digital Holter recorder (Del Mar Reynolds Medical Ltd., UK) was used to record a three-lead ECG throughout exercise and for 10 minute periods pre- and post-exercise. The ECG electrodes were positioned in the modified V5, CC5, modified V5R electrode configuration as shown in Figure 3.1. This system provided ECG data with 12-bit accuracy. Subsequently, each lead was separately analysed using a Reynolds Pathfinder digital analyser (Del Mar Reynolds Medical Ltd., UK). Beat-to-beat cardiac cycle interval of normal sinus origin (RR), QT_a (Q wave onset to T wave apex) and QT_e (Q wave onset to T wave end) interval data were measured for each sinus beat and exported for further analysis using the Reynolds Research Tools software (Del Mar Reynolds Medical Ltd., UK).

5.4.1.3 Data analysis

The QT and RR data sets were linearly interpolated with a sampling frequency of 2Hz; this facilitated easier separation of the data into specific time segments and ensured a uniform number of points within each analysis period. Mean values were calculated for consecutive one-minute windows for both QT and RR data sets for all three ECG leads. Subsets of the averaged QT and RR data sets were then defined that represented these parameters within specified intervals during exercise and during the pre- and post-exercise phases:

1. Consecutive one-minute periods during the pre-exercise resting phase (denoted p1 to p10)
2. The final one-minute period of each exercise stage, in order to represent physiologically steady-state conditions (denoted e1 to e6 in males and e1 to e3 in females)

3. Consecutive one-minute periods during the post-exercise recovery phase (denoted r1 to r10)

The QT data were adjusted using the Bazett correction formula (section 2.3.2.1), in an attempt to provide heart rate-independent QT values. All group mean QT_c data presented here were calculated using the QT_a interval only since it was more reliably detected by the analysis software. Linear regression analysis using the method of least squares was used to quantify the relationship between QT_c and work rate during exercise.

QTVI was calculated for the periods defined above according to the equation described by Berger *et al.* (1997) (section 2.3.2.3). QT variance (QT_v) normalised for mean QT (QT_m) (QTVN) was also quantified according to the definition by Haigney *et al.* (2004):

$$QTVN = QT_v / (QT_m)^2$$

Prior to calculating QT_v and RR_v the QT and RR data were linearly detrended within consecutive one-minute segments in order to remove the potential influence on variability of time-dependent trends in the data. Two sets of data were calculated for each of the above indices (QT_aVI and QT_eVI, QT_aVN and QT_eVN), in order to compare the relative influence thereon of using either QT_a or QT_e intervals.

The Lilliefors test for goodness of fit to a Normal distribution confirmed that all parameters were Normally distributed at each stage of the measurement protocol.

One way analysis of variance (ANOVA) was used to compare the mean pre-exercise values of QT, QT_c, QT_aVI, QT_eVI, QT_aVN and QT_eVN with their respective mean values during each stage of exercise and recovery. ANOVA was also used to perform between-stage comparison of parameters during each stage of exercise and recovery. Multiple pairwise comparisons were performed for these data using Tukey's honestly significant difference criterion, and differences were considered significant for $p<0.05$. Student's two-sample t-tests for unequal variance (heteroscedastic data) were used to assess between-lead and between-gender differences for all parameters at each stage of the protocol. Student's two-sample t-tests for paired samples were used to assess differences between the indices QT_aVI and QT_eVI and the indices QT_aVN and QT_eVN at each stage of the protocol. All data quoted in the text represent mean ± SD.

5.4.2 Study 2

5.4.2.1 Subjects

Eight males (age 20.7 ± 0.4 years, mass 78.4 ± 7.7 kg [mean ± SD]) volunteered to take part in the investigation. Individual maximal oxygen uptake ($\dot{V}O_{2max}$) values confirmed the homogeneity of aerobic fitness within the group (50.7 ± 4.9 ml kg^{-1} min^{-1} [mean ± SD]). Subjects were initially at rest and were asked to breathe in accordance with a metronome set to a rate of 15 breaths per minute (0.25 Hz) for a period of six minutes. At the end of this period they were instructed to breathe spontaneously for a further six minutes at rest. Subjects then immediately performed a progressive exercise test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport; Lode, Netherlands). The exercise protocol employed was similar to that described in section 3.3, with the difference that the work rate was increased until volitional fatigue. Breath-by-breath oxygen uptake ($\dot{V}O_2$) data were recorded throughout using an Oxycon Pro respiratory gas

analysis system (Jaeger, Germany) to enable estimation of subjects' aerobic capacity.

5.4.2.2 Physiological measurement

A Reynolds Lifecard CF digital Holter recorder (Del Mar Reynolds Medical Ltd., UK) was used to record a three-lead ECG continuously throughout the pre-exercise, exercise and post-exercise periods. The ECG leads were positioned in the modified V5, CC5, modified V5R electrode configuration (Figure 3.1). This system provided ECG data with a sample accuracy of 2.5 μV (magnitude of least significant bit; 12-bit resolution) and 128 Hz sampling frequency. All analyses in these studies were performed using data from a single ECG lead (lead 1 for each subject) as the between-lead differences in RR and QT data using this ECG system had been previously quantified (preliminary study, chapter 3). The ECG recordings were analysed using a Reynolds Pathfinder digital analyser (Del Mar Reynolds Medical Ltd., UK). All ECG data used for subsequent analysis in this study were free of any form of morphologically abnormal beat, and this was verified by both the Holter system and by human observation. Beat-to-beat cardiac interval (RR) and QT interval data were automatically measured for each sinus beat and exported for further analysis using the Reynolds Research Tools software (Del Mar Reynolds Medical Ltd., UK). The QT and RR data also underwent human visual examination in order to verify the accuracy of the data prior to subsequent analysis. When either the RR or QT intervals were considered to be anomalous, both the RR and QT data points were removed from the data set. This occurred infrequently (and mainly during exercise), resulting overall in fewer than 1% of the data being removed. All QT analysis in this investigation was based on the QT_e interval (Q wave onset to T wave end), resulting from the previous observation that the detection performance for T_a and T_e of the Holter system used were considered broadly equivalent.

The Task Force Haemodynamic Monitor (CNSystems Medizintechnik GmbH, Austria) was used to record beat-to-beat cardiac ventricular performance parameters (via ICG) and beat-to-beat blood pressure (via photoplethysmography, employing the vascular unloading technique at the finger) (Figures 5.1-5.6).

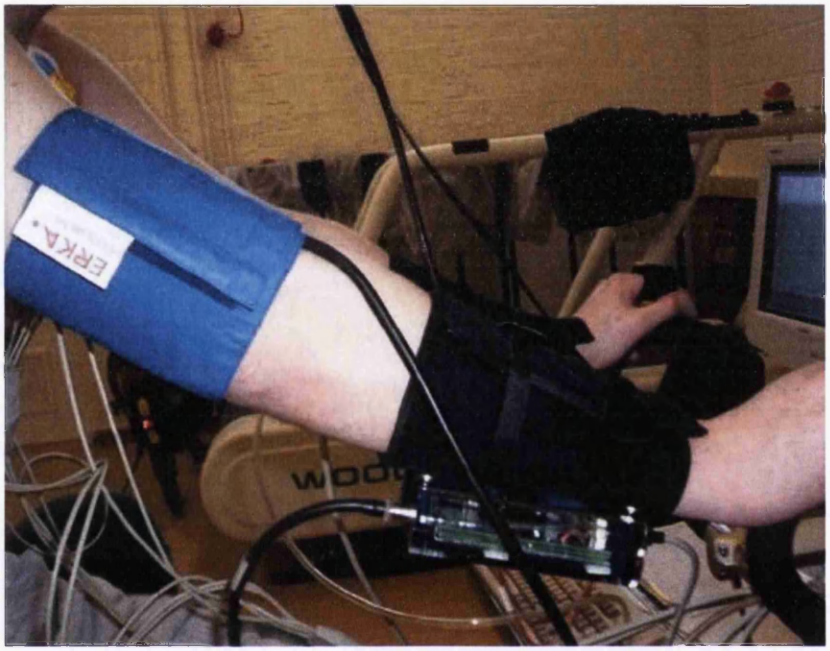


Figure 5.1 (a) Task Force Monitor oscillometric blood pressure cuff

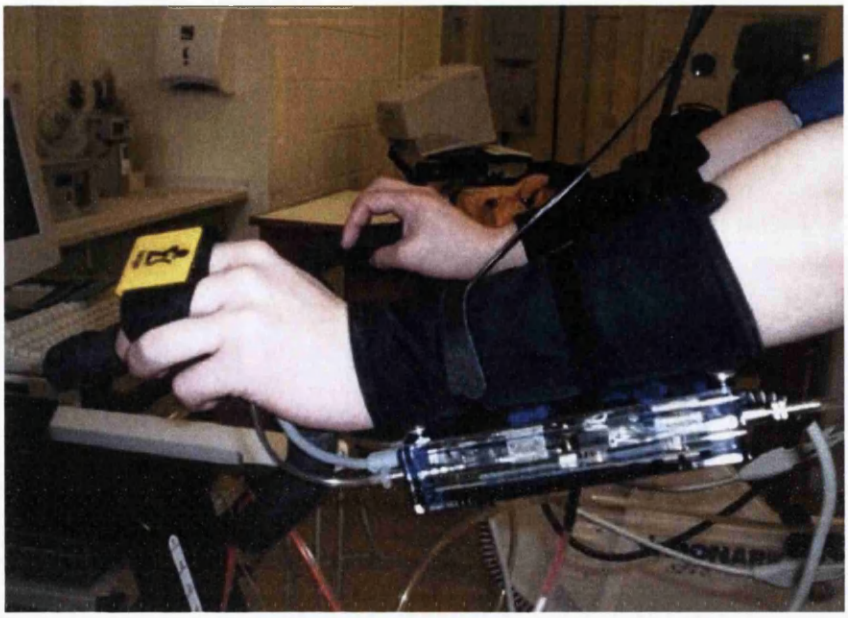


Figure 5.1 (b) Task Force Monitor photoplethysmographic blood pressure cuff

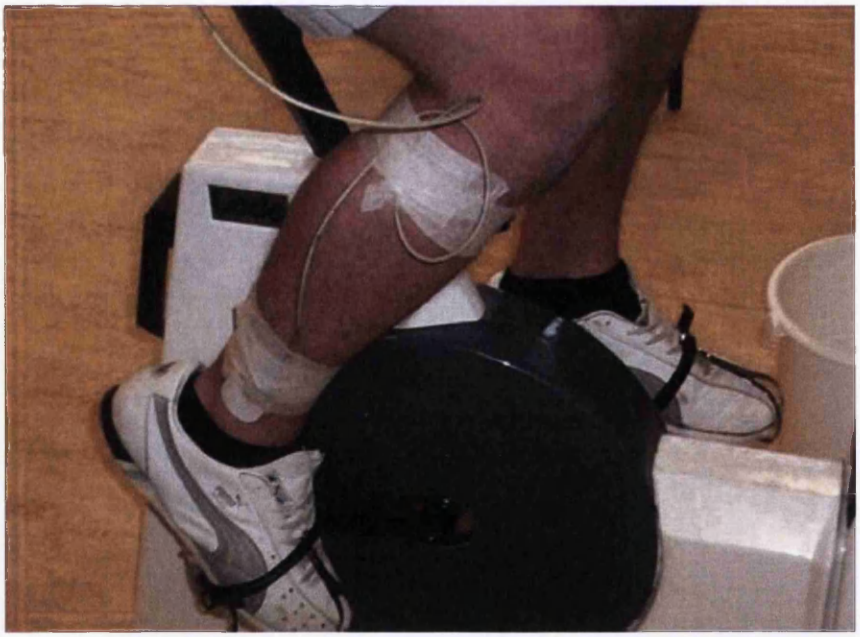


Figure 5.1 (c) Task Force Monitor ankle electrode

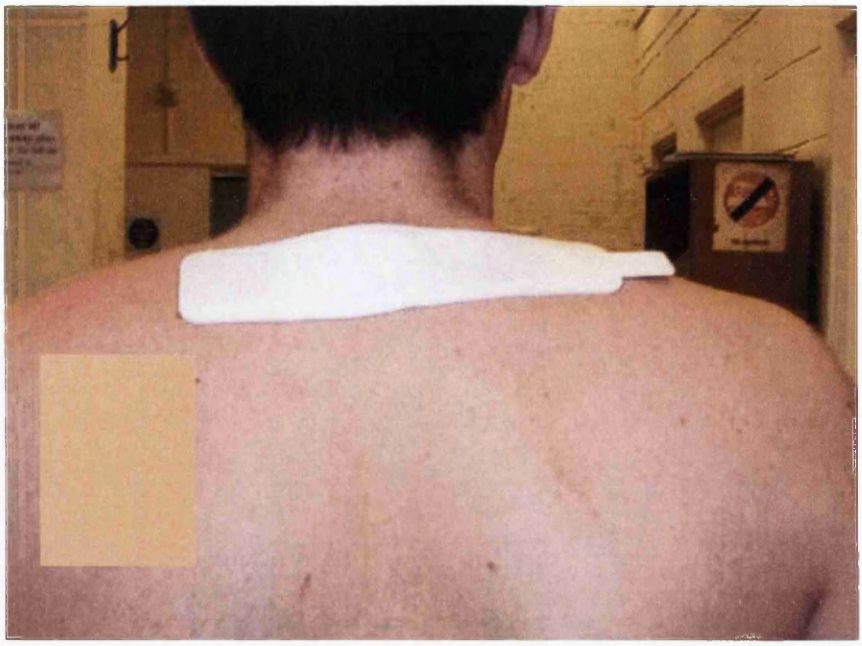


Figure 5.1 (d) Task Force Monitor ICG neck electrode

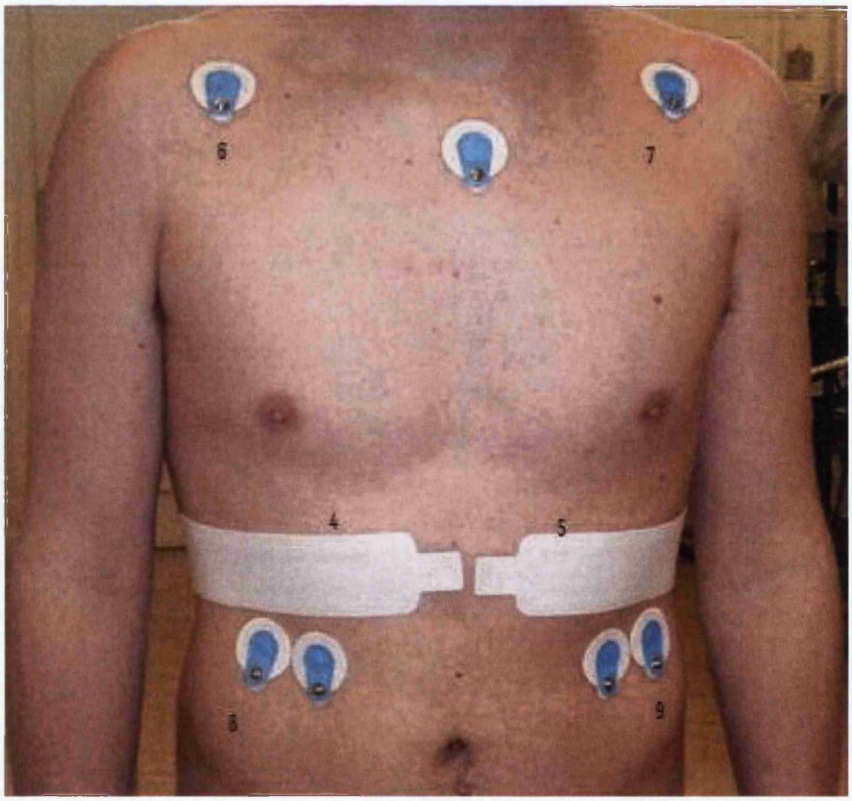


Figure 5.1 (e) Task Force Monitor ICG abdominal electrodes (electrodes 4-5) and ECG electrodes (electrodes 6-9)

Figure 5.1 (f) Task Force Monitor

Using these methods the following parameters were determined (all defined in section 2.3.3.2): stroke volume index (SVI), cardiac output index (CI), left ventricular ejection time (LVET), end diastolic volume index (EDVI), ejection fraction (EF), index of contractility and acceleration index (IC and ACI, both of which are measures of the inotropic state of the myocardium), total peripheral resistance (TPRI), left ventricular work index (LVWI), systolic and diastolic blood pressure (BP) and pulse pressure (PP). The term 'index' in several of these measures indicates that the original parameter values were normalised by dividing the individual values by the subject's body surface area (mean ± SEM body surface area = 1.97 ± 0.03 m^2).

