Differences in autonomic function between high and low cardiovascular disease risk patients

DIFFERENCES IN AUTONOMIC FUNCTION BETWEEN HIGH AND LOW CARDIOVASCULAR DISEASE RISK PATIENTS

A thesis submitted for the degree of Doctor of Philosophy

by

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ABSTRACT

Autonomic function is altered in many cardiovascular disease (CVD) states and altered function is associated with increased morbidity and mortality. Early research assessed the autonomic nervous system (ANS) using invasive methodology. The sample sizes were small and the technique was impractical for routine clinical work. Advances in technology have facilitated the ability to non-invasively quantify autonomic function using heart rate variability (HRV) methodology. However, these techniques have poor reproducibility and consequently, non-invasive assessment of autonomic function is rarely applied or utilised in the clinical setting.

The aim of this thesis was to identify differences in autonomic function, measured non-invasively by HRV, in a large cohort of patients with well-defined CVD risk at rest and during pharmacological stress with dobutamine. Patients suffering with cardiovascular disease risk and individuals at high risk include those with heart failure, diabetes, chronic kidney disease, hypertension, and coronary artery disease (CAD). Low risk individuals include those who had a normal dobutamine stress echocardiogram (DSE), were not receiving pharmacological medication and who had no CVD risk factors including those stated above.

Patient demographic characteristics, autonomic function, resting and stress echocardiography, haemodynamics, and haematological data were recorded and analysed accordingly. This study used the Task Force® Monitor (TFM), a new commercially available and validated non-invasive monitoring system for the quantification of autonomic function. The TFM is able to record and quantify autonomic and haemodynamic parameters continuously online, on a beat-to-beat basis. Three hundred and fifty non-selected patients who were referred for a dobutamine stress echocardiogram in a London district general hospital were recruited for the study.

The results in this thesis have demonstrated that significant differences exist in autonomic function at rest in patients with declining systolic and diastolic cardiac function, diabetes, chronic kidney disease and hypertension compared to normal patients. Indeed, deteriorating cardiac and renal function was associated with declining autonomic function.

In patients with coronary artery disease there are no differences in autonomic function compared to normal patients at rest, however, in response to dobutamine stress, ischaemic patients demonstrated autonomic responses that were diametrically opposed to those detected in non-ischaemic patients.

Non-invasive analysis of autonomic function using HRV methodology at rest and during stress provides great potential to further risk stratify high CVD risk populations.

ACKNOWLEDGMENTS

I would like to take this opportunity to thank my director of studies, Dr Rajan Sharma for providing me with both excellent training and with the opportunity to pursue a topic I love. Your exemplary scholarship, dedication, support, and hard work remain an inspiration to me. In many ways this thesis is the culmination of your investment and I am forever indebted to you. I would like to thank Professor Sibel Roller and Dr David Chappell for their academic support and valued advice during the completion of this thesis. Thanks to Dr Stuart Rosen for providing me with the necessary equipment needed to record autonomic function.

I would like to thank my wife Rachel and parents William and Jacqueline for their collective support and encouragement throughout my academic endeavours. Without their support I would not have found the motivation to complete this thesis.

A final thank you to all the volunteer participants, patients, and their families for their consent and involvement in this scientific study. It is my aim that the findings presented in this thesis and those that follow will add to published knowledge and encourage new therapies in the fight against cardiovascular disease.

Jamie O'Driscoll

AUTHOR DECLARATION

I hereby declare that this thesis has been composed by myself, that the work is the result of my own investigations except where assistance has been otherwise acknowledged, that the work has not been previously submitted in candidature for any other degree, that all sources of information have been specifically acknowledged by means of references, and that consent is provided for the thesis to be made available for photocopying and for inter-library loan.

Jamie O'Driscoll

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LIST OF ABBREVIATIONS

%	PERCENTAGE
ANS	AUTONOMIC NERVOUS SYSTEM
AR	AUTOREGRESSIVE
BRS	BARORECEPTOR REFLEX SENSITIVITY
BP	BLOOD PRESSURE
BPV	BLOOD PRESSURE VARIABILITY
BMI	BODY MASS INDEX
DAN	DIABETIC AUTONOMIC NEUROPATHY
CRP	C-REACTIVE PROTEIN
CI	CARDIAC INDEX
CO	CARDIAC OUTPUT
cTnT	CARDIAC TROPONIN T
CAN	CARDIOVASCUALAR AUTONOMIC NEUROPATHY
CVD	CARDIOVASCULAR DISEASE
CHF	CHRONIC HEART FAILURE
CKD	CHRONIC KIDNEY DISEASE
CV	COEFFICIENT OF VARIATION
contBP	CONTINUOUS BLOOD PRESSURE
CHD	CORONARY HEART DISEASE
dBP	DIASTOLIC BLOOD PRESSURE
DSE	DOBUTAMINE STRESS ECHOCARDIOGRAM
EF	EJECTION FRACTION
ECG	ELECTROCARDIOGRAM
ESRD	END STAGE RENAL DISEASE
ESR	ERYTHROCYTE SEDIMENTATION RATE
eGFR	ESTIMATED GLOMERULAR FILTRATION RATE

FFT ..... FAST FOURIER TRANSFORM

FBC ..... FULL BLOOD COUNT

ICG ..... IMPEDANCE CARDIOGRAPHY

HDL ..... HIGH DENSITY LIPOPROTEIN

HF_{DBP} ..... HIGH FREQUENCY OSCILLATIONS DIASTOLIC BLOOD PRESSURE

HF_{SBP} ..... HIGH FREQUENCY OSCILLATIONS SYSTOLIC BLOOD PRESSURE

HF (ms) ..... HIGH FREQUENCY IN ABSOLUTE UNITS

HFnu ..... NORMALISED UNITS HIGH FREQUENCY

HR ..... HEART RATE

HRV ..... HEART RATE VARIABILITY

LDL ..... LOW DENSITY LIPOPROTEIN

LA ..... LEFT ATRIAL SIZE

LV ..... LEFT VENTRICLE

LVEF ..... LEFT VENTRICULAR EJECTION FRACTION

LVEDD ..... LEFT VENTRICULAR END DIASTOLIC DIAMETER

LVEDV ..... LEFT VENTRICULAR END DIASTOLIC VOLUME

LVESD ..... LEFT VENTRICULAR END SYSTOLIC DIAMETER

LVESV ..... LEFT VENTRICULAR END SYSTOLIC VOLUME

LVH ..... LEFT VENTRICULAR HYPERTROPHY

LVMI ..... LEFT VENTRICULAR MASS INDEX

LVDD ..... LEFT VENTRICULAR DIASTOLIC DYSFUNCTION

LVSF ..... LEFT VENTRICULAR SYSTOLIC FUNCTION

LVSD ..... LEFT VENTRICULAR SYSTOLIC DYSFUNCTION

LF_{DBP} ..... LOW FREQUENCY OSCILLATIONS DIASTOLIC BLOOD PRESSURE

LF_{SBP} ..... LOW FREQUENCY OSCILLATIONS SYSTOLIC BLOOD PRESSURE

LF (ms) ..... LOW FREQUENCY IN ABSOLUTE UNITS

LFnu ..... NORMALISED UNITS LOW FREQUENCY

MAC ..... MITRAL ANNULAR CALCIFICATION

MI ..... MYOCARDIAL INFARCTION

MSNA ..... MUSCLE SYMPATHETIC NERVE ACTIVITY

NICE ..... NATIONAL INSTITUTE FOR HEALTH AND CLINICAL EXCELLENCE

NA ..... NORADRENALINE

NYHA ..... NEW YORK HEART ASSOCIATION

oscBP ..... OSCILLOMETRIC BLOOD PRESSURE

PNS ..... PARASYMPATHETIC NERVOUS SYSTEM

PSA ..... POWER SPECTRAL ANALYSIS

PSD ..... POWER SPECTRAL DENSITY

PI ..... PULSE INTERVAL

PWTD ..... END DIASTOLIC POSTERIOR WALL THICKNESS

RWMSI ..... REGIONAL WALL MOTION SCORE INDEX

RAN ..... RENAL AUTONOMIC NEUROPATHY

RHR ..... RESTING HEART RATE

RPM ..... REVOLUTIONS PER MINUTE

RV ..... RIGHT VENTRICLE

SI ..... STROKE INDEX

SV ..... STROKE VOLUME

SCD ..... SUDDEN CARDIAC DEATH

SNS ..... SYMPATHETIC NERVOUS SYSTEM

sBP ..... SYSTOLIC BLOOD PRESSURE

STD ..... END DIASTOLIC SEPTAL WALL THICKNESS

TDI ..... TISSUE DOPPLER IMAGING

TEB ..... THORACIC ELECTRICAL BIOIMPEDANCE

TFM ..... TASK FORCE® MONITOR

TD ..... THERMODILUTION METHOD

TPR ..... TOTAL PERIPHERAL RESISTANCE

TPRI ..... TOTAL PERIPHERAL RESISTANCE INDEX

UK ..... UNITED KINGDOM

VLF (ms) ..... VERY LOW FREQUENCY

WMA ..... WALL MOTION ABNORMALITY

WMSI ..... WALL MOTION SCORE INDEX

CHAPTER 1: INTRODUCTION

1.1: AIM

This thesis aims to examine differences in autonomic function, as assessed non-invasively by heart rate variability (HRV), in a cohort of patients characterised as high and low cardiovascular disease risk at rest and during pharmacological stress.

1.2: HISTORICAL BACKGROUND AND CURRENT APPLICATION

In 1996, the European Society of Cardiology and the North American Society of Pacing and Electrophysiology composed a writing Task Force which produced the seminal paper: Heart rate variability: standards of measurement, physiological interpretation and clinical use (Malik et al. 1996). Since its publication, HRV has been intensively studied and the number of annual publications reporting HRV has risen to approximately 500, with diverse applications (Taylor and Studinger 2006). Despite this vast amount of research, at present there is limited application of HRV in the clinical setting. The lack of clinical application is due to low population sample sizes and diverse methods used to quantify HRV (Malik et al. 1996), making standardisation and comparison between studies difficult and therefore quantification of norms impossible.

Heart rate (HR) continuously fluctuates around its mean (Mancia et al. 1983) and is under the control of complex neural and endocrine mechanisms aimed at maintaining cardiovascular stability. A healthy heart is symbolised by significant fluctuations around

its mean HR, or rather significant beat-to-beat variability. Conversely, medical conditions that are associated with and accelerate cardiovascular disease morbidity and mortality are characterised by a significant attenuation of this beat-to-beat variability (Routledge et al. 2002; Sandercock et al. 2005).

Rather than being undesirable noise, it appears that HRV reflects the activity of cardiovascular control mechanisms, and has since evolved to become a widely applied tool and as a non-invasive index of the autonomic nervous system (ANS). Although still the subject of discussion, it is suggested that high frequency (HF) oscillations (0.15 – 0.4 Hz) are a marker of parasympathetic modulation and low frequency (LF) oscillations (0.04 – 0.15 Hz) are a marker of sympathetic activity (Berger et al. 1989; Notarius and Floras 2001; Parati et al. 1995; Saul et al. 1991; Taylor and Studinger 2006). However, other researchers contend that HRV reflects the ability with which post-junctional sino atrial node receptors react to oscillations in sympathetic and parasympathetic nerve discharge, rather than the absolute magnitude of neurotransmitter release. Therefore, HRV should be considered a marker of neural outflow modulation, rather than of the intensity of the stimulus (Floras 2009; Notarius and Floras 2001).

The non-invasive technique used in the present thesis has previously provided valuable information and is arguably the most important application of HRV is in diagnosing and predicting outcome in a number of disease states that affect the ANS either directly or indirectly (Malik et al. 1996). Indeed, a cardinal feature of disease states characterised by high cardiovascular disease (CVD) risk is a reduced HRV.

Up until recently, measuring autonomic function has relied upon invasive procedures, which are impractical in the outpatient setting and research using such methodology were hindered by small sample sizes. Non-invasive techniques have progressively evolved, however, earlier methods exhibited poor reproducibility (Sandercock et al. 2005). The growth in the popularity of investigating HRV has not only developed from its appeal as a measure of cardiac autonomic modulation for researchers and clinicians, but also from advances in technology. The Task Force® Monitor (TFM) is a well validated (Fortin et al. 2001) non-invasive monitoring system for the evaluation of autonomic and haemodynamic function. The TFM enables the determination of real time beat-to-beat HRV, baroreceptor reflex sensitivity (BRS), stroke volume (SV) and continuous blood pressure (BP) monitoring, providing complete non-invasive autonomic and haemodynamic assessment (Fortin 1998; Gratze et al. 1998). All functions of the TFM have been assessed previously (Fortin et al. 2006a; Fortin et al. 2006b; Gratze et al. 1998), with the instrument implemented in a number of clinical studies (Beitzke et al. 2002; Braun et al. 2005; Gratze et al. 1999; Parati et al. 2003; Skrabal 2004).

1.3: APPLICATION OF HEART RATE VARIABILITY WITHIN THIS THESIS

Preliminary research techniques used to analyse autonomic modulation and providing inferences into its association with cardiovascular disease were initially invasive and as a consequence were restricted by small sample sizes. Advances in technology have provided a means to study autonomic function non-invasively using HRV methodology, however, reliability of data obtained from early equipment is poor, with CV ranging

from 1-235% (Sandercock et al. 2004). In addition, despite the use and availability of non-invasive equipment, studies have been performed with small sample sizes. Furthermore, previous studies failed to standardise cardiovascular disease groups or risk stratify groups using modern tools, such as echocardiography.

The purpose of this thesis is to assess autonomic function in a large group of patients with well-defined cardiovascular disease risk. Autonomic function was quantified non-invasively with HRV technology using the TFM. The groups selected to study comprised those patients diagnosed with systolic and diastolic heart failure, chronic kidney disease (CKD), diabetes, hypertension, and ischaemic heart disease as indicated by dobutamine stress echocardiography (DSE). Cardiac function was examined by detailed echocardiographical analysis whereas diabetes and/or CKD were assessed by utilisation of haematological parameters. Non-selected, consecutive patients who were referred on clinical grounds for a dobutamine stress echocardiogram from Ealing Hospital, a district general hospital, were recruited for the study.

This thesis is subdivided into chapters, and each experimental chapter represents a discrete study of a distinct population. Each of these chapters is linked to the central research hypothesis and to other study chapters by a common theme. In all empirical chapters a common methodology was used as described in Chapter 3: General Methods. A detailed breakdown of the patient populations studied, is detailed in Chapter 4: Baseline Population.

Chapter 5 presents empirical work analysing differences in HRV at rest in patients with declining cardiac function (systolic and diastolic dysfunction). Heart failure is characterised by an increased sympathetic drive and reduced HRV, which are associated with increased risk of morbidity and mortality. Previous research analysed changes in HRV in patients with chronic heart failure, largely based on New York Heart Association (NYHA) functional classification. However, although this is an acceptable means of defining a population, there is some overlap in cardiac function when patients are characterised using these criteria and from a pathophysiological view, heart failure begins with impaired diastolic as well as impaired systolic function. In this chapter patients were categorised according to systolic and diastolic function using detailed echocardiography analysis and associations with their respective HRV measurements were investigated. To the researchers knowledge, this has not been previously performed.

Chapter 6 presents empirical work analysing differences in HRV at rest in diabetic and CKD patients. The data derived from the investigative studies of these patients has been presented associatively, since both disease states are similar in presentation with respect to signs and symptoms as well as the prognosis of cardiovascular disease (CVD). This evaluation is in addition to the finding that the elevated CVD risk is not completely accounted for by traditional risk factors. Previous research has ascertained that a reduced HRV and elevated sympathetic drive is associated with an increased risk of cardiovascular morbidity and mortality in diabetic and CKD patient populations, however the pathological link between these associations is not well understood.

Therefore this chapter aimed to investigate any associations with HRV and echocardiographic and inflammatory parameters, exhibited by these patients.

Chapter 7 presents empirical work analysing HRV differences at rest and during dobutamine stress in patients with and without hypertension (HTN). A plethora of research has demonstrated that HTN is associated with elevated sympathetic drive and attenuated HRV, which culminates into elevated cardiovascular disease risk. It has also been established that functional cardio-dynamics are impaired in patients with HTN. This chapter aimed to assess changes in HRV and associated frequency oscillations at rest and during the haemodynamic challenge induced by dobutamine in patients with and without HTN. Such a comparison may provide useful information regarding the autonomic control mechanisms employed to buffer the haemodynamic challenge.

Chapter 8 presents empirical work analysing HRV differences at rest and during dobutamine stress in ischaemic and non-ischaemic responders. Patients with coronary artery disease (CAD) are at increased risk of CVD morbidity and mortality. Sympathetic activation is considered one of the factors implicated in life-threatening dysrhythmias. In combination with echocardiography, this study enabled the assessment of HRV and associated frequency oscillations during real time ischaemic and non-ischaemic responses to dobutamine stress. The results may provide useful clinical information regarding autonomic control and further insight into the mechanisms responsible for the increased risk of mortality seen in patients with CAD.

Dobutamine infusion was the functional stress modality selected in chapters 7 and 8 since it does not compromise the essential technical consideration for HRV analysis, which is stationary recording. Dobutamine also guarantees a controlled and appropriate haemodynamic workload, which cannot be guaranteed with exercise. Observed changes in autonomic modulation, assessed non-invasively using HRV methodology during dobutamine stress are unique in this sample size and may enhance understanding of the mechanisms underlying the increased risk of CVD morbidity and mortality seen in hypertensive and coronary artery disease patients.

Chapter 9 summarises the findings of the thesis, allowing conclusions to be drawn and proposes future directions of research endeavours.

CHAPTER 2: REVIEW OF LITERATURE

2.1: INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of premature morbidity and mortality in the United Kingdom (UK) (Allender 2008) and is estimated to remain the leading cause of death worldwide through to 2020 (Murray and Lopez 1997). Fatality rates remain low under the age of 35 years and then increase exponentially until the age of 75 years, with men experiencing higher mortality rates (Allender 2008). While mortality rates have decreased in the UK by approximately 40% in the last ten years, predominantly through improved knowledge, life style modifications and advances in medical therapy, a large number of individuals will die suddenly of CVD cause, having had no previous symptoms of their disease (Haskell and Durstine 2005).

Cardiovascular disease not only affects the myocardium and circulatory network, but can also result in system wide dysfunction. Furthermore, disease of other organ systems in the human body can adversely affect the cardiovascular system and lead to significant disease. Epidemiological research has evolved our understanding of the aetiology of CVD through consistency of observed associations and biological probability in experimental studies. However, CVD causation is complex and not completely understood.

Cardiovascular disease can cause significant pathophysiological and morphophysiological alterations to the structure and function of vital organ systems and in particular the inotropic, chronotropic, lusitropic, and dromotropic functions of the myocardium and essential cardiovascular control feedback operations, such as the baroreceptor reflex. As such haemodynamic control and stability is directly compromised, which gradually exacerbates symptoms and progression of disease. This compromised haemodynamic system has encouraged scientists to research how autonomic dysfunction impacts on cardiovascular control and progression of disease.

The purpose of this chapter is to review the current literature regarding cardiac function and autonomic modulation in health and disease. In addition, this chapter will address the impact of disease of other organ systems, which are associated with elevated CVD morbidity and mortality, namely chronic kidney disease and diabetes. In order for the concepts of cardiac function and autonomic modulation to be fully comprehended, the first part of this review will describe the cardiovascular system, structure and function of the myocardium and autonomic modulation in healthy conditions. The subsequent section of the review will focus on literature examining the impact of CVD, diabetes, and chronic kidney disease on autonomic modulation. Finally, the remainder of the chapter will provide a summary of the review, highlighting areas that require further investigation, and clarify the central aims, objectives, and hypothesis of the present thesis.

2.2: THE CARDIOVASCULAR SYSTEM: A BRIEF OVERVIEW

The cardiovascular system is a continuous closed-loop organ system that consists of the heart, two circulatory systems (systemic and pulmonary), and transport medium (blood). The primary function of the cardiovascular system is to deliver oxygen and nutrients (e.g., glucose, free fatty acids, and amino acids) to metabolising tissue, remove waste products from tissue (e.g., carbon dioxide, urea, and lactate) for elimination or reuse, transport hormones and enzymes for physiological regulation, maintain fluid volume to prevent dehydration, absorb and redistribute heat to maintain thermal balance, and regulate pH to control acidosis and alkalosis (Ehrman et al. 2010). Importantly, to perform these roles and maintain homeostasis, the cardiovascular system is extremely responsive and coordinated in response to changing circumstances of endogenous and exogenous origin, by adjusting the functions of various organs (Pagani 2003). However, the cardiovascular system is also fragile and vulnerable to disease (Birch et al. 2005).

Regulation of the cardiovascular system, namely the heart and vasculature is complex and relies upon neural and endocrine (neurohumoral) influences. The heart is a vital organ, capable of maintaining rhythmic contractions autonomously with an intrinsic rate of approximately 100 - 105 $b \cdot min^{-1}$ in humans (Levick 2003; Wilmore et al. 2008). However, for optimal function, electrical and contractile performance needs to be modulated. A highly organised control system, which includes a sensory (afferent) system relaying information to the central nervous system (brain stem), an example of which is stretch receptors in arterial walls (baroreceptors), an integrating control centre

that assimilates all relevant information, and an efferent (response) system (e.g., sympathetic and parasympathetic pathways) that relays neural activity to specific target organs, such as the myocardium (heart muscle), the pacemaker and conducting cells of the heart, and the smooth muscle cells of the blood vessels to initiate a response in order to maintain homeostasis (Birch et al. 2005; Bolis 2003; Milnor 1990).

The neural system is a fast acting coordinated control mechanism. Derangement in neural control as a consequence of disease can initiate cardiovascular damage. Indeed, remodelling of the cardiac nervous system plays a critical role in the development of symptoms associated with specific cardiac disease, instead of merely being a response to the disease process itself (Armour and Ardell 2004). Consequently, loss of homeostatic control due to defective neural mechanisms is associated with increased morbidity and mortality.

2.3: NORMAL CARDIAC STRUCTURE AND FUNCTION

The myocardium is highly organised tissue, composed of smooth muscle cells, fibroblasts, and cardiac myocytes. The essential contractile cell of the myocardium is the myocyte (Opie 2004). A specialised structure of the myocyte is the sarcolemma, which is composed of a lipid bilayer allowing interaction with the intracellular and extracellular environment (hydrophobic core causes the sarcolemma to be impermeable to charged molecules). The sarcolemma forms the intercalated discs and the transverse tubular system. The intercalated discs are a specialised cell-to-cell junction, which

serves as a strong mechanical linkage between myocytes and as a path of low resistance that allows for rapid conduction of action potentials between myocytes. Transverse tubules (T tubules) are inward foldings (invaginations) in the sarcolemma, allowing a communication link between extracellular and intracellular spaces. The T tubules allow close proximity of L-type calcium (Ca^{2+}) channels and the sarcoplasmic reticulum Ca^{2+} stores and are therefore important structural components in the excitation-contraction coupling (Katz 2006; Levick 2003; Opie 2004) (Figure 2.1).

The essential contractile unit within the myocyte is the sarcomere. The sarcomere contains the contractile apparatus and is composed of thick and thin intertwined protein filaments. The key proteins of the contractile apparatus are myosin, actin, tropomyosin, and the troponin complex (Katz 2006; Levick 2003; Opie 2004). Myosin contains the site for actin binding (globular head) as well as a catalysing site for ATPase activity. Actin is the major contractile protein, and the interaction between the myosin globular head and actin in the presence of adenosine triphosphate (ATP) results in cross-bridge formation and sarcomere shortening.

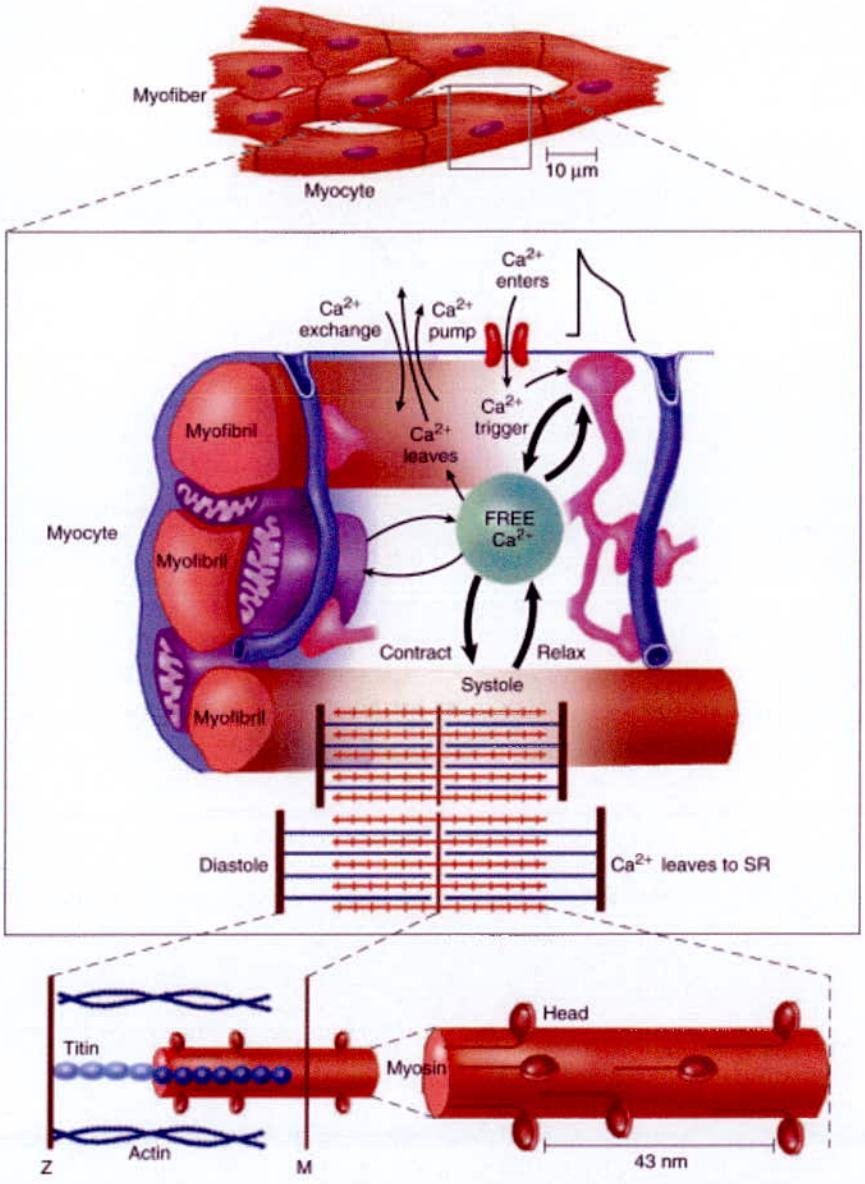

Figure 2.1: The crux of the contractile process lies in the changing concentrations of Ca^{2+} ions in the myocardial cytosol. **Upper panel**, Difference between the myocardial cell or myocyte, and the myofiber, composed of many myocytes. **Middle and lower panels**, Ca^{2+} ions are schematically shown as entering via the calcium channel that opens in response to the wave of depolarization that travels along the sarcolemma. These Ca^{2+} ions trigger the release of more Ca^{2+} from the sarcoplasmic reticulum (SR) and thereby initiate a contraction-relaxation cycle. Eventually, the small amount of calcium that has entered the cell will leave predominantly by a Na^{+}/Ca^{2+} exchanger, with a lesser role for the sarcolemmal calcium pump. The varying actin-myosin overlap is shown for systole, when Ca^{2+} ions arrive, and diastole, when Ca^{2+} ions leave. The myosin heads, attached to the thick filaments, interact with the thin actin filaments (cross-bridge cycling). Taken from Libby et al. (2008).

Tropomyosin influences actin-myosin cross-bridge formation by physically blocking the actin-myosin binding site, thus preventing Ca^{2+} binding. The troponin complex is composed of three proteins, troponin T (TnT), I (TnI), and C (TnC), all of which are important in regulating the extent of cross-bridge formation and structural integrity of the sarcomere. Troponin T binds the troponin complex to tropomyosin, TnC is a Ca^{2+} binding protein, and TnI exerts an inhibitory action to prevent myosin cross bridge cycling (Katz 2006; Levick 2003; Opie 2004).

Excitation-contraction coupling refers to the mechanism by which an action potential leads to a contraction of the myocyte. The fundamental ion for inducing this coupling is Ca^{2+} and is achieved through increases in cytosolic Ca^{2+} levels. An action potential reaching the myocyte activates L-type Ca^{2+} channels and results in an influx of Ca^{2+} ions into the myocyte. This triggers greater Ca^{2+} release from the sarcoplasmic reticulum (SR). As Ca^{2+} ions bind to TnC, the activity of TnI is inhibited, which induces a conformational change in troponin and exposes the active site between actin and myosin and therefore enabling cross-bridge formation. Contraction and shortening of the sarcomeres occurs through the sliding of acting and myosin cross-bridges (sliding filament theory) via the hydrolysis of ATP (Katz 2006; Levick 2003; Opie 2004). On completion of contraction, Ca^{2+} ions are transported back into the SR primarily by the sarcoendoplasmic reticulum Ca^{2+} ATPase pump (SERCA), which increases its activity in response to beta-adrenergic (sympathetic) stimulation. As cytosolic Ca^{2+} ion concentration fall and calcium ions dissociate from TnC, tropomyosin inhibits the actin-myosin interaction, leading to relaxation of the contracted cell. The excitation-contraction cycle can then repeat with the next action potential (Levick 2003).

Importantly and as discussed in greater detail later, cardio-dynamic function is regulated by the autonomic nervous system, which is capable of modulating the speed of myocyte depolarisation and force of contraction through increased Ca^{2+} release and therefore greater cross-bridge formation.

2.4: CARDIAC CYCLE

The cardiac cycle consists of precisely timed electrical and mechanical events that are responsible for rhythmic atrial and ventricular contractions. The initiation of ventricular depolarisation on the electrocardiogram (ECG) is considered the starting point of the cardiac cycle (Lewis 1920; Penny 1999; Wiggers 1915). When a cardiac action potential depolarises the ventricles, orchestrated opening and closing of ion channels occurs and mechanical systole ensues. Ventricular contraction causes intra-ventricular pressure to rise and this pressure rapidly exceed intra-atrial pressures, causing forced closure of the mitral and tricuspid valves. This sequence is followed by a period of isovolumetric contraction (an increase in pressure with no change in volume) and when intra-ventricular pressure exceeds aortic and pulmonary arterial pressures, the aortic and pulmonary valves open and blood is ejected from the ventricles into the pulmonary and systemic circulation. At the conclusion of ventricular ejection, the intra-ventricular pressures fall below those of the pulmonary artery and aorta, which results in closure of the aortic and pulmonary valves. This is followed by isovolumetric relaxation (a decrease in pressure with no change in volume) until intra-ventricular pressures falls below intra-atrial pressures. As the intra-ventricular pressure falls below intra-atrial

pressures, the mitral and tricuspid valves open and early ventricular filling ensues due to an atrioventricular pressure gradient, which is followed by late diastolic filling provided by atrial contraction (figure 2.2 and 2.3).

Invasive (catheterisation) and non-invasive (imaging) methods can be employed to determine cardiac structure and function as well as blood flow velocity during systole and diastole, all of which can provide information regarding cardiac contractility and compliance.

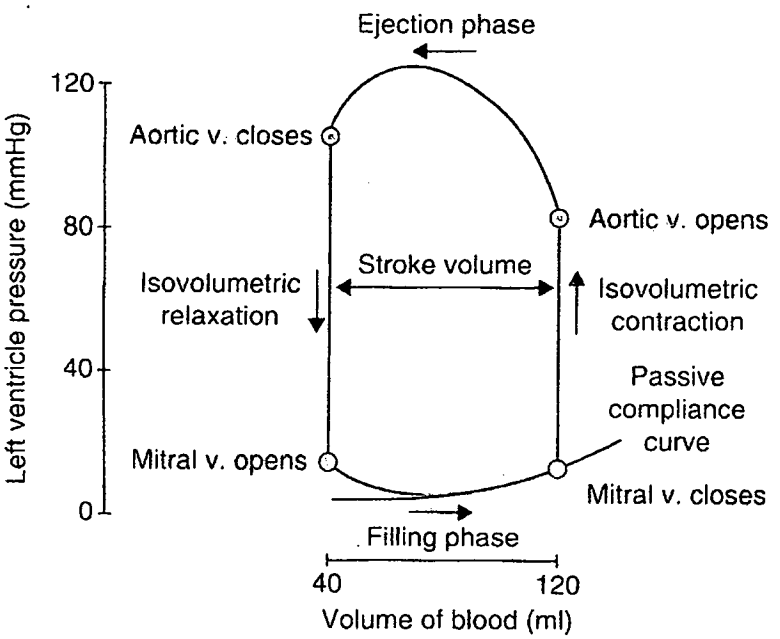

Figure 2.2: Pressure volume loop. Left ventricular volume is graphed on the horizontal axis, with pressure on the vertical axis. The temporal direction of pressure-volume changes is shown with arrows. During diastole, volume increases with little rise in pressure. After mitral valve closure, isovolumic contraction results in a rapid rise in pressure with no change in volume. At the onset of ejection, the aortic valve opens with a rapid decrease in left ventricular volume during systole. Aortic valve closure is followed by isovolumic relaxation. Taken from Levick (2003).

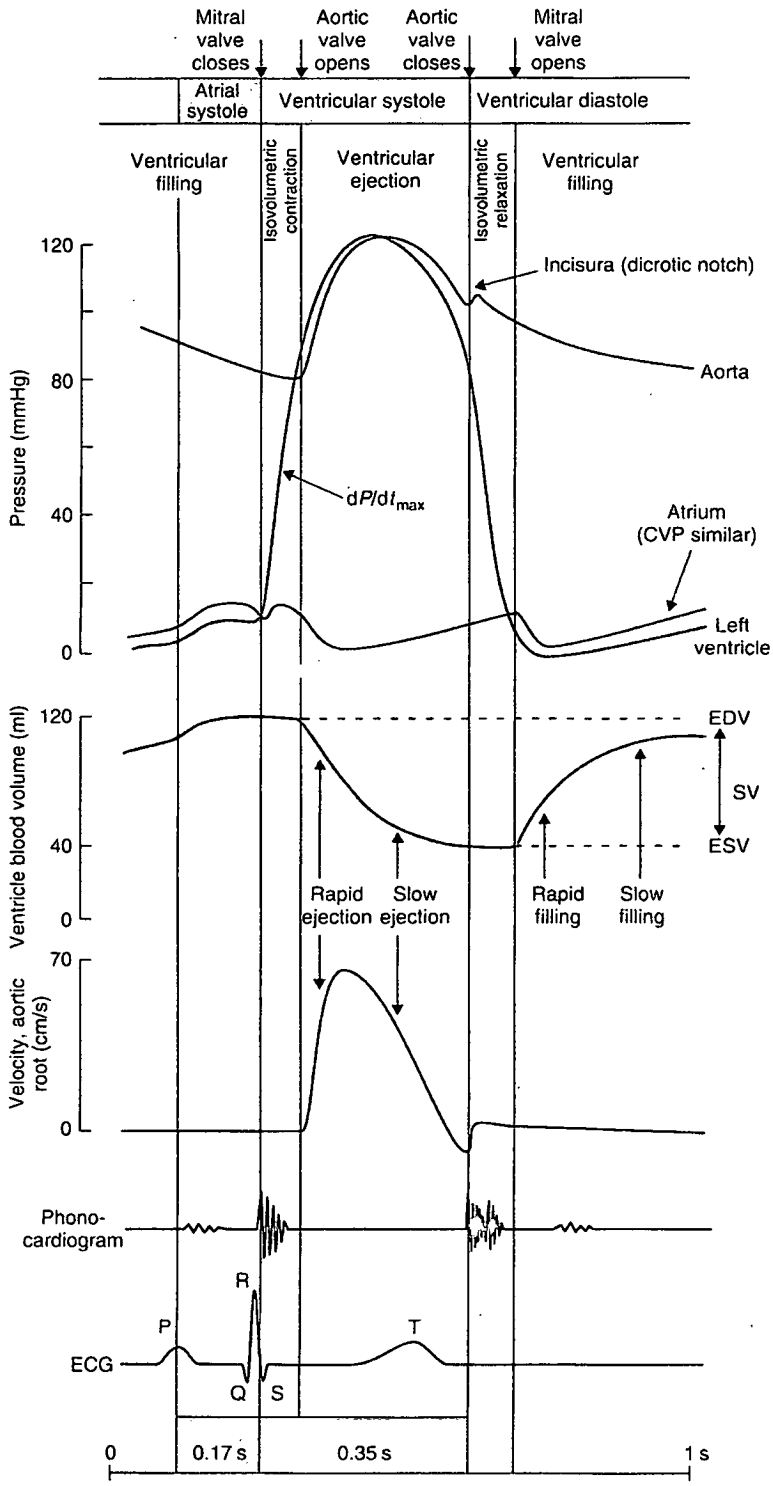

Figure 2.3: The mechanical events in the cardiac cycle, first assembled by Lewis (1920), but conceived earlier by Wiggers (1915). Taken from Levick (2003).