5.4.2.3 Data analysis

Subsets of the QT and RR data sets were defined that represented these data within specified intervals during exercise and during the pre- and post-exercise phases:

1. Consecutive one-minute intervals during the six minute period of metronomic breathing
2. Consecutive one-minute intervals during the six minute period of spontaneous breathing
3. The final one-minute period of each exercise stages (in order to represent physiologically steady-state conditions)
4. Consecutive one-minute intervals during the post-exercise resting phase, which lasted for 15-minutes

Prior to further data processing the QT and RR data were linearly detrended within consecutive one-minute segments (corresponding to the subsets defined above) in order to remove any time-dependent trends in the data.

Variability of the RR interval was quantified in the time domain (RMSSDNN: square root of the mean of the sum of the squares of differences between adjacent RR intervals, and SDNN: standard deviation of all RR intervals) according to the Task Force guidelines on HRV (Task Force ESC & NASPE 1996). (In these definitions "NN" indicates that the RR intervals are measured from normal beats of sinus origin). RMSSDNN and SDNN represent the high-frequency variability and the total variability of the RR interval data respectively (Task Force ESC & NASPE 1996). Equivalent time domain parameters were calculated to describe

the variability of the QT interval (RMSSDQT and SDQT). QTVI was calculated according to the equation described by Berger *et al.* (1997) (section 2.3.2.3).

Each of the measured parameters was separately averaged to provide single representative values for the discrete physiological states during pre-exercise (metronomic breathing and spontaneous breathing states) and during post-exercise (0-5, 5-10 and 10-15 minute post-exercise recovery periods). (Exercise values for each parameter were already defined for representative one-minute stages.) All data quoted in the text represent mean ± SD. Error bars in the figures represent the SEM (standard error in the mean) for the plotted parameters.

5.4.2.4 Statistical analysis

The Stepwise method of multiple linear regression was used to establish whether a significant predictor model could be obtained for the following dependent variables: RR, QT_e, RMSSDNN, SDNN, RMSSDQT, SDQT and QTVI. This was done separately for pre- and post-exercise data (not using exercise data). Each of the independent (predictor) variables was first visually checked for the presence of outliers and to assess linearity using scatterplots. Each of the predictor variables obeyed these assumptions and so each was standardised (giving its z score) prior to subsequent regression analysis. Parameter inclusion was carefully considered with regard to underlying physiology; any parameter that was a direct linear product of other (included) fundamental parameters was excluded from subsequent analysis to avoid singularity. Multicollinearity was minimised by the judicious selection of predictor variables, examination of the predictor variable correlation matrix (Table 5.1), and by manually examining the influence of variable inclusion on the resulting R^2 values (proportion of explained dependent parameter variance for the models). These procedures resulted in the selection of

the following subset of predictor variables: SVI, LVET, EDVI, IC, ACI, TPRI, LVWI, PP. Standardised (Beta) coefficients were used to report the contribution of each predictor variable to the multiple regression models. Adjusted R^2 ($adjR^2$) values were reported as this was considered to be the most appropriate measure of R^2 for small samples. A value of $p<0.05$ for a predictor variable indicated its statistically significant unique contribution to the model. The standard assumptions of multiple regression (Normality, linearity and homoscedasticity) were verified via scatterplots of the residuals (differences between the observed values of the dependent variable and those predicted by the regression model) and using Normal probability plots, separately for pre- and post-exercise states.

5.5 Results

5.5.1 Study 1

5.5.1.1 Characterisation of RR, QT and QT_c as a function of exercise work rate

The QT interval was measurable for exercise intensities up to 68±8% of WR_{max} in males and 75±10% of WR_{max} in females, equivalent to mean heart rates of 152±9 bpm (males) and 157±4 bpm (females). There were significant differences in RR values between males and females during both the pre-exercise and exercise stages, and also during the initial stages of recovery. Gender differences in QT occurred infrequently during recovery only. Significant between-lead differences in QT_a (Figure 5.2 (a) & (b)) and QT_e were observed during both exercise and recovery periods in males and females. Bazett-corrected QT_c values displayed significant between-lead differences at various stages throughout the pre-exercise, exercise and recovery periods (Figure 5.2 (c) & (d)).

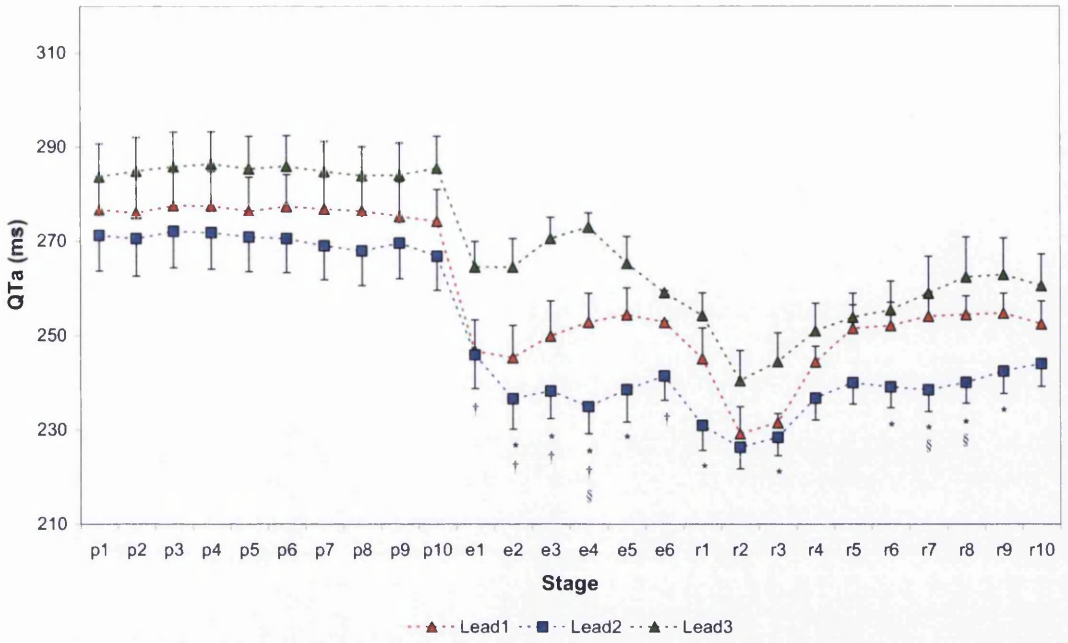


Figure 5.2 (a) Values of QT_a obtained from the three ECG leads (male group) (§ = significant difference between leads 1 and 2; † = significant difference between leads 1 and 3; * = significant difference between leads 2 and 3)

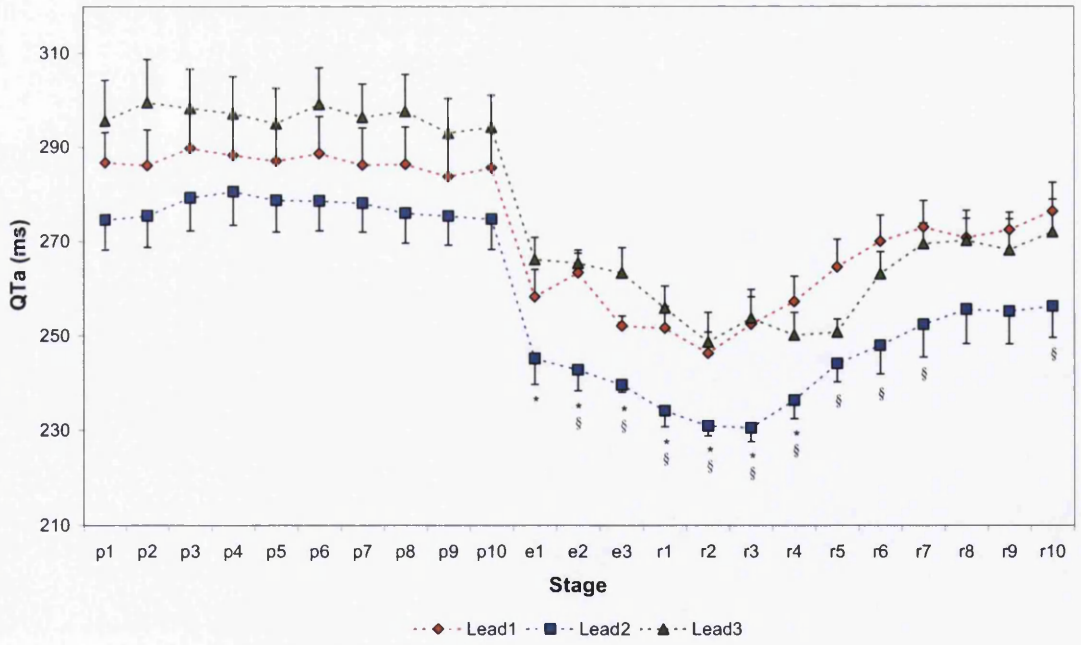


Figure 5.2 (b) Values of QT_a obtained from the three ECG leads (female group) (§ = significant difference between leads1 and 2; * = significant difference between leads 2 and 3)

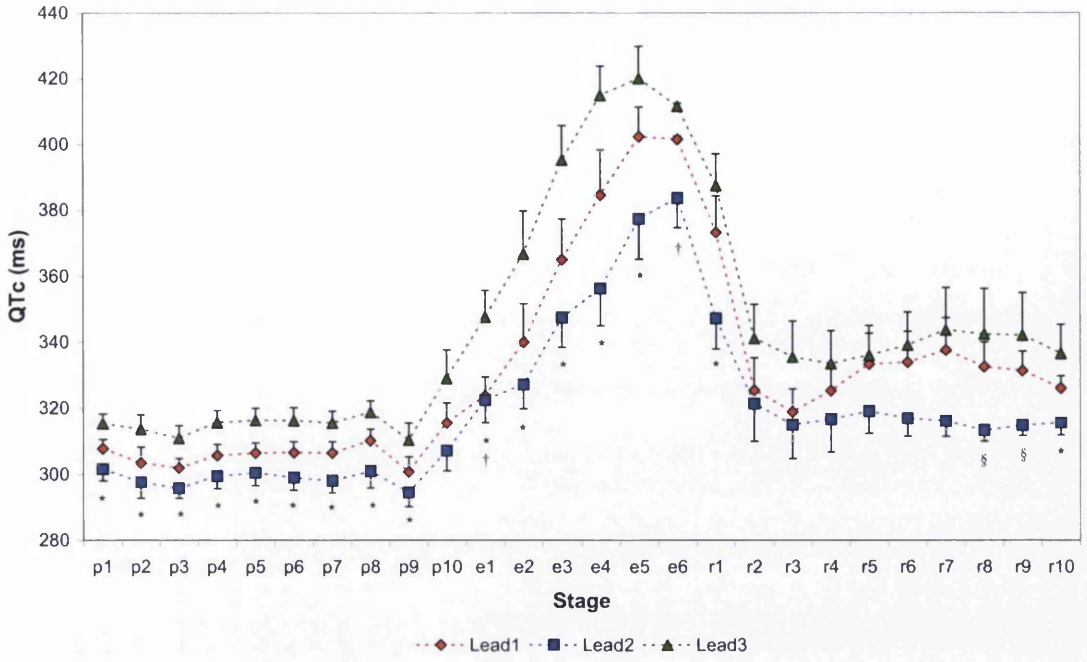


Figure 5.2 (c) Bazett-corrected QT_a (QT_c) values for the three ECG leads (male group) (§ = significant difference between leads 1 and 2; † = significant difference between leads 1 and 3; * = significant difference between leads 2 and 3)

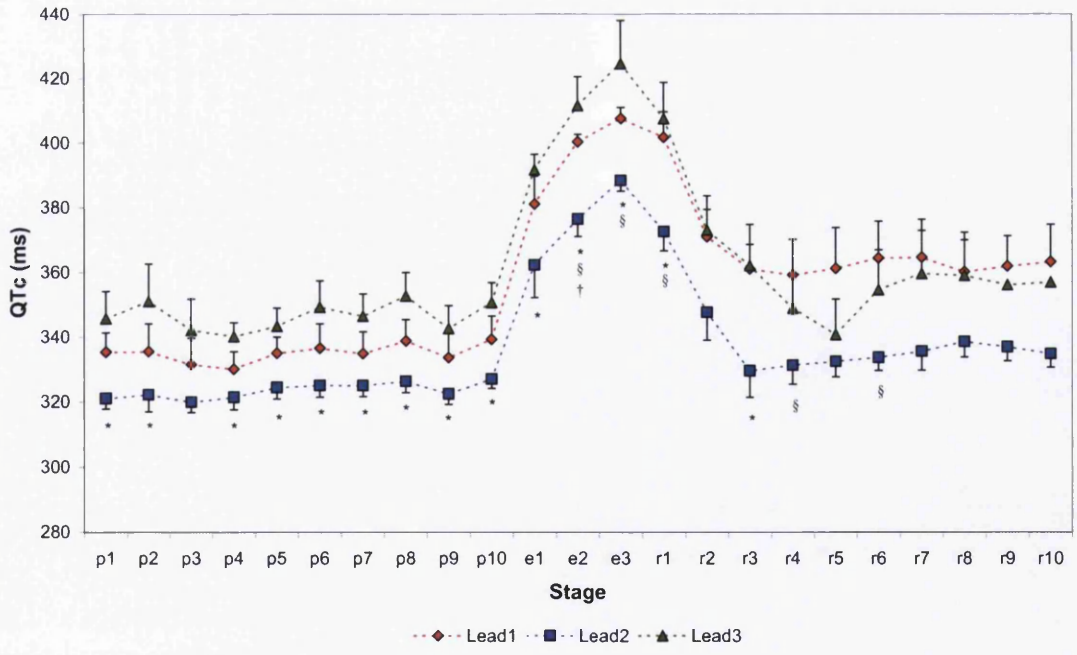


Figure 5.2 (d) Bazett-corrected QT_a (QT_c) values for the three ECG leads (female group) (§ = significant difference between leads 1 and 2; † = significant difference between leads 1 and 3; * = significant difference between leads 2 and 3)

Figures 5.2(a)-(d) show that Bazett-correction reversed the trend of QT reduction as a function of exercise work rate. This over-correction of QT resulted in an almost linear increase in QT_c as a function of work rate. Table 5.1 presents the results of linear regression analysis of group mean QT_c as a function of work rate for each of the three ECG leads.

Table 5.1 Parameters of the linear regression fit of QT_c (heart rate-corrected QT_a interval) against work rate during exercise

Lead	Gradient ($ms \cdot W^{-1}$)	Intercept (ms)	R^2
Female			
1	0.442	357	0.937
2	0.435	337	0.998
3	0.550	360	0.986
Male			
1	0.673	282	0.996
2	0.464	291	0.965
3	0.644	312	0.960

5.5.1.2 QT variability indices (QTVI and QTVN)

Figure 5.3 illustrates the effect of linearly detrending the RR, QT_a and QT_e time-series data within consecutive one-minute segments, performed prior to calculating the QT variability indices.

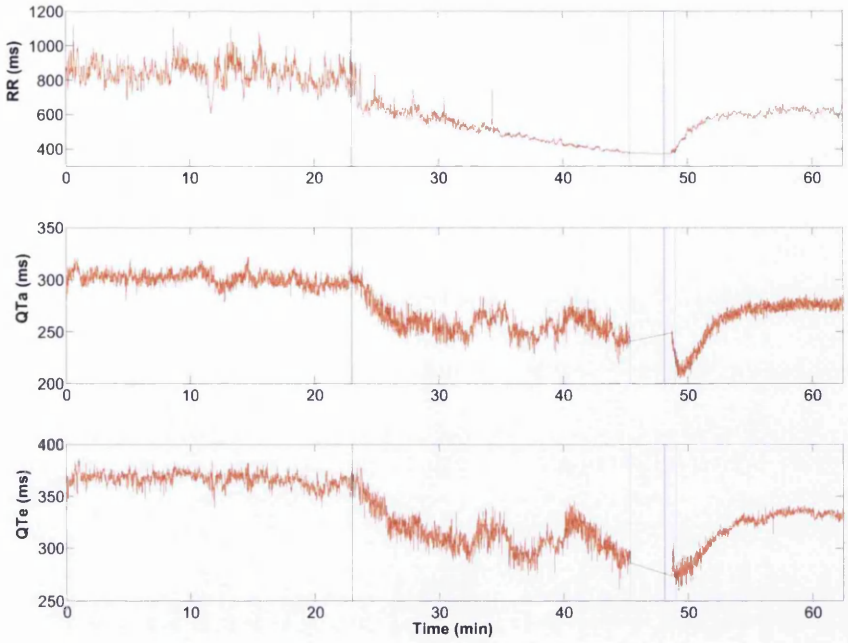


Figure 5.3 (a) RR, QT_a and QT_e data from a single subject (ECG lead 1). Vertical bars: solid (interval) = exercise period; dashed (interval) = period during which QT could not be measured (high intensity exercise)

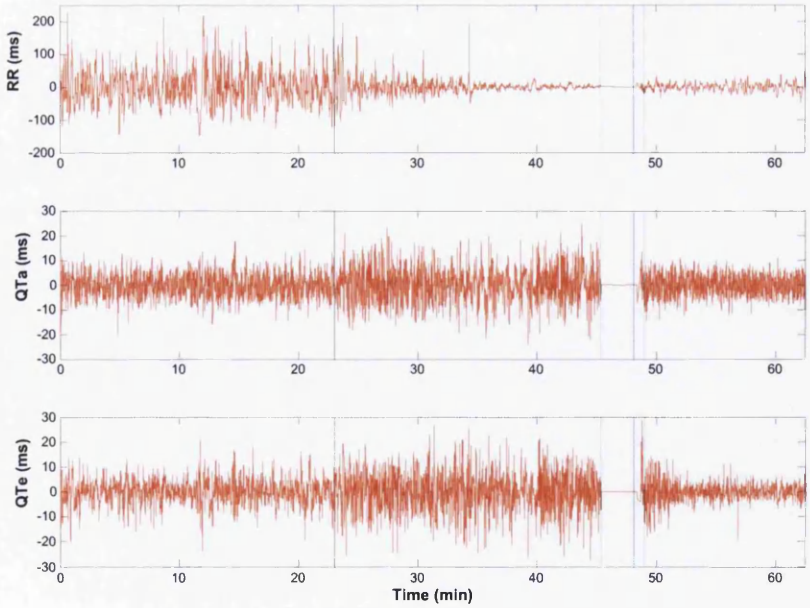


Figure 5.3 (b) RR, QT_a and QT_e data from a single subject (ECG lead 1) following detrending within consecutive one minute blocks. Vertical bars: as above

The QT variability indices are presented in Figures 5.4 (a) & (b) and 5.4 (c) & (d) for males and females, respectively. Figures 5.4 (a) and 5.4 (c) show the variability ratios (QT_aVI) calculated using the QT_a interval, whilst Figures 5.4 (b) and 5.4 (d) show the variability ratios (QT_eVI) calculated using the QT_e interval.

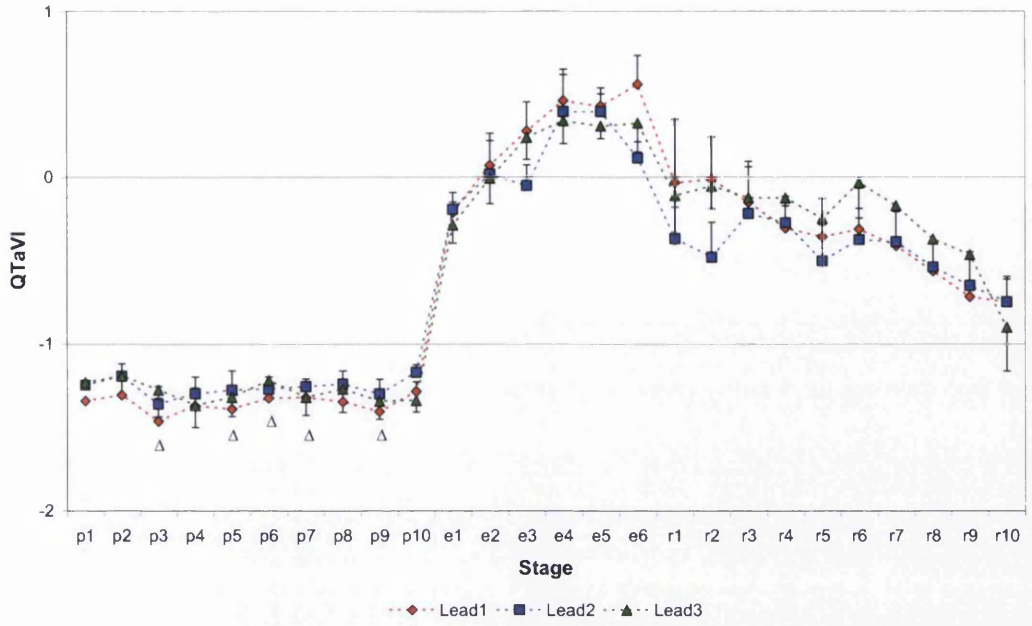


Figure 5.4 (a) QT variability index (QT_aVI) calculated from the three ECG leads (male group) (Δ = significant difference between male and female groups in at least one lead)

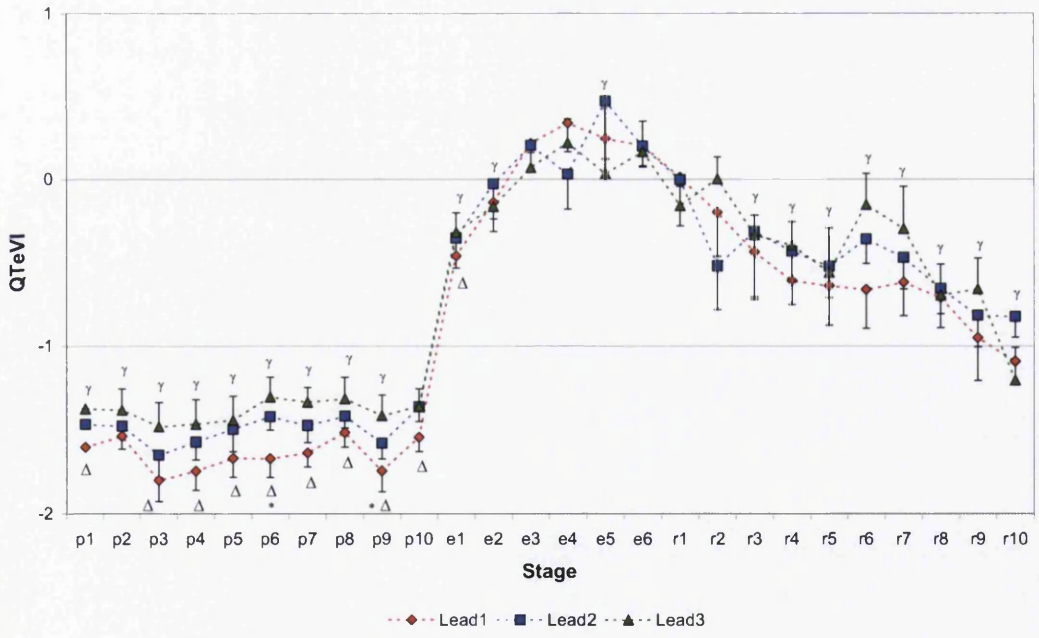


Figure 5.4 (b) QT variability index (QT_eVI) calculated from the three ECG leads (male group) (Δ = significant difference between male and female groups in at least one lead; * = significant difference between two or more leads; γ = significant difference compared with QT_aVI in at least one lead)

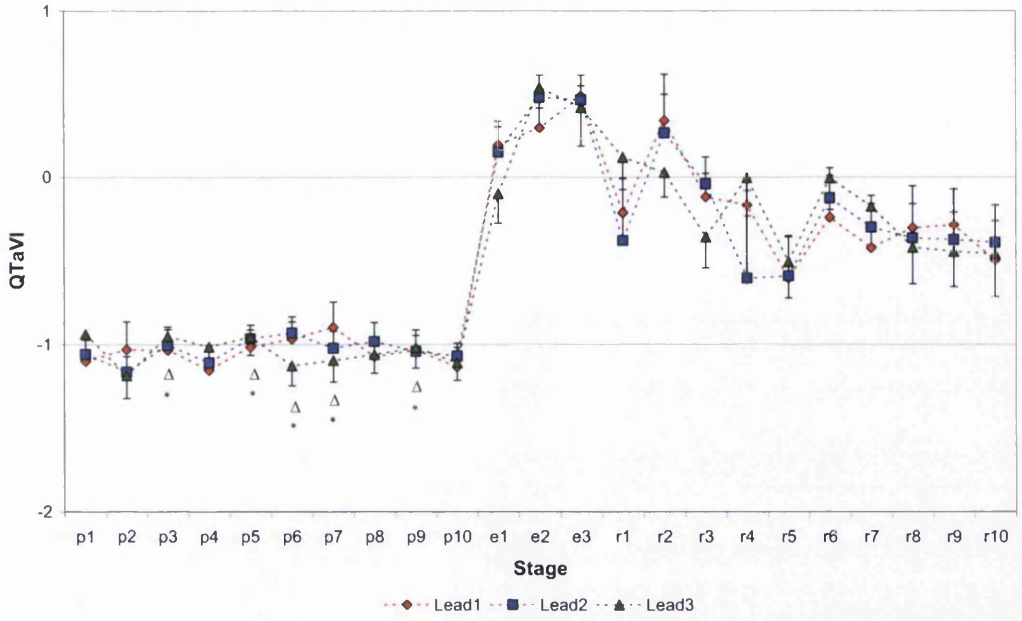


Figure 5.4 (c) QT variability index (QT_aVI) calculated from the three ECG leads (female group) (Δ = significant difference between male and female groups in at least one lead; * = significant difference between two or more leads)

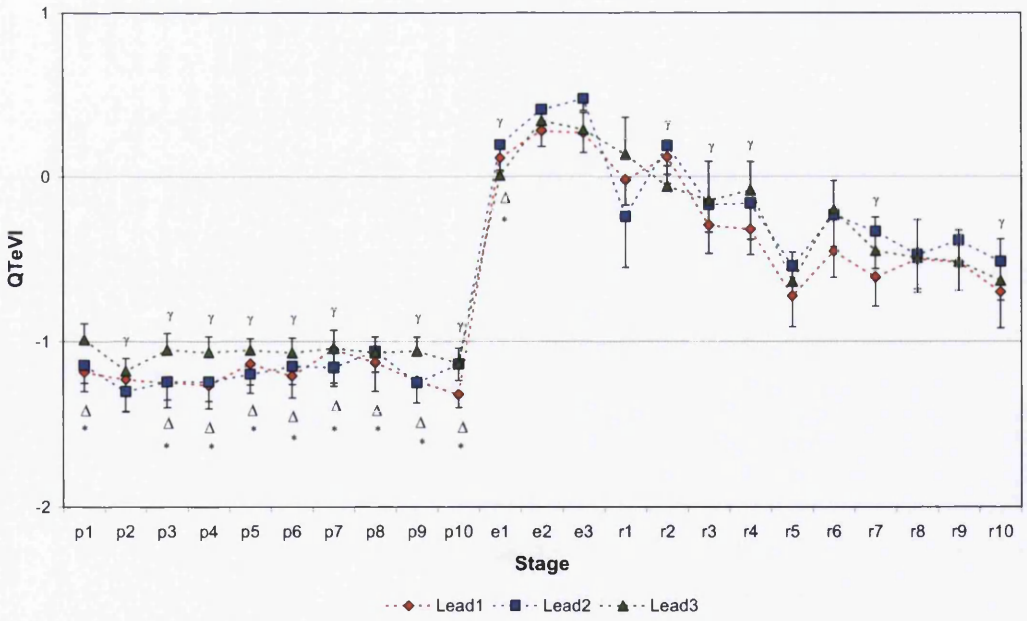


Figure 5.4 (d) QT variability index (QT_eVI) calculated from the three ECG leads (female group) (Δ = significant difference between male and female groups in at least one lead; * = significant difference between two or more leads; γ = significant difference compared with QT_aVI in at least one lead)

There were no appreciable between-lead differences for either QT_aVI or QT_eVI in males, but there were between-lead difference in these indices for females during rest. Both QT_aVI and QT_eVI were significantly smaller (more negative) in males compared with females in at least one lead during the majority of pre-exercise, but there were no appreciable gender-related differences during the exercise and recovery periods. There were significant differences between QT_aVI and QT_eVI in at least one lead during all phases of the experimental protocol for males and females.

There was an initial rapid increase in both QT_aVI and QT_eVI (of approximately one order of magnitude) within the first minute of exercise in both males and females. This was generally followed by progressively smaller (and non-significant) increases in these parameters for each increment in work rate. Compared with the mean pre-exercise value, QT_aVI was significantly increased throughout exercise and during the first nine minutes of recovery in males ($p<0.001$) and females ($p<0.01$). Similarly QT_eVI was significantly increased throughout exercise and during the first nine minutes of recovery in males and females (both $p<0.001$). QTVI values greater than zero indicate a greater variability of QT compared with RR. In males both QT_aVI and QT_eVI were greater than zero from the third minute of exercise until the end of the exercise period. In females both QT_aVI and QT_eVI were greater than zero throughout exercise and during the initial stages of recovery.

When expressed as a simple non-logarithmic ratio, It was observed that both QT_aVI and QT_eVI displayed a strong linear relationship with exercise work rate up to moderately high intensities in males and females (QT_aVI: $R^2 = 0.94$ for males, 0.95 for females; QT_eVI: $R^2 = 0.95$ for males and females). This linearity extended up to work rates of 52±8% of WR_{max} in males (equivalent to a mean HR

of 141±11 bpm) and 48±11% of WR_{max} in females (equivalent to a mean HR of 156±3 bpm).

Normalised QT variance (QTVN) indices are presented in Figures 5.5 (a) & (b) and 5.5 (c) & (d) for males and females respectively. Figures 5.5 (a) and 5.5 (c) show QTVN normalised using the QT_a interval (QT_aVN), whilst Figures 5.5 (b) and 5.5 (d) show QTVN normalised using the QT_e interval (QT_eVN).

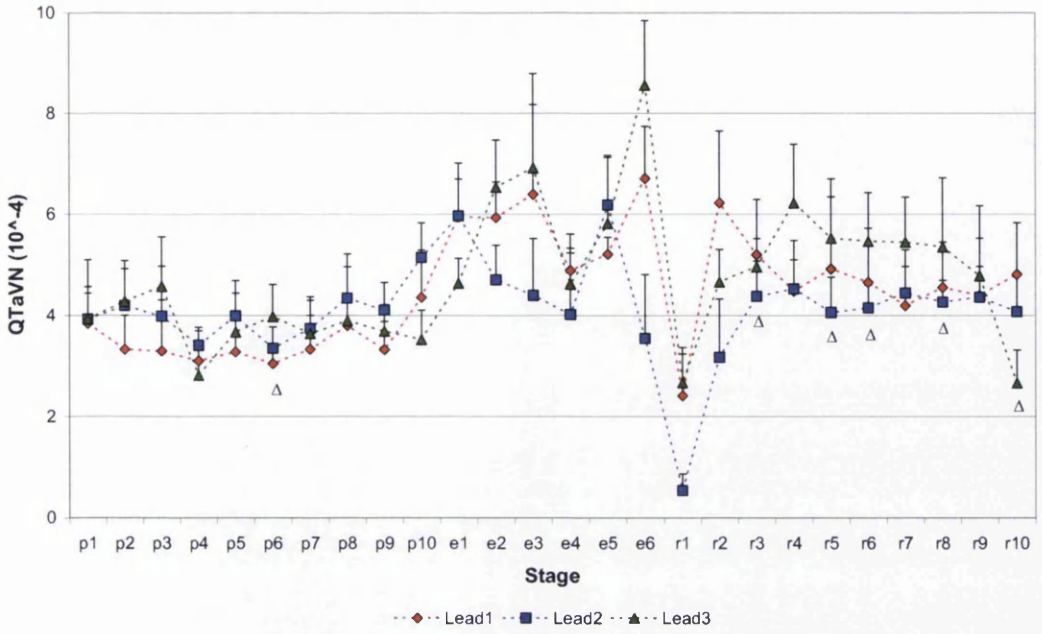


Figure 5.5 (a) QT variance normalised by mean QT_a, calculated from the three ECG leads (male group) (Δ = significant difference between male and female groups in at least one lead)

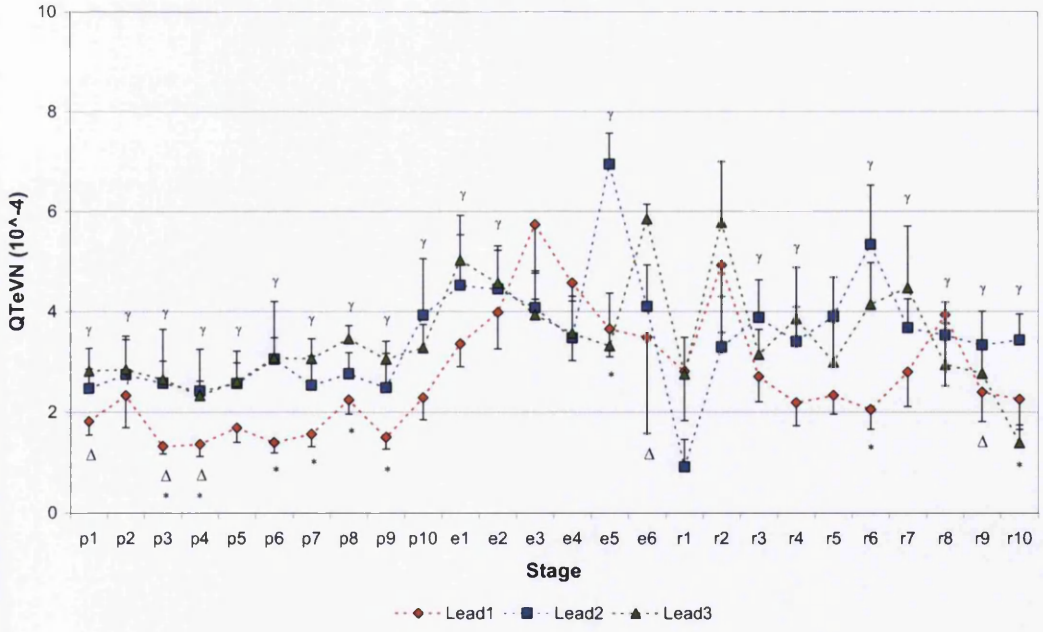


Figure 5.5 (b) QT variance normalised by mean QT_e, calculated from the three ECG leads (male group) (Δ = significant difference between male and female groups in at least one lead; * = significant difference between two or more leads; γ = significant difference compared with QT_aVN in at least one lead)