2.5: MYOCARDIAL PERFORMANCE

The performance of the myocardium and functioning of the entire cardiovascular system are determined by preload, afterload and contractility (von Spiegel et al. 1998). Preload of the heart is the end-diastolic wall tension, which is the load present before the onset of systole. Increasing preload through augmented venous return causes greater sarcomere stretch and thus increases the length-tension relationship. The stretching of the muscle fibres increases the affinity of TnC for calcium, causing a greater number of cross-bridges to form and augmentation in stroke volume (SV), otherwise know as the Frank-Starling law. Afterload is the wall tension during the ejection phase and according to the law of Laplace, wall tension of the ventricle is proportional to intraventricular pressure and ventricular diameter and inversely proportional to ventricular wall thickness. An increase in afterload counters ejection; therefore greater contraction (increased intraventricular pressure generation) is required to maintain SV (Penny 1999; von Spiegel et al. 1998).

Contractility is independent of preload and afterload. Therefore, an increase in contractility or inotropic state means an increase in force or increase in shortening for the same preload and afterload. Assessment of contractility in vivo is difficult, since dynamic alterations in SV could be due to changing loading conditions or contractility. Contractility can be derived from the pressure-volume relationship with the end-systolic elastance (E_{es}) slope being an index of contractility (Gorcsan et al. 1997; Opie 2004), which is independent of loading conditions (von Spiegel et al. 1998) (figure 2.4).

However, limitations of E_{es} as an indicator of ventricular contractility have been identified (Penny 1999).

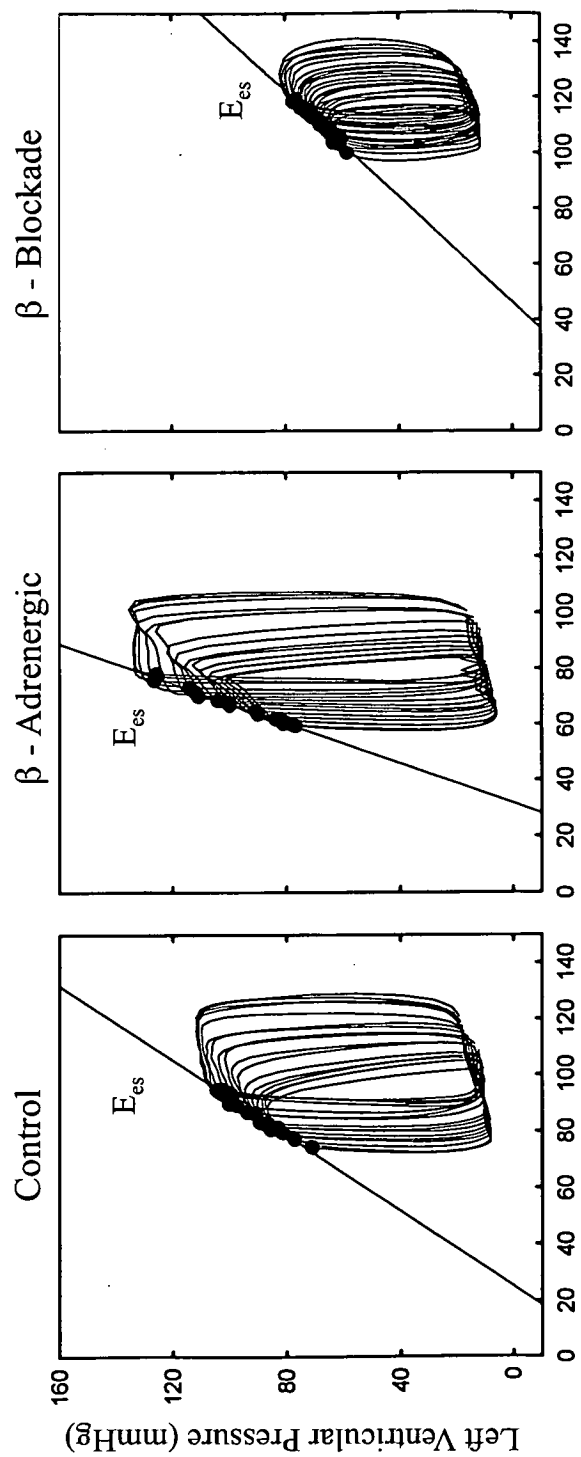

Figure 2.4: Contractility changes demonstrated on pressure-volume loops. Control (Left) versus β-adrenergic stimulation by dobutamine (middle) versus β-blockade by esmolol (right). Note the much steeper E_{es} (end systolic pressure-volume relationship) slope during β-adrenergic stimulation than during β-blockade. These data provide evidence of the use of E_{es} as an inotropic index. Data taken from Gorcsan et al. (1997).

The autonomic nervous system and particularly the sympathetic limb govern the inotropic status of the myocardium. In the resting state, the calcium ion concentration in the cardiac cytosol is only sufficient to activate approximately 40% of the potential cross-bridge sites, which allows the myocardium to have considerable contractile reserve. This large contractile reserve can be exploited by increasing Ca^{2+} occupancy of TnC (Levick 2003; Opie 2004). An increase in sympathetic activity, mediated through the neurotransmitters noradrenaline and adrenaline (catecholamine's), which activate β-adrenoceptors, cause an increased chronotropic, dromotropic, inotropic, and lusitropic effect as well as shortening the myocyte action potential duration (Levick 2003).

When endogenous catecholamine's (or synthetic β-adrenergic agonists) bind to β-adrenoceptors, it causes a conformational change of the receptor (Katz 2006). This change causes intracellular signalling to activate guanine nucleotide-binding proteins (G-stimulating proteins [Gs proteins]), which results in adenylate cyclase stimulation and subsequent increases in cyclic adenine monophosphate (cAMP) production from ATP. Increased cAMP activates cAMP-dependant protein kinase A (PKA), which in turn phosphorylates specific sites within the myocyte, such as the L-type Ca^{2+} channel, phospholamban, and TnI, which are important in the excitation-contraction coupling process (Katz 2006; Opie 2004). Enhanced contractility is generated via an increased influx of extracellular Ca^{2+} in conjunction with an increased affinity of the SR pumps for Ca^{2+}, which increases the size of the SR Ca^{2+} store. The enlarged Ca^{2+} store and increased rate of depolarisation cause a greater systolic free Ca^{2+} transient, which activates more cross-bridges and increased force of contractility (Levick 2003).

Preservation of the diastolic phase of the cardiac cycle is essential for refilling of the heart. Diastole is energy dependant and the stimulated Ca^{2+} ATPase pumps in the SR remove sarcoplasmic Ca^{2+} and phosphorylation of the thin filament TnI speeds up cross-bridge cycling, allowing a faster relaxation (Levick 2003; Opie 2004; Zhang et al. 1995) and ensuring adequate cardiac filling. Activation of inhibitory G-proteins (Gi-proteins) through muscarinic receptor activation (parasympathetic modulation), decreases cAMP and PKA activation, decreases Ca^{2+} entry and release and therefore reduces chronotropic, dromotropic, inotropic, and lusitropic status (Katz 2006).

The myocardium and vasculature are physiologically matched so that under basal conditions, pressure-volume work is generated with minimal myocardial oxygen consumption and therefore maximal efficiency (Burkhoff and Sagawa 1986; Hoeft et al. 1991). Myocardial efficiency varies depending on preload, afterload and contractility, with previous research demonstrating that at rest, maximal myocardial efficiency is achieved when the ejection fraction is in the range of 60% (von Spiegel et al. 1998).

2.6: CARDIAC INNERVATION

Regulation of cardiac neural activity is highly integrated and complex. In addition to an efferent (sympathetic and parasympathetic) and afferent nerve supply, the heart possesses an intrinsic (intracardiac) nerve supply (Armour and Ardell 2004; Ter Horst 2000). Information accumulated in the last decade has exposed the complexity and functional importance of intracardiac neurons in modulating heart function (Armour et

al. 1997; Arora et al. 2001; Baptista and Kirby 1997), with recent research detailing that cardiac performance is modulated by circuitry at multiple levels in a hierarchical fashion (Armour 2004; Kukanova and Mravec 2006; Verrier and Antzelevitch 2004) (Figure 2.5).

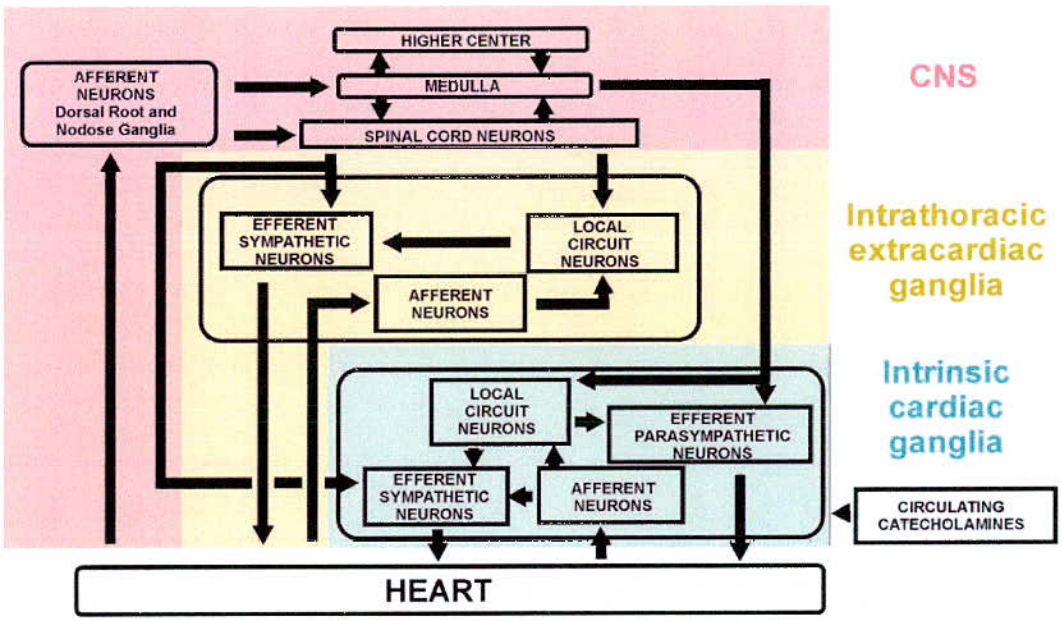

Figure 2.5: Concept of hierarchical organisation of cardiac modulation illustrating complex neuronal interconnections of the nervous system regulating heart activity. Cardiac sensory information is transduced by afferent neuronal somata in intrathoracic and extrinsic cardiac ganglia via intrathoracic local circuit neurons to cardiac motor neurons. Cardiac sensory information is also transduced centrally to generate longer-loop medullary and spinal cord reflexes. Note: CNS = central nervous system. Taken from Armour (2004).

Indeed, there is increasing structural and functional evidence indicating that intrinsic cardiac neurons, which are concentrated within epicardial fat pads and consistently identified in five atrial and five ventricular locations (figure 2.6 and 2.7), interact with extracardiac intrathoracic ganglia and the central nervous system in a complex fashion to help maintain adequate cardiac performance (Armour et al. 1997; Hou et al. 2007).

Furthermore, in vivo and in vitro histochemical, electrophysiological, and pharmacological studies strongly suggest that neurons form functional afferent, efferent, and local circuits within the cardiac nerve plexus (interconnecting nerve network) and it is now believed that this plexus processes and integrates sensory input with the final outcome being effective modification (fine-tuning) of cardiac dynamics (Pauziene et al. 2000). This complex cardiac nerve plexus; also known as 'heart brain' provides modulation of myocardial activity on a beat-to-beat basis in both physiological and pathological conditions.

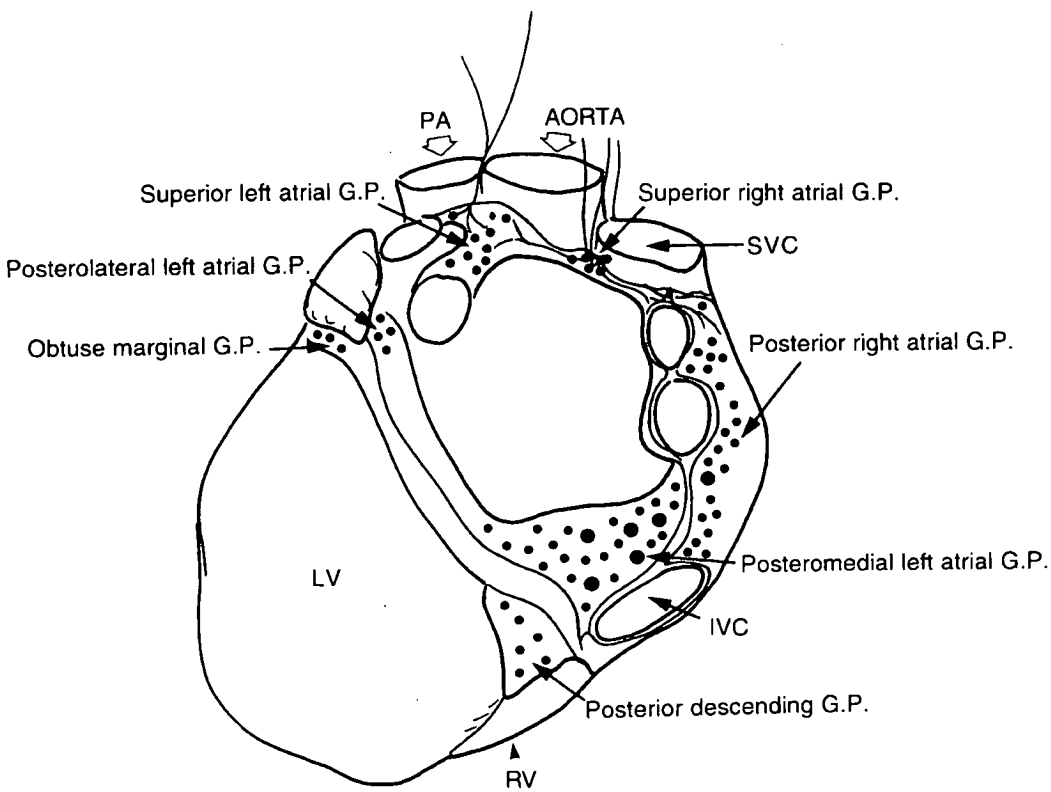

Figure 2.6: Drawing of a posterior view of the human heart and major vessels illustrating the locations of posterior atrial and ventricular ganglionated plexuses. Note: PA = pulmonary artery; SVC = superior vena cava; IVC = inferior vena cava; RV = right ventricle; LV = left ventricle; G.P. = ganglionated plexuses. Taken from Armour et al. (1997).

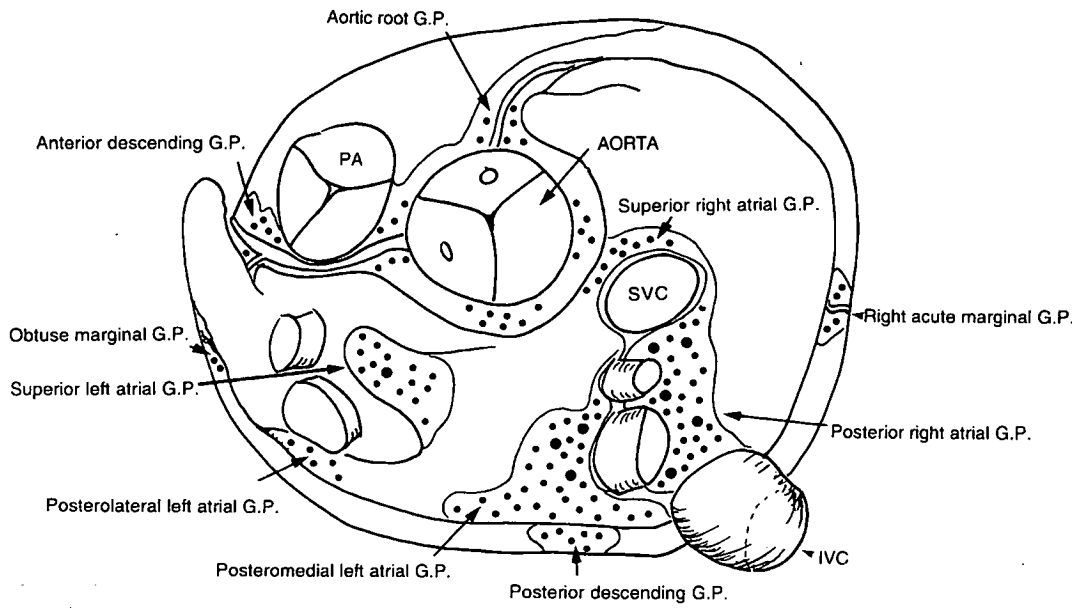

Figure 2.7: Drawing of a superior view of the human heart illustrating the distribution of ganglionated plexuses on the surface of the atria and ventricle. Note: PA = pulmonary artery; SVC = superior vena cava; IVC = inferior vena cava; G.P. = ganglionated plexuses. Taken from Armour et al. (1997).

2.7: THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system (ANS) arises from the central and peripheral nervous systems and consists of two subsystems, the sympathetic (SNS) and parasympathetic nervous system (PNS). Generally, the ANS regulates the activities and functions of organ systems not normally under voluntary control. The SNS and PNS are in many ways counteracting systems, with efferent sympathetic nerves enhancing cardiac indexes and parasympathetic efferent neurons depressing them (Armour 2004), but in other instances are synergistic, for example, although neural innervation is not necessary to initiate the heartbeat, the SNS and PNS modulate the frequency of sino-atrial node depolarisation.

The SNS and PNS are tonically active and innervate a number of essential organ tissues. Since the SNS and PNS typically have opposing effects on a given tissue, increasing the activity of one system while decreasing the activity of the other results in very rapid and precise control of a tissues function. At rest the PNS predominates (vagal tone) with the overall effect to conserve and store energy and to regulate basic bodily functions such as digestion and urination. Conversely, the SNS predominates during emergency 'fight-or-flight' reactions and during exercise (figure 2.8). The overall effect is to increase haemodynamic output and direct oxygen rich blood to tissues that need it, such as skeletal muscle.

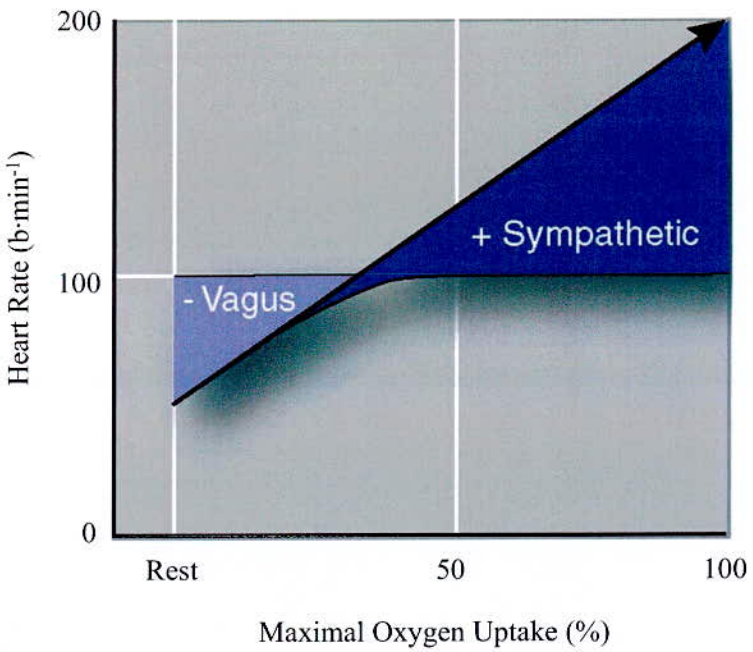

Figure 2.8: The relative contribution of the parasympathetic and sympathetic nervous system to the rise in heart rate. The initial increase in heart rate to 100 b·min^{-1} is governed by vagal withdrawal, whereas increasing activity of the sympathetic nervous system allows the heart to increase above 100 b·min^{-1}.

The SNS and PNS are essential for maintaining internal stability or homeostasis of human haemodynamic function, namely the cardiovascular system (Zhong et al. 2006). It has been proposed that the function of efferent neurons coordinating regional cardio dynamics is influenced to a considerable extent on the synergistic interactions among neurons located in higher centres through to the intrinsic cardiac nervous system (cardiac neuronal hierarchy) (Armour 2004). Through this complex interaction chronotropic, inotropic, and dromotropic functions as well as vascular tone are continually adjusted on a beat-to-beat basis to meet the changing needs and demands of the human body.

2.7.1: PARASYMPATHETIC NERVOUS SYSTEM

The parasympathetic nervous system originates centrally from the medulla oblongata (brain stem) and reaches the heart through the vagus nerve (cranial nerve X). The vagus nerve divides into the superior and inferior cardiac nerves, which traverse the epicardial vascular structure of the heart until the atrio-ventricular (AV) node where they then plunge intramurally projecting their terminal axons in the subendocardium (Vaseghi and Shivkumar 2008; Zipes 1990). Vagus neurones are heterogeneously distributed throughout the myocardium with greater density innervating the sino-atrial (SA) node and AV node compared to surrounding myocardial tissue. In addition, the right vagus nerve affects the SA node more than the AV node and the left vagus nerve affects the AV node more than the SA node (Vaseghi and Shivkumar 2008; Zipes 1990).

The vagus nerve carries impulses to the SA and AV nodes and releases the neurotransmitter acetylcholine (ACh). Acetylcholine is stored in vesicles and is released by parasympathetic stimulation, activating primarily postsynaptic muscarinic and preganglionic nicotinic receptors. The effects are terminated by rapid degradation by acetylcholinesterase (AChE) (Vaseghi and Shivkumar 2008). In ventricular myocardium, the primary post-synaptic receptor of ACh is the type M_2 muscarinic receptor and activation of this receptor decreases intracellular cAMP production, causing an inhibitory chronotropic, dromotropic, and inotropic effect (Opie 2004). This emphasises the information described previously, which detailed that the PNS predominates at rest and has a depressant effect on the heart, slowing impulse generation and conduction and thus decreases heart rate (figure 2.9).

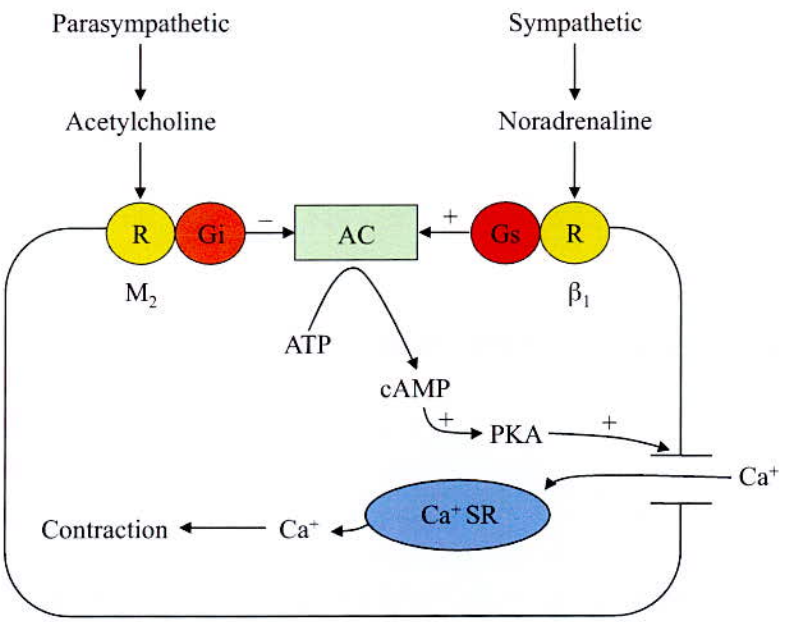

Figure 2.9: Signal transduction pathway for regulating cardiac dynamics through parasympathetic and sympathetic modulation. Note: R = receptor; Gi = inhibitory G-protein; Gs = stimulating G-protein; AC = adenylyl cyclase; ATP = adenosine triphosphate; cAMP = cyclic adenosine monophosphate; PKA = protein kinase A; Ca^{+2} = calcium ions; M_2 = muscarinic receptor; β_1 = beta-receptor. Adapted from Katz (2006); Libby et al. (2008); Opie (2004).

2.7.2: SYMPATHETIC NERVOUS SYSTEM

The preganglionic neurons of the sympathetic nervous system arise from the thoracic and lumbar regions of the spinal cord (segments T_1 through to L_2). Compared to the vagus nerve, sympathetic neurons are short and synapse with postganglionic neurons within ganglia found in the sympathetic ganglion chains. This divergence results in coordinated sympathetic stimulation (mass sympathetic discharge) to tissues throughout the human body. Indeed, 8% of the nerve fibres that constitute the spinal nerve are sympathetic fibres, which allow distribution of sympathetic nerve fibres to the skin, blood vessels, and sweat glands, therefore regulating vascular tone and thermoregulation.

Sympathetic innervation to the myocardium originates mainly from the left and right stellate ganglia. Although significant overlap and complex patterns exist, in general the right sympathetic nerves affect the SA node more than the left and the left affect the AV node more compared to the right, similar to parasympathetic innervation. However, unlike parasympathetic neurons (sub-endocardium), sympathetic neurons travel along the epicardial vasculature of the heart and penetrate into the underlying myocardium similar to coronary artery vessels (sub-epicardium) and end as sympathetic nerve terminals reaching the endocardium (figure 2.10).

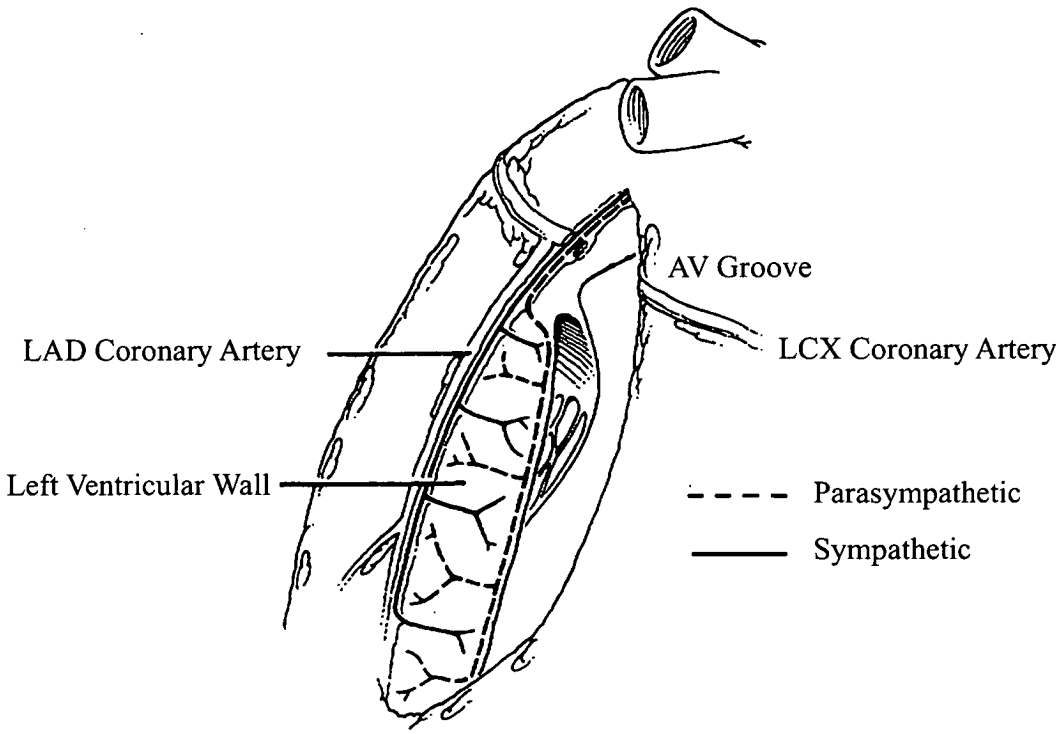

Figure 2.10: A schematic of the sagital view of the left ventricular wall showing pathways of parasympathetic (vagal) and sympathetic afferent and efferent nerves. Postganglionic sympathetic axons are located superficially in the sub-epicardium and postganglionic parasympathetic axons cross the AV groove in the sub-epicardium and dive intramurally at which point they are located in the sub-endocardium. Note: LAD = Left anterior descending; LCX = Left circumflex; AV = atrioventricular. Taken from Zipes (1990).

The major sympathetic neurotransmitter is noradrenaline, which is stored along the length of the terminal axons within swellings known as varicosities, where each varicosity acts as a specialised site of noradrenaline storage and release. Neuronal stimulation leads to noradrenaline release through fusion of vesicles with the neuronal membrane. Noradrenaline binds postsynaptically to myocardial β-adrenergic receptors and within the human heart both β_1 and β_2 subtypes are present with a ratio of approximately 5:1 (Bristow 1993). β-adrenergic receptors are linked intracellularly to the enzyme adenylate cyclase and stimulation of adenylate cyclase increases

intracellular levels of cAMP, which activates protein kinase A (PKA), which phosphorylates L-type calcium channels resulting in an influx of Ca^{2+} into myocytes. This influx releases stores of intracellular Ca^{2+} from the SR, further increasing Ca^{2+} available for cross-bridge formation as described previously. This and other intracellular signalling mechanisms result in enhanced myocardial contractility in addition to the enhanced chronotropic and dromotropic effects of sympathetic activation as already described (figure 2.9). In addition, sympathetic activation reduces the ventricular action potential and refractory period, which may have deleterious consequences in patients with cardiovascular disease and sympathetic activation.

2.8: REFLEX CARDIOVASCULAR CONTROL

Cardiovascular reflexes are critical integrating neural pathways, which are essentially negative and positive feedback control mechanisms that operate to maintain physiological homeostasis. The cardiac and vascular milieu is enriched with mechanoreceptors, chemoreceptors, and baroreceptors, which are stimulated by changing internal and external perturbations. Instantaneous adjustments to cardiac function and the vascular system are made accordingly.

Afferent neurones with sensory neurites in cardiac and vascular tissue are located throughout the cardiac and vascular tissue. These afferent neurons influence parasympathetic and sympathetic efferent postganglionic neurons, which synapse with cardiac efferent neurons. As such, sensory afferent neurons transmit information to

higher neural centres where the neural information is integrated and a response is elicited through efferent neural pathways (sympathetic and parasympathetic), which produces modifications to cardiovascular dynamics (Armour 1999, 2004).

Mechanosensory afferent neurons monitor changes in mechanical deformation and ventricular dynamics, such as the phasic mechanical changes during each cardiac cycle (Armour 2004). Therefore, each period of myocardial contraction and relaxation is monitored, with information transmitted to higher control centres to ensure appropriate cardiac output for haemodynamic stability and vascular perfusion (Armour 1999, 2004).

Chemosensory afferent neurons monitor alterations in the chemical milieu surrounding their sensory neurites, such as adenosine, ATP, and bradykinin and transmit information to elicit a response. They are stimulated by a fall in arterial partial pressure of oxygen (PO_2), a rise in partial pressure of carbon dioxide (PCO_2), or a fall in pH, these changes occurring together or independently. They are also stimulated by an increase in sympathetic activity (Vatner and Hittinger 1996). Importantly, the majority of cardiac afferents can monitor multimodal stimuli and are able to simultaneously sense local mechanical and chemical alterations (Armour 2004).

Baroreceptors located in some of the major systemic arteries are sensory receptors that monitor changes in pressure. If blood pressure falls the number of sensory impulses transmitted from the baroreceptors to the higher control centres decreases and as a

result, efferent activity is adjusted to increase heart rate and vascular resistance so that blood pressure increases to within normal limits (Levick 2003).

It is becoming increasingly evident that the cardiovascular reflexes are not only important in maintaining homeostasis under physiologic conditions, but are also critical in the response to disease.

2.9: OVERVIEW

Regulation of the cardiovascular system is complex and cardiac function is continually modified on a beat-to-beat basis in order to match systemic demand. The chronotropic, dromotropic, inotropic, and lusitropic status of the myocardium is continually monitored and modified by complex circuitry in order to provide appropriate haemodynamic output and therefore oxygen delivery to tissue. The central nervous system, autonomic nervous system, internal cardiac nervous system and feedback receptors are integral for optimal cardiovascular control and it is the coordinated function of these control systems that are integral for continual health.

Malfunction or dysfunction of appropriate subsystem interaction (imbalance in the neural hierarchy) is associated with an increased risk of cardiovascular disease (CVD). Indeed, deteriorating autonomic nervous system modulation has been implicated in the development and progression of adverse CVD, which can be attributed to disease directly affecting myocardial tissue, such as an acute myocardial infarction (AMI),

disease due to other vital organ systems, such as attenuated kidney function and metabolic disease, such as diabetes. It is now recognised that remodelling of cardiac neural modulation occurs before overt signs of cardiac disease become evident, therefore directly affecting clinical management and outcome (Armour and Ardell 2004). This next section of the review of literature will concentrate on autonomic dysfunction in cardiovascular disease, kidney disease, and diabetes.

2.10: AUTONOMIC DYSREGULATION: AN INTRODUCTION

The autonomic nervous system plays an important role in regulation of the heart and in the maintenance of cardiac output in response to acute and chronic stress. Cardiovascular homeostasis at rest and in response to stress is maintained in part by catecholamine's, both circulating and neurally released, with efferent control of heart rate, contractility, and peripheral vascular tone. Acetylcholine and noradrenaline released from nerve terminals or from circulating sources interact with cardiac and vascular receptors, which initiate a cascade of biochemical and electromechanical events such as increasing the rate and force of myocardial contraction and relaxation, and maintaining adequate cardiac output and arterial perfusion pressure to critical organs such as the heart, kidneys and brain. However, the autonomic nervous system and the biochemical processes that mediate these effects do not function normally in chronic disease states.

Cardiovascular disease, diabetes and declining renal function are known to alter the autonomic nervous systems modulation of cardiac function. This dysregulation of cardiac control is characterised with augmented sympathetic drive and reduced parasympathetic modulation, which has been associated with an unfavourable outcome and promote the occurrence of life-threatening arrhythmias (Du et al. 1999; Du and Dart 1999). On the contrary, an amplified PNS outflow may exert a protective anti-arrhythmic effect (Du et al. 1999; Osaka et al. 1996; Taskforce 1996). Indeed, high vagal tone is associated with cardiac protection and commonly seen in healthy and athletic populations.

The ability to reliably quantify the dynamics of the ANS is crucial for examining ANS dysfunction associated diseases (Zhong et al. 2006). The activity of the ANS can be determined invasively, such as measuring cardiac noradrenaline (NA) spill-over to plasma using isotope dilution (Kingwell et al. 1994), measuring muscle sympathetic nerve activity (MSNA) by microneurography (Grassi et al. 1999) or by measuring total body and cardiac noradrenaline spill over from arterial plasma and the coronary sinus respectively (Azevedo et al. 2000) or non-invasively using heart rate variability (HRV) methodology (Malik et al. 1996).

2.11: CARDIOVASCULAR AUTONOMIC NEUROPATHY

Cardiovascular disease (CVD) is at epidemic status in the United Kingdom (UK) (Dalal and Evans 2003) and accounted for 233,000 deaths in 2003 of which 65,000 were premature (Petersen 2005) with an approximate cost to the UK of £29.1 billion (Luengo-Fernández et al. 2006).

Autonomic dysfunction is evident in CVD conditions, advancing unfavourable prognosis in a number of patient groups and related to premature mortality. It has been recently recognised that remodelling of the ANS plays a critical role in the development of overt signs and symptoms associated with CVD, rather than a response to the disease process itself (Armour and Ardell 2004). Therefore, the ability to recognise dysregulation of cardiac control due to disturbed autonomic function is crucial for early interventional treatment for prolonged subsistence.

2.11.1: MYOCARDIAL INFARCTION

In the UK, the incidence of individuals suffering a myocardial infarction (MI) is approximately 950,000 with a mortality rate of approximately 426,000, which was quantified over a 12-year period (Lampe et al. 2000). Autonomic dysfunction reflected by excessive cardiac sympathetic and/or inadequate cardiac parasympathetic modulation (Malik et al. 1996) is a strong and independent predictor of mortality in patients following a MI (Bigger et al. 1993; Bigger et al. 1992b; Kleiger et al. 1987).

Furthermore, the adrenergic drive post MI has been related to the extent of myocardial damage and associated morbidity and mortality (Karlsberg et al. 1981; McAlpine et al. 1988; Sigurdsson et al. 1993).