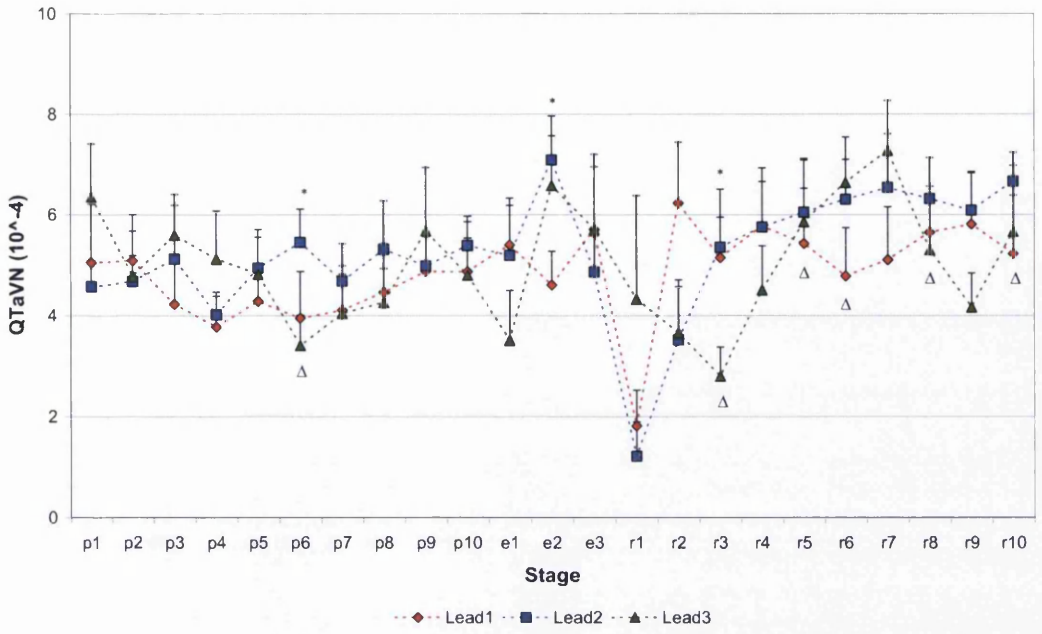


Figure 5.5 (c) QT variance normalised by mean QT_a, calculated from the three ECG leads (female group) (Δ = significant difference between male and female groups in at least one lead; * = significant difference between two or more leads)

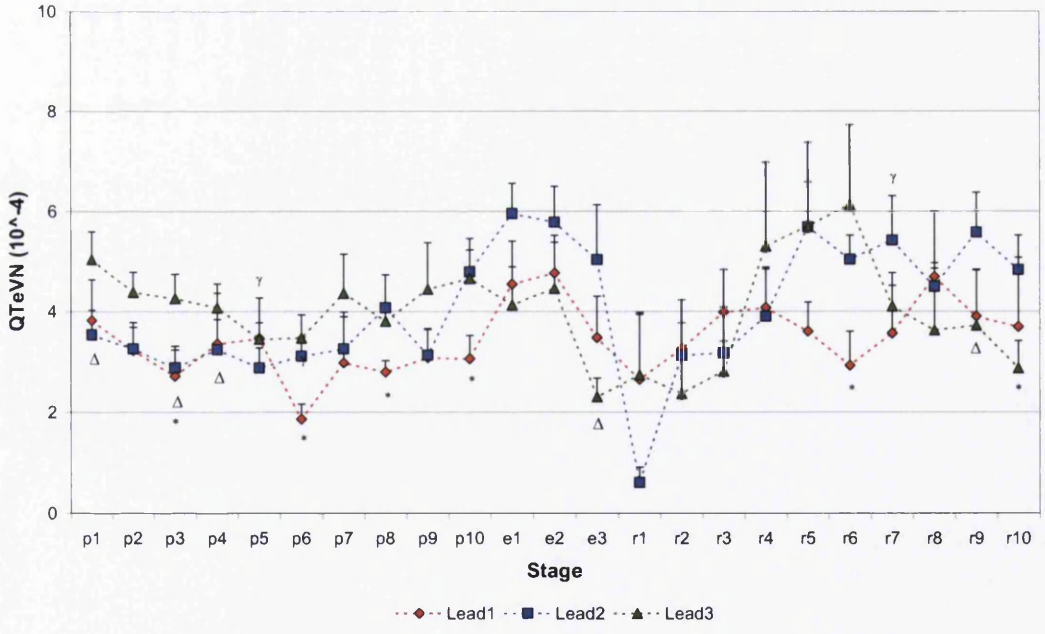


Figure 5.5 (d) QT variance normalised by mean QT_e, calculated from the three ECG leads (female group) (Δ = significant difference between male and female groups in at least one lead; * = significant difference between two or more leads; γ = significant difference compared with QT_aVN in at least one lead)

In comparison with pre-exercise there was a general trend towards elevated values for both QT_aVN and QT_eVN during exercise and recovery in males and females. There were no significant between-stage differences in either index for males or females. There were sporadic between-lead and between-gender differences in QT_aVN and QT_eVN for males and females. Notably there were significant differences between QT_aVN and QT_eVN throughout the experimental protocol for males, but only isolated differences for females.

5.5.2 Study 2

Figure 5.6 (a) presents the characterisation plots of cardiac ventricular function indices during pre-exercise, exercise and post-exercise recovery.

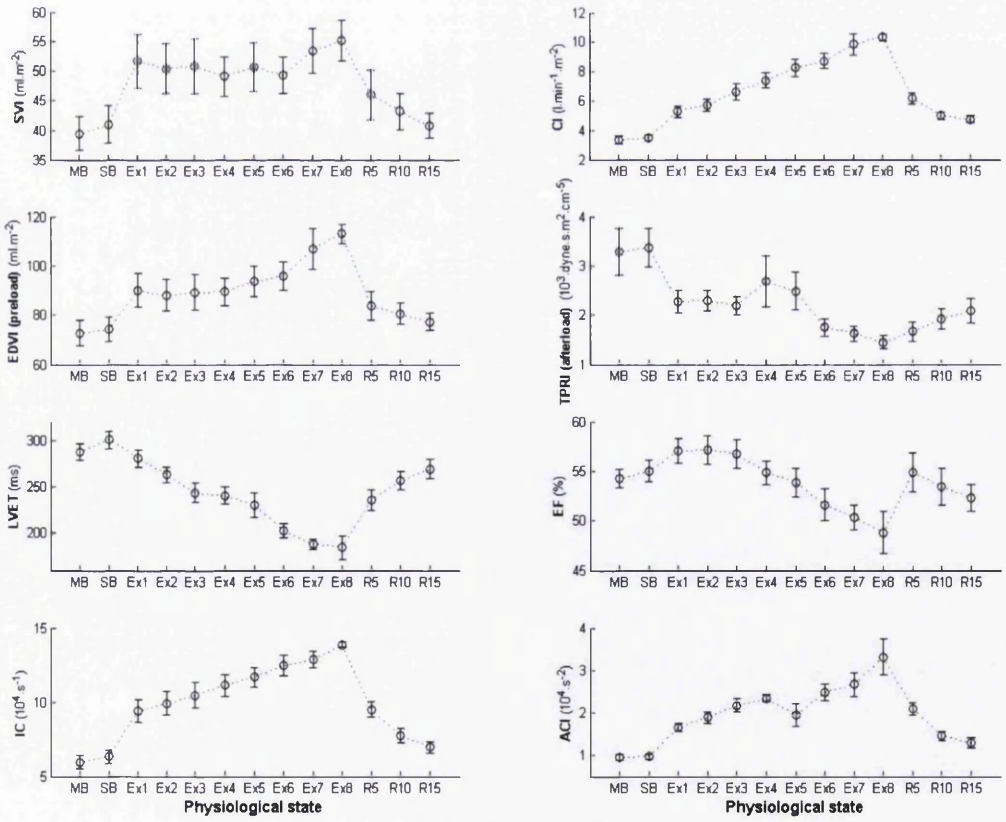


Figure 5.6 (a) Cardiac ventricular performance indices (MB = Metronomic Breathing; SB = Spontaneous Breathing; Ex1-Ex5 = Exercise Stages; R5, R10, R15 = Recovery periods (0-5, 5-10 and 10-15 minutes post-exercise, respectively)

The blood pressure response and associated functional indices are shown in Figure 5.6 (b).

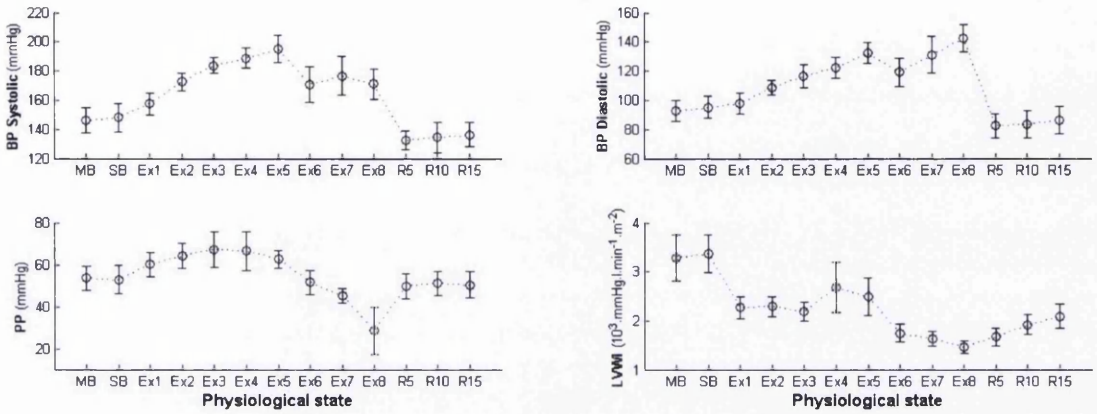


Figure 5.6 (b) Blood pressure response and related performance indices

The plots in Figures 5.6 (a) & 5.6 (b) show substantial differences in parameter values during exercise compared with pre- and post-exercise periods, with parameters exhibiting gradual returns to pre-exercise values within the observation period. The magnitude and trends in the response of each variable were consistent for all subjects. For the same periods, Figure 5.6 (c) presents the absolute values of RR and QT together with indices of their variability and a measure of their relative variability (QTVI).

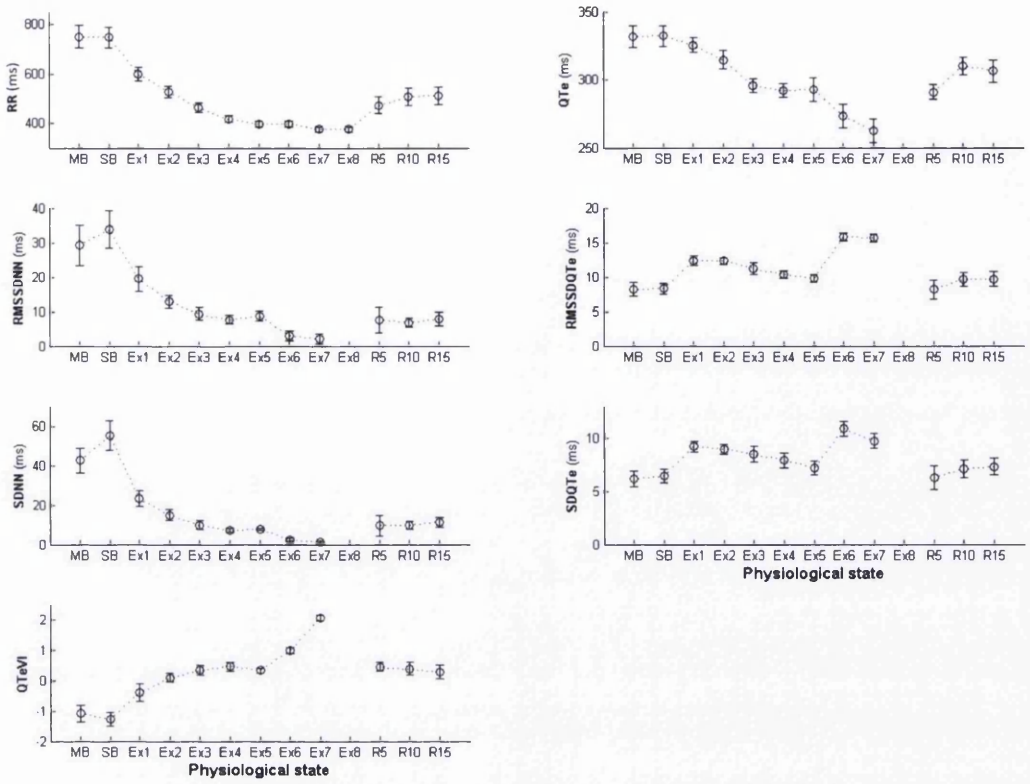


Figure 5.6 (c) RR and QT variability indices, together with QTVI

RR variability parameters (RMSSDNN and SDNN) demonstrated the expected exponential-type decline with increasing exercise work rates (Lewis *et al.* 2007). Conversely, QT variability (RMSSDQT and SDQT) increased at the onset of exercise with a gradual reduction prior to a second increase at the higher exercise work rates. It was not possible to determine QT variability during the highest exercise work rates owing to acknowledged difficulties in accurate QT determination at very high heart rates. Therefore values of QT or RR variability (or QTVI) during exercise stage "Ex8" have not been reported. QTVI demonstrated the rapid and substantial increase in magnitude that was apparent in study 1 (section 5.5.1.2). Reflecting to a degree the observation for QT variability, QTVI was further substantially increased during the highest exercise work rates. QTVI was substantially elevated in comparison with pre-exercise values throughout the post-exercise period.

Multiple linear regression analysis using the stepwise method resulted in significant models for the dependent variables. These models are reported in Tables 5.2-5.10, which also quantify the strength of the model (ANOVA F statistic and p value, together with the adjR^2 value), separately for the pre- and post-exercise states. For comparison between pre- and post-exercise states, each table lists those predictor variables (cardiac performance parameters) that made a significant unique contribution to either the pre- or post-exercise model. Significant predictor variables are highlighted in bold type and have associated p values less than 0.05. Where a predictor variable is listed but did not make a significant unique contribution to the model, the specified Beta coefficient is that which would have resulted from adding that term to the model.