Sympatho-humoral activation as a result of a MI is thought to arise due to an interruption of the fibres that pass across the affected tissue (De La Cruz Torres et al. 2008), which results in derangement in cardiac neural activity (Malik et al. 1996). In essence, myocardial tissue apical to the site of ischaemia or infarction, but not otherwise involved in the process, may lose normal innervation because the nerve fibres serving that tissue travel through ischaemic or infarct tissue located more basally. Thus, myocardial injury, either functional and transient or anatomical and permanent could disrupt autonomic neural transmission. Further, necrosis of infarct cardiac tissue and the ensuing non-contracting segments may change the geometry of cardiac muscle contraction, which in turn may initiate an abnormal firing of sympathetic fibres due to mechanical deformation of the sensory endings (Brown and Malliani 1971; Malliani 1982; Malliani et al. 1973). Indeed, the anatomical region of myocardial injury has proved to reflexively respond differently, characteristically with inferoposterior MI resulting in bradycardia and hypotension (negative feedback, Bezold-Jarisch effect) and anterior MI more frequently evoking tachycardia and hypertension (positive feedback, Bainbridge reflex) (Thames and Minisi 1989; Webb et al. 1972; Zipes 1990). The markedly increased sympathetic activation, which was first recognised in 1967 (Brown 1967) may debilitate the activity of parasympathetic innervations (vagal fibres) directed

to the sinus node. However, an alternative explanation is that the responsiveness of sinus node cells to neural modulations is reduced following a MI (Malik et al. 1996).

Non-invasive quantification of autonomic modulation using spectral analysis of HRV in patients surviving a MI demonstrated a reduction in total and individual power of spectral components (Bigger et al. 1991), which is associated with increased morbidity and mortality. When the power of sympathetic and parasympathetic frequencies were calculated in normalised units an increased low frequency (sympathetic modulation) component and diminished high frequency (parasympathetic modulation) component was observed during resting conditions and over 24-hour electrocardiography (ECG) recordings (Lombardi et al. 1992; Lombardi et al. 1987). These results indicate sympathetic predominance and dysregulation of autonomic cardiac control. In addition, research reported a significant association with a reduced HRV and increased rates of mortality, where patients with a low HRV had a 3.4-fold increased risk of death compared to patients with higher HRV (Kleiger et al. 1987). In addition, invasive methods of determining adrenergic drive post MI demonstrated augmented sympathetic activity through measuring MSNA (Hogarth et al. 2009) and plasma catecholamine levels (Karlsberg et al. 1981; McAlpine et al. 1988; Sigurdsson et al. 1993).

Baroreceptor reflex sensitivity (BRS) is a marker of the capability of the autonomic nervous system to reflexively increase parasympathetic activity and simultaneously reduce sympathetic drive in response to a sudden increase in blood pressure (La Rovere et al. 1995). When reduced, the BRS is significantly associated with an increased risk of

death following a MI (Farrell et al. 1992; La Rovere et al. 1988) and later research supported the fact that when measured together, a reduced HRV and BRS further increases the risk of mortality in patients following a MI (La Rovere et al. 1998).

Although HRV is reduced and MSNA and plasma catecholamine levels are elevated following a MI, over a period of between 6 and 12-months HRV has been shown to improve in this population, but still remains lower than in healthy individuals (De La Cruz Torres et al. 2008) and MSNA and plasma catecholamine levels have been found to return to the level of matched control groups (Hogarth et al. 2009). The pathophysiological consequences of cardiac tissue infarction powerfully influences the activity of the ANS (Zuanetti et al. 1996), and therefore those patients who are at an increased risk of life threatening arrhythmias (Billman et al. 1982; Farrell et al. 1991a; Schwartz et al. 1988). Importantly, the consequences may be anatomically determined (Figure 2.10) and results from previous research suggest that invasive and non-invasive measures of autonomic modulation may be useful for risk evaluation of post-MI patients (Bigger et al. 1992a, b; Farrell et al. 1991a; Hogarth et al. 2009; Kleiger et al. 1987).

2.11.2: CHRONIC HEART FAILURE

Chronic heart failure (CHF) is a complex condition (McKee et al. 1971), which is difficult to manage in clinical practice, with mortality rates exceeding 10% in patients with mild to moderate CHF (Carson et al. 1996; CONSENSUS-Trial 1987; Nolan et al. 1998; Rector and Cohn 1994; SOLVD-Investigators 1991) despite therapies that

improve prognosis (CONSENSUS-Trial 1987; SOLVD-Investigators 1991). Although only a small number of the overall population of patients diagnosed with CHF, mortality rates of more than 40% exist in New York Heart Association (NYHA) functional classification IV patients (Cohn et al. 1993; Johnson et al. 1993; Nolan et al. 1998).

Major abnormalities of autonomic cardiovascular control mechanisms are associated with CHF (Hoyer et al. 2008; La Rovere et al. 2003; Nolan et al. 1998), with signs of sympathetic hyperactivity (Cohn et al. 1984; Floras 1993) and parasympathetic withdrawal (Binkley et al. 1991b; Eckberg et al. 1971; Floras 1993). This transition in sympathovagal neural control may play an important role in both predicting survival of CHF patients (Stein et al. 1995) and the pathophysiology of cardiac death (Nolan et al. 1998). Indeed, it has been suggested that CHF should be viewed as a neurohormonal model, in which CHF progresses as a result of the over expression of biologically active molecules, such as cytokines that are capable of exerting deleterious effects on the heart and circulation (Mann and Bristow 2005) (figure 2.11).

The most relevant clinical predictors of CHF patient outcome are their NYHA functional classification, left ventricular ejection fraction (LVEF), systolic arterial pressure (sBP), and peak maximal oxygen uptake ($\dot{V}O_{2peak}$) (Aaronson et al. 1997; La Rovere et al. 2003). These factors largely reflect the pathophysiologically reduced performance of the myocardium. Therefore, under such pathological conditions, a compensatory remodelling of autonomic and neurohumoral cardiovascular control mechanism is necessary for maintaining homeostasis.

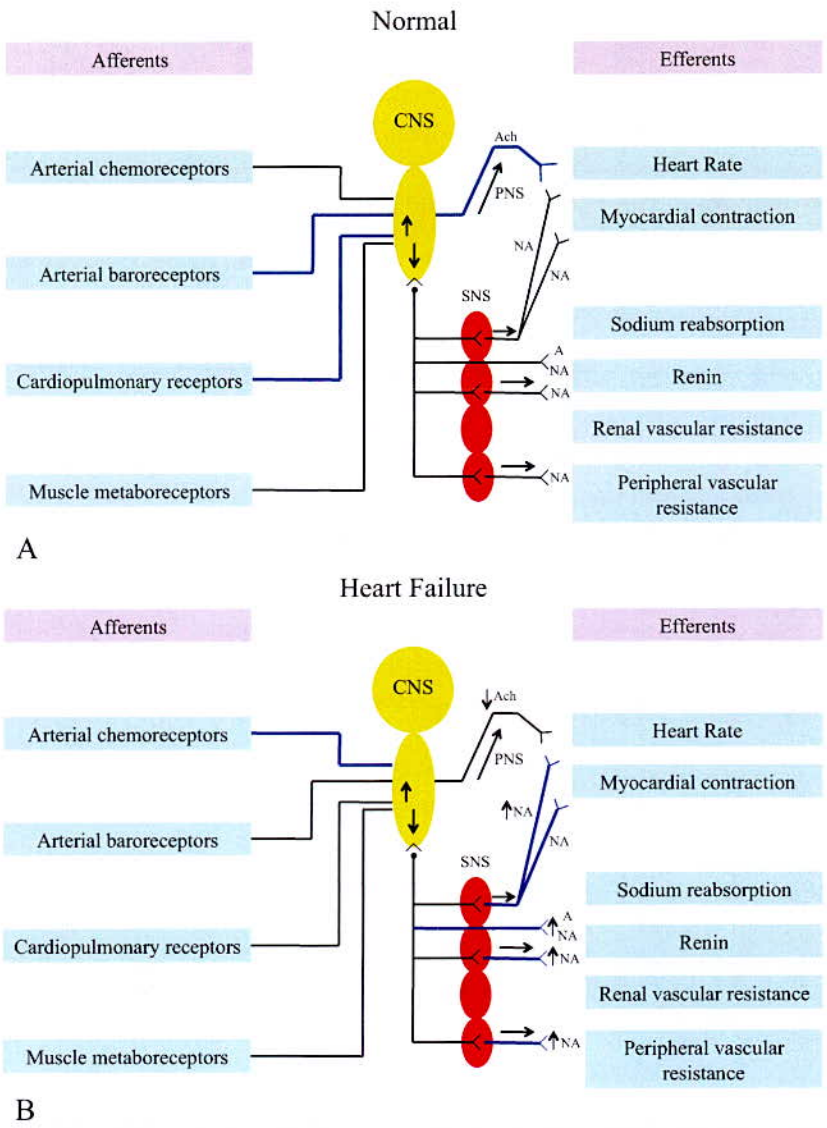

Figure 2.11: Mechanisms for generalised sympathetic activation and parasympathetic withdrawal in heart failure (HF). **A**, Under normal conditions, input from arterial and cardiopulmonary baroreceptor afferent nerves (blue thick line) restrain sympathetic outflow. Parasympathetic control of heart rate is also under arterial baroreflex control. Efferent sympathetic traffic and arterial catecholamine's are low, and heart rate variability is high. **B**, As HF progresses, inhibitory input from arterial and cardiopulmonary receptors decreases and excitatory input increases (blue thick line). The net response to this altered balance includes a generalised increase in sympathetic nerve traffic, blunted parasympathetic and sympathetic control of heart rate, and impairment of the reflex sympathetic regulation of vascular resistance. Note: PNS = Parasympathetic nervous system; SNS = Sympathetic nervous system; Ach = acetylcholine; CNS = central nervous system; A = adrenaline; Na+ = sodium; NA = noradrenaline. Taken from Libby et al. (2008).

The dysautonomia associated with CHF, which is often interpreted as essential in order to maintain haemodynamic output (Gaffney and Braunwald 1963; Guzzetti et al. 1995) contributes to disease progression and is associated with poor prognosis (Cohn et al. 1984; Packer 1992; Yamada et al. 2003). Historically, the first evidence that sympathetic activity is enhanced in CHF was based on the findings that urinary catecholamine's and their metabolites are increased in NYHA functional classification III and IV patients (Braunwald 1984). A further demonstration and one that has become the best predictor of clinical status, prognosis, and severity of disease in patients with CHF is serum noradrenaline (NA) levels (Cohn et al. 1984; Packer 1988). In addition, the application of isotope dilution methods for measuring cardiac noradrenaline release to plasma demonstrated that in untreated heart failure patient's cardiac noradrenaline spill over is increased as much as 50-fold, which is similar to levels of release seen in the healthy heart during near maximal exercise (Hasking et al. 1986).

Non-invasive assessment and quantification of autonomic dysfunction through analysis of HRV may provide additional important prognostic value for patients with CHF (Nolan et al. 1998; Stein et al. 1995), since it has been shown to predict clinical outcome in such patient groups (Bonaduce et al. 1999; Brouwer et al. 1996; Fauchier et al. 1999; Nolan et al. 1998; Ponikowski et al. 1997). Power spectral analysis (PSA) of HRV may provide clinicians with valuable information regarding deterioration of disease. Indeed, previous research demonstrated that the higher the sympathetic activity the lower the HRV in patients with CHF (van de Borne et al. 1997). These methods of autonomic assessment each have their strengths and limitations; however, they could be

implemented to help guide the intervention required to preserve and/or slow disease progression in order to maintain functional capacity. Such interventions include pharmacological optimisation and exercise training, which have both been shown to modulate the ANS, by reducing sympathetic drive and increasing parasympathetic modulation (Malfatto et al. 2002; Packer 1992).

2.11.3: HYPERTENSION

Scientific research has provided considerable evidence, which supports the suggestion that the ANS plays an important role in both blood pressure (BP) regulation and in the development of hypertension (Julius 1991; Singh et al. 1998). Hypertension increases further risks of cardiovascular morbidity and mortality (Lantelme et al. 2002; MacMahon et al. 1990; Petersen 2005) and affects approximately 34% and 30% of men and women respectively, in England (Petersen 2005). Meta-analysis of prospective data has demonstrated that an increase of 20 mmHg and 10 mmHg in systolic (sBP) and diastolic (dBP) BP respectively over normotensive BP levels, doubles the risk of death from coronary heart disease (CHD) (Lewington et al. 2002). Furthermore, individuals with a history of hypertension are at almost twice the risk of suffering a MI (Yusuf et al. 2004). These risk are associated with the development of atherosclerosis (figure 2.12).

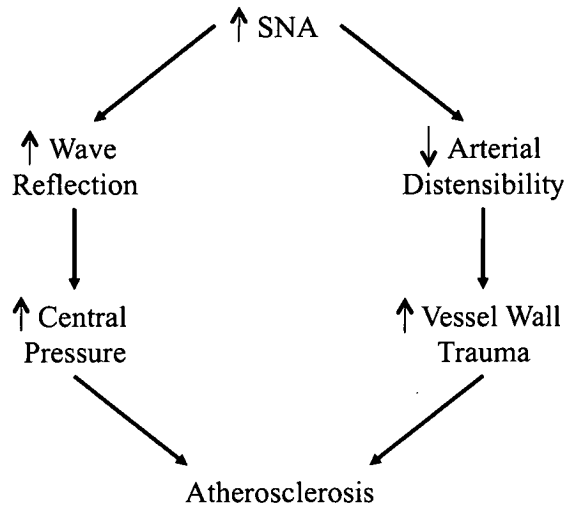

Figure 2.12: Mechanisms responsible for the atherogenic effects of an increase in sympathetic cardiovascular drive. Note: SNA = Sympathetic nerve activity. Taken from Grassi and Mancia (2003).

Autonomic dysfunction has been demonstrated previously in patients with systemic hypertension, where an increased sympathetic drive combined with decreased levels of parasympathetic modulation is described (Chakko et al. 1993; Guzzetti et al. 1988; Huikuri et al. 1996; Julius 1991; Liao et al. 1996; Takalo et al. 1994; Tsuji et al. 1996b) signifying altered efferent neural modulation. In addition, sympathetic hyper-activity has been demonstrated in early hypertension (Julius 1991), which highlights the significant effects early disease has on the ANS.

Although adrenergic drive was initially thought to represent a compensatory adjustment to support the functioning of a mechanically overloaded heart, enhanced sympathetic drive is now perceived as an inappropriate response, which is thought to both initiate and maintain the elevated blood pressure levels and additionally contribute to adverse cardiovascular events (Grassi and Esler 1999). The adverse cardiovascular effects and

functional deterioration of cardiac dynamics exerted from this unfavourable adrenergic drive range from compensated left ventricular hypertrophy (LVH), to left ventricular (LV) dysfunction, heart failure and eventually death (Perlini et al. 2006).

Measurement of nerve firing in postganglionic sympathetic efferent's directed to the skeletal muscle vasculature with microneurography and regional rates of noradrenaline spill over using isotope dilution techniques demonstrates activation of the sympathetic nerves of the heart, kidneys, and skeletal muscle vasculature. In addition, spectral analysis of heart rate (HR) fluctuations demonstrated that a reduced HRV is present in patients with systemic hypertension, and among normotensive patients, a lower HRV was associated with greater risk of developing hypertension (Singh et al. 1998). This is consistent with the findings that dysautonomia is present in the early stage of hypertension (Julius 1991; Singh et al. 1998) and it is thought that HRV analysis may provide clarification of the pathogenesis of systemic hypertension and the role of the ANS (Bootsma et al. 1994; Malik et al. 1996; Malik and Camm 1993; Singh et al. 1998).

Sympathetic over activity seems to particularly influence sBP by increasing the rate of left ventricular ejection, reducing aortic compliance through increased neural vascular tone, and via arteriolar vasoconstriction (Esler 2003). Sympathetic mediated vasoconstriction in skeletal muscle vascular beds, which reduces glucose delivery to muscle may be a basis for insulin resistance and hyperinsulinaemia (Bray et al. 1989), and the high renal sympathetic tone contributes to the development of hypertension by

stimulating renin secretion and through promoting renal tubular reabsorbtion of sodium (Esler 2003). Furthermore, research has detailed that hypertension can impair the cardiac baroreflex (Siche et al. 1995) and cause vascular alterations (Lantelme et al. 1994), which will impact on short term BP control (Lantelme et al. 2002) and increase cardiovascular risk factors further.

2.12: DIABETIC AUTONOMIC NEUROPATHY

Diabetic autonomic neuropathy is a heterogeneous disorder (Boulton et al. 2005), which affects different parts of the nervous system and therefore organ systems, such as the cardiovascular system and gastrointestinal tract (Vinik et al. 2003b) and presents with diverse clinical symptoms (Boulton et al. 2005).

Diabetic autonomic neuropathy (DAN), is a common complication, which is positively related to an unfavourable outcome (Pagkalos et al. 2008) and carries an increased risk of morbidity and mortality (Boulton et al. 2005; Vinik et al. 2003a; Ziegler 1994, 2001). Diabetic autonomic neuropathy increases with age (diabetes duration) and affects between 28.5-73% of all diabetic patients (Bellavere 1995; Dyck et al. 1993; Low et al. 2004; Young et al. 1993) with approximately 20% presenting symptoms (Dyck et al. 1993). Furthermore, DAN is rarely recognised and poorly understood, which in part may be attributed to asymptomatic autonomic neuropathy and without examination may go undetected (Hurwitz et al. 1994). These unsuspecting clinical features significantly impacts upon survival and quality of life in patients with diabetes (Bellavere 1995;

Vinik and Erbas 2001; Vinik et al. 2003b) as well as contributes to an increased cost (Vinik et al. 2003a) of caring for the approximate 2.5 million adult diabetic patients in the UK (Petersen 2005).

Cardiovascular autonomic neuropathy (CAN) is the most researched and clinically important form of DAN (Boulton et al. 2005), which is frequently overlooked (Maser et al. 2003; Maser 2000; Vinik et al. 2003b) and has prevalence rates of between 7.7-90% in diabetic patients (Vinik et al. 2003b). However, the heterogeneous methodology makes it difficult to compare CAN epidemiology across different research studies (Vinik and Ziegler 2007). Cardiovascular autonomic neuropathy is a frequent degenerative complication in diabetes and is associated with an increased risk of cardiovascular disease (Balcioğlu et al. 2007; Chanudet et al. 2007; Pagkalos et al. 2008). Indeed, diabetic patients with impaired autonomic function had approximately double the risk of mortality when compared to non-diabetic patients (Gerritsen et al. 2001).

Cardiovascular autonomic neuropathy results from damage to the autonomic nerve fibres that innervate the myocardium and vasculature, which causes abnormalities in HR control and central and peripheral blood vessel dynamics (Manzella and Paolisso 2005; Maser and Lenhard 2005; Maser et al. 2003; Vinik and Ziegler 2007). The metabolic disorders of diabetes lead to diffuse and widespread damage of peripheral nerves and small vessels (Vinik et al. 2003b). Damage to small myelinated and unmyelinated nerve fibres is manifested by impairment of vagally controlled HRV with diminished

peripheral sympathetic tone that leads to increased blood flow together with reduced thermal and pain sensation, which gives rise to DAN (Watkins 1999; Watkins and Thomas 1998). Small nerve fibre damage can occur selectively or together with impairment of other sensory modalities due to the loss of large nerve fibres (Watkins and Thomas 1998). Indeed, the ubiquitous distribution of the ANS and the fact that the vagus nerve is the longest of the ANS (accounting for approximately 75% of all parasympathetic activity), typically renders almost all organs susceptible to dysautonomia and results in DAN to be a potential system wide disorder (Vinik et al. 2003b).

Parasympathetic nervous dysfunction causes an above normal resting heart rate (RHR), which is possibly attributed to vagal impairment that results in unopposed sympathetic nervous outflow, with RHR reaching up to 130 $b \cdot min^{-1}$ (Maser and Lenhard 2005; Vinik and Ziegler 2007). Parasympathetic nervous dysfunction occurs earlier than sympathetic nervous dysfunction in CAN (Maser and Lenhard 2005), however, advancement of disease with combined sympathetic and parasympathetic nervous impairment causes a slower HR (Vinik and Ziegler 2007). Advanced dysautonomia causes an apparent fixed HR, which means the determination of HR alone is not a reliable indication of CAN, whereas a reduction in HRV is the earliest indicator (Maser and Lenhard 2005; Vinik and Ziegler 2007).

Research has demonstrated that diabetic patients diagnosed with dysautonomia via analysis of HRV, have increased mortality rates of 20-27% (Ewing et al. 1991; Maser et

al. 2003; Rathmann et al. 1993; Ziegler 1994) compared to 4-5% in diabetic patients with no dysautonomia (Maser et al. 2003; Ziegler 1994), with the rates of sudden death higher in the former group (Ziegler 2001). Indeed, power spectral analysis (PSA) of HRV demonstrated a reduction of both parasympathetic and sympathetic activity and an alteration in the sympathovagal balance in diabetic patients (Bellavere et al. 1992). Furthermore, a recent study demonstrated that diabetic patients showed distinct changes in HR and BP variability (BPV) with reduced BRS, which decreased from minimal to severe according to disease status. In addition, the sensitive methods used to collect and analyse the data highlighted patients with no previous evidence of dysautonomia according to conventional testing, which may represent the early stages of CAN (Ziegler et al. 2001). Previous studies have demonstrated that sympathetic dominance is associated with a higher cardiovascular mortality in diabetic patients, which may be related to cases of sudden death (Ewing et al. 1976; Kleiger et al. 1987; Manzella and Paolisso 2005; Tsuji et al. 1994), despite the absence of documented pre-existing heart disease (Tsuji et al. 1994).

2.13: RENAL AUTONOMIC NEUROPATHY

Renal autonomic neuropathy is a prominent characteristic of renal dysfunction (Rubinger et al. 1999), which involves both the sympathetic and parasympathetic nervous system (Kurata et al. 2000; Oikawa et al. 2008; Rubinger et al. 2004). In addition, research has reported that an altered sympathovagal balance is paralleled with a reduced BRS, which is important in the overall integrity of autonomic control (Lazarus

et al. 1973; Pickering et al. 1972; Robinson and Carr 2002). However, symptoms of RAN are often vague and non-specific (Vita et al. 1990).

Renal autonomic neuropathy (RAN) is a complication that is related to a poor outcome (Hausberg et al. 2002; Oikawa et al. 2008; Zoccali et al. 2002). Patients presenting with chronic kidney disease (CKD) or end stage renal disease (ESRD) are characterised with extreme cardiovascular morbidity and mortality (Coquet et al. 2005; Hausberg et al. 2002; Kotanko 2006; Oikawa et al. 2008; Rostand et al. 1991), which is not completely explained by traditional cardiovascular disease risk factors. Indeed, for patients aged between 15-30 years of age on haemodialysis, the incidence of cardiovascular death is 150 times greater than the general population (Robinson and Carr 2002). In the UK, the prevalence of patients diagnosed with CKD is approximately 5,554 patients per million population (pmp) (Rodriguez-Puyol 1998) and ESRD affects approximately 1,479,000 individuals globally, of which approximately 393,000 in Europe (298,000 in the European Union) (Moeller et al. 2002). Research has demonstrated that moderate-to-severe RAN can be present in up to 63% of patients with renal dysfunction (Vita et al. 1999).

Patients with mild to moderate renal dysfunction or ESRD, display increased levels of sympathetic activity compared with healthy subjects (Blankestijn 2004; Converse et al. 1992; Koomans et al. 2004; Ligtenberg et al. 1999; Neumann et al. 2004), with evidence detailing that the diseased kidneys themselves may be a trigger of sympathetic hyperactivity (Campese 2000; Converse et al. 1992; Ye et al. 1998). Signals arising

from the kidney appear to mediate sympathetic over-activity (Campese and Kogosov 1995; Hausberg et al. 2002) that may be the result of circulating uraemia-related toxins, which are present in renal dysfunction and more pronounced in patients with ESRD regardless of effective dialysis treatment (Recordati et al. 1981; Rubinger et al. 1999). The circulating toxins may produce sustained activation of sympathetic nervous activity through stimulation of renal afferent signals (Hausberg et al. 2002; Weise et al. 1995), which has been shown to be irreversible through long-term dialysis (Agarwal et al. 1991; Vita et al. 1999; Vita et al. 1992). However, as described above the sympathetic over-activity may result from stimuli independent of toxins arising in the diseased kidney (Hausberg et al. 2002). In addition, reports have detailed that lesions may occur in both the afferent and efferent limbs of the ANS in conditions of chronic uraemia (Vita et al. 1999; Weise et al. 1995), which may also contribute to renal dysautonomia.

A raised level of sympathetic nervous activity is now recognised as an important mechanism that contributes to an increased morbidity and mortality (Hausberg et al. 2002; Zoccali et al. 2002; Zoccali et al. 2003) as well as the incidence of sudden cardiac death (SCD) (Boero et al. 2001; Coquet et al. 2005; Ranpuria et al. 2008) in RAN patients. Indeed, research has stated that high levels of circulating catecholamine's adjunct with sympathetic nervous hyperactivity renders patients with renal dysfunction vulnerable to a series of severe cardiovascular complications, ranging from left ventricular hypertrophy (LVH), arterial remodelling, atherosclerosis and arrhythmias (Mancia et al. 1999; Orth et al. 2001; Rozanski et al. 1999; Zoccali et al. 2002). These combined diseases culminate with a 92% excess risk of cardiovascular complications

(Zoccali et al. 2002), even in the absence of pre-diagnosed CVD (Rubinger et al. 2004; Rubinger et al. 1999).

The presence and severity of RAN does not appear to be associated with either the duration of renal dysfunction or dialysis treatment (Vita et al. 1999). Therefore, the ability to use sensitive, non-invasive, and reproducible equipment that has the ability to rapidly evaluate autonomic nervous control in patients with renal dysfunction is advantageous for early intervention and prevention of premature morbidity and mortality.

2.14: SUMMARY AND RATIONALE

Neural regulation of the cardiovascular system is highly integrated and complex. Cardio-dynamic function is monitored and modified on a beat-to-beat basis through a coordinated and sophisticated interplay in the neural hierarchy. This neural hierarchy provides precise modulation or fine-tuning of the cardiovascular system, matching the rate and force of myocardial work as well as vascular dynamics to that of systemic demand and therefore maintaining bodily homeostasis. However, as has been demonstrated within this review of literature, dysfunction or imbalance of the synergistic interactions of cardiac neural modulation is associated with increased risk of cardiovascular disease.

Autonomic dysfunction, characteristically coupled with raised sympathetic activity is now recognised as an important mechanism involved in cardiovascular complications in humans. There is consistent empirical research, which demonstrates that elevated sympathetic tone measured invasively and non-invasively is associated with and predicts mortality in a variety of diseases of the cardiovascular system, such as myocardial infarction, chronic heart failure, and hypertension as well as metabolic diseases, such as chronic kidney disease and diabetes. The measurement of cardiac autonomic modulation in diseases associated with high cardiovascular disease risk may help to refine the prognosis and may be useful for patient stratification in intervention studies aimed at reducing cardiovascular complications.

2.15: HYPOTHESIS AND AIM OF WORK UNDERTAKEN

The central hypothesis of this thesis is that autonomic function measured indirectly and non-invasively by heart rate variability is significantly attenuated in disease states characterised by high compared to low cardiovascular disease risk. The forthcoming chapters involve testing this hypothesis in different cardiovascular disease groups, namely systolic and diastolic heart failure, diabetes and chronic kidney disease, hypertension, and ischaemic heart disease. These groups were characterised according to demographic, echocardiographic, and haematological parameters.

This thesis reports its findings on a population of unselected patients with well-defined cardiovascular disease risk that were referred on a clinical basis for a dobutamine stress

echocardiogram in a district general hospital. All procedures documented were performed on all patients recruited and for this reason a general methods (Chapter 3) and population chapter (Chapter 4) was constructed to provide the reader with a detailed breakdown of the common methodology and patient population studied. Each empirical study is constructed with separate aims, hypothesis, and objectives, which are related to the central hypothesis.

CHAPTER 3: GENERAL METHODS

3.1: RESEARCH DESIGN AND PROCEDURE

The research within this thesis was conducted on human subjects, and as such appropriate ethical procedures were completed in full before recruitment commenced (see section 3.2.1: Study Population). The populations were recruited from a clinical setting and important decisions were taken and procedures were performed by clinicians, for example patient assessment, referral, and interpretation of echocardiography results. Data collected and interpreted by clinicians has been used within this thesis and acknowledgement is made accordingly (see figure 3.1 and 3.2). The patients selected for study were those referred for a dobutamine stress echocardiogram (DSE). This enabled the study of heart rate variability (HRV), an index of autonomic function, at rest and during a functional stress test. Importantly due to the methodological requirement of stationary recording for accurate HRV acquisition, the DSE procedure was ideal since the patient's adopt a supine position and are stationary throughout the period of data acquisition. A further advantage is the reduced noise observed that would otherwise be heightened by skeletal muscle contraction during exercise. In addition, the DSE procedure provided a reproducible haemodynamic workload.

All patients studied were referred on clinical grounds from Ealing Hospital National Health Service (NHS) trust's (EHT) chest pain clinic with atypical chest pain. The PhD

candidate met each patient on the day of his or her DSE and recruited him or her for the study. This procedure included providing the patient with an information sheet, answering any questions or concerns regarding the test and research and obtaining their signed consent to participate in the study. Physical characteristics and demographic data, including drug history were collected (from case notes and via a brief interview) from each patient by the PhD candidate. Cannulation and haematological sampling as well as all application, analysis, and storage of HRV and haemodynamic parameters were performed by the PhD candidate. All echocardiography acquisition and data analysis was performed by Dr Rajan Sharma (Consultant Cardiologist and Director of Studies) who was blinded to all other data. Personnel from the Pathology Department at EHT performed all haematological analyses using validated assays. Chapter design was undertaken by the PhD candidate with advice from the supervisory team (figure 3.1 and 3.2).

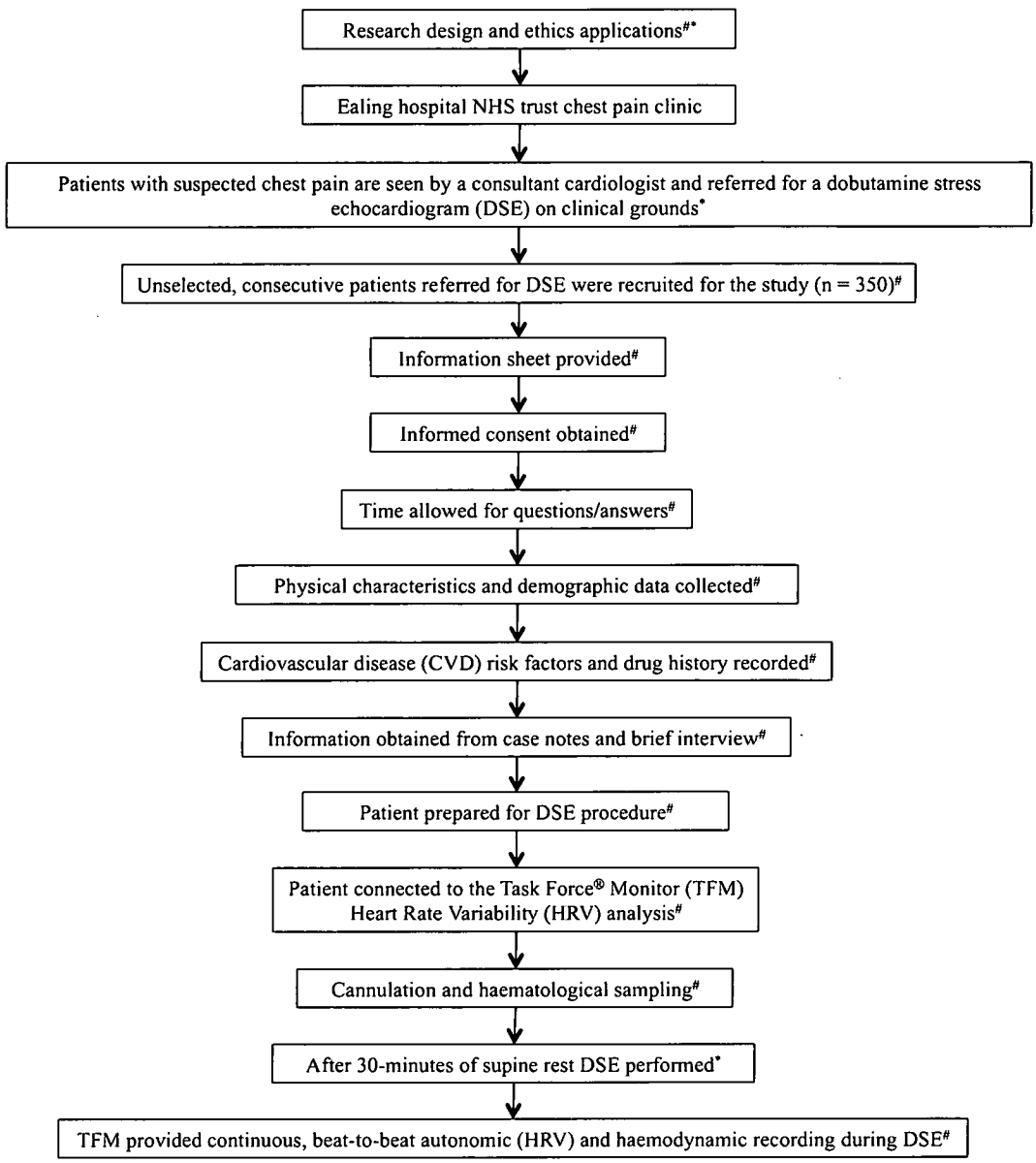

Figure 3.1: A flow diagram to illustrate patient recruitment and study protocol. Note: # = procedures performed by Jamie O'Driscoll (JO'D) (Ph.D candidate); * = procedures performed by Dr Rajan Sharma (RSH) (director of studies); #* = procedures performed by both JO'D and RSH.

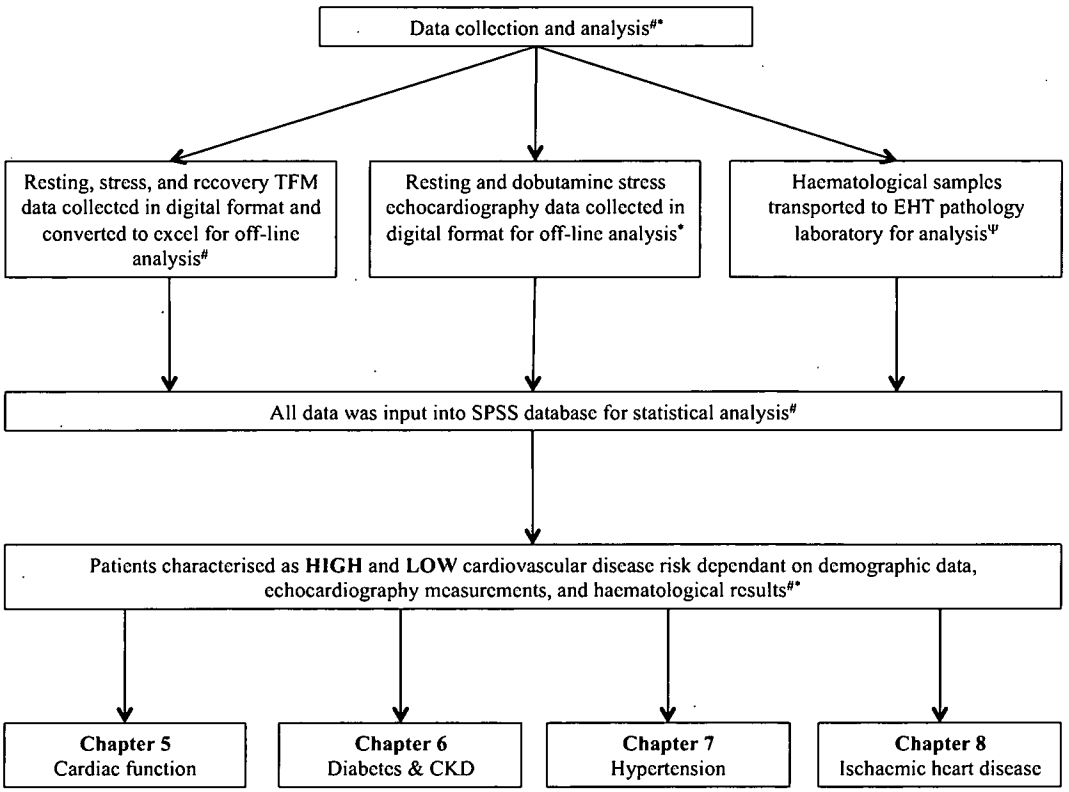

Figure 3.2: A flow diagram to illustrate data collection, analysis, and Ph.D thesis construction. Note: # = procedures performed by Jamie O'Driscoll (JO'D) (Ph.D candidate); * = procedures performed by Dr Rajan Sharma (RSH) (director of studies); #* = procedures performed by both JO'D and RSH; TFM = Task Force® Monitor; EHT = Ealing hospital NHS Trust; Ψ = procedures performed by Ealing hospital NHS trust's pathology laboratory; CKD = Chronic kidney disease; RSH was blinded to patient demographic, haematological, and TFM data; JO'D was blinded to echocardiography data.