Table 5.2 Pearson correlation coefficient matrix for the selected predictor variables

Predictor variable	**SVI**	**LVET**	**EDVI**	**IC**	**ACI**	**TPRI**	**LVWI**	**PP**
SVI	1.00	0.65	0.97	0.88	0.34	-0.30	0.61	0.66
LVET		1.00	0.49	0.48	-0.18	0.36	0.33	0.34
EDVI			1.00	0.88	0.44	-0.46	0.59	0.68
IC				1.00	0.45	-0.40	0.66	0.63
ACI					1.00	-0.51	0.20	0.16
TPRI						1.00	-0.01	-0.20
LVWI							1.00	0.73
PP								1.00

Table 5.3 Multiple linear regression model for RR

Stage	Pre-Exercise			Post-Exercise		
Model	$adjR^2 = 0.912$; $F_{5,90} = 200.5$; $p<10^{-4}$			$adjR^2 = 0.839$; $F_{5,114} = 125.9$; $p<10^{-4}$		
Dependent variable = RR	Predictor variable	Beta	p	Predictor variable	Beta	p
	SVI	0.65	0.0003	**SVI**	1.51	0.0000
	EDVI	0.61	0.0011	**EDVI**	-0.50	0.0000
	IC	-0.18	0.0176	**IC**	-0.82	0.0000
	TPRI	0.93	0.0000	**TPRI**	-0.20	0.0004
	LVWI	0.56	0.0000	**LVWI**	-0.16	0.0173

Table 5.4 Multiple linear regression model for QT

Stage	Pre-Exercise			Post-Exercise		
Model	$adjR^2 = 0.856$; $F_{6,89} = 96.4$; $p<10^{-4}$			$adjR^2 = 0.570$; $F_{3,116} = 32.9$; $p<10^{-4}$		
Dependent variable = QT	Predictor variable	Beta	p	Predictor variable	Beta	p
	SVI	1.32	0.0000	SVI	0.29	0.1838
	EDVI	0.45	0.0636	**EDVI**	0.68	0.0000
	IC	-0.20	0.0416	IC	-0.28	0.0680
	ACI	0.21	0.0000	**ACI**	-0.45	0.0001
	TPRI	0.65	0.0000	**TPRI**	-0.25	0.0005
	LVWI	-0.59	0.0000	LVWI	-0.04	0.6889
	PP	-0.20	0.0025	PP	-0.05	0.7067

Table 5.5 Multiple linear regression model for RMSSDNN

Stage	Pre-Exercise			Post-Exercise		
Model	$adjR^2 = 0.654$; $F_{2,93} = 92.0$; $p<10^{-4}$			$adjR^2 = 0.099$; $F_{1,118} = 15.2$; $p=0.0001$		
Dependent variable = RMSSDNN	Predictor variable	Beta	p	Predictor variable	Beta	p
	SVI	0.14	0.2662	**SVI**	0.34	0.0002
	LVET	0.69	0.0000	LVET	-0.07	0.5060
	TPRI	0.25	0.0002	TPRI	0.14	0.1491

Table 5.6 Multiple linear regression model for SDNN

Stage	Pre-Exercise			Post-Exercise		
Model	$adjR^2 = 0.602$; $F_{1,94} = 147.3$; $p<10^{-4}$			$adjR^2 = 0.297$; $F_{2,117} = 18.2$; $p<10^{-4}$		
Dependent variable = SDNN	Predictor variable	Beta	p	Predictor variable	Beta	p
	SVI	0.07	0.3838	**SVI**	0.71	0.0000
	LVET	0.78	0.0000	LVET	-0.16	0.2844
	IC	0.09	0.2139	**IC**	-0.28	0.0218

Table 5.7 Multiple linear regression model for RMSSDQT

Stage	Pre-Exercise			Post-Exercise		
Model	$adjR^2 = 0.253$; $F_{2,94} = 17.8$; $p<10^{-4}$			$adjR^2 = 0.183$; $F_{3,116} = 10.3$; $p<10^{-4}$		
Dependent variable = RMSSDQT	Predictor variable	Beta	p	Predictor variable	Beta	p
	SVI	-0.98	0.0000	SVI	0.24	0.3435
	EDVI	-0.32	0.3686	**EDVI**	0.39	0.0044
	IC	0.62	0.0010	**IC**	-1.02	0.0000
	ACI	0.14	0.1735	**ACI**	0.51	0.0005

Table 5.8 Multiple linear regression model for SDQT

Stage	Pre-Exercise			Post-Exercise		
Model	$adjR^2 = 0.248$; $F_{2,93} = 17.3$; $p<10^{-4}$			$adjR^2 = 0.220$; $F_{3,116} = 12.6$; $p<10^{-4}$		
Dependent variable = SDQT	Predictor variable	Beta	p	Predictor variable	Beta	p
	SVI	0.98	0.0000	SVI	-0.24	0.3725
	EDVI	-0.45	0.2116	**EDVI**	0.40	0.0017
	IC	0.64	0.0008	**IC**	-1.21	0.0000
	ACI	0.12	0.2132	**ACI**	0.68	0.0000

Table 5.9 Multiple linear regression model for QTVI

Stage	Pre-Exercise			Post-Exercise		
Model	$adjR^2 = 0.411$; $F_{2,93} = 35.0$; $p<10^{-4}$			$adjR^2 = 0.270$; $F_{2,117} = 23.6$; $p<10^{-4}$		
Dependent variable = QTVI	**Predictor variable**	**Beta**	**p**	**Predictor variable**	**Beta**	**p**
	SVI	-0.68	0.0000	**SVI**	-0.43	0.0000
	ACI	0.38	0.0000	**ACI**	0.51	0.0000

Table 5.10 Multiple linear regression model for EF

Stage	Pre-Exercise			Post-Exercise		
Model	$adjR^2 = 0.975$; $F_{2,93} = 1885.2$; $p<10^{-4}$			$adjR^2 = 0.967$; $F_{2,117} = 1741.3$; $p<10^{-4}$		
Dependent variable = EF	Predictor variable	Beta	p	Predictor variable	Beta	p
	SVI	3.88	0.0000	**SVI**	2.33	0.0000
	EDVI	-3.65	0.0000	**EDVI**	-1.91	0.0000

The regression models for both RR and QT included significant unique contributions from a large number of the predictor variables, and consequently accounted for a large proportion of the variance in these parameters (91% and 86% during pre-exercise and 84% and 57% during post-exercise for RR and QT, respectively). However, whereas the predictor variables included in the model for RR were consistent for pre- and post-exercise, fewer predictor variables contributed to the post-exercise QT model compared with those during pre-exercise.

The ability of the regression model to describe the variability of RR (RMSSDNN and SDNN) was substantially reduced post-exercise (accounting for 65% and 10% of variability in RMSSDNN and 60% and 30% of variability in SDNN, pre- and post-exercise respectively). Moreover, the predictor variables making a unique contribution to the model were different (and mutually exclusive) for the pre- and post-exercise models for both RMSSDNN and SDNN.

The predictive abilities of the regression models for RMSSDQT and SDQT were low-to-moderate but were very similar to each other, and also did not differ greatly for pre- and post-exercise states (25% and 25% during pre-exercise and 18% and 22% during post-exercise for RMSSDQT and SDQT, respectively). The

predictor variables included in the models for RMSSDQT and SDQT were identical when compared for either the pre- or post-exercise states. There was partial consistency between the predictor variables included in the pre- and post-exercise models for RMSSDQT and SDQT, with IC providing a significant unique contribution in both.

The predictive abilities of the regression models for QTVI were moderately good (41% and 27% for pre- and post-exercise, respectively) and there was good consistency between the predictor variables included in the pre- and post-exercise models, with SVI and ACI providing significant unique contributions in both.

In order to test and validate the chosen methods of multiple regression modelling, an additional multiple regression analysis with ejection fraction (EF) as the dependent variable was also performed. All possible predictor variables were as previously stated. The predictive abilities of the regression models for EF were very high (98% and 97% for pre- and post-exercise, respectively) and there was good consistency between the predictor variables included in the pre- and post-exercise models, with SVI and EDVI providing significant unique contributions in both. The magnitude of the Beta coefficients for SVI and EDVI were large, indicating a strong contribution to the model, and were appropriately signed in accordance with the theoretical relationship between these parameters:

$$(EF = SVI/EDVI)$$

5.6 Discussion

5.6.1 Study 1

5.6.1.1 RR and QT characterisation during exercise and recovery

Moderate-to-high intensity exercise predictably caused significant changes in heart rate, RR, QT and QT_c intervals compared with pre-exercise resting values in both males and females. Heart rate did not recover to pre-exercise values within the observed ten minute recovery period, consistent with the known persistence of autonomic disturbance following exercise. The prolonged (lagging) reduction in QT observed during the first two minutes of recovery is attributable to QT-RR hysteresis (main study, chapter 3). The QT_c interval returned to the mean pre-exercise value within the first minute of recovery for males and females, whilst QT remained significantly altered for a further three to eight minutes. There were significant gender differences in QT duration only during the recovery period. However, QT_c was significantly different between males and females in at least one lead during every stage of the protocol, and this provides some evidence that there are gender differences in the relationship between QT and heart rate.

5.6.1.2 Use of the Bazett correction formula

The results of study 1 confirm that the Bazett correction formula adequately accounts for the relationship between QT and RR during the pre- and post-exercise periods (heart rate ranges for males and females during pre-exercise were 70-79 and 78-85 bpm, respectively, and during post-exercise were 100-139 and 102-153 bpm, respectively). However, during exercise this relationship was valid only for work rates up to 90 W (31±5 $\%WR_{max}$) in males and up to 60 W (32±8 $\%WR_{max}$) in females. The Bazett formula substantially over-corrected for the influence of heart rate on QT during all but the lowest exercise intensities. In fact the highly non-linear reduction in QT with increasing exercise intensity was

reversed following Bazett correction, yielding QT_c values that increased substantially with progressive exercise. Ahnve & Vallin (1982) observed a similar "trend reversal" when QT was adjusted using the Bazett correction formula during atrial pacing at various rates. This substantial over-correction was explained by the suggestion that the normal QT-RR relationship (assumed to be adequately described by the Bazett formula) depends on the parallel influence of autonomic tone on heart rate and ventricular repolarisation, and that the "artificial" increase in heart rate during atrial pacing abolishes this relationship. The results of this study suggest that physical exercise might represent a similar perturbation of the QT-RR relationship via the substantially altered ANS influence that occurs during exercise conditions.

Ahnve & Vallin (1982) also noted that the magnitude of QT shortening is not influenced by the sympathetic nervous system since propanolol (beta adrenoceptor antagonist) did not change QT at constant-paced heart rates. However, cholinergic blockade using atropine caused a reduction in QT, and the authors suggested that this was evidence of a direct cholinergic influence on depolarisation-repolarisation of the ventricular myocardium. More recently, Magnano *et al.* (2002) showed that parasympathetic modulation of the ventricular myocardium has a major influence on the dynamic adaptation of QT to changing heart rate. These authors examined QT duration and the QT-heart rate slope during heart rate elevation following the administration of atropine, isoproterenol (beta adrenoceptor agonist) or physical exercise. For equivalent heart rates, QT was longest and the QT-heart rate slope was smallest (indicating impaired QT reduction per unit increase in heart rate) for isoproterenol compared with either exercise or atropine, both of which gave similar results. This provided evidence that parasympathetic withdrawal has a substantial and dominant influence on QT shortening at elevated heart rates. Moreover, the authors noted

that this parasympathetic modulation must occur directly on the ventricular myocardium since there were no chronotropic differences between the tested conditions in their study.

5.6.1.3 QT variability indices

Previous research therefore leads to the hypothesis that the apparent breakdown in validity of the Bazett correction formula during exercise might reflect differing autonomic modulation of the atrial and ventricular myocardium. The relative influence of the ANS on QT and heart rate was therefore examined in the present work by quantifying the previously described RR and QT variability indices, with particular emphasis on the QTVI.

To reiterate, the QTVI is expressed as the base-10 logarithm of the ratio between the squared coefficients of variance for the QT and RR intervals. The QTVI therefore compares the magnitude of temporal variability in these two parameters, whilst QTVN quantifies the extent of repolarisation lability (squared coefficients of variance of QT) without adjustment for heart rate variability (Haigney *et al.* 2004).

In this work, gender differences in both the magnitude and dynamic properties of the QTVI indices (QT_aVI and QT_eVI) were observed. QT_aVI and QT_eVI were lower (more negative) in males compared with females during pre-exercise rest and during moderate intensity exercise, indicating a more marked dominance of RR variability over QT variability in males compared with females. This suggests that there is a greater relative chronotropic (atrial myocardium) influence from the ANS during these conditions in males compared with females. Post-exercise

recovery values of both QTVI indices were similar in males and females, suggesting similar relative levels of autonomic influence on the atrial and ventricular myocardium during this time.

There was a substantial and rapid increase in both QTVI indices within the first minute of exercise in males and females. This behaviour might be caused by the similarly large and rapid withdrawal of parasympathetic influence on heart rate that occurs during this time (Yamamoto *et al.* 1991, Tulppo *et al.* 1996). Subsequently, positive QTVI values were observed during the majority of exercise, indicating a shift towards dominance of QT variability over RR variability. It is possible that this may be explained by the persistent parasympathetic modulation of the ventricular myocardium, but not of the atrial myocardium, during these physiological conditions. It is also notable that Atiga *et al.* (1998) suggested a QTVI value of 0.1 or greater to be a discriminator for higher risk of arrhythmic events. In this work both QTVI indices exceeded 0.1 at work rates greater than 120 W (42 ± 7 $\%WR_{max}$) in males and greater than 90 W (32 ± 8 $\%WR_{max}$) in females, equivalent to heart rates of 128 ± 14 bpm and 145 ± 6 bpm respectively. Whether this can be interpreted as indicating an elevated risk of arrhythmia during physical exercise at intensities greater than these values is a matter for further investigation.

Compared with pre-exercise values, both of the calculated QTVI indices (QT_aVI and QT_eVI) remained elevated until the tenth minute post-exercise in males and females. This indicates a sustained dominance of QT variability over RR variability, and it is possible that this is associated with a sustained imbalance of autonomic influence on the atrial and ventricular myocardium. This is in accord with the diminished parasympathetic modulation of HRV that is observed for several hours post-exercise (Hautala *et al.* 2001).

5.6.1.4 Choice of QT interval measurement

To reiterate, the QT interval is commonly measured as the interval between the Q wave onset and either the apex of the T wave (QT_a) or the end of the T wave (QT_e). Several authors have indicated that QT_a is an adequate index of ventricular repolarisation time as no meaningful information is lost from the T_a-T_e interval, at least during rest (Merri *et al.* 1989, Kligfield *et al.* 1996, Bidoggia *et al.* 2000, Chauhan *et al.* 2002). However, other authors have suggested that there is variability in the T_a-T_e interval in individuals who are exercising or who have cardiac disease (O'Donnell *et al.* 1985, Davey 1999). For example, Davey (1999) found significant differences in QT_e but not in QT_a between patients with heart failure and both controls and patients with left ventricular hypertrophy. It was concluded that QT_e contained a variability component not present in QT_a, suggesting that QT_e should be used to quantify the ventricular repolarisation interval.

It is worthy of note that there were significant differences between QT_aVI and QT_eVI during the majority of the experimental protocol for males and females. It should therefore be remembered that QT_a represents only a part of the complete ventricular depolarisation-repolarisation period and that, assuming equivalent reliability in their measurement, QT_e might be the more valid index of this interval. Moreover, the results of this study suggest that the T_a-T_e interval contains important information about the autonomic influence on the ventricular myocardium during rest and physical exercise. It is therefore advisable to calculate the QT variability indices during these physiological conditions using either QT_e alone or both QT_a and QT_e.

Chauhan *et al.* (2002) also noted that the QT_e wave cannot be reliably determined at rapid heart rates owing to the fusion of the T wave and the subsequent P wave. In this work, it was found that both QT_a and QT_e intervals could be reliably measured by the Holter system's detection algorithm, even for quite high heart rates. However, it was not possible to measure QT intervals using either method for heart rates greater than around 160 bpm (equivalent to between 75% and 85% of maximal work rates in males and females, respectively).

5.6.2 Study 2

5.6.2.1 Characterisation of cardiac ventricular performance indices

Cardiac performance indices were measured reliably and continuously during rest, exercise and recovery using the Task Force Monitor. These indices provided a comprehensive characterisation of the cardiac and haemodynamic responses to the progressive exercise protocol. Variability in electrocardiographic RR and QT intervals was reliably quantified throughout the protocol apart from during the final stage of exercise, during which time the substantially elevated heart rates made it impossible to measure the QT interval.

5.6.2.2 Quantification and interpretation of ventricular repolarisation variability

The QT interval is known to vary in part as a function of heart rate or RR interval (Porta *et al.* 1998). However, Almeida *et al.* (2006) found that the RR-independent contribution to QT variability in healthy subjects was substantial (greater than 40%). Previous studies had also observed that QT shortening during exercise has a substantial component that is not controlled by heart rate (Rickards & Norman 1981, Milne *et al.* 1982). A complete understanding of the temporal

variability of electrical activity in the ventricular myocardium therefore requires consideration of both RR and QT variabilities.

5.6.2.2.1 QT variability

The magnitude of QT variability is of great clinical significance as it is related to the risk of SCD, caused by sustained ventricular tachycardia or fibrillation (Antzelevitch & Oliva 2006, Piccirillo *et al.* 2007). Temporal variability of the QT interval of the ECG is caused by transmural dispersion of myocardial repolarisation (Piccirillo *et al.* 2007): the duration of repolarisation varies between subendocardial, subepicardial and intermediate layer (M) cells, being delayed in the M cells owing to ion channel population differences (Rosen & Cohen 2004). This myocardial repolarisation inhomogeneity might be accentuated owing to pathological structural changes or notably as a result of ANS changes such as sympathetic hyperactivity or reduced vagal activity (Tomaselli & Zipes 2004, Piccirillo *et al.* 2007). Furthermore, since the ANS has a direct influence on the ventricular myocardium, it could influence ventricular repolarisation independently of SA node modulation (Magnano *et al.* 2002). Nevertheless, despite this understanding of the mechanistic origins and the diagnostic and prognostic utility of QT variability measures, there is no evident previous study that has assessed the relationship between QT variability and standard measures of ventricular performance.

5.6.2.2.2 The QT variability index (QTVI)

QTVI is a marker of temporal inhomogeneity in myocardial repolarisation (Piccirillo *et al.* 2007). Several studies have demonstrated that QTVI, in preference to other indices of either RR or QT variability, has utility as a risk factor or discriminator for cardiac pathologies related to diminished or abnormal

ventricular function. Also, as previously reported, QTVI is highly sensitive to changes in physiological states including rest, exercise and recovery. For example, a substantial and rapid increase in QTVI within the first minute of exercise at relatively low work rates has been noted (section 5.5.1.2). Subsequently, elevated QTVI values were observed throughout a progressive exercise protocol and during an extended period of recovery, indicating a sustained shift towards dominance of QT variability over RR variability.

The potential clinical utility of QTVI, yet the lack of a clear functional interpretation of this index, led to the rationale for this section of the thesis. It was thought that it would be of great interest to determine the relationship between QTVI and standard measures of cardiac ventricular performance and to compare this relationship with that for other indices of cardiac (QT and RR) temporal variability. Since substantial differences in QTVI during exercise and recovery compared with during pre-exercise rest were observed in the first study of this chapter, it was considered important to investigate whether exercise alters these relationships.

5.6.2.3 Relationship between cardiac performance indices and RR and QT variability

Differences between the subsets of included predictor variables, and in their pre/post exercise magnitudes, were observed in several of the pre- and post-exercise models. This suggests that exercise had a substantial influence on the relationship between measures of RR and QT variability and the indices of cardiac ventricular performance.

On the basis of the reported regression models, RMSSDNN appears to reflect LVET and TPRI during rest and to reflect SVI following exercise. SDNN appears to reflect LVET during rest and both SVI and IC following exercise. The results suggest that RMSSDQT and SDQT are equivalent with regard to their relationships with the indices of cardiac ventricular performance: RMSSDQT and SDQT reflect SVI and IC during rest and reflect EDVI, IC and ACI following exercise. SVI and ACI (measures of cardiac output per contraction and the force of contraction, respectively) are the strongest predictors of QTVI, and notably the relationship between QTVI and these indices of cardiac ventricular performance is not altered following high-intensity exercise.

5.7 Limitations

The relationship between cardiac ventricular performance parameters and RR and QT variables during exercise was not quantified because the relatively short periods during which physiological steady states could have been assumed would have been an insufficient basis for performing meaningful regression analysis. Future work might attempt to evaluate such relationships by employing longer, single periods of exercise at uniform work rates.

It is also worthy of note that the majority of variables considered in this chapter remained substantially altered at the end of the recovery period (15 minutes post-exercise) compared with those at rest. Future studies should therefore employ longer recovery periods to analyse the complete recovery behaviour of these variables.

In the first study of this chapter, gender differences in both the magnitude and dynamic properties of the QTVI index during conditions of rest and exercise were observed. As a result of availability and time considerations, only males were considered in the second study of this chapter and a comparison of these results for females would be a useful future addition to this work.

It should be emphasised that the data reported in these studies were obtained from young, physically active, healthy subjects. The direct applicability of this work to clinical populations (such as those with cardiac pathology), and the influence of age and physical fitness thereon, remains questionable. In this context, the results of this chapter should be interpreted with caution.

5.8 Conclusions

5.8.1 Study 1

QT and RR interval variabilities have been quantified during rest and dynamic physical exercise. This work has shown that QTVI undergoes a rapid and substantial increase at the onset of exercise, and that it remains elevated throughout exercise and subsequent recovery. This reflects a sustained relative increase in QT variability compared with RR variability during this time. These changes might be explained by a simultaneous withdrawal of autonomic modulation of the atrial myocardium and an accentuation of autonomic modulation of the ventricular myocardium during exercise. This further suggests that differential autonomic control of the atrial and ventricular myocardium might be related to heart rate-dependent and heart rate-independent modulation of the QT interval.

It is also notable that both QT_c and the non-logarithmic ratio of QT and RR variabilities (10^{QTVI}) displayed linear relationships with work rate, at least up to moderate exercise intensities, and this association requires further investigation. The magnitude of the increase in QTVI during exercise is also worthy of particular note: even moderately elevated heart rates were associated with QTVI values previously shown to represent a risk factor for the development of arrhythmia. Future investigations of QTVI should include an assessment of the clinical implications of elevated values for this parameter during physical exercise.

5.8.2 Study 2

In quantifying the relative variability of RR and QT intervals, the QTVI index appears to be strongly influenced by both SVI and ACI and this relationship is maintained following a period of high-intensity physical exercise. Each of the other regression models of electrocardiographic RR and QT interval magnitudes and variabilities was altered following the performance of physical exercise. QTVI therefore is a reasonably consistent measure of cardiac ventricular performance during pre- and post-exercise physiological conditions, and as such is a more useful index than other parameters based on RR or QT interval alone. These results therefore suggest that QTVI could have utility as an easily measured, non-invasive marker of cardiac performance that is robust to differing physiological conditions.

Chapter 6 Conclusion

The work presented in this thesis was motivated by the need for general characterisation of physiological parameters throughout the full range of heart rates during exercise and post-exercise recovery. This included the assessment of the ability of available apparatus to measure these data reliably, along with the examination of which different (complementary) types of information is extractable from the data and establishment of their properties and sensitivities to differing physiological conditions. This was done:

- To provide a baseline of data for further comparison, as the work detailed here characterises the normal responses for young, healthy adults
- To validate the use of the apparatus and data analysis methods
- To provide examples of best practice

The work of chapter 3 aimed to characterise the Reynolds Lifecard Holter system. The results indicated that the magnitude of paired-lead bias and the associated LOA of the bias for this system are substantially greater for QT data compared with RR data in both males and females. The difference in paired-lead bias for RR and QT being related to differences in the orientation of the cardiac vector during these two phases of the cardiac cycle. The difference in LOA for RR and QT is likely related to the differing accuracy and precision with which these two parameters can be measured from the ECG. Although employed specifically to the Reynolds Lifecard Holter system, this methodology could similarly be used with other Holter systems. The need for the quantification of the between-lead bias and associated LOA for electrocardiographic RR and QT data in future investigations using multi-lead ambulatory ECG systems has therefore been highlighted. Interpretation of the results of such studies should take adequate

account of the between-lead variation in these parameters, and should specify each subject's physiological state.

The relationship between QT and RR intervals for the three different electrode lead configurations of the Reynolds Lifecard Holter system following dynamic physical exercise was then characterised and compared. It was found that the locations of the paired electrodes used to record the surface ECG, significantly affects the calculated magnitudes of QT_a-RR hysteresis. This emphasises the need for ECG electrode placement standardisation in investigations of QT_a-RR hysteresis. There was also evidence of differences in hysteresis between genders, highlighting the need for a set of standard ECG electrode locations for each gender. Standardised ECG recording methodologies could help to elucidate the hypothesised association between QT_a-RR hysteresis and differential autonomic control of atrial and ventricular myocardia.

The results of chapter 4 indicated that the sample entropy of RR time-series data is sensitive to differing physiological conditions when an appropriate choice of parameters is used for its calculation. The work suggests that values of r=0.1-0.15 and M=2 represent an optimal choice for the consistent estimation of SampEn for RR and QT data. However, it appears that the ability of SampEn to discriminate between resting and exercise conditions is poorer than that of the common linear measures of HRV. SampEn was also unable to differentiate between the work loads used.

SampEn of RR data was negatively correlated with normalised LF and LF/HF parameters in cardiac control, which confirms that the complexity of cardiac interval time-series is associated with ANS functional status. Changes in SampEn

for RR or QT data in terms of the altered ANS control of either the atrial or ventricular myocardium (or both) during discrete physiological states are interpreted as system decoupling from external inputs or a reduction in the influence of these inputs. Further investigations might usefully examine the utility of a combined analysis of SampEn for RR and QT data during specific types of pathology.

The DFA scaling exponent values for RR data in chapter 4 were in general agreement with those observed previously during rest and exercise. Post-exercise recovery values of the scaling exponents had not previously been documented; it was observed that recovery values reverted to pre-exercise values within the observation period. During rest and post-exercise recovery, the RR data displayed strong scaling characteristics tending towards those of brown noise (α=2). However, as exercise work loads increased, the RR data tended to demonstrate a 1/f scaling behaviour (α=1).

Scaling exponent values for QT data had not previously been reported. It was observed that α values for QT were significantly lower than those for RR data during all physiological states. Moreover, the α values for QT approached 0.5 during the heaviest exercise work loads, suggesting that the QT data had the characteristics of uncorrelated white noise during these periods.

Short-term (α_1) and long-term (α_2) values for RR data differed significantly during the resting period and the initial stages of exercise, but were comparable thereafter, whilst α_1 and α_2 values for QT data differed only for parts of the rest and recovery stages. However, α_1 for RR was found to be the better discriminator between different physiological states. These differences suggest that it is

preferable to report both α_1 and α_2 exponents for RR and QT data. However, to reiterate, the ability of α to discriminate between resting and exercise conditions is poorer than that of the majority of common linear and non-linear measures of HRV. The α_1 exponent for RR was found to be negatively correlated with various linear and non-linear indices of HRV, further confirming that the fractal characteristic of cardiac interval time-series data is associated with ANS functional status.

This work suggests that RR and QT have dissimilar fractal structures, possibly reflecting differences in the mechanisms of temporal modulation of these two aspects of cardiac electrical activity. Further investigations might usefully compare the fractal characteristics of both RR and QT data in cases of cardiac or neural pathology.

The results of chapter 5 indicated that QTVI undergoes a rapid and substantial increase at the onset of exercise, remaining elevated throughout exercise and recovery. This reflects a sustained relative increase in QT variability compared with RR variability during this time. These changes might be explained by a simultaneous withdrawal of autonomic modulation of the atrial myocardium and an accentuation of autonomic modulation of the ventricular myocardium during exercise. This further suggests that differential autonomic control of the atrial and ventricular myocardium might be related to heart rate-dependent and heart rate-independent modulation of the QT interval.

Both QT_c and the non-logarithmic ratio of QT and RR variabilities (10^{QTVI}) presented linear relationships with work rate, up to moderate exercise intensities, although this association requires further investigation. Even moderately elevated

heart rates were associated with QTVI values previously shown to represent a risk factor for the development of arrhythmia. Future investigations of QTVI should include an assessment of the clinical implications of elevated values for this parameter during physical exercise.

In quantifying the relative variability of RR and QT intervals, QTVI appears to be strongly influenced by both SVI and ACI, this relationship being maintained following a period of high-intensity physical exercise. Each of the other utilised regression models of electrocardiographic RR and QT interval magnitudes and variabilities was altered following physical exercise. QTVI therefore is a reasonably consistent measure of cardiac ventricular performance during pre- and post-exercise physiological conditions, and as such is a more useful index than other parameters based on RR or QT intervals alone. These results therefore suggest that QTVI could have utility as an easily measured, non-invasive marker of cardiac performance that is robust to differing physiological conditions.

As an expansion on the knowledge gained in this work, further investigations could be performed:

1. In older adults in order to assess the effect of aging on the degradation of physiology as reflected in the parameters
2. In children and adolescents to assess whether developmental changes are reflected in the parameters
3. In individuals with pathology
4. In athletes and exercising individuals in order to assess the relative influence of different training types (including the comparison of aerobic and anaerobic training effects) and over-training

The above could potentially lead to information regarding SCD which is seemingly prevalent in active apparently healthy young adults.

For the first time this work has shown:

- That studies should take adequate account of the between-lead variation in RR and QT data, and should specify each subject's physiological state
- That ECG electrode placement standardisation is necessary in investigations of QT-RR hysteresis, highlighting the need for a set of standard ECG electrode locations for each gender
- That RR and QT interval data series have dissimilar fractal structures, which requires further investigation in cases of cardiac or neural pathology
- That the QTVI could have utility as an easily measured, non-invasive marker of cardiac performance that is robust to differing physiological conditions

Appendix I Basic central nervous system anatomy

A1.1 The central nervous system

The vertebrate nervous system coordinates musculature activity, monitors organ function, collects and also inhibits input from the senses and initiates appropriate reaction to stimuli, hence providing protection for the body. The basic unit of the nervous system is the neuron. All neurons have two fibre types which extend from the cell body, the dendrite carrying impulses toward the cell body and the axon carrying impulses away. Each neuron is part of relay system carrying information through the nervous system. The nervous system is initially divided into the central nervous system (CNS) and the peripheral nervous system (PNS). A neuron which transmits impulses towards the CNS is classed as a sensory neuron whilst neurons which transmit impulses away from the CNS are termed motor neurons. Individual neuron fibres exist in bundles which, if part of the PNS, are classed as nerves. A collection of cell bodies along the pathway of a nerve is known as a ganglion and these provide intermediary connections between different neurological structures in the body, such as the PNS and CNS. The PNS is then further subdivided. This is illustrated in Figure A1.1. The CNS represents the largest part of the nervous system and includes the brain and the spinal cord. Together with the PNS, it fundamentally controls behaviour.

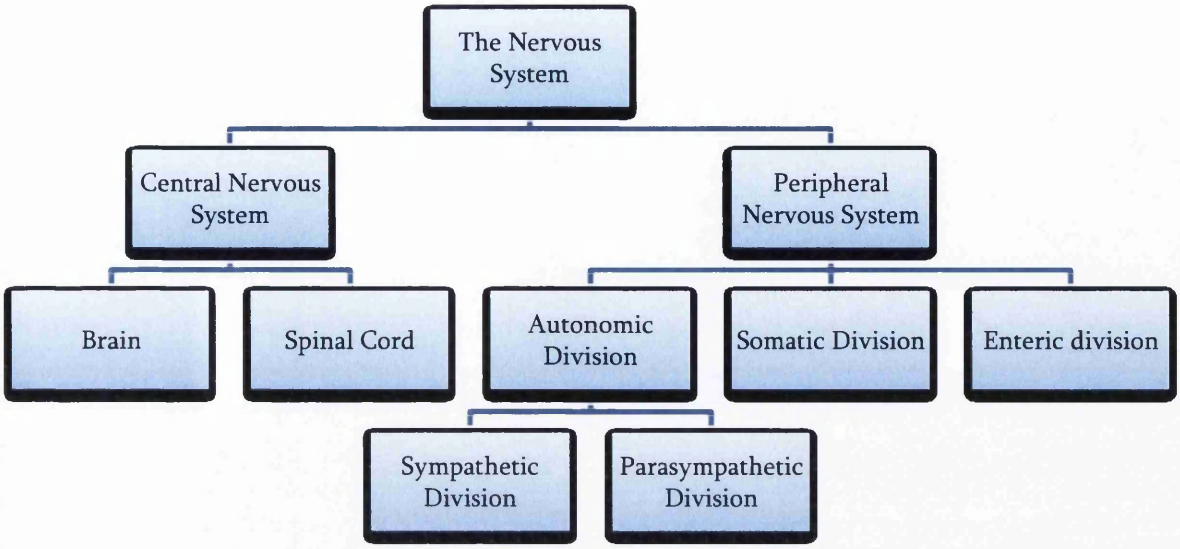


Figure A1.1 Schematic diagram of the vertebrate nervous system

A1.2 The brain

The brain has three main areas of interaction with the nervous system; the cerebrum, the cerebellum, and the brainstem.

The cerebrum's tasks include high-order thinking and learning, voluntary movement and the perception and interpretation of conscious sensations such as pain or heat. It is the largest part of the brain and consists of the left and right cerebral hemispheres. It is composed largely of white matter (myelin) with a thin outer layer of unmyelinated grey matter, the cerebral cortex. Within this cortex, higher brain functions of memory, reasoning and thought take place.

The cerebellum is beneath the cerebrum and manages learned automatic bodily functions through the coordination of muscular activity. It maintains balance and the equilibrium of the body through the subconscious control of muscle tone and

posture. It is referred to as the small brain as its structure is similar to that of the cerebrum.

The brainstem has three major areas of interaction: the mesencephalon or midbrain, which functions as the "relay station" of the brain, transmitting messages between the spinal cord, the cerebrum and the cerebellum; the pons variolii, consisting of nerve fibres which bridge the space between the hemispheres of the cerebrum and also send messages to and from the spinal cord, the cerebrum and the medulla oblongata. This has four centres of control:

1. The cardiac control centre (composed of two neural pools, a cardio-acceleratory centre and a cardio-inhibitory centre) which regulates cardiac output
2. The respiratory control centre which regulates breathing by integrating afferent signals from mechanical and neuro-mechanical sources, (information from both these sources, combined with voluntary and involuntary CNS control, effects stimulation of the respiratory muscles)
3. The vasomotor centre controlling vasoconstriction and dilation
4. The reflex centre which responds to irritants and hence regulates reflex actions such as vomiting

Situated beneath the midbrain, the hypothalamus links the nervous system to the endocrine system via the pituitary gland. It has a role in the regulation of basal metabolic rate (energy expenditure whilst at rest), temperature and hydration, although "drives and emotions" such as pleasure and appetite are also controlled by the hypothalamus, through the synthesis and secretion of neuro-hormones.