3.2: DEMOGRAPHIC DATA

3.2.1: STUDY POPULATION

The study population was composed of a series of consecutive patients who were physician referred for a DSE between October 2005 and September 2008 to EHT's cardiology department. The sample size, including exclusions, was 350. Exclusion criteria included age < 18 years, patients unable to consent, unstable angina, severe aortic stenosis, inaccurate data consistent with excessive noise, patients in atrial fibrillation, patients who develop ectopic heart beats, patients who require atropine to achieve target heart rate, poor echocardiographic image quality, and hospital inpatients. Due to incomprehensible autonomic trace recordings, poor image quality, and/or the need to use atropine to achieve target heart rate, 36 patient recordings were excluded from the final data analysis, leaving a sample size of 314. Thames Valley University, Ealing Hospital NHS Trust, and the National Research Ethics Service (Ealing and West London Research Ethics Committee) approved the study protocol and all patients provided signed consent before testing.

3.2.2: CLINICAL PARAMETERS ASSESSED

Clinical characteristics and indications for testing were obtained from direct communication with each patient, hospital records, request forms, and Ealing NHS trust hospitals electronic database. Parameters assessed included:

- Age, gender, diabetes (including type), renal dysfunction, hypertension, hypercholesterolaemia, positive family history of cardiovascular disease, past history of ischaemic heart disease or heart failure, previous coronary interventions, and evidence of valvular disease.
- All medication documented.
- Presence of cardiac symptoms.
- Haematological parameters.

3.3: NON-INVASIVE AUTONOMIC ASSESSMENT

The Task Force® Monitor (TFM) (CNSystems, Graz, Austria) is a recently developed and well-validated commercially available non-invasive monitoring system for evaluation of the cardiac autonomic nervous system (ANS) (figure 3.3). In the present thesis the TFM was used for the continuous non-invasive beat-to-beat monitoring and automatic online calculation of all cardiovascular haemodynamic and HRV parameters (Fortin 1998; Valipour et al. 2005).

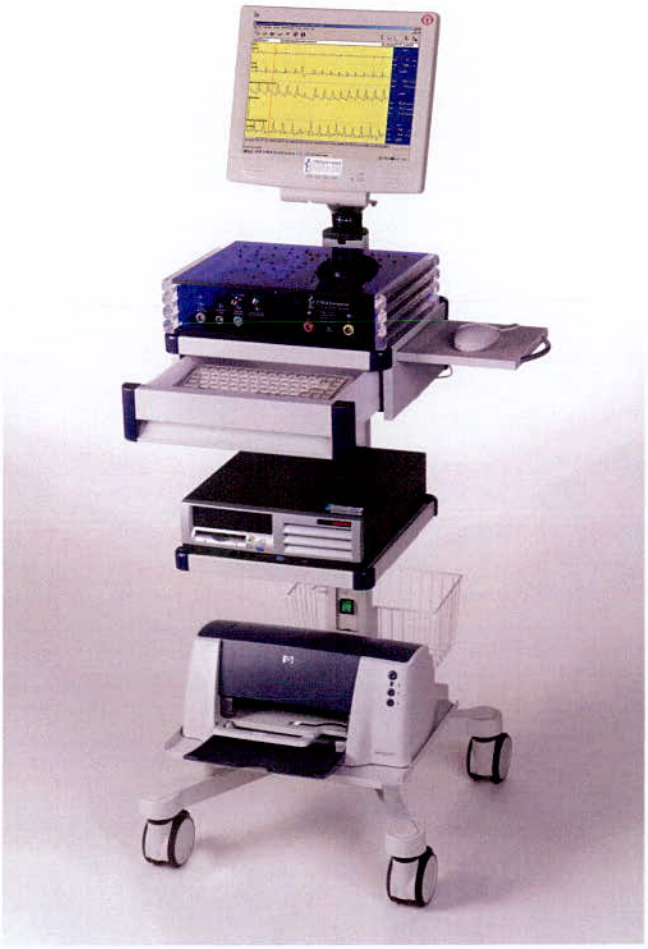

Figure 3.3: A picture of the Task Force® Monitor used to record autonomic and haemodynamic parameters.

The TFM enables the continuous measurement of blood pressure (contBP) by use of the vascular unloading technique (Fortin 1998; Gratze et al. 1998), and beat-to-beat stroke volume (SV) measurement with impedance cardiography (ICG) (Fortin 1998; Gratze et al. 1998; Kubicek et al. 1966; Sramek 1983b). Continuous blood pressure is automatically corrected to oscillometric blood pressure (oscBP) values obtained at the brachial artery of the contralateral arm. A 6-channel ECG is included for R-R interval

determination (Fortin 2001; Valipour et al. 2005) and the beat-to-beat values are used for the real-time calculation of HRV by an autoregressive model (Bianchi 1997; Fortin 2001; Schloegl 1997) and are displayed as 3-dimensional sliding power spectra (Fortin 2001). In addition, baroreceptor reflex sensitivity (BRS) is automatically evaluated via the sequence method (Parati 1992) and displayed on-line. The TFM meets the requirements of the CE mark (CE 0408, TUeV Austria, Vienna) and the Food and Drug Administration (FDA) clearance 510(k) (n°:K014063).

3.3.1: CONTINUOUS BLOOD PRESSURE MONITORING

Carefully measuring BP with a sphygmomanometer is sufficient for routine clinical evaluation and multiple measures can provide an adequate assessment of the typical BP response for an individual e.g. from supine to standing (Wieling 1999). However, this conventional method of BP assessment is not suitable for evaluation of conditions with sudden transient changes in the circulation (beat-to-beat fluctuations in arterial pressure) and therefore for the evaluation of patients with disturbances in cardiovascular control mechanisms (Benditt et al. 1996; Low 1996).

Intra-arterial measurements are not used routinely in cardiovascular laboratories due to potential complications, such as bleeds (Hirshberg et al. 1989) and intravascular instrumentation has the disadvantage of affecting autonomic tone (Harms et al. 1999; Stevens 1966; Wieling 1999). The TFM measures conBP at the proximal limb of the index or middle finger by an improved version of the vascular unloading principle

(Hirschl et al. 1996; Parati et al. 1989; Parati et al. 2003; Peñáz et al. 1976; Schmidt et al. 1992; van Egmond et al. 1985). It is known that the arteries located in the fingers are responsible for thermoregulation and are therefore susceptible to vasoconstriction and vasodilation according to environmental temperature and the volume state of the subject. Therefore, the pressure measured in the finger arteries may or may not correspond to the pressure in the large arteries. For this reason, the contBP is automatically corrected to oscBP values obtained at the brachial artery of the contralateral arm, thereby resulting in true arterial BP values as opposed to finger arterial pressure (Fortin 1998; Fortin et al. 2006b; Habenbacher 2002) (figure 3.4).

The contBP device of the TFM has been systematically tested against both the Finapres™ and intra-arterial BP monitoring. The results were comparable between all methods of contBP measurement during rest as well as during the process of autonomic function testing. The systematic comparison highlighted the advantage of the TFM's non-interrupted BP recording compared to the Finapres™, which requires recalibration during testing (Fortin 1998; Fortin et al. 2006b; Habenbacher 2002). In addition, the oscillometric blood pressure device used on the TFM has been evaluated against the protocol of the American national standard for electronic or automated sphygmomanometers (ANSI AAMI SP10-1992) and the quality mark for the German hypertension league (Fortin 2001) as well as other validated blood pressure devices such as the Dinamap® blood pressure monitor (Fortin 2001).

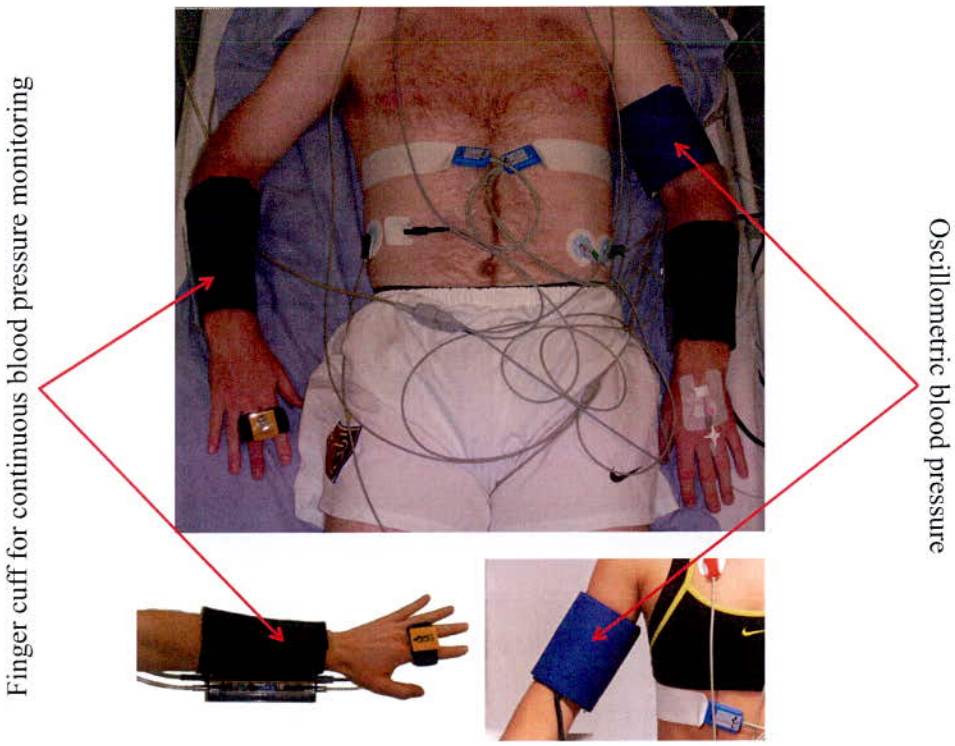

Figure 3.4: A picture of the finger cuff for beat-to-beat blood pressure monitoring and oscillometric blood pressure cuff (contralateral arm).

3.3.2: IMPEDANCE CARDIOGRAPHY

Impedance cardiography works by measuring changes in thoracic electrical bioimpedance (TEB) over changes in time in relation to the cardiac cycle (Fortin et al. 2006a; Ventura 2000), which enables SV, CO, and thoracic fluid content to be determined (Drazner et al. 2002; Harms et al. 1999). Traditionally, beat-to-beat CO is measured invasively via the thermodilution (TD) method, which is considered the gold standard (Fegler 1954; Fortin et al. 2006a; Swan et al. 1970), despite non-invasive measures of CO being commercially available (Drazner et al. 2002; Harms et al. 1999). The TD method (also known as the Swan-Ganz catheter) is expensive, requires trained

personnel, and can only be performed in a hospital intensive care unit or catheterisation laboratory and is therefore not available in the outpatient setting (Parrott et al. 2004; Weiss et al. 2003). In addition, the TD method of CO assessment has inherent risks associated with the procedure, which include infection sepsis, arrhythmias, and increased morbidity and mortality (Connors et al. 1996; Guyatt 1991; Mermel et al. 1991).

An estimate of real-time continuous beat-to-beat stroke volume (SV) was obtained using an improved method of transthoracic impedance cardiography (ICG) (Fortin 1998; Fortin et al. 2006a; Gratze et al. 1998). Impedance cardiography was first advanced by Nyboer et al. (1940) who by using the resistivity of blood (p) and the length (L) of the chest, established the relationship of impedance change (ΔZ) and base impedance (Z_0) to the volume change (ΔV) of the tissue under measurement as described below in figure 3.5.

$$\Delta V = p \frac{L^2}{Z_0^2} \Delta Z$$

Figure 3.5: First non-invasive method of calculating cardiac output and systemic vascular resistance using impedance cardiography.

Further research later developed the Minnesota Impedance Cardiograph by modelling the thorax as a cylinder (Kubicek et al. 1966), which incorporated the maximum value of the first derivative of the impedance waveform (dZ/dt_{max}) and left ventricular ejection time (LVET) and therefore developed a new equation for calculating SV as detailed in figure 3.6.

$$SV = p \frac{L^2}{Z_0^2} \left(\frac{dZ}{dt} \right)_{max} LVET$$

Figure 3.6: New impedance cardiography equation for stroke volume that incorporates the impedance waveform and left ventricular ejection time.

Development of a less cumbersome ICG device together with a new formula for calculating SV was developed in the 1980's (Sramek 1983a). The new method substituted the cylindrical model of the chest (Kubicek et al. 1966) with that of a truncated cone. At the same time, through direct measurement of normal adult volunteers and analysis of chest x-rays, the circumference (C) of the lower part of the thorax at the level of the xiphoid was measured at approximately three times the measured distance (L) between the electrodes (Sramek 1983a), which was used to estimate the electrical participating volume of the thorax (V_{th}). However, this method overestimated SV and subsequent research reported that L was approximately 17% of body height (H) (Sramek 1983a), which yielded the following equation, with the factor that calculates V_{th} highlighted in blue (figure 3.7).

$$SV = \left(\frac{(0.17\,H)^3}{4.2} \right) \frac{\left(\frac{dZ}{dt} \right)_{max}}{Z_0} LVET$$

Figure 3.7: Impedance cardiography calculation of stroke volume modelling the chest as a truncated cone.

Later, researchers modified the equation further by introducing the term δ (the actual body weight divided by the ideal weight), which accounted for deviations from ideal body weight to more accurately determine the volume of the thorax (Bernstein 1986) as follows (figure 3.8).

$$SV = \delta \times \left(\frac{(0.17\,H)^3}{4.2} \right) \frac{\left(\frac{dZ}{dt} \right)_{max}}{Z_0} \quad LVET$$

Figure 3.8: Modification of the equation in figure 3.7 introducing actual weight divided by ideal weight to more accurately determine the volume of the thorax.

The shape of the human thorax changes according to weight, such that an obese individual will have a more frustum shaped thorax and lean individuals will have a more cylindrical thorax (Fortin et al. 2006a). Each individual's body composition or classification of lean, normal, or obese can be estimated by the individual's body mass index (BMI) (WHO 1997; Xavier Pi-Sunyer 1998). For this reason the TFM incorporates the calculation of each individual's BMI in order to more accurately quantify SV measurements. Body mass index is defined as weight (kg) divided by the height (m^2), and the TFM's software therefore incorporates the following equation (Fortin et al. 2006a) (figure 3.9).

$$SV = C_1 \cdot \frac{\left(\frac{W}{H^2}\right)^n}{Z_0^m} \cdot H^3 \cdot LVET \cdot \frac{\left(\frac{dZ}{dt}\right)_{max}}{Z_0} = C^1 \cdot \frac{W^n \cdot H^{3-2n}}{Z_0^{m+1}} \cdot LVET \cdot \frac{\left(\frac{dZ}{dt}\right)_{max}}{Z_0}$$

Figure 3.9: Task Force® Monitors equation for calculating stroke volume.

The equation described above (figure 3.9) enables the TFM to estimate changes in blood volume in the aorta and changes in fluid volume in the thorax (Fortin et al. 2006a) and thus utilize ICG methodology. The online and continuous quantification of left ventricular SV is calculated by measuring the maximum rate of thoracic electrical impedance during ventricular ejection, dividing it by the base impedance, and multiplied by the left ventricular ejection time and a volume constant of the chest, which is determined by the individual's age, height, weight, and body surface area (Fortin et al. 2006a; Thomsen 1979; Valipour et al. 2005). Indeed, the reproducibility of CO recordings from the TFM were comparable to the TD method, in addition to being non-invasive, patient-friendly, and risk free (Fortin et al. 2006a).

Traditionally, the electrodes used to measure ICG are either two ring-electrodes consisting of either silver or aluminium, which are wrapped around the body or ECG spot electrodes (Fortin et al. 2006a). The electrodes are placed on the neck and below the thorax in order to create a non-invasive uniform high frequency alternating current field within the thorax as needed for ICG measurement (Fortin et al. 2006a). The two methods of electrode placement described above have disadvantages; firstly, for the ring-electrodes the individuals body has to be manoeuvred to apply them, which can be

dangerous if they have inner or outer wounds, secondly, due to moving the subject, the electrodes could easily loose contact with the skin and thirdly, the patients breathing may be obstructed or made difficult due to the electrodes. For the spot electrodes, the major disadvantage is the low reproducibility, since the locations of the electrodes are rarely ever identical in consecutive measurements (Fortin et al. 2006a). The TFM uses newly designed electrodes, which consist of two electrode bands manufactured at predetermined distances and set onto an adhesive strip. For ICG measurements, three such electrodes are used; one applied to the nape of the neck and two others placed on the thorax in line with the xiphoid process (Fortin et al. 2006a). This method generates a homogenous field within the thorax since the electrodes cover a large area, with limited patient disturbance and due to the electrode design there is high reproducibility and therefore superior comparability of consecutive measurements compared to other ICG methods (Fortin et al. 2006a). Furthermore, the TFM's ICG measurements have high reproducibility and have a strong correlation with the TD method of recording haemodynamic parameters (Alber 2003; Drazner et al. 2002; Greenberg et al. 2000; Parrott et al. 2004). The measurement current used for ICG is low and safe for all subjects. Indeed, the TFM uses a lower measurement current (400 μA) than other available non-invasive ICG devices, namely BioZ (4 mA) and meets the standard EN 60601-1 for class CF devices (Fortin et al. 2006a). Figure 3.10 illustrates the placement of the three TFM electrodes; one applied to the nape of the neck and two others placed on the thorax in line with the xiphoid process (Fortin et al. 2006a).

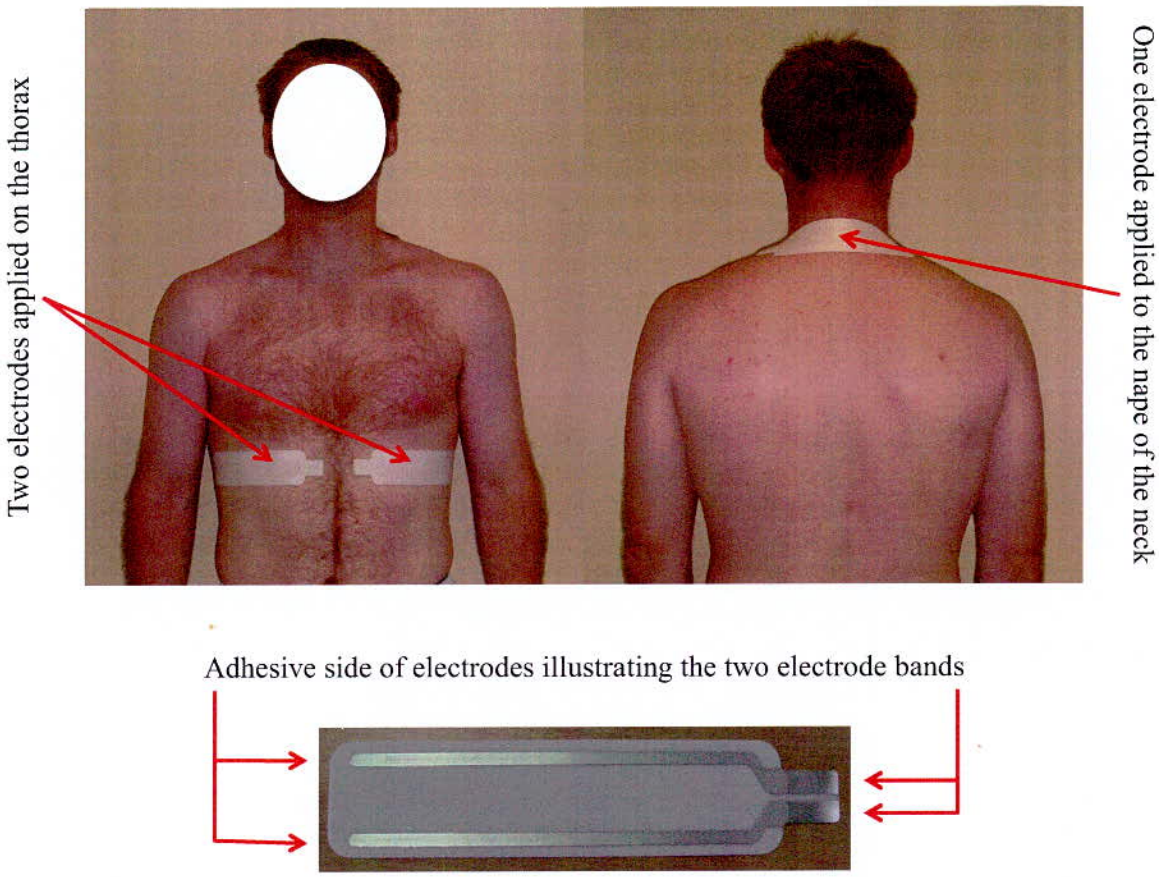

Figure 3.10: Task Force® Monitors impedance cardiography electrode placement.

The TFM calculates the total peripheral resistance index according to Ohm's law, where total peripheral resistance index equals mean BP divided by cardiac index (Valipour et al. 2005).

3.3.3: BARORECEPTOR REFLEX SENSITIVITY

The cardiac baroreflex is a feedback loop with cardiac, vascular, and cerebral components, which is involved in short-term BP regulation (Lantelme et al. 2002). The baroreflex regulates BP by adjusting HR, myocardial contractility, and peripheral resistance to transient increases or decreases in BP (Laitinen et al. 1999).

Baroreceptor reflex sensitivity was traditionally quantified by measuring the slope of the relationship (linear regression line) between the increase in sBP and the reflex lengthening of the pulse interval (PI), due to the intravenous administration of a vaso-active drug (alpha-adrenoreceptor stimulant), such as phenylephrine (Kardos et al. 2001; Korner et al. 1974; Pinna et al. 2000; Smyth et al. 1969). The relationship between sBP and PI lengthening represents the strength of the BRS (Pinna et al. 2000). This technique enabled researchers to identify that BRS decreases with age (Gribbin et al. 1971; Laitinen et al. 1998), hypertension, in the presence of cardiovascular disease, namely CHD and heart failure (Mortara et al. 1997; Osculati et al. 1990) and as an important independent predictor of total cardiac mortality and SCD (Schwartz et al. 1992).

Administration of a vaso-active drug, such as phenylephrine to measure BRS is performed by cannulation of the radial or brachial artery in order to record beat-to-beat arterial blood pressure (Smyth et al. 1969). Therefore widespread application is impossible since it is not available in the outpatient setting (Parati 1992; Pinna et al. 2000).

Advances in computer technology and sophisticated algorithms have enabled BRS to be quantified non-invasively (Malliani et al. 1991; Panerai 1995; Parati et al. 1995; Robbe et al. 1987). The TFM uses the widely employed sequence technique method (Bertinieri et al. 1985; Bertinieri et al. 1988; Parati et al. 1988) to investigate spontaneous baroreflex control of the heart (Di Rienzo et al. 2001).

The sequence method is based on computer identification of a series of successive increases (hypertension / bradycardia or +PI / +sBP sequences) or decreases (hypotension / tachycardia or -PI / -sBP sequences) in sBP and lengthening of the PI (Di Rienzo et al. 2001; Fritsch et al. 1986; Mathias 1999; Parati 1992; Valipour et al. 2005). The slope of the regression line between the sBP and PI values in each sequence is taken as an index of the BRS control of the heart (Di Rienzo et al. 2001; Smyth et al. 1969; Valipour et al. 2005). Research has demonstrated that the interactive sequences of sBP and PI are real physiological events rather than chance interactions (Blaber et al. 1995).

3.3.4: HEART RATE VARIABILITY

Heart rate variability (HRV) is considered one of the most promising non-invasive markers of cardiac autonomic activity (Bolis 2003). Basic (electrocardiography) and advanced (power spectral analysis) methods are currently utilised to measure HRV to provide an index of autonomic modulation (Akselrod et al. 1981; Malik et al. 1996; Pagani et al. 1997).

The concept of circulatory homeostasis refers to the nature of an organism to maintain a relatively regular HR and BP with varying environmental conditions, whether endogenous or exogenous. Indeed, it was generally acknowledged that a regular HR is a sign of cardiovascular health (Sandercock et al. 2005). However, a healthy heart is symbolised by significant beat-to-beat variability (Routledge et al. 2002; Sandercock et al. 2005).

Heart rate variability reflects autonomic modulation through phasic changes in sinus node stimulation associated with sympathetic and parasympathetic inputs. Importantly, HRV represents an end-organ response localised in the sinus node, which is determined by nerve firing, cardiac adrenergic receptor sensitivity, and post-synaptic signal transduction (Kurata 2003). Therefore, HRV is a marker of neural efferent activity and cannot quantify the intensity of stimulus (Floras 2009; Notarius and Floras 2001).

In healthy individuals the neural hierarchy maintains cardiovascular homeostasis through fine modifications in myocardial performance, vascular tone, and R-R intervals (Lanfranchi and Somers 2002; van Ravenswaaij-Arts et al. 1993). The oscillating changes in R-R intervals are caused by continuous alterations in sympathetic and parasympathetic mediated neural impulses; all of which contributes to the cardiovascular control system (Akselrod et al. 1981). The sympathetic and parasympathetic nervous activity is assessed by the oscillating fluctuations in the frequency and amplitude of each R-R interval (Akselrod et al. 1981). R-R intervals from an ECG recording oscillate around two main frequencies; high and low (Ditor et al.

2005). The low frequency (LF) component ranges between 0.04 and 0.15 Hz and has been shown to predominantly represent sympathetic outflow to the heart (Montano et al. 1994; Pomeranz et al. 1985), whereas the high frequency (HF) component ranges between 0.15 and 0.40 Hz and corresponds to parasympathetic outflow to the heart via the vagus nerve (Pomeranz et al. 1985). Thus, the ratio of LF-to-HF (LF:HF Ratio) for HRV is an accepted measure of cardiac sympathovagal balance (Ditor et al. 2005).

The TFM uses power spectral analysis (PSA) for quantification of HRV. Power spectral analysis involves degeneration of each R-R interval into a sum of waves (sinusoidal) of different amplitude and frequencies, thus the results can be displayed with the magnitude of variability as a function of frequency (power spectrum) (Akselrod et al. 1981; Weise et al. 1995). Therefore, the power spectrum reflects the amplitude of R-R interval fluctuations at different oscillation frequencies (Akselrod et al. 1981).

The main advantage of PSA of signals is the possibility to study frequency specific oscillations (Davidson et al. 1976; Luczak and Laurig 1973; Malliani et al. 1991). Therefore, not only can the degree of variability from the measurements be obtained, but the oscillating frequency can also be obtained (van Ravenswaaij-Arts et al. 1993). To understand this process, signals contain information that ranges from components that change very slowly (low frequency [LF]) to components that fluctuate rapidly (high frequency [HF]). Rapid modulation of HR control occurs through the parasympathetic limb of the autonomic nervous system. Conversely, the sympathetic nervous system is unable to mediate HF components because the sino-atrial node response to changes in

noradrenaline interacting with βeta adrenergic receptors is slower than that of acetylcholine interacting with muscarine receptors. Due to the differences in neurotransmitter function, the two subsystems of the autonomic nervous system operate at different frequencies. Therefore HR fluctuations related predominantly to sympathetic or parasympathetic activity can be quantified (Pumprla et al. 2002) (Figure 3.11).

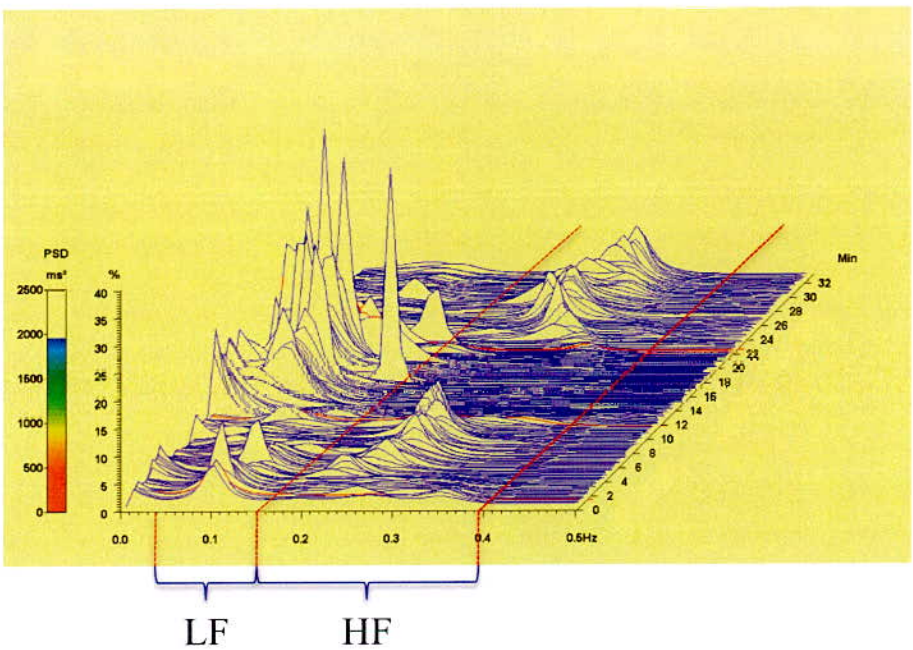

Figure 3.11: Power spectral analysis of a healthy adult during autonomic function testing. The figure is used in this thesis to illustrate the different frequency oscillations in R-R intervals and the specific input from sympathetic and parasympathetic limbs of the autonomic nervous system during physiological stress challenges. Note: LF = Low frequency; HF = High frequency.

The TFM combines several published detection algorithms (Cuiwei 1995; Pan 1986) with the subsequent values used to calculate real-time HRV by an autoregressive model (Di Rienzo 1992) and displayed as three-dimensional sliding power spectra (Fortin 2001; Gratze et al. 1998).

The TFM computes the total power of the frequency bands (low and high frequency [LF and HF respectively]) and the power density of each spectral component in both absolute values (ms^2) and normalised units (nu), with the LF and HF ratio determined. The use of normalisation provides a formal means of reducing the uncertainty in assessing the distribution of spectral power when variance, or the very low frequency (VLF) component, undergoes divergent changes with the autonomic balance as frequently observed in experimental conditions (Pagani 2003). Normalised units are computed by subtracting the VLF (noise) component from the total power and then dividing the power of every LF and HF component by total power and multiplying the ratio by 100 (Pagani 2003).

The TFM's QRS-algorithm was evaluated with the MIT/BIH database, which contains 24-hours of real world ECG data, including the broadest possible range of waveforms, including ambiguous cases (Moody 2001) and the detection rate of all included data was 98.21% (Fortin 1998; Fortin 2001). Figure 3.12 demonstrates the process of calculating the online and beat-to-beat integrative autonomic and haemodynamic parameters through the TFM.

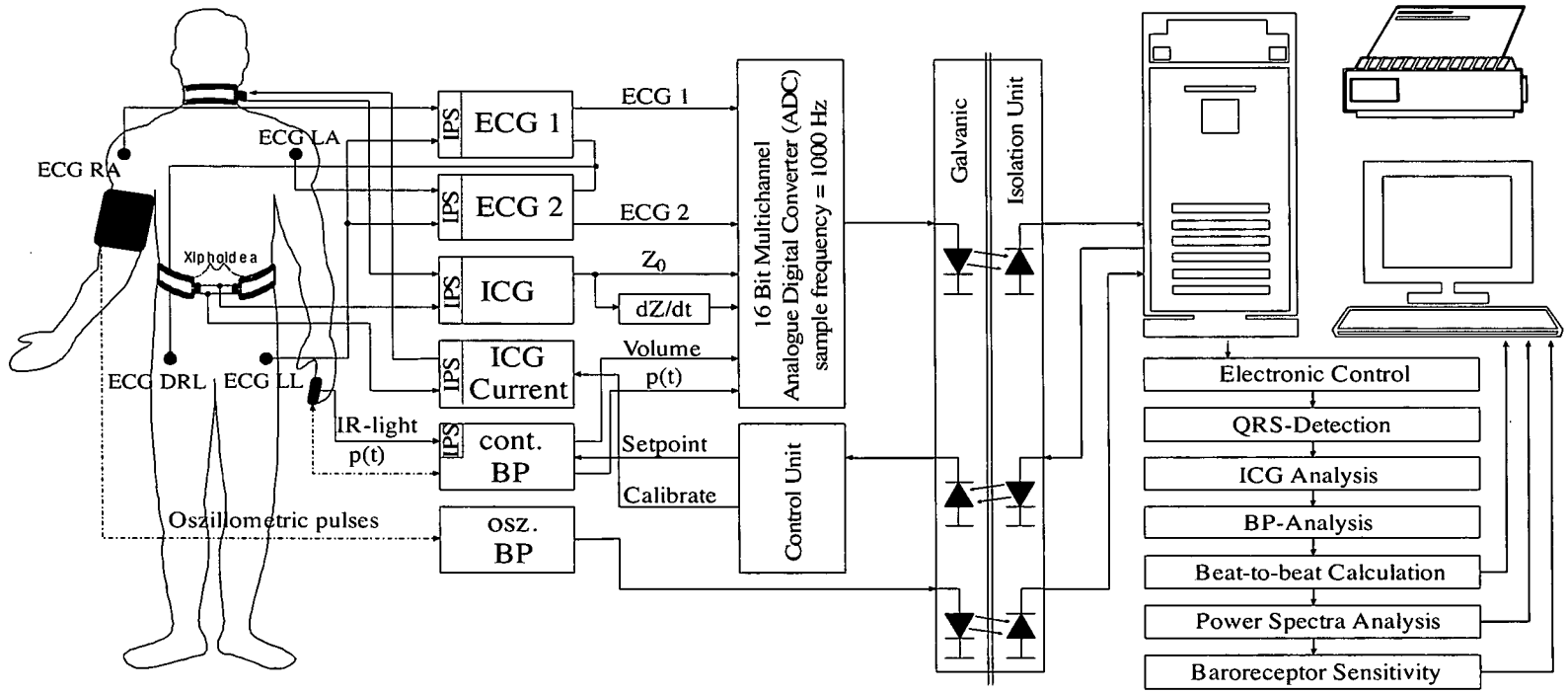

Figure 3.12: Task Force® Monitors algorithm for calculating beat-to-beat autonomic and haemodynamic parameters.

3.3.5: VALIDITY AND RELIABILITY OF THE TASK FORCE® MONITORS MEASUREMENT

Traditionally, measuring autonomic function has relied upon invasive procedures, which is impractical in an outpatient setting and research using such methodology had small sample sizes. Heart rate variability is a non-invasive technique, which has evolved in recent time and provides an index of cardiac autonomic modulation. However, earlier tools had poor reproducibility with coefficient of variation results ranging from 1-235% (Sandercock et al. 2004). In addition, HRV methodology requires stationary, ectopic free recording periods to reduce measurement error. Therefore, populations that may benefit from HRV assessment who have electrocardiographic disturbances, such as frequent ectopic beats or atrial fibrillation are excluded. This limitation is evident within this research due to the large number of patients excluded during data analysis (see figures 3.13, 3.14, 3.15, and 3.16).

The inter-individual and intra-individual reliability of the TFM, measured over four separate trials at 2-week intervals has been previously performed and demonstrated low (intra-individual) and moderate (inter-individual) CV (Goswami et al. 2009). In healthy volunteers measured over 3-separate recordings, our own group have recorded CV values of 10.7% for BP, 7.2% for BRS, 3.9% for SV (ICG), and 5% for HRV measurements using the TFM. The distinction of low, medium, or high CV has been used by some researchers to quantify the meaning of value, where a CV of less than 10% is considered low, 10-20% is considered modest, and above 20% is considered high (Reland et al. 2005; Toyry et al. 1995).