A1.3 The spinal cord

The spinal cord extends from the base of the medulla oblongata through the spinal vertebrae ending at the first lumbar vertebra. 31 pairs of spinal nerves exit the spinal cord along its length. The afferent and efferent nerve fibres associated with these nerves transmit signals to and from the brain. Efferent motor neurons innervate skeletal muscle via the anterior horn whilst afferent sensory nerve fibres enter the spinal cord via the posterior horn of the spinal cord. The grey core of the spinal cord is surrounded by a sheath of white matter containing these ascending and descending nerve tracts.

Appendix II Clinical applications of HRV analysis

A2.1 Introduction

Extensive effort has been made to date in describing changes in cardiovascular variability in a plethora of applications (both physiological and pathological) although this clinical research has continued in some cases without reflection and understanding of the fundamental causes of the variability and also without reference to the possibility that not all changes in cardiovascular variability result from changes in autonomic function (Malpas 2002). Although cardiovascular risk stratification using HRV has been researched widely in terms of myocardial infarction, angina pectoris, congestive heart failure, malignant arrhythmia, sudden cardiac death and essential hypertension, other non-cardiac pathologies have also been investigated with regard to potential HRV changes. Research areas include: obstructive sleep apnoea, endocrinological and metabolic disease, respiratory disease including asthma and COPD, diabetic autonomic neuropathy, end stage renal disease, Parkinson's disease, Chagas' disease, multiple sclerosis, perinatal pathology, sudden infant death syndrome and clinical depression. However, as HRV deals with RR interval variations, its measurement is limited to individuals in sinus rhythm and to those with a low number of ectopic beats, resulting in approximately 20 - 30% of high risk post-myocardial infarction patients being excluded from any HRV analysis (Sztajzel 2004). In addition, loss of spectral power of HRV may be used to confirm the cessation of brainstem function in clinical practice (Gang & Malik 2003). Altered HRV has also been associated with cirrhosis of the liver, eclampsia and tetanus, although assessment in these areas has not been systematically explored (Gang & Malik 2003). Several major areas of current research into the clinical influences on HRV are detailed in the following sections in order to demonstrate the variety of systemic conditions that can result in alterations in HRV.

A2.2 Cardiovascular risk stratification

The major area of agreement in the clinical applicability of HRV analysis has been in the prediction of mortality and malignant arrhythmias in post acute myocardial infarction patients, which has been linked to lowered HRV (Kleiger *et al.* 2005). Although most studies have been conducted in the sub-acute phase of infarction, others have been performed up to a year post-event with HRV remaining significantly associated with subsequent mortality (Bigger *et al.* 1993). Myocardial ischaemia and autonomic nerve activity are closely related and moreover, sympathetic activation may precipitate ischaemia which in turn may lead to increased release of noradrenaline from cardiac sympathetic nerves resulting in a "vicious circle" (Forslund *et al.* 2002).

A2.2.1 Sudden cardiac death

Sudden cardiac death (SCD) is defined as an unexpected natural death resulting from cardiac aetiology preceded by a sudden loss of consciousness (Ranpuria *et al.* 2008). The pathophysiology of SCD is not clearly understood, although there is around a seventeen-fold increase in risk during and immediately following physical exercise (Kannankeril & Goldberger 2002) and hence the case has been made for pre-exercise participation screening (Corrado *et al.* 2003). A possible mechanism for SCD could involve an abnormally depressed parasympathetic control, resulting in a failure to counteract the sympathetically mediated QT interval prolongation (Sundaram *et al.* 2008).

The significance of time domain HRV analysis in predicting SCD is controversial (Kudaiberdieva *et al.* 2007) particularly in individuals with congestive heart failure (Nolan *et al.* 1998). Prevalent LF power (suggesting sympathetic dominance) of the PSD has been shown to have prognostic significance in

predicting SCD in individuals with congestive heart failure however (Galinier *et al.* 2000, La Rovere *et al.* 2003, Guzzetti *et al.* 2005). The abnormal (narrow or complex) Poincaré plot (section 2.5.4.2.2) has been described as a significant predictor of SCD in individuals with congestive heart failure (Brouwer *et al.* 1996, Makikallio *et al.* 2001). However, the positive predictive value of HRV analysis in SCD remains low (Lombardi *et al.* 2001, Kudaiberdieva *et al.* 2007).

A2.2.2 Hypertension

There is a decline in HRV relatively early in the development of hypertension and decreases in autonomic function precede the development of clinical hypertension (Schroeder *et al.* 2003). Abnormalities in the adrenergic regulation of either cardiac output or peripheral vascular resistance possibly represent the pathophysiological basis of the hypertensive state (Grassi & Mancia 2004). There is difficulty in determining the degree to which events in ANS dysfunction are a cause or a consequence of hypertension and further studies are needed to isolate the various contributions of sympathetic over-activity and parasympathetic withdrawal, as well as the role of various antihypertensive treatments on changes in HRV (Schroeder *et al.* 2003). HRV (and BRS) contribute importantly and additionally to risk stratification for hypertension, particularly in the presence of reduced left ventricular ejection fraction as increased mortality is associated with autonomic imbalance in hypertensive individuals (La Rovere *et al.* 2001).

A2.2.3 Myocardial infarction and coronary artery disease

HRV analysis has had its greatest cardiological use in post myocardial infarction (MI) risk stratification (Kleiger *et al.* 2005). ANS imbalance may trigger mechanical and electrical complications, leading to sudden death both in the acute and chronic phases of MI (Carpeggiani *et al.* 2005). 10% of individuals die

within one year post-MI (Sanz *et al.* 1981); the majority of these are attributable to ventricular tachyarrhythmias, whilst the remainder result from re-infarction and heart failure (Rosenthal *et al.* 1985). Kleiger *et al.* (1987) presented the initial study which suggested the independent and long-term predictive value of HRV analysis after MI. Their study showed that reduced SDNN was linked with low ejection fraction, poor exercise performance, high New York Heart Association (NYHA) functional class (I-IV, the latter being most severe) and short RR intervals. Subsequent to this work, multiple studies (Pipilis *et al.* 1991, Bigger *et al.* 1993, Valkama *et al.* 1994) confirmed the value of HRV analysis in risk stratification post-infarction through a general decrease in spectral measures post event. The study conducted by Bigger *et al.* (1993), concluded that all frequency bands of the power spectrum are significant predictors of all-cause cardiac mortality, with the VLF band having most predictive value. Lombardi *et al.* (1987) showed a predominance of sympathetic activity two weeks post-MI which progressively normalised over the following year, whilst Rothschild *et al.* (1988) showed a decrease in cardiac vagal tone post-MI which contributed to the relative sympathetic over-activity. The HRV normalisation in the 12 months following infarction has created controversy as there has been disagreement as to whether recovery is partial or complete (Di Micco *et al.* 2000) with some individuals exhibiting a progressive deterioration in HRV with eventual sudden death (Nakagawa *et al.* 1994). Cripps *et al.* (1991) suggested that the relative risk of sudden death or ventricular tachycardia was seven times greater in post-MI patients with reduced HRV than in those with normal HRV. Whilst the observed autonomic changes have a short term adaptive effect through the maintenance of blood pressure, their longer term consequences are clearly maladaptive, resulting in increased myocardial oxygen consumption, adverse cardiac remodelling and renin-angiotensin system activation (Frenneaux 2004).

Coronary artery disease (CAD) arises from narrowed coronary arteries following the build up of plaque formed from fat, cholesterol, calcium and other substances (atherosclerosis) leading to reduced HRV (Casalo *et al.* 1995). Initial studies have suggested that CAD is independently associated with specific parasympathetic impairment which occurs without sympathetic dysfunction (Rich *et al.* 1988, Hayano *et al.* 1990, Hayano *et al.* 1991), with the severity of parasympathetic dysfunction being proportional to CAD severity (Nolan *et al.* 1994) and with elevated sympathetic activity (McCance *et al.* 1993). Several epidemiological studies (Okin *et al.* 2000, Robbins *et al.* 2003, Dekker *et al.* 2004) have indicated that QT interval prolongation predicts the risk of CAD. Likewise, cross-sectional studies have highlighted a positive association between QT interval prolongation and sub-clinical arterial disease (Maebuchi *et al.* 2008).

A2.2.4 Congestive heart failure

Congestive heart failure (CHF) is characterised by low cardiac output with sympathetic activation (Cohn *et al.* 1984), renin-angiotensin system activation (Cody 1984) and impaired baroreflex gain (Ferguson *et al.* 1992) ultimately leading to death. The mode of death is dependent on NYHA classification, individuals with less advanced CHF more often die suddenly, whilst those with NYHA class IV typically die from pump failure (Carson *et al.* 2005). CHF has an incidence in the general population of between 6-10% in individuals over 65 years of age (Sandercock & Brodie 2006) and approximately 50-60% of affected individuals will die within five years of diagnosis (Cygankiewicz *et al.* 2008). Norepinephrine levels increase in individuals with CHF, this correlating with disease severity (Levine *et al.* 1982) and with the prognosis of heart failure (Rector *et al.* 1987), suggesting a close relationship between the ANS and CHF (Soejima *et al.* 2000). Numerous studies have shown reduced HRV in individuals with CHF (Saul *et al.* 1988, Casalo *et al.* 1989, Binkley & Cody 1992, Nolan *et al.*

1992) resulting from increased sympathetic drive and/or reduced vagal activity, but little attention has been given to the relationship between HRV and CHF severity (Casalo *et al.* 1995).

The study by Saul *et al.* (1988) compared individuals with CHF of NYHA classes III and IV (that is with marked to severe limitations in physical activity) with normal control subjects, with reduced HRV being found in all PSD bandwidths. Soejima *et al.* (2000) highlighted a clear correlation between HRV, left ventricular function and NYHA classification. They also indicated that LF HRV showed a progressive decrease with worsening NYHA classification, but HF HRV decreased to NYHA class II and did not decrease thereafter. They concluded that in their study, abnormal HF HRV enabled only mid-term prognosis, whereas abnormal LF HRV enabled both mid- and long-term prognoses. Agreement on the prognostic role of HRV in SCD prediction in individuals with CHF however remains controversial, with the majority of studies indicating no prognostic significance owing to the difficulty in categorising the sudden or arrhythmic nature of death (Cygankiewicz *et al.* 2008).

Individuals with CHF have increased QT interval variability compared with age-matched healthy subjects (Berger *et al.* 1997) which predisposes to electrical instability of the myocardium and may favour certain arrhythmias (Cygankiewicz *et al.* 2008). Abnormal QT interval variability may help in the identification of individuals with high risk of SCD among asymptomatic patients with only mild to moderate left ventricular dysfunction (Piccirillo *et al.* 2007).

A2.2.5 Cardiovascular surgery

HRV analysis has been utilised to evaluate ANS function post cardiovascular surgery, decreased HRV being an independent predictor of prolonged hospitalisation post surgery (Stein *et al.* 2001). Chase *et al.* (2004) reported that HRV can provide an improved method of assessing agitation in ICU patients, enabling improved sedation management through optimised drug delivery when compared with traditional methods.

A2.3 Neurological application

Cerebrovascular trauma represents one of the main causes of death and disability in Western countries. Furthermore, impaired cardiovascular autonomic regulation has been described in stroke patients, often complicating the determination of the clinical source of the pathology (D'Addio *et al.* 2006). Spectral and time domain methods have described HRV reduction post-stroke although some individuals with poor outcome have shown random complex HRV behaviour, which these techniques are unable to quantify (Korpelainen *et al.* 1999). However, the level of autonomic imbalance in the months following acute ischemic stroke has not been thoroughly investigated and available data are incomplete (Lakusic *et al.* 2005). Transient autonomic dysfunction exists in individuals with acute medullary stroke, contrasting with those with non-medullary stroke owing to the role of the medulla oblongata in autonomic function (Meglic *et al.* 2001). The quantification of autonomic imbalance during the acute phase of stroke would allow the identification of individuals with further high risk of malignant arrhythmias and SCD (Lakusic *et al.* 2006).

Autonomic modulation of circulation is the primary response to traumatic haemorrhage and post-event individuals with adequate autonomic responses are

better able to maintain peripheral resistance and arterial pressure compared with those whose autonomic responses are blunted (Cooke *et al.* 2006). Acute brain injury or trauma can also result in decreased HRV and BRS, indicating an "uncoupling" of the autonomic and cardiovascular systems proportional to the degree of neurological injury, especially at the low end of the LF and HF bands, whilst in brain dead individuals, all spectral components are virtually non-existent (Papioannou *et al.* 2006). A positive correlation between autonomic cardiovascular control and the subsequent outcome post-trauma has also been shown (Haji-Michael *et al.* 2000).

A2.4 Renal disease

The end stage renal disease population has one of the highest mortality rates, even when adjusted for age, race, sex and co-morbid conditions (Gussak & Gussak 2007). Chronic kidney disease is a progressive condition that results in significant morbidity and mortality. As the kidneys maintain the homeostatic balance of bodily fluids through the filtration and secretion of metabolites, chronic kidney disease can affect almost every body system (Snively & Gutierrez 2004). Circulating angiotensin II physiologically connects the cardiovascular, endocrine and renal systems and also plays a role in disease development in some forms of hypertension, obesity and diabetes, each of these pathologies being linked with altered autonomic function. One of the major causes of death in end-stage renal disease patients under maintenance of haemodialysis is ventricular arrhythmias (Lung Wen *et al.* 2007) although there are numerous other factors implicated in the vulnerability of chronic dialysis patients to cardiovascular causes of death including sympathetic activation (Furgeson & Chonchol 2008).

HRV significantly increases following dialysis (Lung Wen *et al.* 2007). Frequency domain analysis indicates that HF components are almost lost in individuals with chronic renal failure, with the LF mean power being around three times greater than HF (compared with healthy subjects where the power in both frequency bands is comparable (Lerma *et al.* 2004)). In this case, the LF/HF ratio at rest in chronic renal failure patients is around three times greater than in healthy subjects.

Decreasing the activity of the renin-angiotensin system by angiotensin converting enzyme inhibition or angiotensin II receptor blockade reduces sympathetic activity which is suggestive of a cause and effect relationship between cardiac disease and sympathetic over-activity in chronic renal disease patients (Neumann *et al.* 2007). However, the chronic interactions among the renin-angiotensin system, cardiac reflexes and arterial baroreflexes that impact on sympathetic nerve activity, and the link between the sympathetic nervous system and long term blood pressure regulation, need further research (Lohmeier *et al.* 2001).

A2.5 Diabetes Mellitus

The American Diabetes Association has defined diabetes mellitus as a group of metabolic diseases characterised by hyperglycaemia resulting from defects in insulin[1] secretion, insulin action or both. It is characterised by widespread neurological degeneration affecting the small nerve fibres of the sympathetic and parasympathetic branches of the ANS (Schroeder *et al.* 2005). Diabetes mellitus reduces life expectancy by between five and ten years, it is the most common

[1] Insulin is a pancreatic hormone which affects metabolic rate through the regulation of glucose uptake by cells from the blood.

reason for renal replacement therapy worldwide, the most common cause of blindness in individuals under the age of 65 and the most common cause of non-traumatic amputation (Marshall & Flyvbjerg 2006).

Autoimmune-mediated destruction of insulin producing β-cells in the Islets of Langerhans in the pancreas characterises type 1 diabetes (also known as insulin dependent diabetes). Destruction of β-cells leads to progressive decline in the body's insulin secretory capacity, with destruction of 80% of β-cells resulting in clinical symptoms. Currently this is considered a result of a combination of environmental factors, such as viral infections, superimposed on a genetic susceptibility (Fowler 2007).

Similar to type 1, type 2 diabetes (or non-insulin dependent diabetes) involves both genetic susceptibility and environmental initiators, the genetic component being greater than in type 1 diabetes (Fowler 2007). The prevalence of type 2 diabetes is estimated at 2-6% in most European countries (Rana *et al.* 2005). Approximately 90% of diabetes incidence is type 2 and it results from a combination of insulin resistance or relative insulin deficiency with increased heptatic glucose production, insulin resistance is generally thought to precede insulin deficiency. Type 2 diabetes in mild cases can be unnoticed for several years. In this case secondary complications may result such as renal failure, diabetic autonomic neuropathy, diabetic retinopathy, cardiovascular disease, cerebrovascular disease and peripheral vascular disease leading to MI, stroke and amputation (Watkins 2003). In diabetic individuals, the risk of cardiovascular disease approaches that in non-diabetic people with previous MI and even with appropriate therapy, 70% of cardiovascular events still occur (Reckless 2006).

A2.5.1 Diabetic autonomic neuropathy

A common and potentially serious complication of diabetes, although among the least recognised and understood, is diabetic autonomic neuropathy (DAN). This is initially an asymptomatic condition which has a significant negative impact on survival and quality or life, initially through abnormalities in heart rate control, as well as through defects in central and peripheral vascular dynamics (Vinik & Ziegler 2007). Of individuals with DAN, 25-50% die within 5-10 years of diagnosis and the 5-year mortality rate in these individuals, is three times higher than in those without DAN (Vinik & Erbas 2001). DAN can involve all parts of the ANS and manifests as dysfunction of one or more organ systems. The metabolic disorders of diabetes lead to widespread damage of peripheral nerves and small vessels. DAN initially manifests in longer nerves, and since the vagus nerve (cranial nerve X) accounts for approximately 75% of all parasympathetic activity, even the early effects of DAN are widespread.