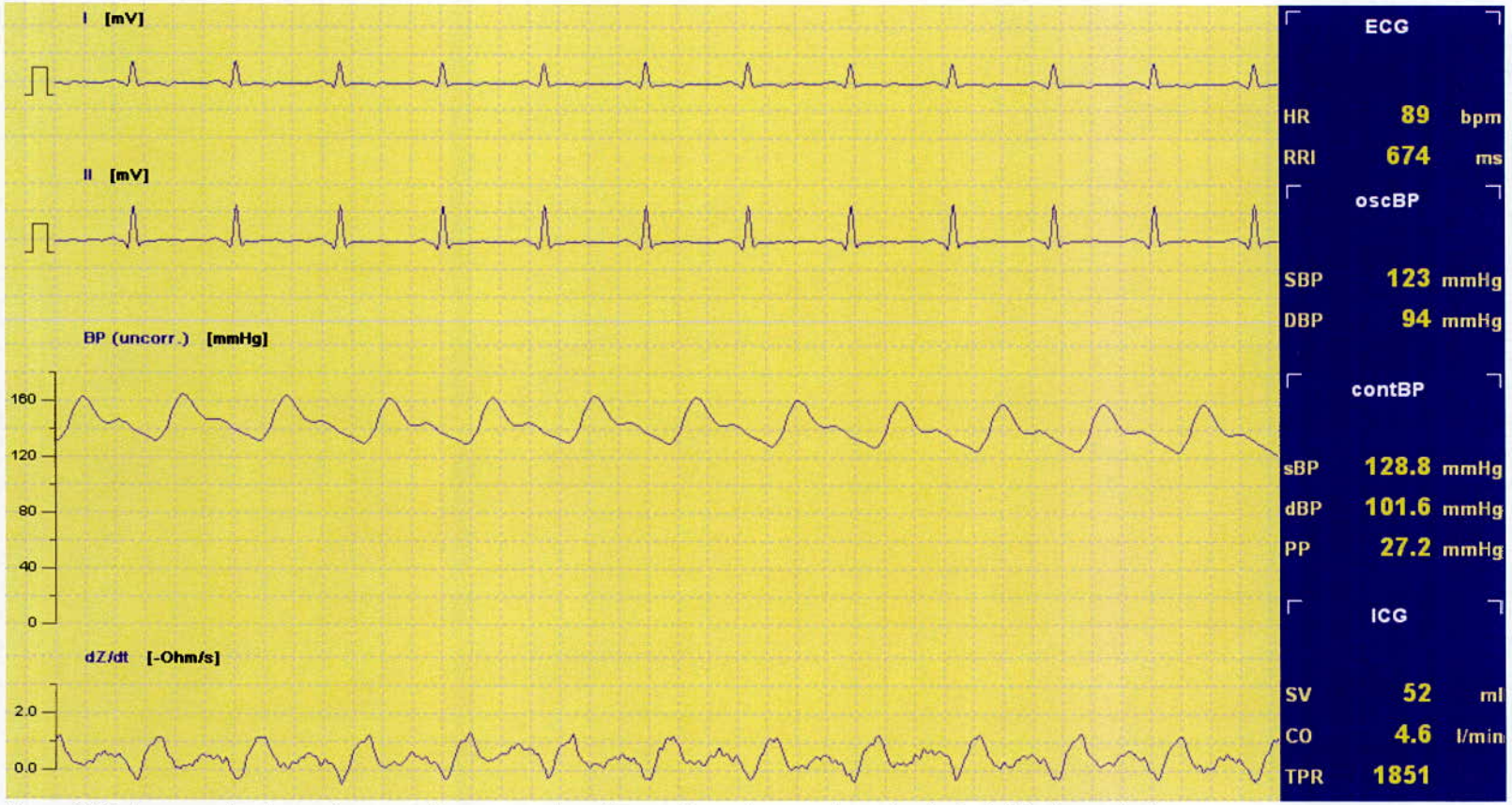

Figure 3.13: An example of a continuous online autonomic and haemodynamic trace of a patient included in data analysis.

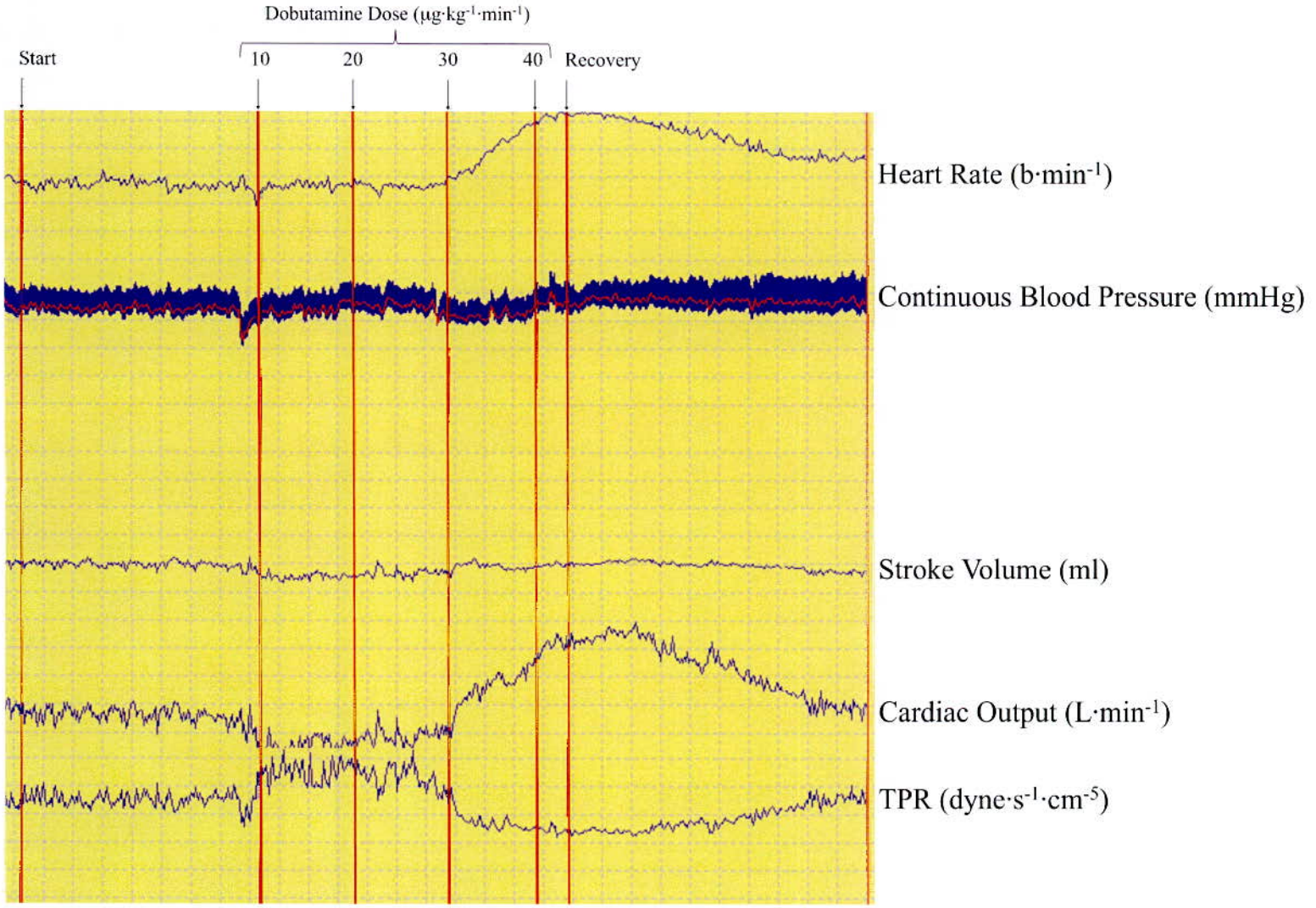

Figure 3.14: An example of a continuous online autonomic and haemodynamic trace during a complete study of a patient included in data analysis.

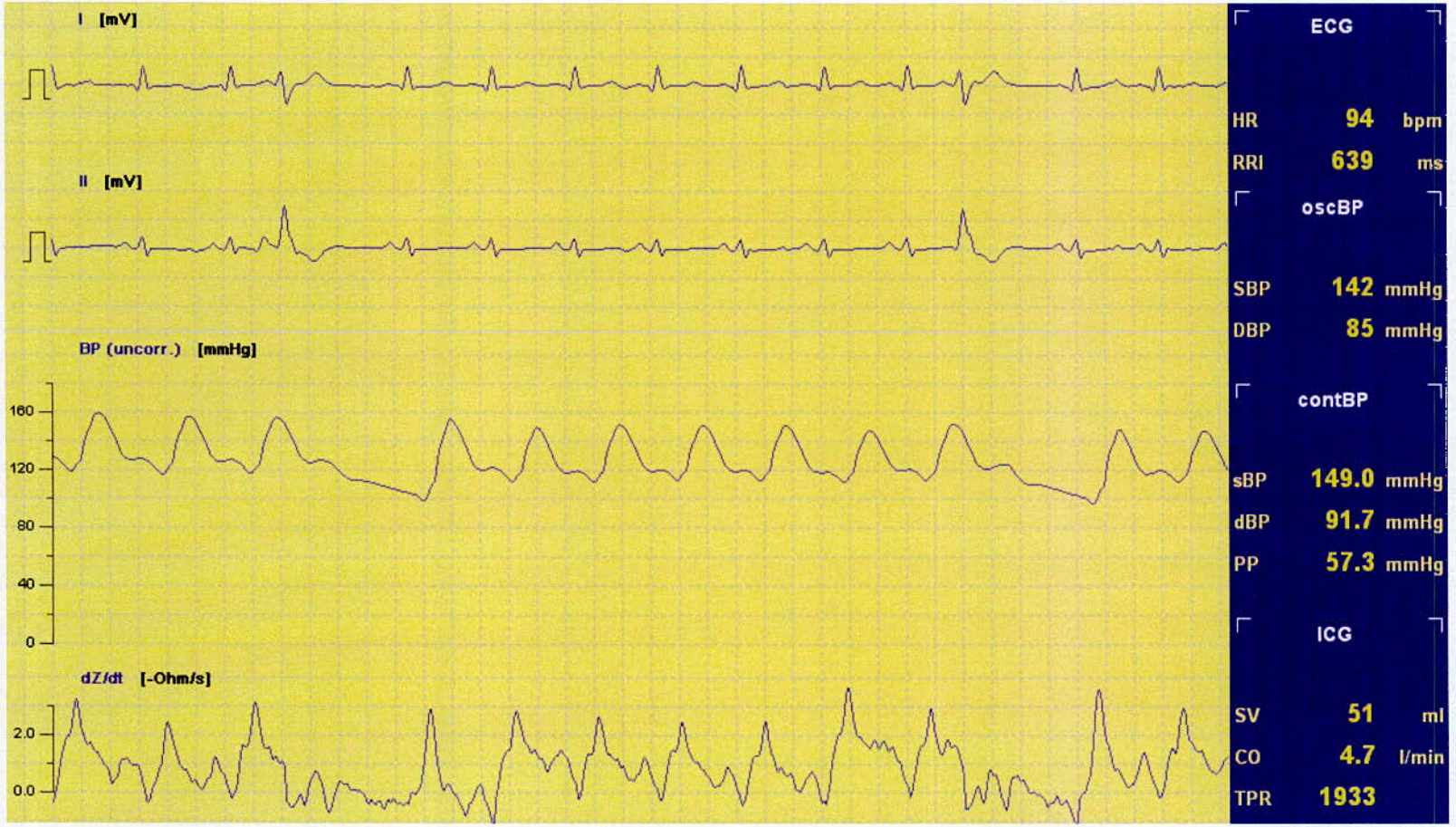

Figure 3.15: An example of a continuous online autonomic and haemodynamic trace of a patient with numerous premature ventricular contractions who was excluded from data analysis.

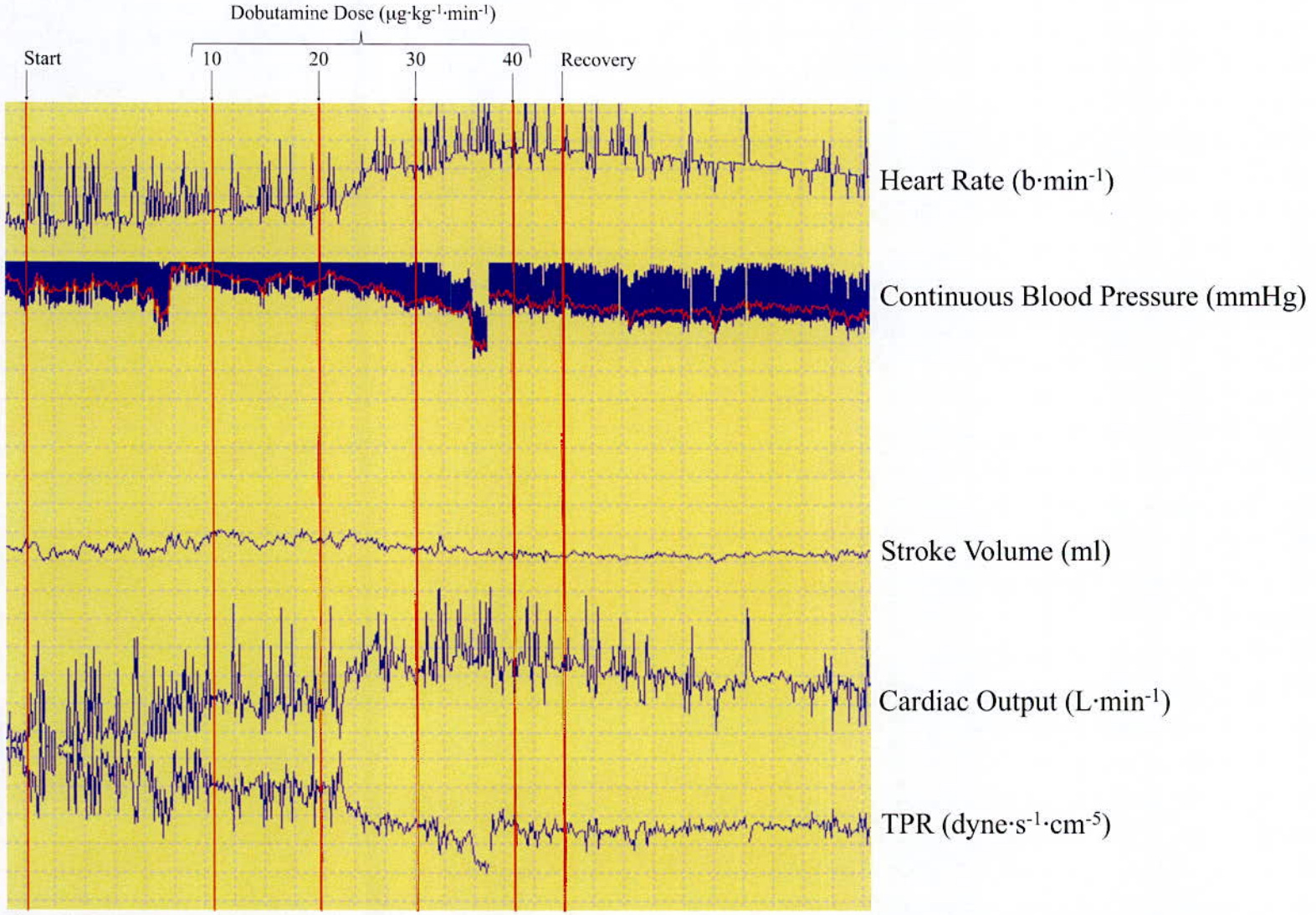

Figure 3.16: An example of a continuous online autonomic and haemodynamic trace during a complete study of a patient with numerous premature ventricular contractions who was excluded from data analysis.

3.4: TRANSTHORACIC ECHOCARDIOGRAPHY

Echocardiography (cardiac ultrasound) is currently the most widely used imaging technique to evaluate cardiovascular anatomy, function, and haemodynamic properties of the heart and great vessels (Libby 2008; Ryan et al. 2008; Sharples et al. 2007). The portability and relative affordability of the equipment has led to wide acceptance of the technique by medical physicians and research scientists (Sharples et al. 2007). Transthoracic echocardiography (TTE) is the standard method of obtaining echocardiographic images of the cardiac structures, where a piezoelectric transducer is placed on the patient's thorax and images are obtained non-invasively through the chest wall using sound wave (ultrasound) technology.

Clinical application of echocardiography includes two-dimensional (2D), motion-mode (M-mode), pulsed and continuous wave Doppler colour-flow imaging of the heart and great vessels (Ryan et al. 2008). Two-dimensional and M-mode applications allow real-time visualisation of cardiac structures from multiple tomographic planes (Libby 2008) enabling quantification of cardiac systolic function, such as ejection fraction (EF) (Ryan et al. 2008) and Doppler and colour flow imaging enable the quantification of cardiac haemodynamic variables and flow disturbances such as gradients or pressure (Libby 2008; Ryan et al. 2008). High risk patients can be identified according to those with impaired left ventricular systolic function, impaired left ventricular diastolic function and significant valvular disease (Ommen et al. 2000).

In the present thesis, a full cross sectional study was performed at baseline using a General Electric Vingmed System 7 ultrasound machine (figure 3.17). All images were stored in digital format and off line measurements made by two experienced observers blinded to the rest of the study. All measurements were averaged over three cardiac cycles. Data capture was successful in all cases.

Figure 3.17: General Electric Vingmed System 7 ultrasound machine used to acquire all echocardiography images.

3.4.1: LEFT VENTRICULAR SIZE AND SYSTOLIC FUNCTION

Left ventricular (LV) end diastolic diameter (LVEDD) and LV end systolic diameter (LVESD) were measured from parasternal long axis M mode recordings of the LV, with the cursor at the tips of the mitral valve leaflets. From this, LV fractional shortening was calculated (Schiller et al. 1989). Left ventricular end systolic and diastolic volumes were determined from apical 4-chamber view by modified biplane Simpson's technique (figure 3.18) and the standard formula applied to give LV ejection fraction (LVEF) (Schiller et al. 1989).

Table 3.1 illustrates the formulae used for assessing LV systolic function (Schiller et al. 1989), LV volumes (Teichholz et al. 1976), and LV mass index (Devereux and Reichek 1977).

Table 3.1: The Formulae used for Assessing Left Ventricular (LV) Systolic Function, LV Volumes and LV Mass Index.

Parameter	Formula Calculation
Fractional Shortening (FS) (%)	FS = 100 x (LVEDD - LVESD) / LVEDD
Ejection Fraction (EF) (%)	EF = 100 x (LVEDV - LVESV) / LVEDV
Teichholz formula for volume (cm^3)	Volume = (7 / 2.4 + LVEDD) x $(LVEDD)^3$
Devereux formula for LV mass (g)	LV mass = 0.8 {1.04 (STD + LVEDD + PWTD)^3 - $LVEDD^3$} + 0.6

Note: LVEDD = Left ventricular end diastolic diameter; LVESD = Left ventricular end systolic diameter; LVEDV = Left ventricular end diastolic volume; LVESV = Left ventricular end systolic volume; STD = End diastolic septal wall thickness; PWTD = End diastolic posterior wall thickness.

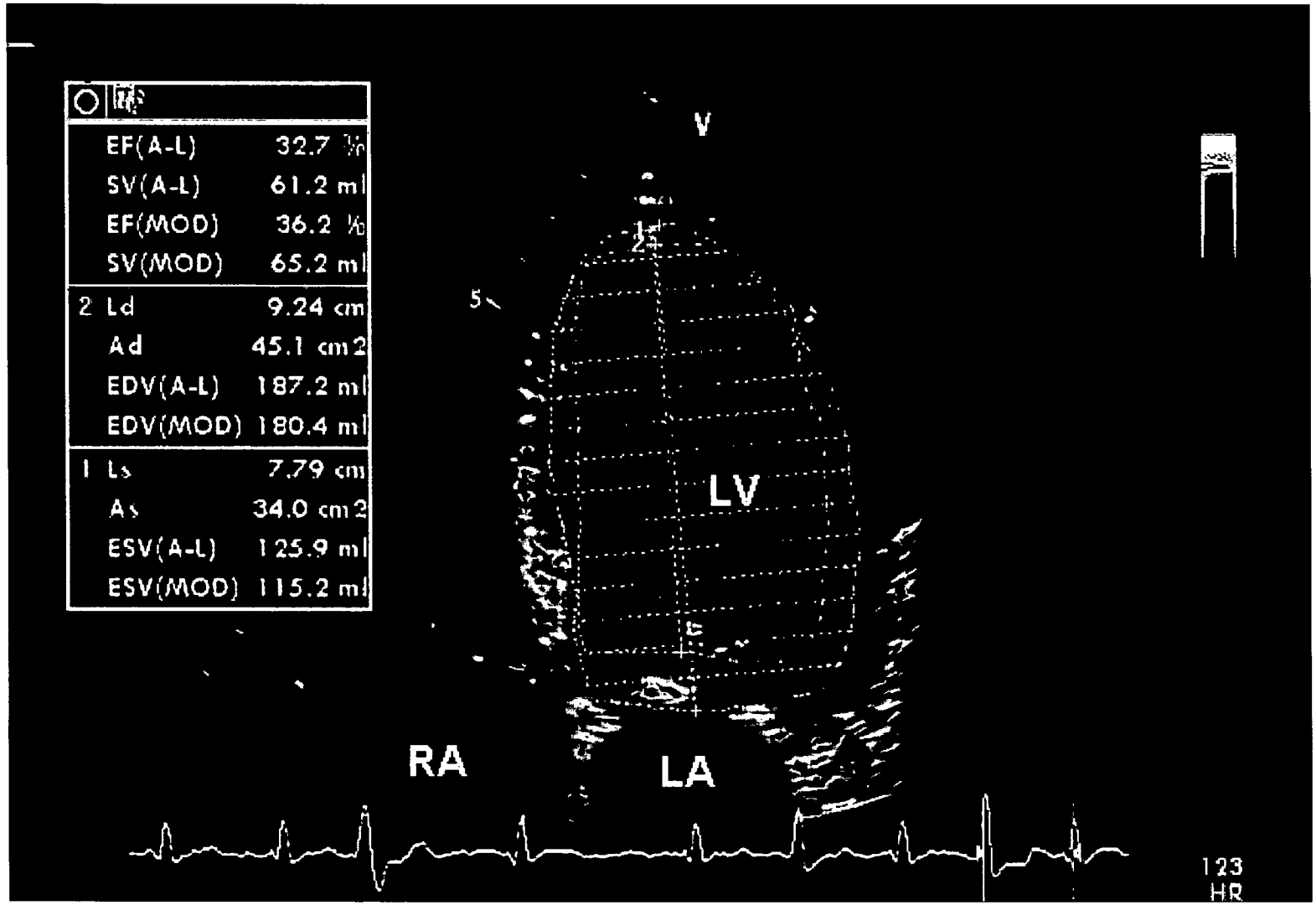

Figure 3.18: Left ventricular ejection fraction (LVEF) calculated by biplane Simpson's method. This technique requires planimetry of the endocardial border in systole and diastole and assumes ventricular volume is the sum of the volumes of adjacent sets of discs of varying depth and cross-sectional area. This is the most accurate echocardiographic technique for LVEF and was used for all patients in our study.

3.4.2: LEFT VENTRICULAR DIASTOLIC FUNCTION

Transmitral inflow was recorded using pulsed wave Doppler recordings at the mitral valve leaflet tips in the apical 4-chamber view. Peak velocity of early filling (E), peak velocity of atrial filling (A), the E/A ratio, and E-deceleration time (ms) were measured. Pulsed wave Doppler of pulmonary venous flow in apical 4-chamber view was measured. Peak pulmonary venous systolic (S) and diastolic (D) velocities, S/D ratio, peak pulmonary venous atrial reversal velocity and the difference between pulmonary venous atrial wave duration and mitral A wave duration (A Dur) were calculated. Flow propagation velocity (Vp) was calculated from the colour M-Mode in the apical 4-chamber view. Left ventricular filling pressure was assessed from A Dur, (Rossvoll and Hatle 1993) and E/Vp ratios (Moller et al. 2000; Ommen et al. 2000). An Adur > 30ms or E/Vp ratio > 1.8 suggest raised LV filling pressure. Left ventricular diastolic dysfunction was defined from the transmitral Doppler pattern as abnormal relaxation (E/A ratio <1, E deceleration time > 220ms), restrictive filling (E/A > 2.0, E/A between 1 and 2 with E deceleration time < 150ms), or pseudonormal filling (Ea < 0.08ms, Vp < 0.45m/s, Adur > 30ms, Arev ≥ 0.35m/s in the context of normal transmitral Doppler) (Appleton et al. 1988; Klein et al. 1989; Sohn et al. 1997).

3.4.3: TISSUE DOPPLER IMAGING TO ASSESS LONGITUDINAL FUNCTION

For assessment of LV longitudinal function, on line pulsed wave tissue Doppler velocities were measured at the lateral, septal, anterior, inferior, posterior and anteroseptal sites of the mitral annulus. This was obtained from apical 2, 3, and 4 chamber views. An example of a mitral annular velocity trace is shown in figure 3.19. Here the sample volume was placed on the septal site of the mitral annulus.

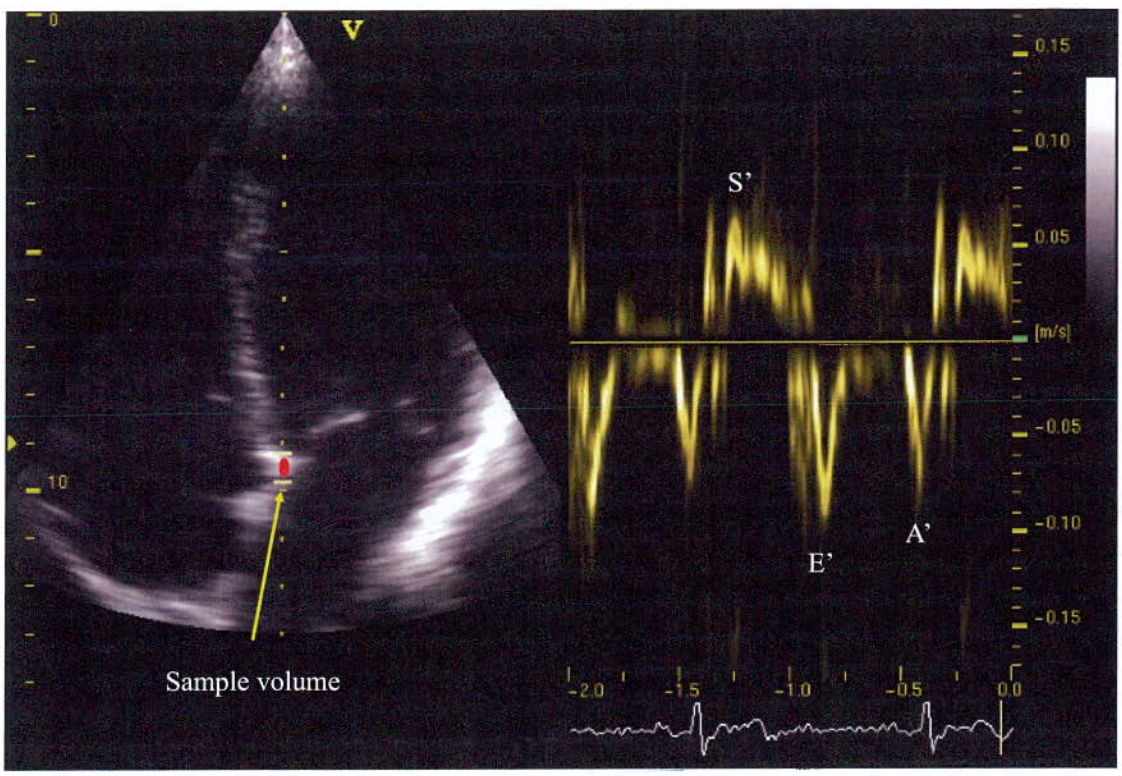

Figure 3.19: Mitral annular velocity trace with the sample volume positioned on the septal site of the mitral annulus.

Peak systolic (S'), early diastolic (E') and late diastolic (A') annular velocities were determined at the 6 points of the mitral annulus and the values averaged. E/E' ratio was determined as a measure of LV filling pressure (Nagueh et al. 1997; Ommen et al. 2000), with a value >15 suggesting significant elevation (figure 3.20).

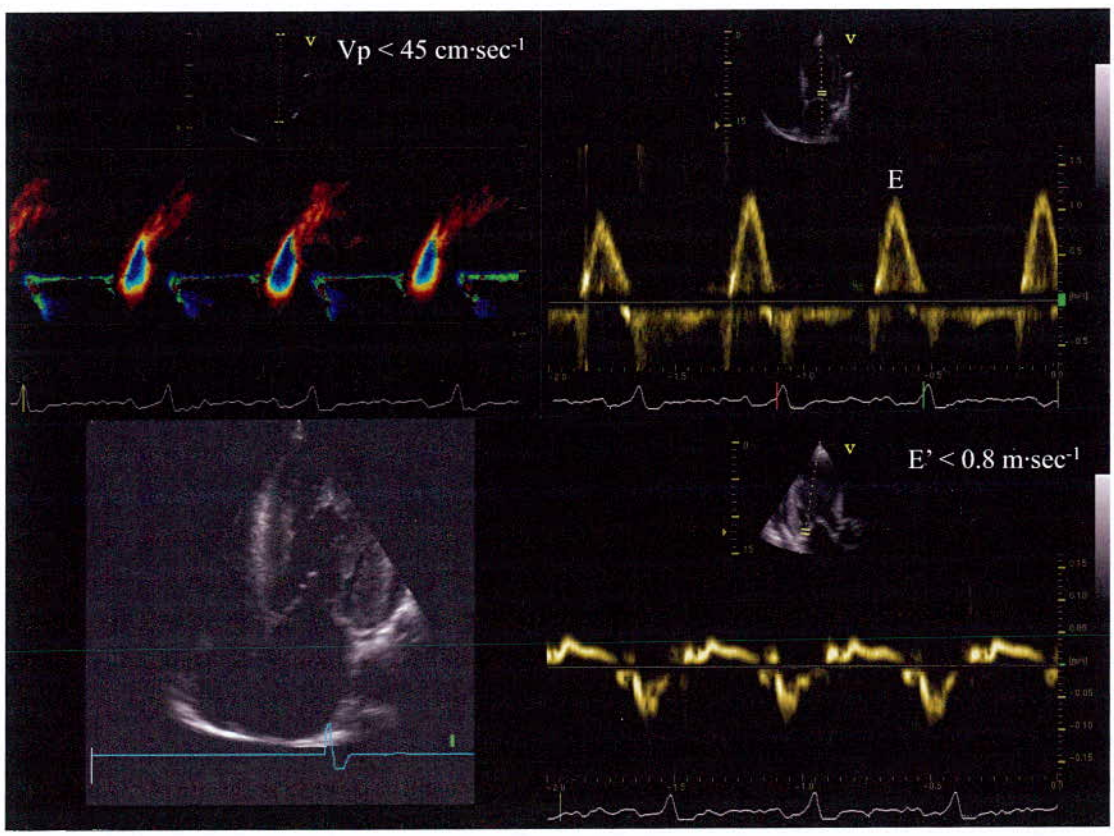

Figure 3.20: Illustration of how mitral E/E' and mitral E/Vp measurements were taken in order to estimate left ventricular filling pressure.

3.4.4: LEFT VENTRICULAR WALL THICKNESS & MASS INDEX

From the parasternal long axis view, interventricular, and LV posterior wall thickness at end diastole were measured from parasternal M-mode recordings of the LV, with the cursor just below the tips of the mitral valve leaflets (figure 3.21). Left ventricular mass was calculated from M-mode measurements of septal and posterior wall thickness and LV cavity size at end diastole, according to the method of Devereux (Devereux and Reichek 1977). This was corrected for body surface area to give LV mass index (LVMI). Table 3.1 shows the formula used for LV mass calculation. Left ventricular hypertrophy was defined as a LVMI > 125 g/m^2 according to studies in the general population (Silberberg et al. 1989). The pattern of left ventricular hypertrophy was visually assessed in parasternal and apical views and defined as concentric or asymmetrical.

3.4.5: MITRAL ANNULAR CALCIFICATION

Mitral annular calcification (MAC) was defined from 2D echocardiography as follows: an echo dense band visualised throughout systole and diastole, distinguishable from the posterior mitral valve leaflet, located anterior, and parallel to the posterior left ventricular wall (Benjamin et al. 1992).

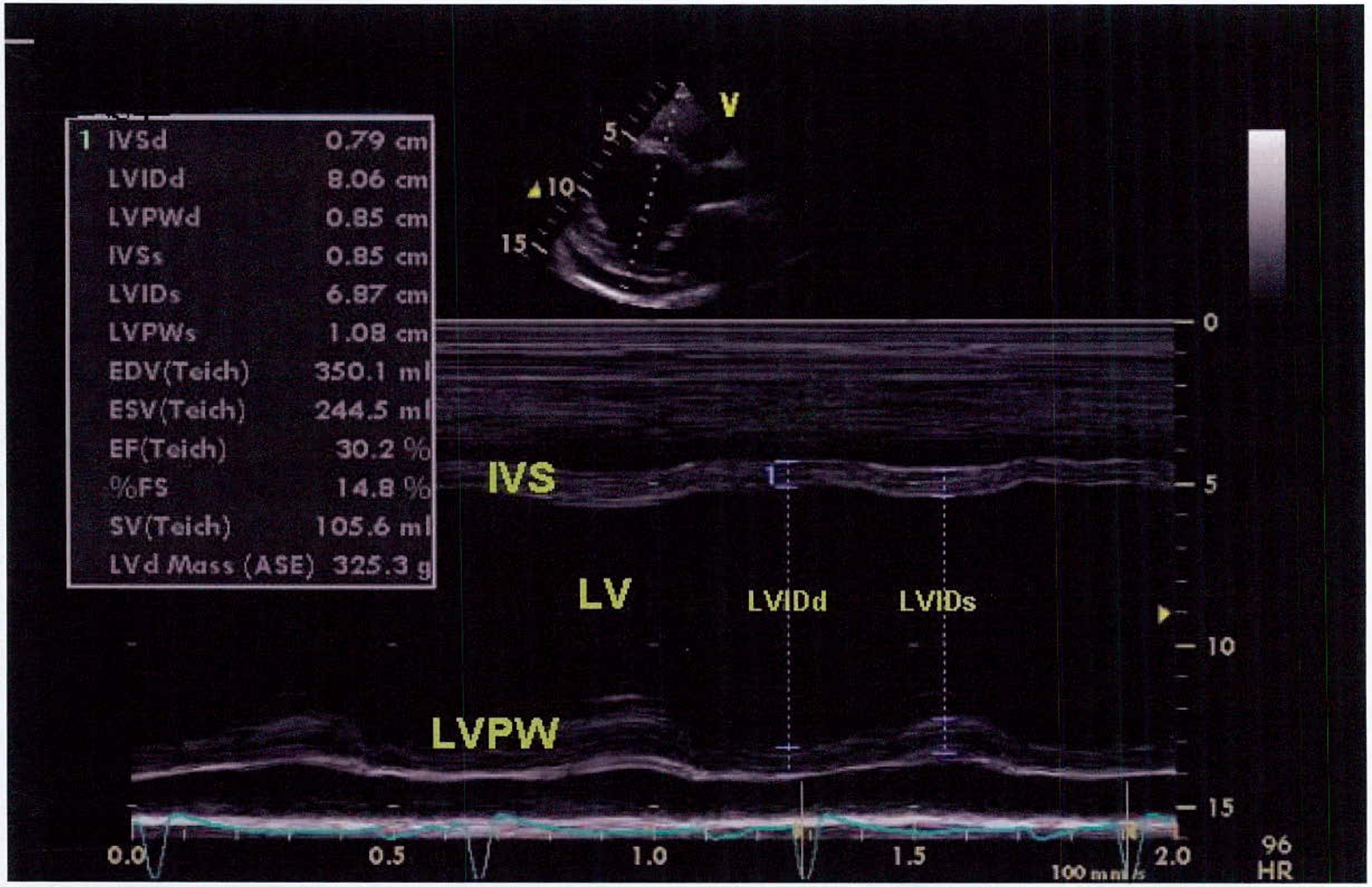

Figure 3.21: Left ventricular assessment is made from linear M-mode measurements in parasternal long axis view. The cursor is placed just beyond the mitral valve leaflet tips and cardiac motion in a single plane displaced over the cardiac cycle. In this way, LVESD, LVEDD, IVSd and LVPWd can be determined and from this LVEF is calculated by converting the LVESD and LVEDD distances into volumes. This technique for LVEF is known as Teicholz formula.

3.4.6: OTHER ECHOCARDIOGRAPHY MEASUREMENTS

Left atrial size (LA) and aortic diameter (Ao) were measured from M-Mode tracings in the parasternal long axis view at end diastole. Left ventricular and pulmonary outflow tract velocities were measured by placing the pulsed wave sample volume just distal to the aortic and pulmonary valves in the apical 5-chamber and short axis views respectively. Right ventricular (RV) size was assessed in the apical 4-chamber view by comparing the ratio of the RV diameter in end diastole to LV diameter in end diastole. Right ventricular function was visually assessed but formal quantification techniques were not used. An estimation of pulmonary artery pressure was made from the tricuspid regurgitation velocity and inferior vena cava diameter.

3.5: STRESS ECHOCARDIOGRAPHY

Stress echocardiography can be performed in conjunction with dynamic exercise (treadmill or cycle) or with pharmacological agents, such as dobutamine, dipyridamole, or adenosine (Armstrong and Zoghbi 2005; Becher et al. 2004; Senior et al. 2005) for patients unable to exercise. Stress echocardiography was introduced in 1979 and has progressively evolved into a functional procedure for identifying patients with coronary artery disease (CAD) (Armstrong and Ryan 2008; Armstrong and Zoghbi 2005; Becher et al. 2004; Libby 2008; Senior et al. 2005; Sharples et al. 2007).

Exercise ECG is the technique most widely used for the non-invasive diagnosis and risk evaluation of patients with suspected or known CAD; however, only approximately 40% of patients can achieve a truly diagnostic exercise stress test (Becher et al. 2004; Geleijnse et al. 1997; Senior et al. 2005). Dobutamine induced stress echocardiography (DSE) is a pharmacological stress, which evaluates the reserve capacity of the myocardium (cardiovascular reserve) by increasing myocardial oxygen demand. In healthy, normal circumstances, an individuals symptoms improve with increased cardiovascular work, demonstrated by improved myocardial muscle thickening. Conversely, in conditions when symptoms deteriorate, such as with induced myocardial ischaemia, the increased demand cannot be met due to underlying pathology, which results in a diminished cardiovascular reserve that can be a direct result of CHD (Libby 2008).

Stress echocardiography images are obtained digitally in the parasternal and apical views at rest and at defined intervals during testing, which captures views of the heart in all three vascular territories (Senior et al. 2005). The diagnosis of CHD during DSE is based on the detection of a deterioration in wall motion during stress (wall motion abnormality [WMA]) (Armstrong and Zoghbi 2005). The WMA may be a subtle or obvious reduction in systolic myocardial muscle thickening (hypokinesis) or development of an area of no thickening or movement (akinesis) or paradoxical motion (dyskinesis) (Sharples et al. 2007). A resting WMA implies either that the patient has suffered a previous MI or that there is enough myocardial ischaemia at rest to cause impaired myocardial systolic function. If a WMA is induced during DSE, this implies

that there is obstructive CAD (Elhendy et al. 2000; Senior et al. 2005; Sharples et al. 2007). Severe worsening of regional ventricular function is associated with a more unfavourable prognosis (Elhendy et al. 2000) and the calculation of a wall motion score index (WMSI) can be valuable for prognosis and risk stratification by quantifying the amount of myocardium at risk (Armstrong and Zoghbi 2005). Furthermore, a wall motion abnormality occurs lower in the ischaemic cascade, before ECG changes as investigated during exercise treadmill testing (Nesto and Kowalchuk 1987).

Dobutamine, which is predominantly a β_1 adrenoceptor agonist (Rang 2007), is the preferred pharmacological stress agent used to increase myocardial oxygen demand and highlight coronary flow discordance, possibly due to cost effectiveness (Armstrong and Zoghbi 2005). The ventricles of the myocardium contain mainly β_1-adrenergic receptors, which causes predominantly inotropic responses, therefore dobutamine may have an apparently dominant inotropic selectivity causing increased myocardial muscle thickening (Opie 2004). At higher doses of dobutamine, blockade of peripheral alpha-receptors also occurs resulting in vasodilatation. Atropine, which is a muscarinic acetylcholine receptor (mAChRs) antagonist that causes blockade of parasympathetic nervous innervations to cardiac muscle (Rang 2007), can be given to patients who have an inadequate response to dobutamine, thereby producing a chronotropic response with the aim of achieving the desired heart rate (Becher et al. 2004).

The diagnostic accuracy of DSE has been reported in a number of studies, with sensitivity ranging from 71% to 97% and specificity ranging from 64% to 90% (Crouse

et al. 1991; Marwick et al. 1995; Sharma et al. 2005), with the highest sensitivity for three vessel disease and lowest for single vessel disease (Armstrong and Zoghbi 2005; Geleijnse et al. 1997). In addition, DSE is a safe method for the functional assessment of CAD, with serious side effects being less than one in 1000 studies (Geleijnse et al. 1997) as well as being cost effective (Senior et al. 2005).

In this thesis DSE was performed using a General Electric Vingmed System 7 ultrasound machine. Beta-blockers were stopped 72-hours prior to the test but all other anti-hypertensive and anti-ischaemic medication were taken up to the day of the examination. All images were stored in digital format and were reported off-line by two experienced observers blinded to the rest of the study.

Images were acquired in standard parasternal long- and short-axis and apical 2-, 3-, 4-chamber views at baseline and during stepwise infusion of dobutamine, which captures views of the heart in all three vascular territories (Senior et al. 2005) (see figure 3.22). This was given according to a protocol based on 3-minute stages of 10, 20, 30, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Atropine was administered up to a total of 2.0 mg intravenously if the target heart rate was not achieved with dobutamine alone. Intravenous metoprolol was available as an antagonist to dobutamine if required. Blood pressure and 12-lead ECG were recorded at each infusion stage.

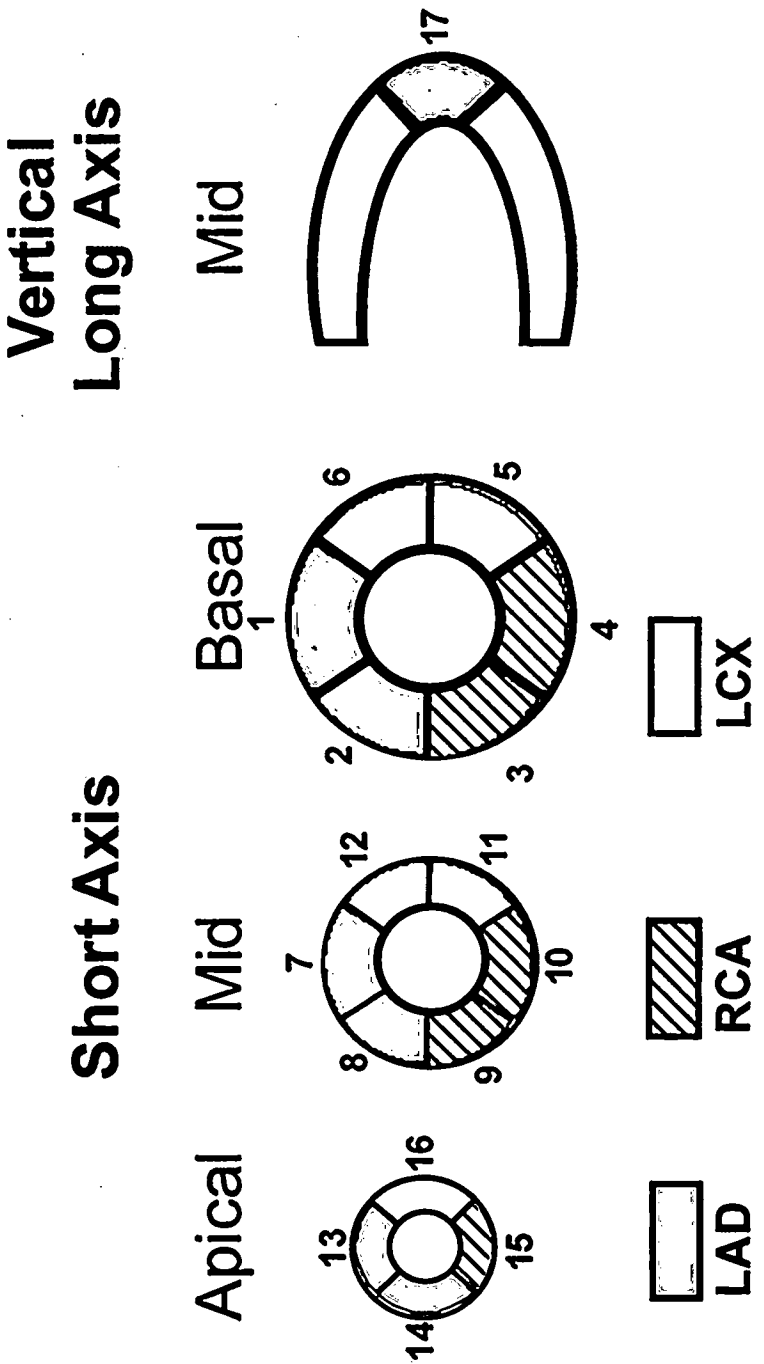

Figure 3.22: Assignment of the 17-myocardial segments to the territories of the left anterior descending (LAD), right coronary artery (RCA), and the left circumflex coronary artery (LCX).

Baseline, low-dose (heart rate 10 – 15 beats above baseline), peak, and recovery (10 minutes after drug infusion terminated) stage images were stored and analysed in digital quad screen format. The test was stopped if: (a) the target heart rate was achieved ([220 – age] x 0.85), (b) ST depression > 2mm occurred, (c) there was a significant tachyarrhythmia (sustained supraventricular tachycardia or a > 3 beat run of ventricular tachycardia), (d) symptomatic severe hypotension occurred, (e) blood pressure exceeded 240 mmHg systolic or 140 mmHg diastolic.

Qualitative analysis was performed with the left ventricle divided into a 17-segment model (see figure 3.23). Regional wall motion was described as hyperkinetic, normal, hypokinetic, akinetic, and dyskinetic. Results were classified as a normal response, with an overall increase in wall motion or an abnormal response. The test was considered positive with the occurrence, under stress, of hypokinesia, akinesia, or dyskinesia in one or more resting normal segments and/or worsening of wall motion in one or more resting hypokinetic segments. The level of agreement, kappa (κ) between the two sonographers was $\kappa = 0.82$. Consensus was obtained in discordant cases.

Regional wall motion score index (RWMSI) at rest and at peak stress was calculated for each patient (Armstrong 1991). The heart is divided into a 17-segment model (Cerqueira et al. 2002a, b) and a score assigned to each segment at baseline and during stress based on the degree of thickening. For this semi-quantitative assessment, each segment is scored according to a four-point scale: 1 = normal, 2 = hypokinesis, 3 = akinesis, 4 =

dyskinesis. A wall motion score index was calculated by adding the numerical value assigned to each segment and dividing by the number of visualised segments.

The extent of ischaemia is based on the number of affected segments, the occurrence of ischaemia at an early stage of the test, (Segar et al. 1992) the wall motion score index at rest and peak stress, (Gunalp et al. 1993) and a slow recovery time (Picano et al. 1989). Global LV ejection fraction and end systolic volumes may also be calculated at baseline and at peak stress. A fall in either of these parameters is an indicator of significant ischaemia.

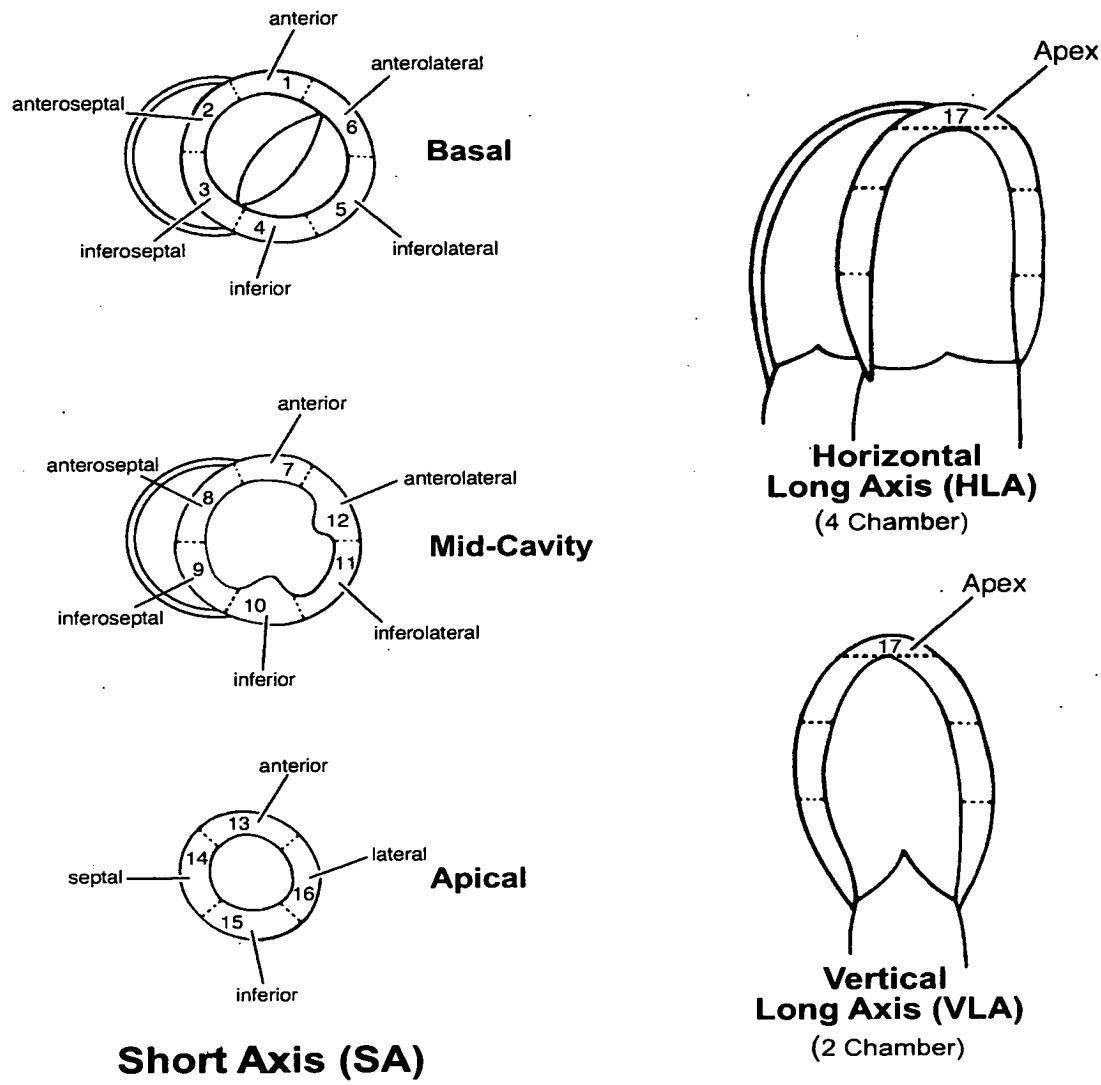

Figure 3.23: Seventeen-segment model of the left ventricle

3.6: ECHOCARDIOGRAPHY MEASUREMENT VALIDITY AND RELIABILITY

Echocardiography is a skilled technique and highly operator dependant. Image quality can vary between individuals due to differences in anatomical orientation of the myocardium, acoustic windows, body composition, and/or disease, such as cardiovascular and respiratory disease. These indiscriminate physical characteristics cause changes in tissue density and the degree of ultrasound penetration, which may introduce error into measurements.

Previous researches have reported small CV values for intra and inter-observer reliability in echocardiography assessment, which demonstrates high reproducibility in the technique (Ladipo et al. 1980; Otterstad et al. 1997; Pollick et al. 1983; Stefadouros and Canedo 1977). The reliability of the echocardiography measurements within this thesis was assessed via repeated measurement of LVEDD, LVESD, E, A, E/A ratio, A Dur, Vp, LVEF; STD, PWTD, PWTS, and LA size in 35 patients (table 3.2).

Table 3.2: Echocardiography Coefficient of Variation Values for Sonographer 1 and 2.

	Coefficient of Variation	
Cardiac Index	Sonographer 1	Sonographer 2
LVEDD	5.6%	5.4%
LVESD	5.2%	5.9%
E	4.1%	4.2%
A	4.2%	4.5%
E/A ratio	4.3%	4.3%
A Dur	4.7%	3.4%
Vp	6.1%	5.2%
LVEF	2.9%	3.3%
STD	2.2%	2.4%
PWTD	3.1%	5.5%
PWTS	4.4%	5.6%
LA	1.7%	1.9%

Note: LVEDD = Left ventricular end diastolic diameter; LVESD = Left ventricular end systolic diameter; E = Early diastolic peak velocity; A = Late diastolic peak velocity; E/A ratio = Ratio of early to late transmitral filling; A Dur = A wave duration; Vp = Flow propagation velocity; LVEF = Left ventricular ejection fraction; STD = End diastolic septal wall thickness; PWTD = End diastolic posterior wall thickness; PWTS = End systolic posterior wall thickness; LA = Left atrium.

3.7: STATISTICAL ANALYSIS

The empirical work within this thesis is deliberately diverse in their methods of data treatment, analysis, and most of all in terms of the populations they represent. As such the statistical tests performed for each study chapter were in line with the hypothesis being tested. All statistical analysis was performed using the statistical package for social sciences (SPSS 17 release version of SPSS for Windows; SPSS Inc., Chicago IL, USA) and an alpha level of 0.05 was considered indicative of a statistically significant difference ($p < 0.05$). Continuous variables were expressed as mean ± SD.

3.8: SUMMARY OF PROTOCOL

On arrival patients were provided with an informed consent form and an information sheet describing the study with an opportunity to ask any questions. Patient physical characteristics, including age, height, and weight were recorded. In addition, cardiovascular disease risk factors and drug history were recorded from case notes and from a brief interview with each participant. Venous cannulation was performed no less than 30-minutes before recording any autonomic data due to the possible confounding influence vena puncture may have on autonomic function and in line with manufacture recommendations. Cannulation is primarily for the DSE protocol, but also provided the opportunity to acquire blood samples (Chapter 6). Following blood sampling, patients were set up and positioned for their DSE and autonomic function study (see figure: 3.24 for experimental setup). Resting autonomic and haemodynamic function were recorded

for 15-minutes before administration of dobutamine in order to obtain true resting measures. Intervention marks were set at baseline, at each incremental dose of dobutamine infusion (10, 20, 30, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$), and in recovery. Heart rate variability and haemodynamic data recorded by the TFM was sampled at the final 10-seconds of each intervention mark, and the mean of this interval data was calculated and subsequently used for statistical analysis. Echocardiography images were recorded at baseline, low dose dobutamine, peak dose dobutamine and in recovery.

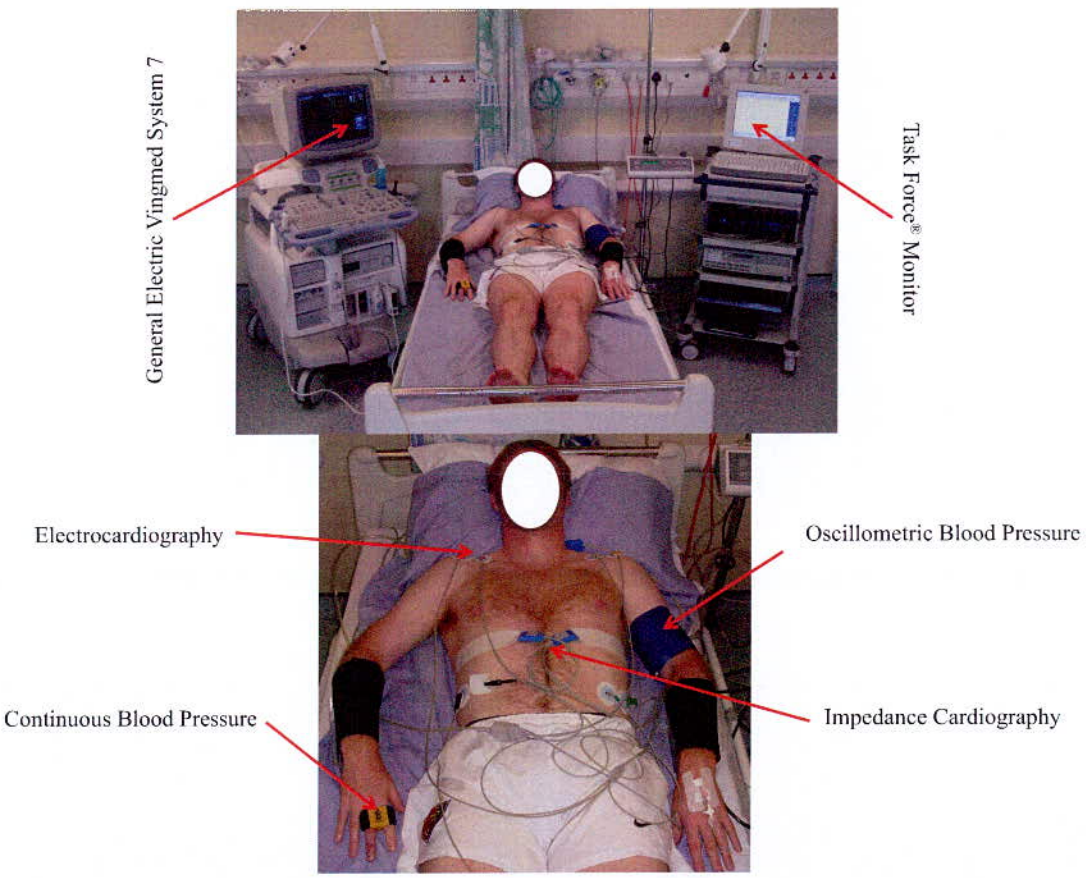

Figure 3.24: Experimental setup.

CHAPTER 4: BASELINE POPULATION

This was a prospective observational study. We recruited three hundred and fifty consecutive patients who were referred for dobutamine stress echocardiography (DSE) from Ealing NHS Trust Hospital's cardiology department. All patients invited participated in the study and there were no exclusions. The baseline characteristics of these patients are described in this chapter. Patient selection and the collection of demographic data are described in Chapter 3: General Methods.

This study complies with the Declaration of Helsinki and had full approval from the local ethical committee. Informed consent was taken from all participating patients.

4.1: PATIENT CHARACTERISTICS

In total, 350-consecutive patients who underwent DSE examination with concomitant autonomic and haemodynamic assessment were studied. Due to incomprehensible autonomic trace recordings, poor echocardiographic image quality, and/or the need to use atropine for clinical test reasons, 36-patient recordings were excluded from the final data analysis, leaving a sample size of 314. Baseline patient characteristics are shown in Table 4.1, coronary angiography results in Table 4.2, and Table 4.3 details the haematological data for all patients. Mean age for all patients was 64.6 ± 11.4 years with 51.9% male (Figure 4.1 illustrates the age and gender distribution of all patients). The

ethnic division of all patients is shown in Figure 4.2 and 4.3, with the majority of patients being Indian Asian (58.3%).

Table 4.1: Baseline Characteristics of all Patients

Parameter	Mean ± SD
Age (Years)	64.6 ± 11 (33 - 89)
Gender	Male: 164 (52.2%)
	Female: 150 (47.8%)
Ethnicity	Asian: 183 (58.3%)
	Caucasian: 101 (32.2%)
	Black: 27 (8.6%)
	Other: 3 (1%)
Height (cm)	164.5 ± 9.7
Weight (kg)	77.9 ± 17.8
Body Surface Area (m^2)	1.8 ± 0.2
Patients with no documented disease	29 (9.2%)
Renal Disease	173 (55.1%)
Hypertension	114 (36.3%)
Hypercholesterolaemia	66 (21%)
Diabetes	59 (18.8%)
FHx of CVD	24 (7.6%)
Exercise Test	Normal 5 (1.6%)
	Abnormal 4 (1.3%)
	Inconclusive 38 (12.1%)
Current Smoker	9 (2.9%)
Ex-smoker	2 (0.6%)
Previous PCI	64 (20.4%)
Previous CABGS	43 (13.7%)
Previous MI	17 (5.4%)
Positive DSE	62 (19.7%)

FHx of CVD = Family history of cardiovascular disease; PCI = Percutaneous coronary intervention; CABGS = Coronary artery bypass graft surgery; MI = Myocardial infarction; DSE = Dobutamine stress echocardiogram.

Table 4.2: Coronary Angiography Results

Parameter	Number
Angiogram	82 (26.1%)
• Normal	19 (23.2%)
• Mild Disease	6 (7.3%)
• Moderate Disease	17 (20.7%)
• Severe Disease	40 (48.8%)
• Normal Coronary Artery Vessels	19 (23.2%)
• Single Vessel Disease	14 (17.1%)
• Double Vessel Disease	25 (30.5%)
• Triple Vessel Disease	24 (29.3%)

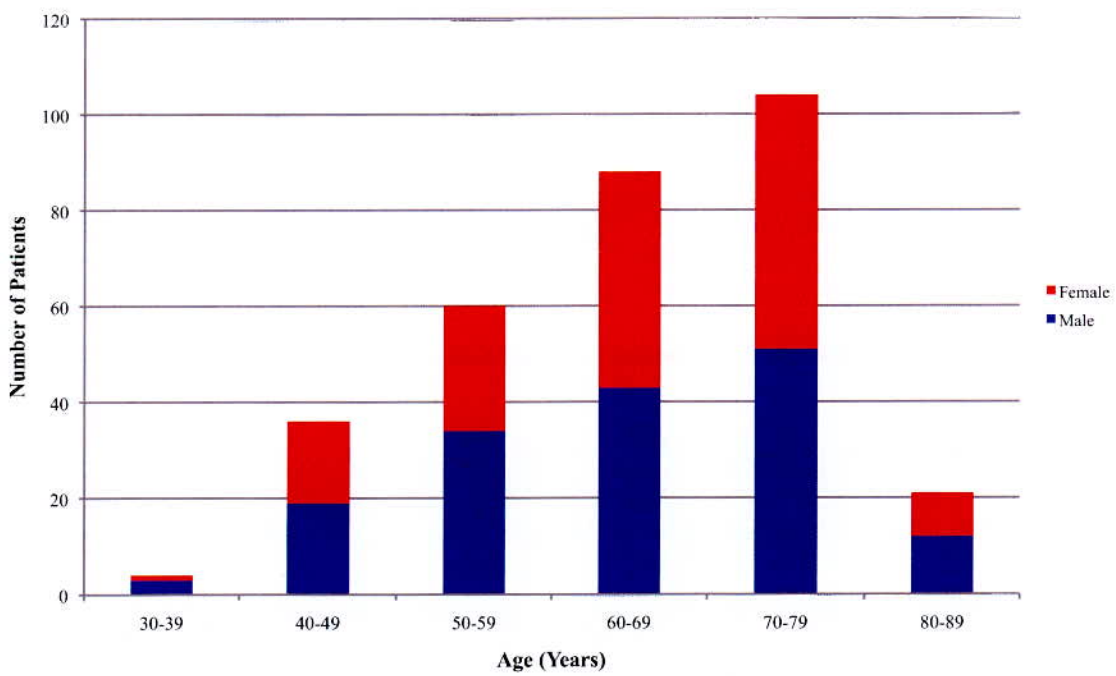

Figure 4.1: A column chart to illustrate the age and gender distribution of all patients.

Table 4.3: Haematological Data for all Patients

Parameter	Mean ± SD
Haemoglobin ($g \cdot dL^{-1}$)	12.6 ± 1.8
White Cell Count ($x10^9 \cdot L^{-1}$)	7.7 ± 2.7
Platelets ($x10^9 \cdot L^{-1}$)	261.3 ± 81
Fibrinogen ($g \cdot L^{-1}$)	3.2 ± 1
INR	1.3 ± 0.8
Sodium ($mmol \cdot L^{-1}$)	138.9 ± 4.5
Potassium ($mmol \cdot L^{-1}$)	4.6 ± 0.5
Urea ($mmol \cdot L^{-1}$)	7.3 ± 4.3
Creatinine ($\mu mol \cdot L^{-1}$)	66 ± 59.8
Estimated GFR ($ml \cdot min^{-1} \cdot 1.73m^2$)	108 ± 42.8
Corrected Calcium ($mmol \cdot L^{-1}$)	2.27 ± 0.1
Glucose ($mmol \cdot L^{-1}$)	5.7 ± 4
Triglycerides ($mmol \cdot L^{-1}$)	1.6 ± 1
Cholesterol ($mmol \cdot L^{-1}$)	4.2 ± 1.2
High Density Lipoprotein ($mmol \cdot L^{-1}$)	1.3 ± 0.5
Low Density Lipoprotein ($mmol \cdot L^{-1}$)	2.4 ± 1
C-Reactive Protein ($mg \cdot L^{-1}$)	8.2 ± 4.3
Erythrocyte Sedimentation Rate (ESR) ($mm \cdot hr^{-1}$)	23 ± 19.3
Thyroid Stimulating Hormone ($mlU \cdot L^{-1}$)	3 ± 4.7
Free Thyroxine ($pmol \cdot L^{-1}$)	15.5 ± 2.9
Haemoglobin A1c (DCCT aligned) (% Total Hb)	6.1 ± 3.6
Serum Creatine Kinase ($IU \cdot L^{-1}$)	123.6 ± 85

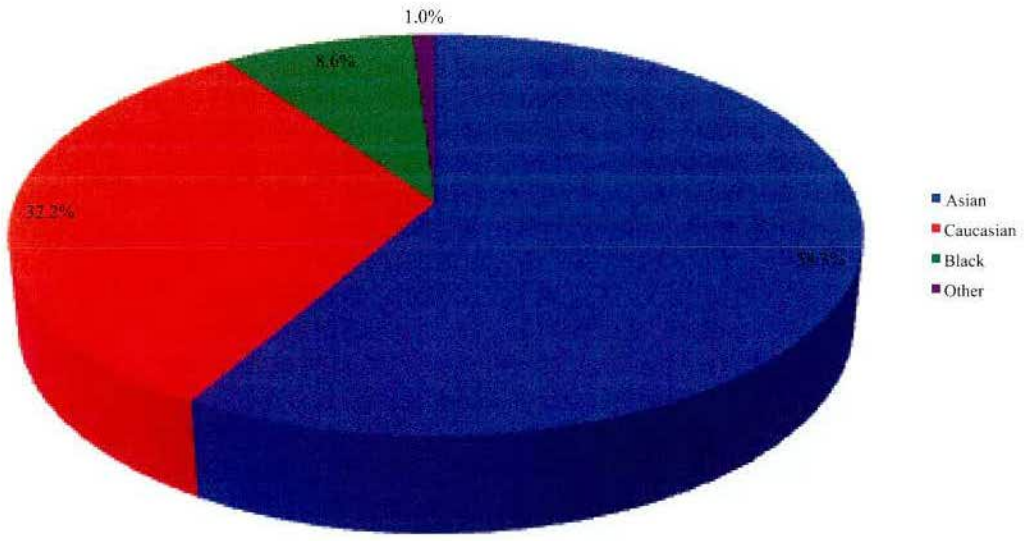

Figure 4.2: A pie chart to illustrate the ethnic division of all patients.

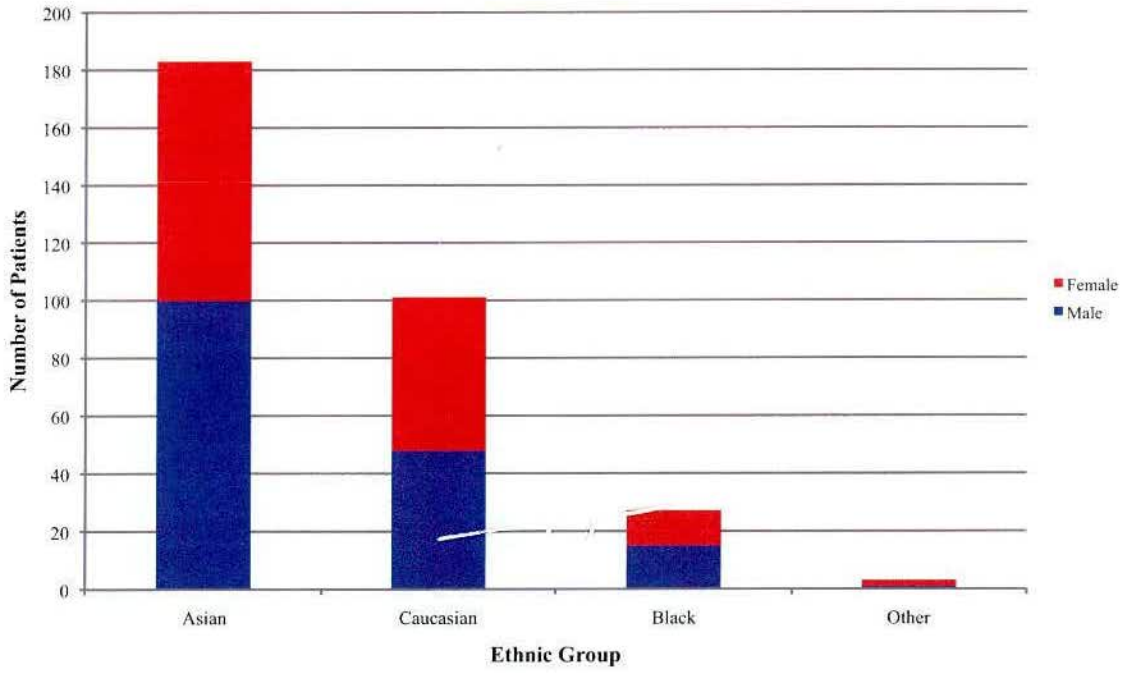

Figure 4.3: A column chart to illustrate the ethnic and gender division of all patients.

Renal disease was the main cardiovascular risk factor (55.1), followed by hypertension (36.3%), raised cholesterol (21%), diabetes (18.8%), a positive family history of cardiovascular disease (7.6%) and current or previous smoker (2.9% and 0.6% respectively). Figure 4.4 illustrates the distribution of cardiovascular disease risk factors for all patients. In addition, 17-patients suffered a previous myocardial infarction (MI) (5.4%), 43-patients had previous coronary artery bypass graft surgery (CABGS) (13.7%) and 64-patients had previous percutaneous coronary intervention (PCI) (20.4%). Figure 4.5 illustrates the number and gender distribution of patients with previous MI, CABGS, and PCI.

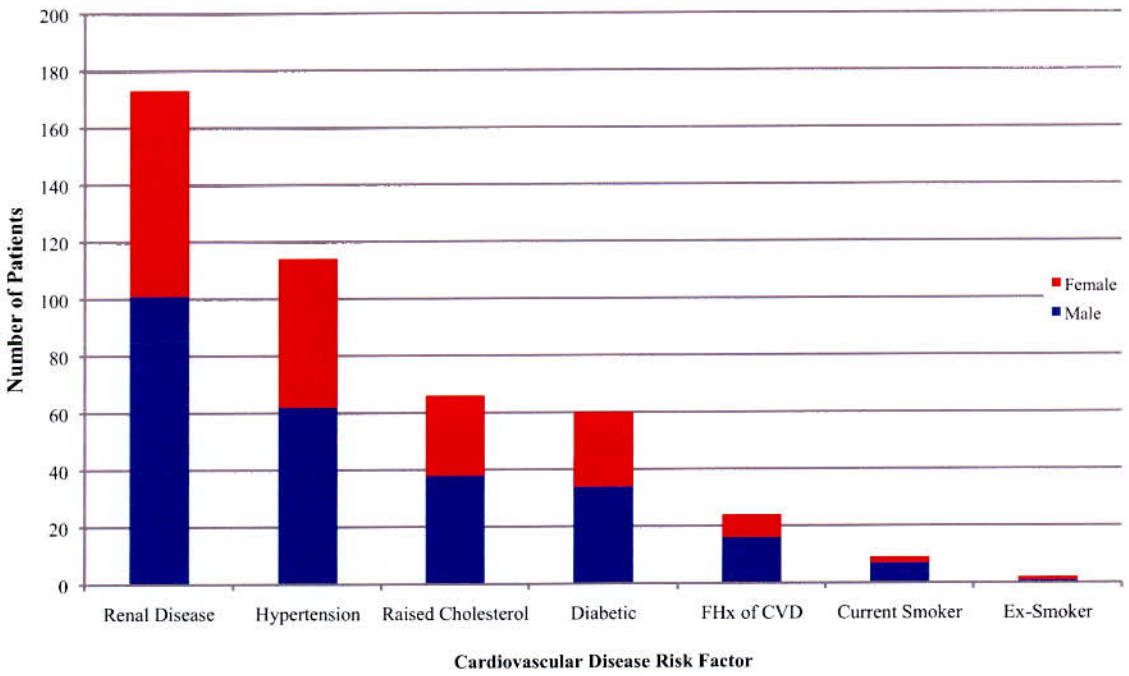

Figure 4.4: A column chart to illustrate the distribution of cardiovascular disease risk factors for all patients. Note: FHx = Family history; CVD = Cardiovascular disease.