The most studied form of DAN but over-looked in patient care is cardiovascular autonomic neuropathy (CAN) which can greatly affect daily activities and produce symptoms such as syncope (Vinik *et al.* 2003). The earliest indicator of CAN is reduced HRV as it results from damage to the autonomic nerve fibres that innervate the heart and blood vessels leading to abnormalities in heart rate control and the 5-year mortality rate from this complication is five times higher for individuals with CAN than for those without (Vinik *et al.* 2003).

Whilst the earliest sign of CAN manifests via reduced HRV at the sub-clinical stage, at more advanced stages, resting tachycardia is evident owing to sympathetic dominance which follows vagus nerve damage (Schönauer *et al.* 2008). Subsequent to this vagal dysfunction, the HF component of HRV is

reduced (Mäkimattla *et al.* 2000). HRV in conjunction with complexity analysis has been suggested as a screening tool for CAN (Khandoker *et al.* 2009). These authors found that compared with conventional HRV indices and Poincaré plot parameters, sample entropy of HRV was able to better distinguish diabetic individuals with CAN from diabetics free of CAN. They suggested that the association between variability and complexity organisation of heart beat fluctuations may be specific to CAN and that the reduced sample entropy of heart rate complexity in individuals with CAN suggests a reduced responsiveness of feedback interactions. The transitions to strongly periodic dynamics in individuals with CAN shown in that study are similar to those observed in Parkinson's disease, obstructive sleep apnoea, SCD, epilepsy and foetal distress syndrome (Vaillancourt & Newell 2002). Type 1 diabetic individuals with CAN may also exhibit QT_c interval prolongation and further autonomic abnormalities such as impaired BRS (Lee *et al.* 2004), although exercise improves BRS sensitivity in type 2 diabetic subjects in addition to improving glucose control (Loimaala *et al.* 2003).

A2.6 Obesity

Obesity is a condition characterised by excess body mass with additional co-morbidities of hyperinsulinemia and insulin resistance causing individuals to suffer from increased mortality and morbidity risk resulting from cardiovascular complications (Avsar *et al.* 2007). Obesity can be defined in absolute terms when referring to the body mass index (BMI) with the World Health Organisation guidelines defining those with BMI ≥ 30 kg m^{-2} as obese (Wolk *et al.* 2003). Obesity in individuals with established CAD worsens the prognosis, is associated with acute coronary syndromes and is related to ANS dysfunction (Piestrzeniewicz *et al.* 2008). Studies of resting muscle sympathetic nerve activity, norepinephrine spill-over from the kidney and plasma norepinephrine indicate

greater sympathetic activation with increasing body weight (Zahorska-Markiewicz *et al.* 1993, Gao *et al.* 1996, Piccirillo *et al.* 1996) and that sympathetic activity is increased in obese subjects compared with non-obese controls (Peterson *et al.* 1988, Rossi *et al.* 1989, Piccirillo *et al.* 1996, Goodfriend & Calhoun 2004). In addition, decreased parasympathetic activity has been reported in obesity (Aronne *et al.* 1995). This sympathetic hyperactivity then impacts on further cardiovascular effects such as elevated blood pressure, heart rate and hence cardiac output, as well as renal effects.

Obese individuals are also more prone to malignant arrhythmias than the non-obese as shown by PSD analysis, obesity being a strong predictor of SCD (Avsar *et al.* 2007). However, weight gain without co-morbidities may not affect cardiac autonomic function; the main reason for cardiac autonomic dysfunction in obesity may be the concomitant disorders. For the overweight or obese, higher levels of vigorous activity are associated with low resting heart rates and HRV levels similar to those in the non-obese who do not participate in vigorous activity, although the positive effects of vigorous activity do not surpass the advantages of a lowered BMI (Rennie *et al.* 2003).

A2.7 Sleep apnoea

Sleep is a dynamic complex process, the stages of which are characterised by autonomic influences on cardiac rhythm and haemodynamics. Sleep studies of young individuals free of cardiac disease show sinus pauses of up to two seconds in duration in association with sinus arrhythmia, the incidence being increased with increasing levels of training but decreased in the elderly (Gula *et al.* 2004). These arrhythmias reflect the changes in autonomic tone that occur during sleep. During non-REM (rapid eye movement) sleep there is an increase in

parasympathetic tone with a reduction in sympathetic tone whilst during REM sleep there is a decrease in parasympathetic tone with surges of sympathetic activity resulting in decreased baroreceptor regulation and control (Gula *et al.* 2004). These authors reported that the highest incidence of non-fatal MI, implanted defibrillator discharges and SCD occur in a non-uniform manner throughout the night with a surge in each of these events occurring between 5.00 and 6.00AM, coinciding with an increased incidence of REM sleep.

Sleep apnoea is characterised as obstructive or central and is reported to be present in 25% of middle-aged men and 10% of middle aged women. Obstructive sleep apnoea (OSA) manifests as a complete or partial obstruction of the pharynx resulting from the relaxation of the musculature, which causes apnoea (complete collapse) or hypopnoea (partial collapse) during sleep. Central sleep apnoea (CSA) results from a cessation of voluntary respiratory drive from the respiratory control centre of the hypothalamus. Apnoeas and hypopnoeas are often accompanied by arterial hypoxia, a rise in carbon dioxide levels and increased sympathetic discharge leading to a repetitive interruption of nocturnal sleep which results in increased cardiac output and fatigue (Chakravorty *et al.* 2006). OSA has numerous co-morbidities including obesity, diabetes, stroke, CHF, cardiac arrhythmia and gastric reflux (Park *et al.* 2008). It is closely related to CAD and is an independent risk factor for hypertension (Peppard *et al.* 2000). Sudden death amongst individuals with OSA occurs predominantly at night in contrast to the general population; almost 50% of this group develop cardiac arrhythmias of some kind during sleep (Baumert *et al.* 2008b).

HRV parameter changes have been reported in individuals with OSA (Park *et al.* 2008) and HRV analysis has been proposed as a screening tool for OSA (Roche *et al.* 1999b). Generally, a decreased HF component and increased LF component,

reflecting decreased parasympathetic and increased sympathetic cardiac modulation has been observed (Narkiewicz *et al.* 1998, Gula *et al.* 2003, Aydin *et al.* 2004, Yang *et al.* 2005) and the LF/HF ratio may be of use in the estimation of OSA severity (Park *et al.* 2008). Continuous positive airway pressure (CPAP) therapy has been shown to restore these indices acutely (Khoo *et al.* 1999) and chronically (Roche *et al.* 1999a).

A2.8 Respiratory disease

A2.8.1 Asthma

The term "asthma" encompasses several distinct disease phenotypes leading to differences in diagnostic classification, the most widely accepted definition being a chronic inflammatory disorder of the airways usually associated with widespread but variable airflow obstruction and an increase in airway response to a variety of stimuli (Lewis *et al.* 2006). Clinically it is defined as a condition in which any of the following are manifest: symptoms prior to treatment are continuous and punctuated by frequent exacerbations or frequent nocturnal symptoms; impairment of lung function demonstrated by spirometry; or there is limitation of daily activities by asthma symptoms (Stirling & Chung 2001).

Individuals with asthma undergo episodes of exaggerated bronchoconstriction in response to a wide variety of exogenous and endogenous stimuli (for example cold air, organic/inorganic allergens including dust or exercise). This hyper-reactivity is generally co-existent with airway inflammation, the pathophysiological mechanism underlying these changes being not fully understood. It is likely that this mechanism is associated with the abnormal ANS control observed in asthmatic subjects (Kaliner *et al.* 1982), the parasympathetic component of the ANS appearing to be implicated. The parasympathetic nervous system is involved

in the bronchoconstriction that occurs during physical exercise in both asthmatic and non-asthmatic subjects (Warren *et al.* 1984) and the bronchoconstriction response to altered airway temperature and/or airway surface osmolarity (McFadden *et al.* 1986). In addition, the existence of altered autonomic function following exercise in asthmatics compared with normal controls has been suggested (Fujii *et al.* 2000). Cardiac vagal activity also appears to be increased in asthma, as demonstrated by the bradycardic response to anticholinergic drugs, methacholine and antigen challenge seen in asthmatic subjects (Crimi *et al.* 1992). It has therefore been suggested that there could be an intrinsic relationship between cardiac and bronchial autonomic control, and that this relationship might be altered in asthmatic individuals (Kallenbach *et al.* 1985). However, vagal regulation of resting bronchomotor tone depends on reflexes initiated in irritant airway receptors (Jammes & Mei 1979), whilst vagal activity to the heart occurs in response to arterial baroreceptors (Kollai *et al.* 1994). This apparent independence of vagal control suggests that bronchial and cardiac vagal activities would be unrelated. In accordance with this concept of system-independent ANS control, some authors have reported a lack of association between changes in ANS control in the cardiac and respiratory systems. For example, Horváth *et al.* (1995) found no correlation between bronchial and cardiac vagal tone (assessed using airway resistance and heart beat period) in non-atopic healthy adults. However, there is also a considerable body of evidence suggesting that numerous cardiovascular parameters are altered as a result of either pathophysiological changes or the administration of therapeutic medication in asthma. This autonomic and resultant cardiac dysfunction may even contribute to some sudden and often unexpected deaths in individuals with asthma (Lewis *et al.* 2006).

A2.8.2 Chronic obstructive pulmonary disease

According to the definition from the European Respiratory Society, chronic obstructive pulmonary disease (COPD) is a disease characterised by reduced maximum expiratory flow and slow, forced emptying of the lungs owing to varying combinations of diseases of the airways and emphysema resulting from respiratory pollutants, mainly tobacco smoke (Agusti *et al.* 2005). Whilst asthma and COPD are both characterised by airflow limitation, asthma is usually episodic in nature, does not progress, begins in childhood and exhibits a good response to bronchodilators and corticosteroids. COPD by contrast has a slow, progressive onset with little variability in symptoms, most individuals being diagnosed after the age of 60 and showing a poor response to bronchodilators and corticosteroids (Barnes 2006). COPD is recognised as having local respiratory effects and wider systemic influences (Fabbri & Rabe 2007). COPD is an independent risk factor for cardiovascular disease and further co-morbidities include hypertension, diabetes, cancer and pulmonary vascular disease, with sufferers mainly dying of non-respiratory disorders such as cardiovascular diseases or cancer (Fabbri & Rabe 2007).

Impaired autonomic regulation in COPD patients via sympathetic activation and parasympathetic withdrawal are commonly observed during acute exacerbations and these are associated with a worse prognosis in this group (Skyba *et al.* 2007). One explanation for the high levels of sympathetic activation in COPD patients is an increased arterial chemoreflex activation of sympathetic outflow which may have important consequences for respiratory muscle function, pulmonary circulation and right heart failure in these individuals (Heindl *et al.* 2001). In addition to the consistent evidence of augmented sympathetic nervous traffic, there is evidence of elevated catecholamine level and renin-angiotensin system

activity in COPD patients which is in keeping with the similar situation which exists in individuals with heart failure.

BRS is reduced in individuals with COPD although the exact mechanism for this remains unclear. Exercise training is known to improve BRS in healthy subjects (Costes *et al.* 2004) and these workers reported that exercise conditioning in COPD patients with poor initial exercise tolerance is a strong stimulus in increasing the baroreflex gain, although it remains undetermined whether the increase reduces the incidence of cardiovascular events. There are two proposed mechanisms for the alteration of the exercise autonomic response in COPD patients (Bartels *et al.* 2003). The first is a lack of ability to achieve a sympathetic response as the baseline sympathetic tone is already elevated, and the second is that there is an increase in HF power indicating an abnormal level of parasympathetic tone, both implying that an increase in heart rate would be difficult. Bartels *et al.* (2003) demonstrated an increase in parasympathetic activity during exercise in COPD patients, in clear contrast to a healthy control group. They also showed a lack of alteration of sympathetic tone during exercise in the COPD group, in direct opposition to the exercise response in the healthy population. This might have a significant bearing on the ability to perform exercise and on the response to a conditioning programme in COPD patients.

A2.9 Thyroid dysfunction

The thyroid gland is one of the largest endocrine glands in the body and is under the control of the hypothalamus and pituitary gland. It primarily controls metabolic rate, protein synthesis and systemic sensitivity to other hormones. Thyroid hormones affect cardiac tissue and the peripheral vasculature by direct action on these tissues and also indirectly, at least in part, from changes at the

ANS level (Cacciatori *et al.* 2000). Cacciatori *et al.* (1996) evaluated the impact of hyperthyroidism on the cardiovascular system by PSD analysis during "Ewing battery" intervention, finding that hyperthyroid patients have reduced vagal influence at the SA node and consequently a relative hypersympathetic tone. This was in agreement with Northcote *et al.* (1986). Pitzalis *et al.* (1998) however found no association between hyperthyroidism and HRV.

Flynn *et al.* (2006) described all-cause mortality and vascular mortality and morbidity in individuals after treatment for hypothyroidism and hyperthyroidism. They advocated an increased use of statins and ACE inhibitors in this group as amongst individuals with treated and stabilised thyroid disease there may be an increased risk of specific non-fatal vascular events, and ischemic heart disease and dysrhythmias in the case of both hypothyroidism and hyperthyroidism. The impact of thyroid dysfunction on the QT interval has not however been investigated extensively (Dörr *et al.* 2006) and as yet there are inconsistent findings, although QT_c interval prolongation has been associated with hyperthyroidism (Colzani *et al.* 2001, Owecki *et al.* 2006) and also with hypothyroidism (Fazio *et al.* 1992). Hyperthyroidism is also associated with an age dependent increase in atrial fibrillation which commonly normalises post treatment. Wustmann *et al.* (2008) studied potential atrial fibrillation triggers in individuals with hyperthyroidism using time and frequency domain analysis and heart rate turbulence analysis with the finding that elevated thyroid hormones increase electrical atrial activity in young individuals without cardiac disease, which in some cases may trigger atrial fibrillation.

A2.10 Depression and Schizophrenia

Depression is a risk factor for cardiac morbidity and mortality in individuals with coronary heart disease and major depression is associated with a four-fold increase in the risk of mortality during the first six months after acute MI. The prognostic significance of this is comparable to that of left ventricular dysfunction and history of MI. Elevated sympathetic activity and hypothalamus dysregulation have been identified in otherwise healthy individuals with major depression (Carney *et al.* 2001). QT interval variability is significantly higher in depressed post-MI patients when compared with non-depressed controls, the former showing more predisposition to SCD resulting from abnormalities in ventricular repolarisation which arise from increased sympathetic activity (Carney *et al.* 2003).

Life expectancy is reduced by around 20% in schizophrenic individuals and besides suicide, cardiovascular disease has been reported to play a role, with decreased vagal activity being identified in acute schizophrenia, altering cardiovascular regulation and increasing cardiac mortality via decreased BRS (Bär *et al.* 2007). These workers reported that the reduction in BRS in acute schizophrenia leads to a severe impairment of blood pressure and heart rate regulation in acute psychotic individuals and that, taken into account with other risk factors associated with this group, this might play a significant role in cardiovascular morbidity and mortality in this population. Coexistent with increased sympathetic activity, untreated schizophrenics have a reported increased frequency of diabetes, dyslipidemia, hypertension and obesity and a further worsening of vascular risk factors has been assigned as a side effect of neuroleptic drug treatment (Scigliano *et al.* 2008).

A2.11 Parkinson's disease

Parkinson's disease is a movement disorder characterised by loss of dopaminergic neurons in the CNS. Cardiac sympathetic denervation appears to be characteristic of most Parkinson's disease patients, reflecting an abnormality of catecholamine function in the brain and the periphery (Goldstein *et al.* 2000). Reduced autonomic activity induced by the disease is associated with a decreased frequency of vascular risk factors and the occurrence of vascular disease is further reduced by treatment with dopaminergic drugs (Scigliano *et al.* 2008).

The incidence of autonomic involvement in the mechanism of orthostatic hypotension, which is common in Parkinson's disease patients, has not been systematically explored (Biaggioni 2007). Abnormalities in cardiovascular autonomic control might underlie the mechanisms that lead to the decrease of arterial pressure during standing although drugs used to control symptoms in Parkinson's disease may promote orthostatic hypotension (Barbic *et al.* 2006). Spectral analysis may provide a research tool to determine the incidence of autonomic abnormalities in early Parkinson's disease (Biaggioni 2007) and is one of the most promising techniques for the evaluation of Parkinson's disease evolution (García-Sánchez *et al.* 2006). Haapaniemi *et al.* (2001) examined tonic autonomic cardiovascular regulation PSD analysis in individuals with untreated Parkinson's disease with the conclusion that the disease causes dysfunction of the diurnal autonomic cardiovascular regulation, with dysfunction becoming more pronounced with disease severity.

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