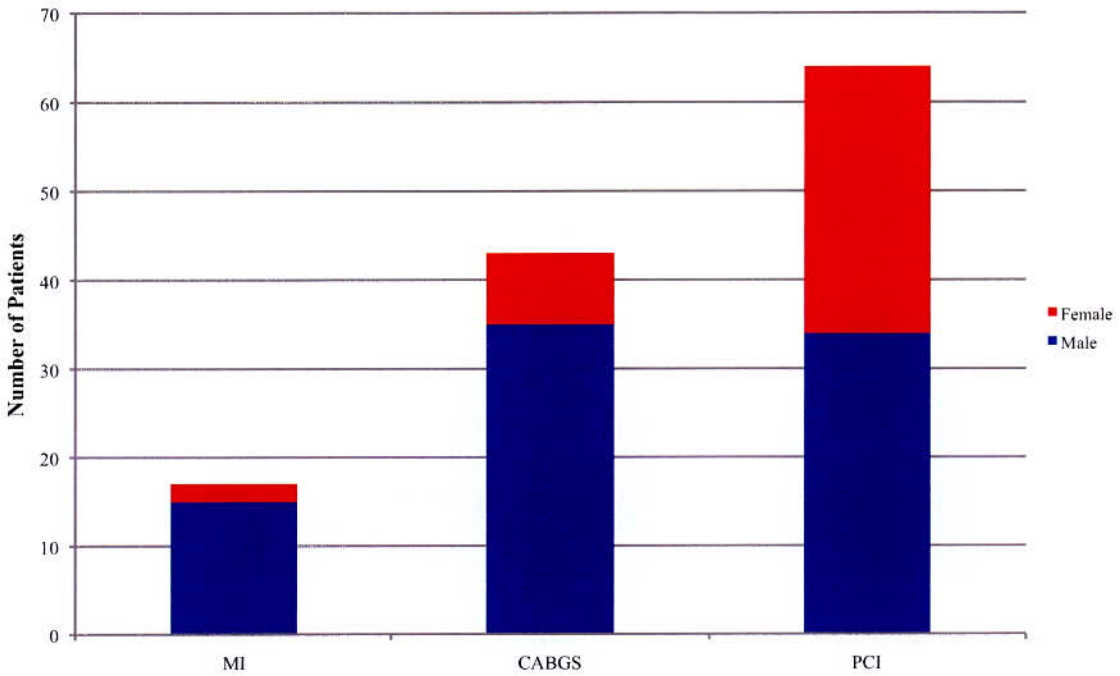

Figure 4.5: A column chart to illustrate the number and gender distribution of patients with previous myocardial infarction (MI), coronary artery bypass graft surgery (CABGS), and percutaneous coronary intervention (PCI).

Eighty-two patients (26.1%) underwent coronary angiography. Nineteen patients (23.2%) had no disease, 6-patients (7.3%) had mild disease, 17-patients (20.7%) had moderate disease and 40-patients (48.8%) had severe disease (Figure 4.6). Of the 57-patients (69.5%) who had coronary artery disease, 14-patients (17.1%) had single vessel disease, 25-patients (30.5%) had double vessel disease, and 24-patients (29.3%) had triple vessel disease (Figure 4.7). Haematological data (table 4.3) demonstrates that the results for all patients are within normal limits with the exception of C-Reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR), which are both markers of inflammation. These results could be due to the large number of patients with renal disease and impact this has on heart rate variability will be addressed in chapter 6.

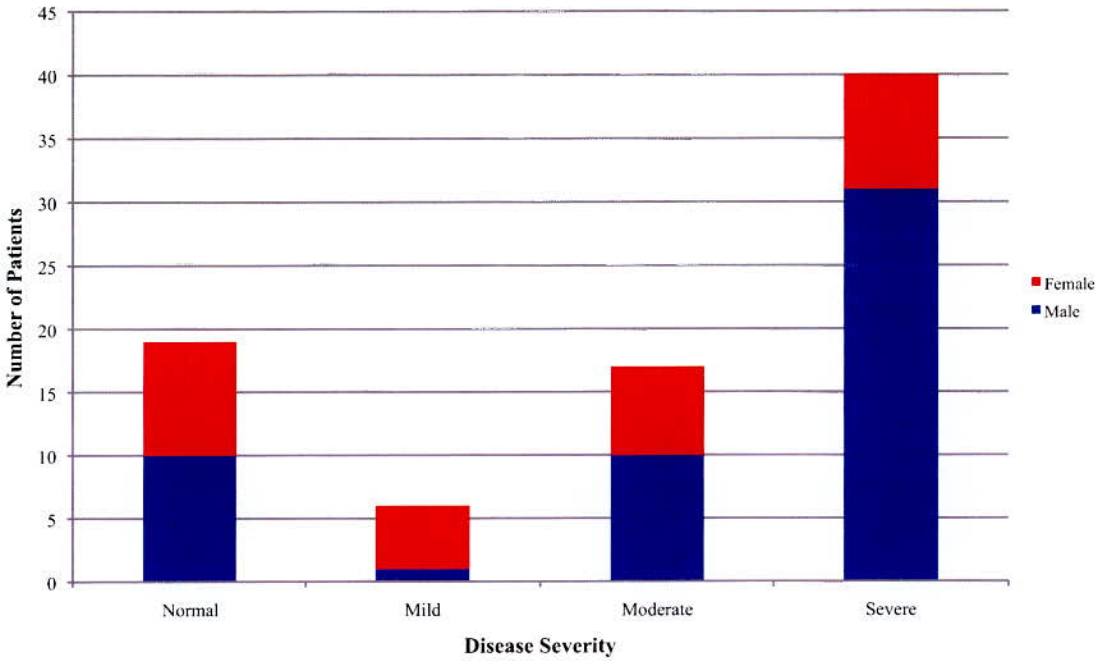

Figure 4.6: A column chart to illustrate the disease severity and gender distribution of all angiogram patients.

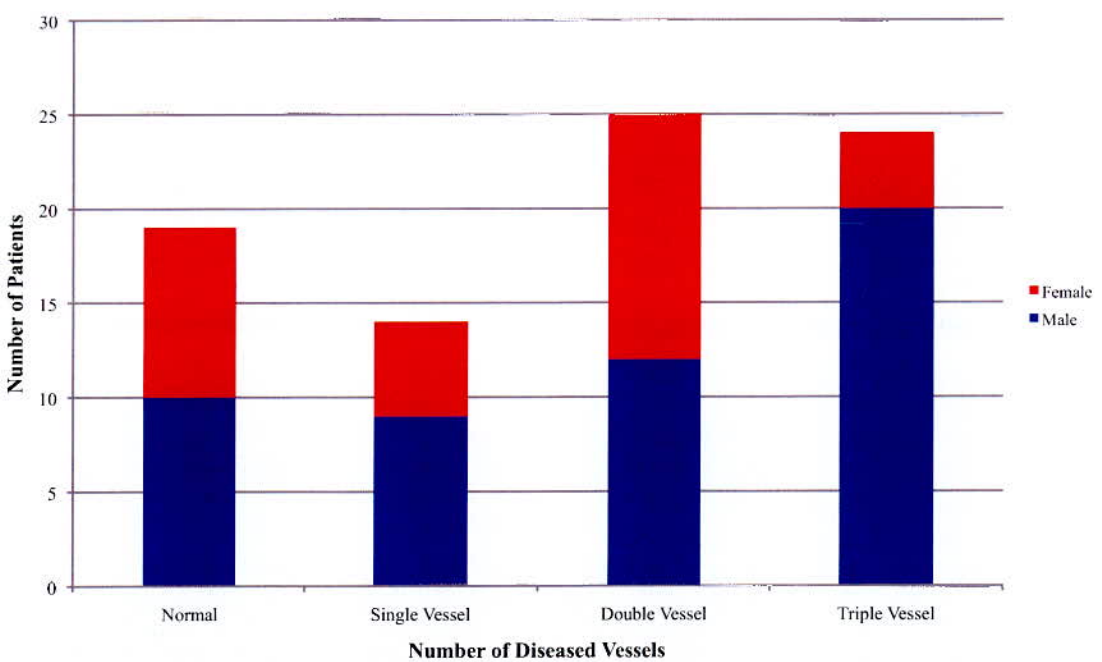

Figure 4.7: A column chart to illustrate the vessel disease and gender distribution of all angiogram patients.

4.2: HAEMODYNAMIC AND AUTONOMIC FUNCTION DATA

Table 4.4 shows the baseline, low dose dobutamine, peak dose dobutamine, and recovery haemodynamic and autonomic function data. Heart rate increased from 74 $b \cdot min^{-1}$ to 143 $b \cdot min^{-1}$ and blood pressure increased from 133/88 mmHg to 154/91 mmHg from baseline to peak pharmacological stress. Heart rate and blood pressure decreased to 98 $b \cdot min^{-1}$ and 139/89 mmHg after 10-minutes of recovery respectively (figure 4.8 and 4.9). Stroke index (SI) decreased from 33.4 $ml \cdot m^2$ to 27.4 $ml \cdot m^2$ and cardiac index (CI) increased from 2.4 $L \cdot min^{-1} \cdot m^2$ to 3.9 $L \cdot min^{-1} \cdot m^2$ from baseline to peak. Stroke index was maintained after 10-minutes of recovery while CI decreased to 2.68 $L \cdot min^{-1} \cdot m^2$ (figure 4.10). Heart rate variability (PSD) decreased from 2887.2 ms^2 to 568.2 ms^2 from baseline to peak dose dobutamine and increased to 622.3 ms^2 at end recovery. Total peripheral resistance index (TPRI) decreased from 3582 $dynes \cdot s \cdot m^2 \cdot cm^5$ to 2281 $dynes \cdot s \cdot m^2 \cdot cm^5$ from baseline to peak dose and then increased to 3311 $dynes \cdot s \cdot m^2 \cdot cm^5$ in recovery (figure 4.11). Low frequency (nu) oscillations of HRV, a reflection of sympathetic modulation, increased from 46.5% to 51% from baseline to low dose dobutamine (10 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and then decreased to 43.9% at peak dose dobutamine. High frequency (nu) oscillations of HRV, a reflection of parasympathetic nervous control, decreased from 53.5% to 49% from baseline to low dose and then increased to 56.1% at peak dose (figure 4.12). These frequency oscillations describe an initial increase in sympathetic activity and withdrawal of parasympathetic modulation from baseline to low dose, and then a withdrawal of sympathetic drive and greater activation of parasympathetic modulation at peak dose dobutamine.

Table 4.4: Haemodynamic and autonomic response to dobutamine stress in all patients.

Parameter	Baseline	10 $\mu g \cdot kg^{-1} \cdot min^{-1}$	Peak	3-min Recovery	10-min Recovery
HR ($b \cdot min^{-1}$)	73.5 ± 14.5	81.6 ± 17.7	142.7 ± 17.9	128.5 ± 18.5	98.2 ± 12.7
RRI (ms)	845.6 ± 156.1	768.4 ± 159.8	428.4 ± 66.4	479 ± 87.9	622.3 ± 88.8
PSD (ms^2)	2887.2 ± 1352.1	2377.3 ± 388.5	568.2 ± 71.7	396.5 ± 41.8	617.7 ± 84.4
sBP (mmHg)	133 ± 38.1	146.7 ± 31.1	154.4 ± 35.1	165.3 ± 32.1	139 ± 25.7
dBP (mmHg)	87.7 ± 16	87.9 ± 19.5	90.6 ± 21.6	93.6 ± 18.9	89.1 ± 16.7
SI ($ml \cdot m^2$)	33.4 ± 9.8	32.5 ± 9.9	27.4 ± 7.4	28.1 ± 8.6	27.4 ± 8.3
CI ($L \cdot min^{-1} \cdot m^2$)	2.42 ± 0.73	2.61 ± 0.84	3.9 ± 1.1	3.59 ± 1.1	2.68 ± 0.84
TPRI ($dyne \cdot s \cdot m^2 \cdot cm^5$)	3582 ± 1230	3369.8 ± 1212	2281 ± 747.5	2606.2 ± 824	3311 ± 1006
LF (nu)	46.5 ± 23.4	51 ± 21.2	43.9 ± 18.6	29.9 ± 19.8	37 ± 29
HF (nu)	53.5 ± 23.4	49 ± 21.2	56.1 ± 18.6	70.1 ± 19.8	63 ± 29
LF (ms^2)	1150 ± 387.2	852.4 ± 349.5	152.9 ± 74.7	105.7 ± 45.2	176 ± 63
HF (ms^2)	1352.2 ± 547.9	805.4 ± 312.1	222.3 ± 91.3	265 ± 63.7	336.7 ± 81.2
LF/HF Ratio	1.06 ± 1.1	1.63 ± 1.97	1.12 ± 0.58	0.73 ± 1.27	2.2 ± 2.57
BRS ($ms \cdot mmHg^{-1}$)	13.9 ± 7.79	10.4 ± 6.18	6.9 ± 5.4	-	8.6 ± 6

Note: HR = Heart rate; RRI = RR interval; PSD = Power spectral density; sBP = Systolic blood pressure; dBP = Diastolic blood pressure; SI = Stroke index; CI = Cardiac index; TPRI = Total peripheral resistance index; LF (nu) = Normalised units low frequency; HF (nu) = Normalised units high frequency; LF (ms^2) = Low frequency power; HF (ms^2) = High frequency power; LF/HF Ratio = Low to high frequency ratio; BRS = Baroreceptor reflex sensitivity.

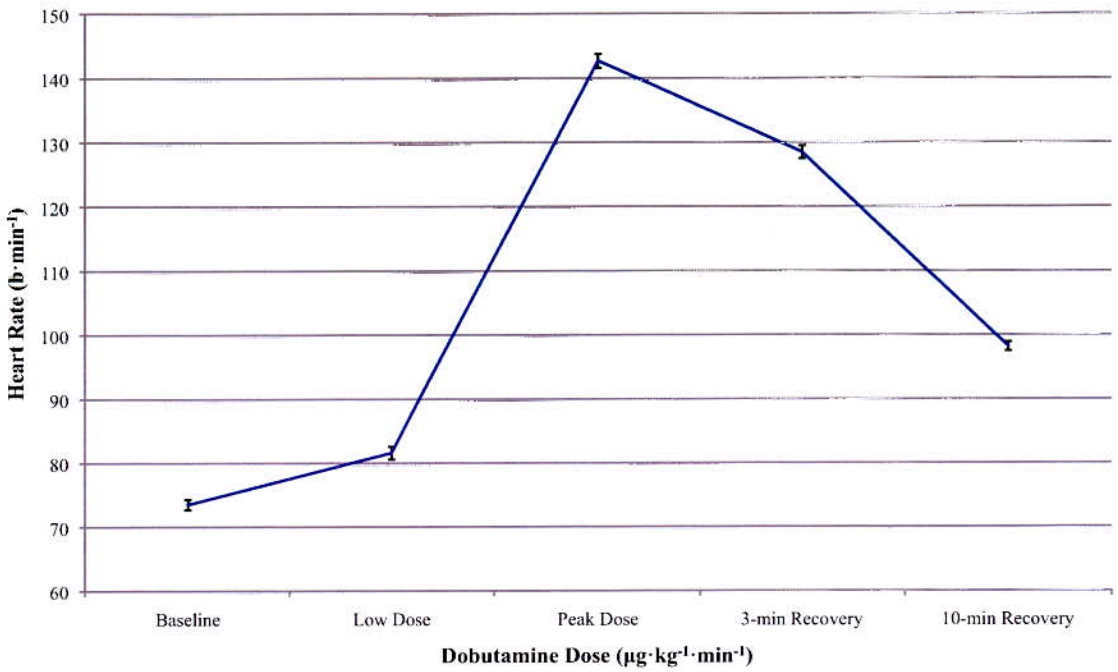

Figure 4.8: Heart rate response to dobutamine infusion in all patients.

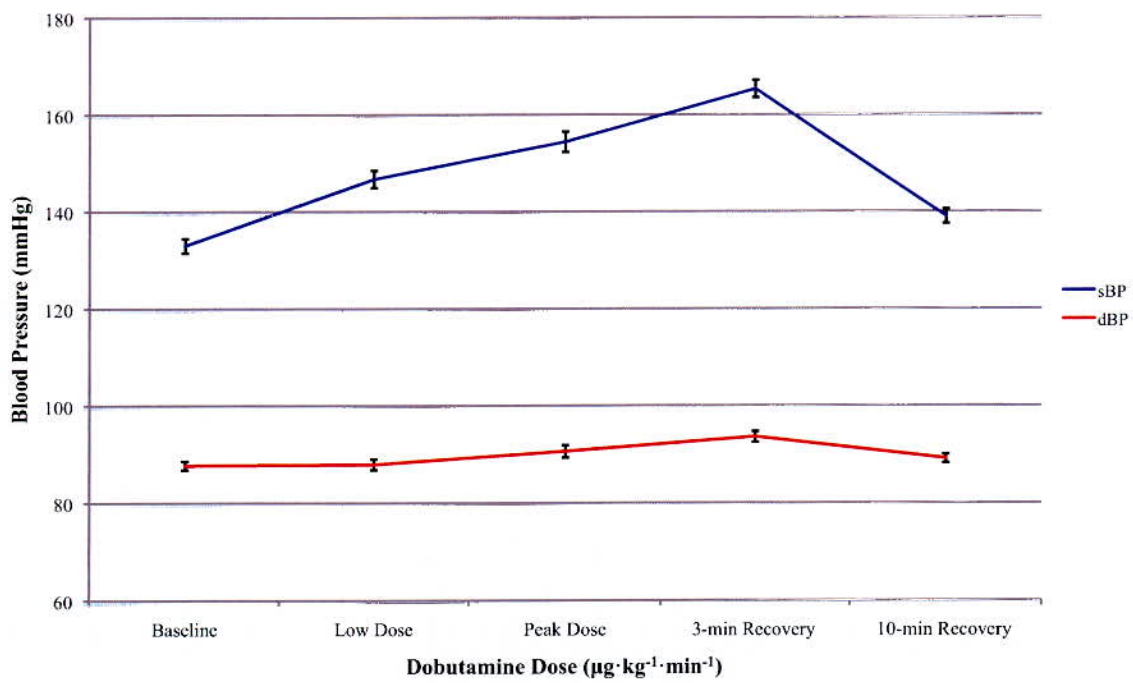

Figure 4.9: Blood pressure response to dobutamine in fusion in all patients.

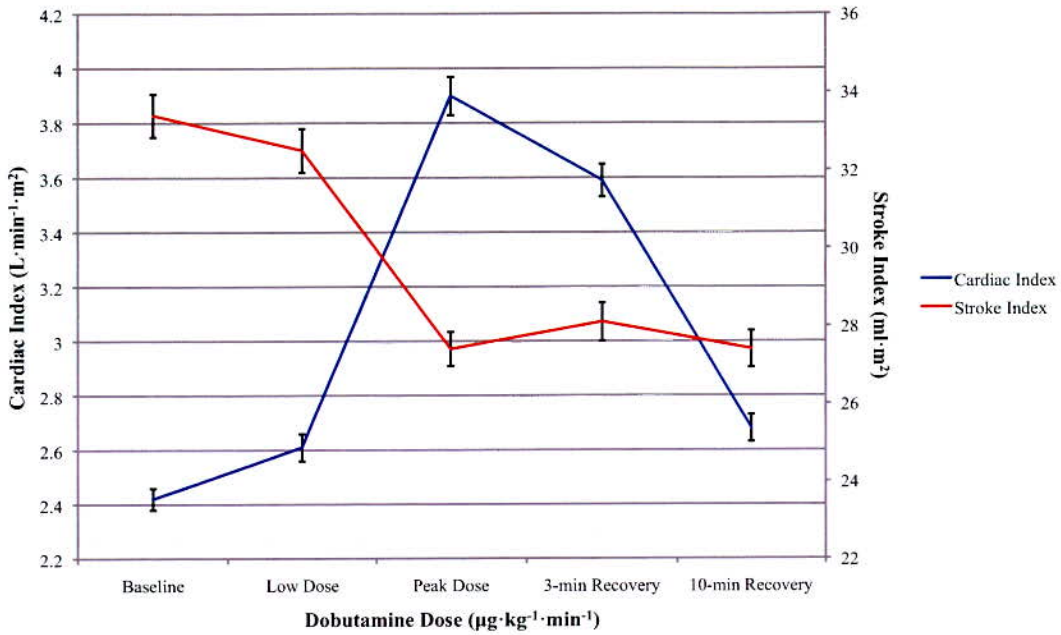

Figure 4.10: Cardiac index and stroke index response to dobutamine infusion in all patients.

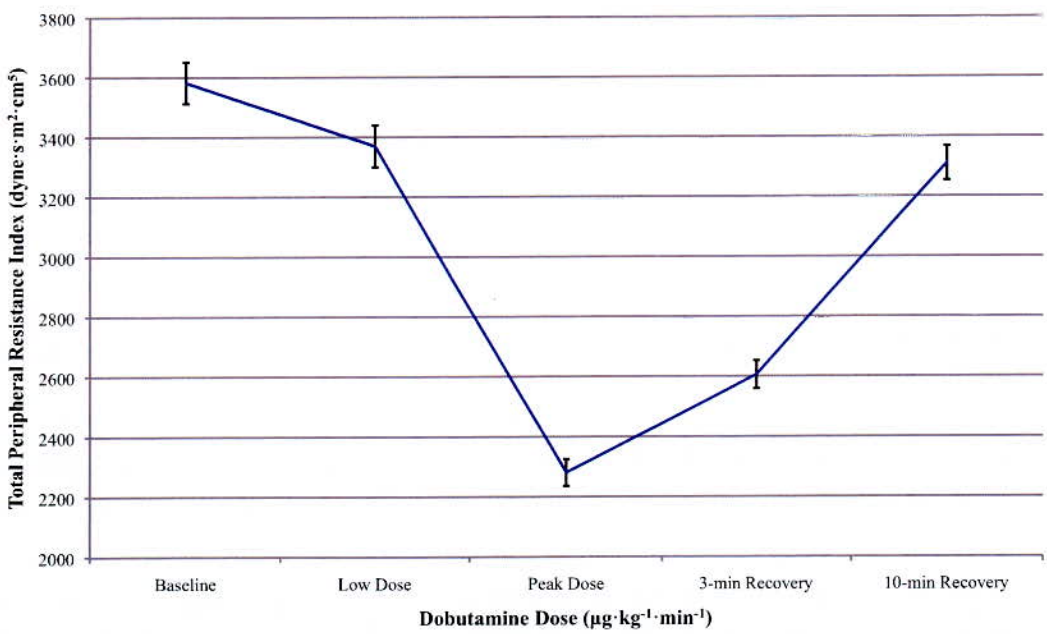

Figure 4.11: Total peripheral resistance index response to dobutamine infusion in all patients.

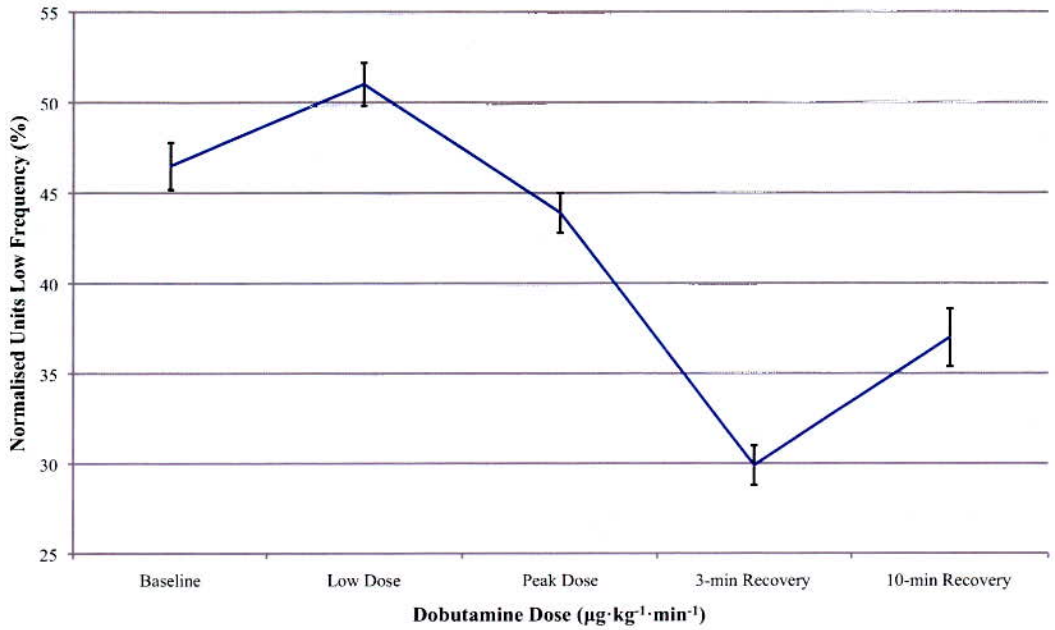

Figure 4.12: Low frequency (nu) response to dobutamine infusion in all patients.

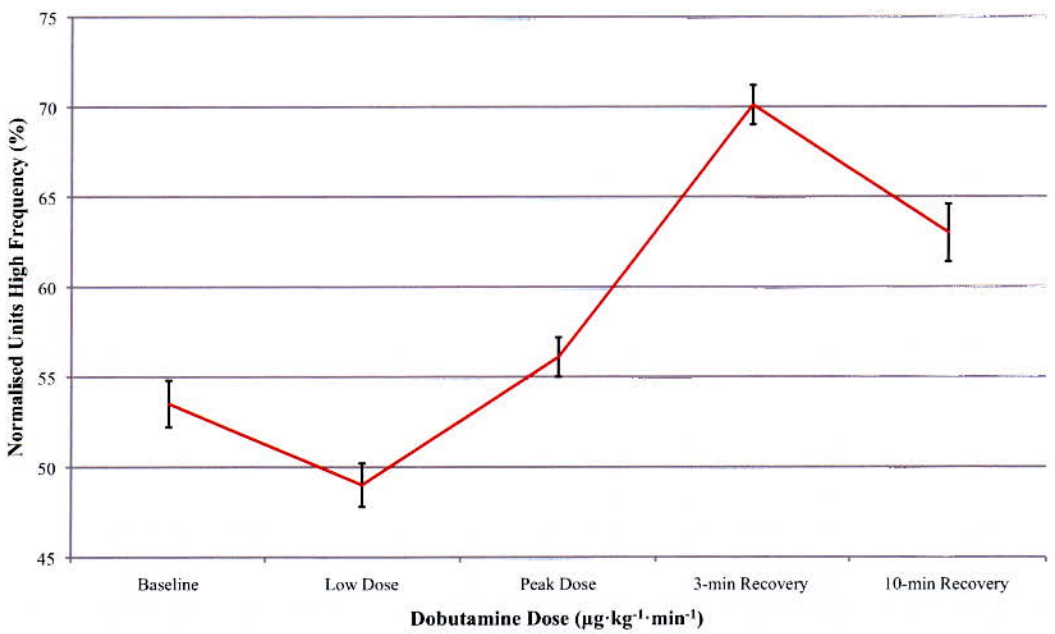

Figure 4.13: High frequency (nu) response to dobutamine infusion in all patients.

4.3: BASELINE AND STRESS ECHOCARDIOGRAPHY DATA

Table 4.5: Baseline Echocardiographic Data

Parameter	Mean ± SD
LVESD (cm)	3.1 ± 0.6
LVEDD (cm)	5.2 ± 1.8
LVFS (%)	26 ± 12
LVEF (%)	58 ± 25
LA (cm)	3.4 ± 1.3
LVMI ($g \cdot m^2$)	116 ± 39
LV wall thickness	1.1 ± 0.5
Mitral E/E'	10 ± 2
Resting WMSI	1.2 ± 0.2

Note: LVESD = Left ventricular end systolic diameter; LVEDD = Left ventricular end diastolic diameter; LVFS = Left ventricular fractional shortening; LVEF = Left ventricular ejection fraction; LA = Left atrium; LVMI = Left ventricular mass index; LV = Left ventricle; Mitral E/E' = Early mitral annular velocity; WMSI = Wall motion score index.

Table 4.6: Stress Echocardiographic Data

Parameter	Mean ± SD
Peak LVEF (%)	69 ± 22
Dobutamine Dose ($\mu g \cdot kg^{-1} \cdot min^{-1}$)	30.5 ± 15
Heart Rate	144 ± 33
Peak WMSI	1.4 ± 0.5

Note: LVESD = LVEF = Left ventricular ejection fraction; WMSI = Wall motion score index.

4.4: SUMMARY

The general haemodynamic response during dobutamine stress echocardiography (DSE) is an increased heart rate (94.1% increase) and systolic blood pressure (16.1%). The modest increase in systolic blood pressure was largely due to a 36.3% drop in total peripheral resistance index. In addition, cardiac index increased (61.2%) and stroke index decreased (18%) during DSE. The decreased stroke index is largely due to a decreased left ventricular filling time and the increased cardiac index is due to an increased heart rate, since cardiac output is equal to heart rate times stroke volume (figure 4.14 and 4.15).

$$\dot{Q} = SV \times HR$$

Figure 4.14: Equation to calculate cardiac output. Note: $\dot{Q}$ = Cardiac Output; SV = Stroke Volume; HR = Heart Rate

$$\frac{\dot{Q}}{BSA} = \frac{SV \times HR}{BSA}$$

Figure 4.15: Equation to calculate cardiac index. Note: BSA = Body Surface Area.

The general response of autonomic nervous modulation during DSE is an initial increase in LF (nu) oscillations (sympathetic activity) and a withdrawal of HF (nu) oscillations (parasympathetic control) from baseline to low dose dobutamine. From low dose dobutamine to peak dose dobutamine there is an increase in HF (nu) oscillations and

decrease in LF (nu) oscillations, which continues into early recovery. At the end of recovery LF (nu) and HF (nu) oscillations begin to stabilise and return to baseline levels.

The relative increased number of patients with significant coronary artery disease in patients who underwent coronary angiography, is reflective of the fact that coronary angiography was requested on clinical grounds and generally requested on the basis of an abnormal DSE or for patients with suspicion of coronary artery disease. Of the 82-patients who underwent coronary angiography 62-patients had significant coronary artery disease and 1-patient had minor disease. The remaining 19-patients had normal coronary arteries. Of the 62-patients who had a positive DSE, 61-patients had significant coronary artery disease. Low risk patients with a normal DSE generally did not have an angiogram.

CHAPTER 5: EFFECT OF DECLINING LEFT VENTRICULAR SYSTOLIC AND DIASTOLIC FUNCTION ON AUTONOMIC MODULATION

5.0: ABSTRACT

BACKGROUND: Disturbances in cardiovascular variability influences both disease progression and survival in patients with declining cardiac function. This study aimed to compare parameters of autonomic function measured non-invasively using heart rate variability (HRV) in a cohort of patients with impaired cardiac function. The results may provide further insight into mechanisms responsible for attenuated HRV in patients with cardiac dysfunction.

METHODS: 86 patients with impaired left ventricular systolic function (LVSF) (32 mild [mean age 68 ± 9 years, 20 male], 29 moderate [mean age 68 ± 11 years, 17 male], and 25 severe [mean age 71 ± 9 years, 19 male] LVSF) and 29 normal patients. Haemodynamic and autonomic data were obtained from beat-to-beat analysis of heart rate and blood pressure using a plethysmographic device, the Task Force® Monitor. We thereby determined total power spectral density (PSD) and associated low frequency (LF) and high frequency (HF) power spectral components in absolute (ms^2) and normalised units (nu). Cardiac structure and function was quantified using echocardiography.

RESULTS: Patients with impaired LVSF had a significantly reduced PSD ($p < 0.001$, for mild, moderate, and severe LVSF) compared to normal patients. Left ventricular end systolic diameter, left ventricular end diastolic diameter, left ventricular ejection fraction, left atrial diameter, mitral E/E', peak systolic velocity, pulmonary arterial reversal velocity, pulmonary-mitral atrial wave duration and flow propagation velocity are all associated with a reduced PSD. However, only peak systolic velocity ($\beta = -7.4 \pm 3.9$, $p < 0.05$) and mitral E/E' ($\beta = 12.2 \pm 4.2$, $p < 0.001$) are independently associated with a reduction in PSD.

CONCLUSION: Power spectral density significantly attenuates as left ventricular (LV) systolic and diastolic function declines. Long axis LV function and LV filling pressure are independently associated with a reduced PSD.

5.1: INTRODUCTION

Heart failure affects millions of individuals worldwide with a mortality rate 4-8 times that of the age-matched population (Kannel 2000; van Jaarsveld et al. 2006). Prognosis remains a major concern for both acute (McMurray et al. 2007) and chronic (Kjekshus et al. 2007) heart failure despite advances in therapeutic options and among patients with severe chronic heart failure (CHF) the risk of death remains 30% to 60% annually (Hosenpud et al. 1994; Nolan et al. 1998; Stevenson et al. 1995). Predictors of mortality in CHF include left ventricular ejection fraction (LVEF) (Gradman et al. 1989), functional capacity (van den Broek et al. 1992), plasma neurohormones (Cohn et al. 1984), renal function (Hillege et al. 2006), ventricular arrhythmias, and heart rate variability (HRV) (Nolan et al. 1998; Smilde et al. 2009). The commonest cause of mortality is sudden cardiac death (SCD) due to ventricular arrhythmias (Deedwania 1994; Zhou et al. 2009). A change in cardiac electrophysiology provides support for the incidence of ventricular arrhythmias in CHF patients. Experimental and clinical findings have demonstrated that ventricular action potential duration and ventricular effective refractory period are increased with CHF (Akar and Rosenbaum 2003; Li et al. 1992). This is related to many factors including scar, electrolyte disturbance, and autonomic dysfunction.

Left ventricular systolic dysfunction (LVSD) affects approximately 3% of the adult population (McDonagh et al. 1997; Mosterd et al. 1997). However, half of this population are asymptomatic and can be identified only by objective methods, normally

echocardiography (Nielsen et al. 2000). Identification and treatment of patients with LVSD improves survival and reduces morbidity (Bristow et al. 1996; CIBIS-II-Investigators 1999; SOLVD-Investigators 1992). Approximately half of all patients with clinically diagnosed heart failure have preserved left ventricular systolic function (LVSF) (Persson et al. 2007), which suggests that left ventricular diastolic dysfunction (LVDD) may be a cause for their clinical symptoms. Indeed, research demonstrated that CHF patients with preserved LVSF have hospitalisation and mortality rates comparable to patients with LVSD (Tsutsui et al. 2001).

Chronic heart failure is a complex multifactorial disease characterised by alterations in autonomic cardiovascular control, principally sympathetic nervous system activation (Floras 2009), which has implications for both disease progression and survival (Cohn et al. 1984; Kaye et al. 1995). Other signs and symptoms of CHF include inadequate tissue perfusion, fluid retention, skeletal muscle abnormalities, abnormal immune response, and other neurohormonal reactions (Adamopoulos et al. 2003; Mann 1999). The enhanced sympathetic outflow directed to the heart and peripheral vasculature with a parallel reduction in parasympathetic (vagal) modulation is frequently interpreted as essential in order to maintain haemodynamics including blood pressure and adequate tissue perfusion (Gaffney and Braunwald 1963; Guzzetti et al. 1995). Chronic sympathetic stimulation severely attenuates HRV and has independent prognostic value for mortality in CHF patients (La Rovere et al. 2003; Nolan et al. 1998; Smilde et al. 2009). Indeed, previous research detailed that the lower the HRV the greater the sympathetic activity in patients with heart failure (van de Borne et al. 1997).

Indices of cardiac structure and function derived from echocardiography provide prognostic information in patients with cardiovascular disease. Diastolic dysfunction is a common cardiac disease and Doppler echocardiography techniques has proved useful in determining cardiac filling pressures and prediction of prognosis in a number of patient groups (Hillis et al. 2004; Ommen et al. 2000; Sharma et al. 2006d). Indeed, the mitral peak Doppler E-wave to peak annulus velocity ratio (E/E') correlates with invasive measures of LV filling pressure (Nagueh et al. 1997; Ommen et al. 2000; Sharma et al. 2006d) and predicts mortality in patients with cardiac disease (Hillis et al. 2004; Wang et al. 2003). However, how LV diastolic dysfunction and cardiac mortality are associated is not entirely clear. Furthermore, although it is clear that a reduced HRV has a negative prognostic impact in patients with heart failure, there have been no studies examining the association with cardiac structure and function.

5.1.1: AIM

The aim of this study is to compare parameters of autonomic function measured non-invasively using HRV methodology in a cohort of patients with impaired cardiac function as measured by echocardiography. The results may provide further insight into mechanisms responsible for attenuated HRV in patients with cardiac dysfunction.

5.1.2: HYPOTHESIS

1. H_1: Heart rate variability is significantly associated with declining left ventricular systolic function measured using detailed echocardiography.

2. H_1: Heart rate variability is significantly associated with declining left ventricular diastolic function measured using detailed echocardiography.

5.2: METHOD

5.2.1: PARTICIPANTS

We studied 86 patients with impaired cardiac function and 29 normal patients. Patient selection, sample size calculation and the collection of demographic data are described in Chapter 3: General Methods, and the baseline characteristics of these patients are described in Chapter 4: Baseline Population. The patients were divided into four groups characterised by their cardiac function; normal, mild, moderate, and severe left ventricular systolic function. The patients were divided into groups according to their measured left ventricular ejection fraction (LVEF) by Biplane Simpson's technique. A LVEF of 45-55, 36-44, and ≤ 35% was mild, moderate and severe left ventricular function respectively (British Society of Echocardiography 2008; Lang et al. 2005). Table 5.1 details the baseline physical characteristics of patient groups.

Table 5.1: Baseline Characteristics of Normal and Left Ventricular Dysfunction Patient Groups

Parameter	Normal (n = 29)	Mild LVEF (n = 32)	Moderate LVEF (n = 29)	Severe LVEF (n = 25)
Age (Years)	62 ± 10	68 ± 9	68 ± 11	71 ± 9
Gender	♂ 10	♂ 20	♂ 17	♂ 19
Ethnicity	Asian = 21 (72.4%)	Asian = 16 (50%)	Asian = 13 (44.8%)	Asian = 11 (44%)
	CAU = 7 (24.1%)	CAU = 12 (37.5%)	CAU = 13 (44.8%)	CAU = 9 (36%)
	Black = 1 (3.4 %)	Black = 4 (12.5%)	Black = 3 (10.3 %)	Black = 5 (20%)
	Other = 0 (0 %)	Other = 0 (0 %)	Other = 0 (0 %)	Other = 0 (0%)
Height (cm)	163 ± 11	168 ± 9.1	168 ± 7.5	168 ± 12
Weight (kg)	74 ± 16.7	83.4 ± 14	73.6 ± 13.5	81.2 ± 18
BSA (m^2)	1.8 ± 0.24	1.93 ± 0.18	1.83 ± 0.19	1.9 ± 0.24

Note: LVEF = Left ventricular ejection fraction; CAU = Caucasian; BSA = Body surface area.

5.2.2: AUTONOMIC ASSESSMENT

The Task Force® Monitor (CNSystems, Graz, Austria) was used for the continuous non-invasive beat-to-beat monitoring and real time calculation of all cardiovascular haemodynamic and autonomic parameters. Parameters included; continuous electrocardiography, continuous blood pressure, stroke index, cardiac index, total peripheral vascular resistance index, baroreceptor reflex sensitivity, and HRV as outlined in Chapter 3 General Methods. After 30-minutes of supine rest, 15-minutes of haemodynamic and autonomic data was recorded and automatically calculated for statistical analysis.

5.2.3: ECHOCARDIOGRAPHY

This was performed using a General Electric Vingmed System 7 ultrasound machine. A full study was performed with all measurements of cardiac size, LV systolic and diastolic function as outlined in Chapter 3 General Methods.

5.2.4: STATISTICAL ANALYSIS

Once the data was collated onto a spreadsheet, it was analysed using the statistical package for social sciences (SPSS 17 release version of SPSS for Windows; SPSS Inc., Chicago IL, USA). Continuous variables were expressed as mean ± SD unless otherwise stated. Differences between groups were determined by independent samples t-test. Linear regression analysis was performed to assess the correlation of power spectral density (HRV) as a continuous variable with echocardiographic parameters. Stepwise multiple logistic regression analysis by forward selection was used to determine independent predictors of a reduced power spectral density. An alpha level of 0.05 was considered indicative of a statistically significant difference ($p < 0.05$).

5.3: RESULTS

5.3.1: AUTONOMIC FUNCTION RESULTS

As shown in table 5.2, patients with a reduced LVEF have significantly attenuated PSD (HRV), LF (ms^2) and LF (nu) oscillatory components of HRV, and BRS compared to normal patients. Patients with a reduced LVEF had significantly elevated HF (nu) frequency power compared to normal patients. Only patients with severe LVEF had a significantly reduced HF (ms^2) oscillatory component of HRV compared to normal patients.

Table 5.2: Autonomic Function of Normal and Left Ventricular Dysfunction Patient Groups

Parameter	Normal	Mild LVEF	Moderate LVEF	Severe LVEF
PSD (ms^2)	3358.1 ± 803.5	1882.5 ± 376.1**	1418.4 ± 514.7**	420.3 ± 374.4**
LF (nu) (%)	59.1 ± 17.1	42.4 ± 18**	30.7 ± 9.9**	16.5 ± 6.6**
HF (nu) (%)	40.9 ± 17.1	57.6 ± 18**	69.3 ± 9.9**	83.5 ± 6.6**
LF (ms^2)	1556 ± 815.6	553.3 ± 339.2**	266.4 ± 188.9**	45.3 ± 56.7**
HF (ms^2)	988 ± 663.5	853.9 ± 465.2	775.5 ± 272.4	307.4 ± 287.8**
BRS ($ms \cdot mmHg^{-1}$)	18 ± 7.9	9.7 ± 2.6**	6.9 ± 2.2**	3.5 ± 1.3**

Note: PSD = Power spectral density; LF (nu) = Normalised units low frequency; HF (nu) = Normalised units high frequency; LF (ms^2) = Low frequency power; HF (ms^2) = High frequency power; BRS = Baroreceptor reflex sensitivity; ** = $p < 0.001$ vs. Normal.

5.3.2: ECHOCARDIOGRAPHY RESULTS

As shown in table 5.3, patients with moderate and severe LVEF have a significantly elevated left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), left atrial (LA) diameter, and peak systolic velocity (PSV) compared to normal patients. All patients with a reduced LVEF had significantly impaired left ventricular diastolic dysfunction as measured by E/Vp and mitral E/E'. Only patients with severe LVEF had significantly elevated myocardial muscle thickness.

Table 5.3: Echocardiographic findings in Normal and Left Ventricular Dysfunction Patient Groups

Parameter	Normal	Mild LVEF	Moderate LVEF	Severe LVEF
LVESD (cm)	2.6 ± 0.7	2.9 ± 0.6	3.4 ± 1.2*	3.8 ± 1.4*
LVEDD (cm)	4.3 ± 0.7	4.7 ± 1.1	5.5 ± 1.3*	5.9 ± 1.6*
Maximal Wall Thickness (cm)	0.8 ± 0.2	0.9 ± 0.3	1.1 ± 0.4	1.2 ± 0.4*
LVEF (%)	61.8 ± 2.2	47.8 ± 2.4**	39.4 ± 1.7**	31 ± 5**
LA diameter (cm)	2.7 ± 0.6	3.1 ± 0.8	4.1 ± 1.6*	4.6 ± 1.2**
Mitral E/E'	6.1 ± 1.2	7.7 ± 2.1*	12.2 ± 3.6**	19.4 ± 4.6**
Mitral E/Vp	1.4 ± 0.6	1.7 ± 0.5	1.9 ± 0.7*	2.2 ± 0.9**
Arev ($m \cdot sec^{-1}$)	16 ± 5	22 ± 14	33 ± 12*	41 ± 16**
Adur (ms)	12 ± 4	18 ± 7	26 ± 9*	37 ± 12**
PSV ($cm \cdot sec^{-1}$)	9.1 ± 2.7	8.8 ± 3.5	7.5 ± 3.1*	6.4 ± 2.1*

Note: LVESD = Left ventricular end systolic diameter; LVEDD = Left ventricular end diastolic diameter; LVEF = Left ventricular ejection fraction; LA = Left atrium; Mitral E/E' = Early mitral annular velocity; E/Vp = Velocity of propagation; Arev = Pulmonary venous flow reversal wave during atrial contraction; Adur = A wave duration; PSV = Peak systolic velocity; * = $p < 0.05$ vs. Normal; ** = $p < 0.001$ vs. Normal.

As shown from table 5.4, HRV was significantly correlated with LVESD, LVEDD, LVEF, Adur, Arev, mitral E/E', mitral E/Vp, LA size, and PSV. Univariate linear regression analysis (Table 5.5) demonstrated that a reduced HRV was associated with increased left ventricular dimensions, filling pressure and a reduced ejection fraction. However, stepwise multiple regression analysis (Table 5.6) with echocardiographic parameters demonstrated that LV filling pressure as measured by mitral E/E' and PSV were the only independent predictors of a reduced HRV.

Table 5.4: Correlation of PSD with Parameters of Cardiac Function

Parameter	r	*p*
LVESD (cm)	0.39	0.04
LVEDD (cm)	0.36	0.05
Maximal Wall Thickness (cm)	0.19	0.21
LVEF (%)	0.89	< 0.001
LA (cm)	0.76	< 0.001
Mitral E/E'	0.84	< 0.001
E/Vp	0.73	< 0.001
Arev ($m \cdot sec^{-1}$)	0.43	0.002
Adur (ms)	0.48	0.001
PSV ($cm \cdot sec^{-1}$)	0.72	< 0.001

Note: LVESD = Left ventricular end systolic diameter; LVEDD = Left ventricular end diastolic diameter; LVEF = Left ventricular ejection fraction; LA = Left atrium; Mitral E/E' = Early mitral annular velocity; E/Vp = Velocity of propagation; Arev = Pulmonary venous flow reversal wave during atrial contraction; Adur = A wave duration; PSV = Peak systolic velocity.

Table 5.5: Univariate Linear Regression Analysis of Echocardiographic Parameters with PSD.

Parameter	β	*p*	95% CI
LVESD (cm)	2.2 ± 0.5	0.002	1.2 to 3.2
LVEDD (cm)	1.6 ± 0.7	0.03	1.1 to 4.1
LVEF (%)	-24.1 ± 8.5	< 0.001	-17.1 to -50.9
LA (cm)	0.3 ± 0.2	0.003	2.3 to 7.9
Mitral E/E'	19.4 ± 7.5	< 0.001	7.4 to 25.4
Mitral E/Vp	7.47 ± 0.5	0.006	4.56 to 12.38
Arev ($m \cdot sec^{-1}$)	4.1 ± 2.1	0.001	2.1 to 6.6
Adur (ms)	3.2 ± 1.3	0.003	1.8 to 7.2
PSV ($cm \cdot sec^{-1}$)	-23.5 ± 6.4	< 0.001	-10.8 to -36.2
LVMI ($g \cdot m^2$)	0.3 ± 0.2	0.35	0.1 to 0.8

Note: CI = Confidence interval; Note: LVESD = Left ventricular end systolic diameter; LVEDD = Left ventricular end diastolic diameter; LVEF = Left ventricular ejection fraction; LA = Left atrium; Mitral E/E' = Early mitral annular velocity; E/Vp = Velocity of propagation; Arev = Pulmonary venous flow reversal wave during atrial contraction; Adur = A wave duration; PSV = Peak systolic velocity; LVMI = Left ventricular mass index.

Table 5.6: Stepwise Multiple Regression Analysis of Echocardiographic Parameters Independently Associated with PSD.

Parameter	β	*p*
Mitral E/E'	12.3 ± 4.2	0.001
PSV ($cm \cdot sec^{-1}$)	-7.4 ± 3.9	0.03

Note: Mitral E/E' = Early mitral annular velocity; PSV = Peak systolic velocity.

5.4: DISCUSSION

This investigation demonstrated that patients with LV dysfunction have a significantly attenuated HRV compared to normal patients. This supports previous research. This is the first study to demonstrate a clear decline in HRV with LVEF. This is also the first study to explore the relationship of HRV with parameters of LV diastolic dysfunction. Indeed, the only independent predictors of a reduced HRV were LV long axis function and mitral E/E', which is the strongest predictor of LV diastolic dysfunction. This study also demonstrated that BRS significantly decreased as LV function deteriorated, which is a finding that has been previously reported (Eckberg et al. 1971). However a large body of evidence has demonstrated that impaired BRS is not the fundamental regulatory defect in human heart failure and that additional mechanisms contribute to the adrenergic stimulation (Floras 2003). There was no significant correlation with LVWT and HRV. However, this apparent anomaly is not a surprise considering there has been no clear-cut association previously. Long axis velocity was more strongly associated with HRV than LVEF. This is not surprising as tissue Doppler imaging (TDI) indices of long axis function are less load dependant than LVEF.

A reduction in LV performance was characterised by a significantly reduced HRV compared to normal patients. The reduction in autonomic modulation was demonstrated in both the HF (ms^2) and LF (ms^2) oscillations of HRV, with a more pronounced reduction in the later as LV function declines. This supports previous research (Guzzetti et al. 1995; van de Borne et al. 1997).

The finding of an association of diastolic dysfunction with HRV is novel. Elevated cardiac filling pressures can increase cardiac noradrenaline spill over early in the course of heart failure by stimulating a cardiac-specific sympathoexcitatory reflex (Floras 2009). Increasing cardiac filling pressure to maintain stroke volume and blood pressure activates the cardiac sympathetic reflex, which itself causes a reduction in arterial baroreflex heart rate control and further intensifies heart failure symptoms (Malliani and Montano 2002). In addition, atrial dilatation, a morphophysiological expression of an increase in cardiac filling pressure, decreases HRV (Horner et al. 1996). This study did demonstrate that as LA size increases HRV decreases. Indeed, activation of left atrial receptors by balloon distension caused an increase in cardiac sympathetic nerve activity (Karim et al. 1972) and in conditions of volume overload, atrial vagal receptors discharge during atrial systole and diastole and this continual firing has been postulated to blunt the capability of generating efficient restraint on sympathetic outflow (Floras 2009; Malliani and Montano 2002; Recordati et al. 1976; Spyer 1990).

Indices of LV diastolic function, including mitral E/E', A Dur, mitral E/Vp derived from Doppler echocardiography have been consistently found to be the best predictor of LV filling pressure (Ommen et al. 2000; Sharma et al. 2006d). Advanced LV diastolic dysfunction predicts a poorer prognosis in patients with cardiovascular disease (Hillis et al. 2004) and this is supported with indices of HRV. Indeed this study has bridged a gap in the research by demonstrating that LV diastolic dysfunction is an independent predictor of a reduced HRV. However, impaired cardiac function is a complex multifactorial disease state and determining causation requires further research.

Reducing cardiac filling pressure in heart failure may attenuate cardiac noradrenaline spill over (Azevedo et al. 2000) and improve HRV, due to a reduced activation of mechanoreceptor efferent's (Floras 2003). The use of diuretics, natriuretic peptides (Abramson et al. 1999), or ultrafiltration (Agostoni et al. 1993) administered carefully to avoid systemic hypotension, baroreceptor unloading, and increased sympathetic activity, might prevent or delay reflexively increased cardiac sympathetic activation (Azevedo et al. 2000) by reducing LV filling pressure.

5.5: SUMMARY

This is the first study to show that HRV declines with worsening LV systolic and diastolic function. Therefore the research hypothesis, which stated that HRV will be significantly associated with LV systolic and diastolic function can be accepted. This study also demonstrated that a parameter of LV diastolic function, mitral E/E', is independently associated with HRV. Impaired cardiac function causes system wide pathological alterations; however, reducing or normalising cardiac filling pressure is a potential therapeutic target for patients with diminishing cardiac function. Patients with diabetes and chronic kidney disease have increased cardiovascular disease morbidity and mortality, which is not entirely due to increased prevalence of cardiomyopathy or coronary artery disease and the role of autonomic dysfunction in this group remains uncertain.

CHAPTER 6: IMPACT OF DIABETES AND CHRONIC KIDNEY DISEASE ON AUTONOMIC CONTROL

6.0: ABSTRACT

BACKGROUND: Autonomic neuropathy is common in diabetic and chronic kidney disease (CKD) patients and is associated with increased cardiac morbidity and mortality. This investigation aimed to correlate autonomic dysfunction assessed non-invasively using heart rate variability (HRV) with markers of inflammation in diabetic and CKD patients.

METHODS: 59 diabetic patients (mean age 66 ± 10 years, 33 male), 173 CKD patients (93 mild [mean age 64 ± 9 years, 56 male], 61 moderate [mean age 65 ± 9 years, 35 male], and 19 severe [mean age 62 ± 14 years, 10 male] CKD) and 29 normal patients (mean age 62 ± 10 years, 10 male). Haemodynamic and autonomic data were obtained from beat-to-beat analysis of heart rate and blood pressure using a plethysmographic device, the Task Force® Monitor. We thereby determined total power spectral density (PSD) and associated low frequency (LF) and high frequency (HF) power spectral components in absolute (ms^2) and normalised units (nu). C-reactive protein, a marker of inflammation and estimated glomerular filtration rate (eGFR), a marker of renal function were analysed from blood samples.

RESULTS: Diabetic and CKD patients had a significantly reduced PSD ($p < 0.05$ and $p < 0.001$ respectively) compared to normal patients. Low frequency (LF) power (ms^2) significantly correlated with baroreceptor reflex sensitivity in diabetic and CKD patients ($r = 0.89$, $p < 0.001$ and $r = 0.84$, $p < 0.001$ respectively). Levels of CRP were negatively associated with PSD in diabetic and CKD patients ($r = -0.82$, $p < 0.001$ and $r = -0.80$, $p < 0.001$ respectively). In CKD patients, eGFR significantly correlated with PSD ($r = 0.7$, $p < 0.001$).

CONCLUSION: Low frequency power (ms^2) was associated with BRS in diabetic and CKD patients. Thus LF power (ms^2) may simply reflect BRS function. C-reactive protein showed a negative association with PSD in diabetic and CKD patients. Thus increased inflammatory activity may represent a new auxiliary mechanism linking decreased HRV to poor prognosis in diabetic and CKD patients. Autonomic function declines with decreasing renal function.

6.1: INTRODUCTION

The previous chapter demonstrated that heart rate variability (HRV) is significantly associated with declining systolic and diastolic function. Patients with diabetes and chronic kidney disease (CKD) have increased cardiovascular disease morbidity and mortality. However, at present there is no clear pathological link since there is no association with an increased prevalence of cardiomyopathy or coronary artery disease in patients with metabolic disease. Dysfunction of the autonomic nervous system (ANS) is a prominent characteristic in patients with diabetes and CKD (Hausberg et al. 2002; Maser and Lenhard 2005), and patients presenting with both of these complex disease states exhibit advanced autonomic dysfunction (Cashion et al. 2000). An important feature of this compromised autonomic function is abnormal regulation of the cardiovascular system, including heart rate control, as well as defects in central and peripheral vascular dynamics (Maser and Lenhard 2005; Vinik and Ziegler 2007), which plays an important role in the pathogenesis, progression, and prognosis of disease (Blankestijn 2004; Rubinger et al. 1999).

Globally, there are approximately 171 million individual's diagnosed with diabetes (Wild et al. 2004) and 1.5 million individual's diagnosed with CKD (Moeller et al. 2002). Over the past decade, heart rate variability (HRV) analysis has emerged as a non-invasive clinical tool for the assessment of the sympathetic and parasympathetic modulation of the ANS (Malik et al. 1996). Cardiac autonomic neuropathy as a result of diabetes and renal disease is positively related to a poor prognosis (Hausberg et al. 2002;

Pagkalos et al. 2008) and the measurement of HRV is a reliable index of cardiac autonomic dysfunction in these multifaceted disease states (Axelrod et al. 1987; Cloarec-Blanchard et al. 1992; Pagkalos et al. 2008).

A depressed HRV is evident in patients with diabetes and CKD and is significantly associated with increased cardiovascular disease morbidity and mortality and a reduced quality of life (Balcioğlu et al. 2007; Hausberg et al. 2002; Maser et al. 2003; Vinik et al. 2003a; Vinik and Ziegler 2007). The elevated cardiovascular disease risk in patients with diabetes and CKD is not completely accounted for by traditional risk factors (Ranpuria et al. 2008) and an imbalance of ANS control is implicated in life-threatening cardiac events such as arrhythmogenesis and sudden cardiac death (SCD) (Coquet et al. 2005; Ranpuria et al. 2008; Sztajzel 2004; Tsuji et al. 1996a).

In addition to a reduced HRV, a reduced baroreceptor reflex sensitivity (BRS) is associated with increased mortality (La Rovere et al. 1998), particularly related to an increased risk of SCD of arrhythmic cause, which has been demonstrated in both experimental (Hull et al. 1994) and clinical studies (Farrell et al. 1991b; Hohnloser et al. 1994a; La Rovere et al. 2001). Indeed, a reduced BRS is evident in patients with diabetes (Loimaala et al. 2003; Pagkalos et al. 2008) and CKD (Lazarus et al. 1973; Pickering et al. 1972).

The enhanced cardiovascular disease risk with autonomic dysfunction in patients with diabetes and CKD could be explained in part by the numerous comorbidities and the

presence of unique metabolic and physiological alterations. An example of this can be observed in patients with end-stage renal disease (ESRD), where successful renal transplantation corrected autonomic function (Rubinger et al. 1999). This suggests that the autonomic abnormalities may be caused by humoral factors, which are reversed with transplantation (Rubinger et al. 1999).

Patients with diabetes and CKD have elevated cardiovascular disease (CVD) risk, particularly SCD, which is not completely accounted for by traditional risk factors and may be associated with ANS disturbances. The complex nature of these disease states makes the management of cardiac risk factors and the prevention of SCD challenging. Therefore, the study of autonomic control and specifically the incidence of sympathetic hyper-activity in these patient groups is a desirable research objective.

6.1.1: AIM

The analysis of autonomic function measured non-invasively using HRV in diabetic and CKD patients may help to refine prognosis and may be useful for patient risk stratification in intervention procedures aimed at reducing cardiovascular disease. Changes in autonomic modulation and a reduced HRV is associated with an increased risk of cardiovascular morbidity and mortality in diabetic and CKD patient populations, but the pathological link between these associations is not well understood. Therefore the aim of the study was to assess HRV in patients with diabetes and CKD during

supine rest and investigate any associations with haemodynamic measurements, echocardiographic parameters, and markers of inflammation.

6.1.2: HYPOTHESIS

1. H_1: Sympathetic neural outflow, represented by low frequency oscillations of heart rate variability will be significantly elevated in patients with diabetes and chronic kidney disease compared to normal patients.

2. H_1: Baroreceptor reflex sensitivity will be significantly attenuated in patients with diabetes and chronic kidney disease compared to normal patients.

3. H_1: Heart rate variability will be significantly associated with C-reactive protein in patients with diabetes and chronic kidney disease.

4. H_1: Heart rate variability is significantly attenuated as kidney function declines as measured using estimated glomerular filtration rate.

6.2: METHOD

6.2.1: PARTICIPANTS

We studied 59 diabetic patients, 173 CKD patients and 29 normal patients. Patient selection and the collection of demographic data are described in Chapter 3: General Methods, and the baseline characteristics of these patients are described in Chapter 4: Baseline Population. The patients were divided into three groups, normal, diabetic, and diabetic and CKD. The CKD patients were divided into groups characterised by their CKD status: mild, moderate, and severe. Patients were classified as diabetic by an oral glucose tolerance test (Gavin et al. 1997) and by two fasting glucose tests of 7 $mmol \cdot L^{-1}$ or more (WHO 1985). The CKD patients were divided into mild, moderate, and severe CKD according to their estimated Glomerular Filtration Rate (eGFR), where an eGFR of 60-89, 30-59, and <30 $ml \cdot min^{-1} \cdot 1.73m^2$ was mild, moderate and severe CKD respectively (Burden and Tomson 2005). Table 6.1 and 6.2 details the baseline physical characteristics of patient groups.

Table 6.1: Baseline Characteristics of Normal, Diabetic, and Diabetic and CKD Patient Groups

Parameter	Normal (n = 29)	Diabetic (n = 59)	Diabetic and CKD (n = 42)
Age (Years)	62 ± 10	66 ± 10*	68 ± 9*
Gender	♂ 10	♂ 33	♂ 27
Ethnicity	Asian = 21 (72.4%)	Asian = 35 (59.3%)	Asian = 23 (54.8%)
	CAU = 7 (24.1%)	CAU = 15 (25.4%)	CAU = 12 (28.6%)
	Black = 1 (3.4%)	Black = 8 (13.6%)	Black = 7 (16.7%)
	Other = 0 (0%)	Other = 1 (1.7 %)	Other = 0 (0%)
Height (cm)	163 ± 11	165 ± 9	166.1 ± 8.9
Weight (kg)	74 ± 16.7	80.7 ± 17.1	81.5 ± 15.7
BSA (m^2)	1.83 ± 0.24	1.87 ± 0.2	1.9 ± 0.2
Ischaemic	0	17 (28.8%)	12 (28.6%)

Note: CKD = Chronic kidney disease; * = $p < 0.05$; CAU = Caucasian; BSA = Body surface area.

Table 6.2: Baseline Characteristics of Normal and CKD Patient Groups

Parameter	Normal (n = 29)	Mild CKD (n = 93)	Moderate CKD (n = 61)	Severe CKD (n = 19)
Age (Years)	62 ± 10	64 ± 9	65 ± 9	62 ± 14
Gender	♂ 10	♂ 56	♂ 35	♂ 10
Ethnicity	Asian = 21 (72.4%)	Asian = 50 (53.8%)	Asian = 33 (54.1%)	Asian = 9 (47.4 %)
	CAU = 7 (24.1%)	CAU = 36 (38.7%)	CAU = 19 (31.1%)	CAU = 4 (21.1%)
	Black = 1 (3.4 %)	Black = 7 (7.5 %)	Black = 7 (11.5 %)	Black = 6 (31.6 %)
	Other = 0 (0 %)	Other = 0 (0 %)	Other = 2 (3.3 %)	Other = 0 (0 %)
Height (cm)	163 ± 11	165 ± 9.1	164 ± 9.9	166 ± 8.9
Weight (kg)	74 ± 16.7	80 ± 17.8	76 ± 14.9	87 ± 21
BSA (m^2)	1.8 ± 0.24	1.88 ± 0.21	1.83 ± 0.22	1.94 ± 0.3
Ischaemic	0	17 (18.3%)	15 (24.6%)	5 (26.3%)

Note: CKD = Chronic kidney disease; CAU = Caucasian; BSA = Body surface area.

6.2.2: AUTONOMIC ASSESSMENT

The Task Force® Monitor (CNSystems, Graz, Austria) was used for the continuous non-invasive beat-to-beat monitoring and real time calculation of all cardiovascular haemodynamic and autonomic parameters. Parameters included; continuous electrocardiography, continuous blood pressure, stroke index, cardiac index, total peripheral vascular resistance index, BRS, and HRV as outlined in Chapter 3: General Methods. After 30-minutes of supine rest, 15-minutes of haemodynamic and autonomic data was recorded and automatically calculated for statistical analysis.

6.2.3: ECHOCARDIOGRAPHY

This was performed using a General Electric Vingmed System 7 ultrasound machine. A full study was performed with all measurements of cardiac size, left ventricular (LV) systolic and diastolic function as outlined in Chapter 3: General Methods.

6.2.4: HAEMATOLOGICAL SAMPLING

Whole blood samples were collected via vena puncture. This was performed according to the local NHS trust hospital guidelines. Cannulation was performed 30-minutes before any autonomic, haemodynamic, or echocardiography measurements were performed. This was primarily performed to reduce any confounding influence cannulation may have on autonomic modulation.

All samples were immediately sent to the laboratory for processing. In total 50 ml of blood per patient was required. C-reactive Protein (CRP) was assayed on the Immulite-1 (Diagnostic Product Corporation). A minimum volume of 110 μL of serum was required. The assay coefficient of variation (CV) was 10% at 4.0 $mg \cdot L^{-1}$, 7.5% at 8.0 $mg \cdot L^{-1}$, and 4.8% at 31.0 $mg \cdot L^{-1}$. The assay was linear up to 250 $mg \cdot L^{-1}$, with an analytical sensitivity of 0.1 $mg \cdot L^{-1}$ and a functional sensitivity of 0.2 $mg \cdot L^{-1}$. There was no high dose hook effect up to 8900 $mg \cdot L^{-1}$.

Estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease study (MDRD) calculation (figure 6.1), which is recommended by the National Institute for Health and Clinical Excellence (NICE) and the UK Renal Association.

$$eGFR\ (ml \cdot min^{-1} \cdot 1.73m^2) = 186 \times (Creatinine / 88.4)^{-1.154} \times (age)^{-0.203}$$

- Multiply by 0.742 if female
- Multiply by 1.210 if black

Figure 6.1: Formula for calculating estimated glomerular filtration rate.

6.2.5: STATISTICAL ANALYSIS

Once the data was collated onto a spreadsheet, it was analysed using the statistical package for social sciences (SPSS 17 release version of SPSS for Windows; SPSS Inc., Chicago IL, USA). Continuous variables were expressed as mean ± SD. Independent samples t-test was used to test the differences between patient haematological results. A Pearson's product-moment correlation coefficient was used to measure the degree of linear relationship between autonomic and haematological data in diabetic and CKD patient groups. An alpha level of 0.05 was considered indicative of a statistically significant difference ($p < 0.05$).

6.3: RESULTS

6.3.1: AUTONOMIC FUNCTION RESULTS

As shown in table 6.3, patients with diabetes alone or in combination with CKD have a significantly lower HRV, low frequency (LF) (ms^2) power, and BRS compared to normal patients. Diabetic patients without CKD had a significantly reduced high frequency (HF) (ms^2) power compared to normal patients.

Table 6.3: Resting Autonomic Function of Normal, Diabetic, and Diabetic and CKD Patient Groups

Parameter	Normal	Diabetic	Diabetic & CKD
RRI (ms)	877 ± 167	823.3 ± 128.5	819.4 ± 120.2
PSD (ms^2)	3358.1 ± 803.5	1582 ± 689*	1287 ± 467.6**
LF (nu) (%)	59.1 ± 17.1	47.6 ± 3.2	44.9 ± 3.7
HF (nu) (%)	40.9 ± 17.1	52.4 ± 3.2	55.1 ± 3.7
LF (ms^2)	1556 ± 815.6	424.6 ± 105.5*	443.5 ± 137.9*
HF (ms^2)	988 ± 663.5	589.1 ± 232.4*	623.1 ± 317.3
BRS ($ms \cdot mmHg^{-1}$)	18 ± 7.9	8.09 ± 3.4*	8.72 ± 3.8*

Note: CKD = Chronic kidney disease; RRI = RR interval; PSD = Power spectral density; LF (nu) = Normalised units low frequency; HF (nu) = Normalised units high frequency; LF = Low frequency (ms^2); HF (ms^2) = High frequency; BRS = Baroreceptor reflex sensitivity; * = $p < 0.05$ vs. Normal; ** = $p < 0.001$ vs. Normal.

As shown in table 6.4, CKD patients have a significantly lower HRV, LF (ms^2) power, and BRS compared to normal patients. Patients with moderate and severe CKD have significantly lower normalised units of LF (LF [nu]) and significantly higher HF (nu) compared to normal patients. These significant autonomic differences result in moderate and severe CKD patients having a significantly lower LF/HF ratio compared to normal patients.

Table 6.4: Resting Autonomic Function of Normal and CKD Patient Groups

Parameter	Normal	Mild CKD	Moderate CKD	Severe CKD
RRI (ms)	877 ± 167	856 ± 165.8	853 ± 170.3	812 ± 128
PSD (ms^2)	3358.1 ± 803.5	2013 ± 457*	1416.6 ± 520**	1206.9 ± 349.5**
LF (nu) (%)	59.1 ± 17.1	45.3 ± 2.2	39 ± 2.9*	36.1 ± 7.8*
HF (nu) (%)	40.9 ± 17.1	54.7 ± 2.2	61 ± 2.9*	63.9 ± 7.8*
LF (ms^2)	1556 ± 815.6	600.9 ± 182.8*	438.4 ± 107.9*	223.3 ± 116.4**
HF (ms^2)	988 ± 663.5	659.9 ± 393.7	496.9 ± 355.8*	387 ± 235.2*
BRS ($ms \cdot mmHg^{-1}$)	18 ± 7.9	9.6 ± 1.3*	8.8 ± 1.3*	8.3 ± 2.2*

CKD = Chronic kidney disease; RRI = RR interval; PSD = Power spectral density; LF (nu) = Normalised units low frequency; HF (nu) = Normalised units high frequency; LF = Low frequency; HF = High frequency; BRS = Baroreceptor reflex sensitivity; * = $p < 0.05$ vs. Normal; ** = $p < 0.001$ vs. Normal.

As shown in figure 6.2 and 6.3, there is significant correlation with BRS and LF (ms^2) power in normal patients and those with diabetes and CKD ($r = 0.89$, $p < 0.001$ and $r = 0.84$, $p < 0.001$; respectively).

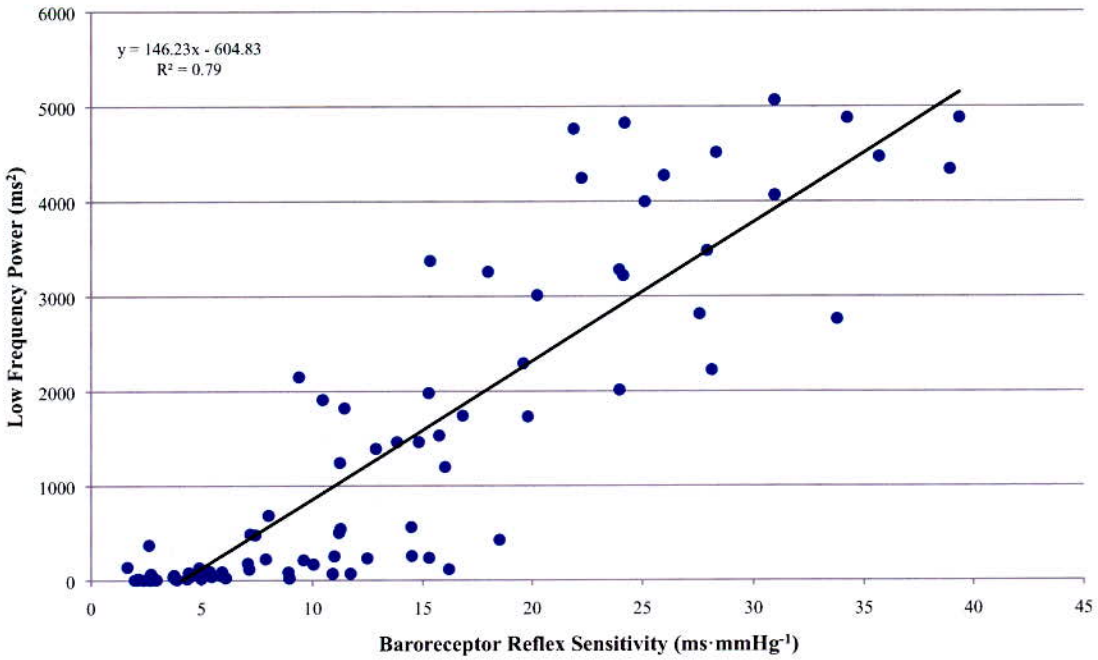

Figure 6.2: Relationship between baroreceptor reflex sensitivity and low frequency (ms^2) power in normal and diabetic patients.

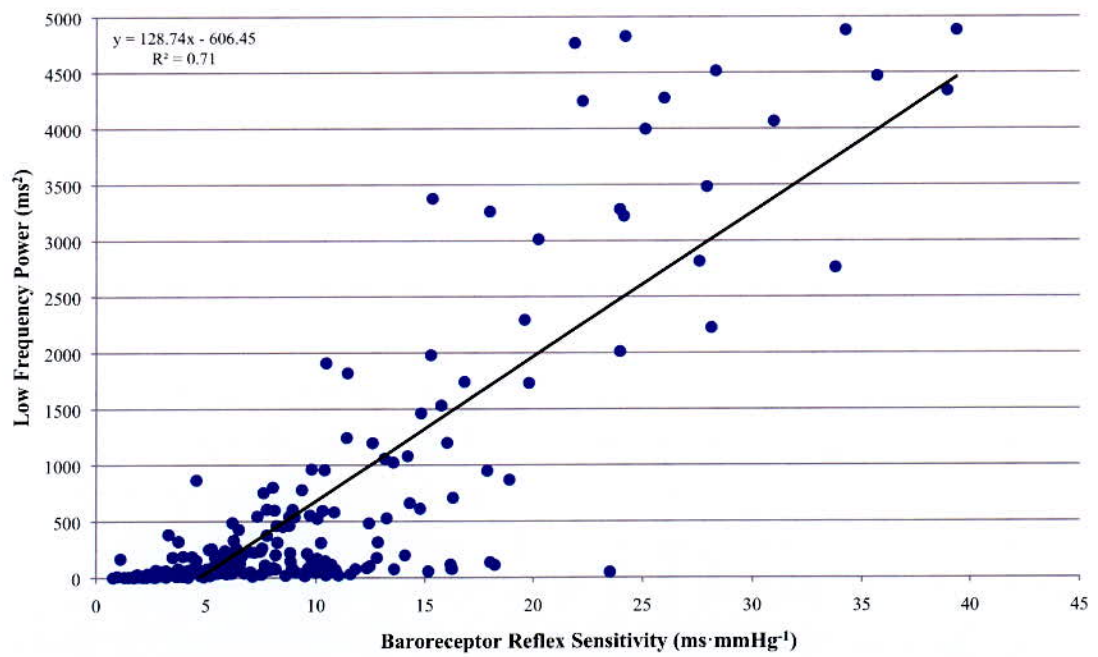

Figure 6.3: Relationship between baroreceptor reflex sensitivity and low frequency (ms^2) power in normal and chronic kidney disease patients.

6.3.2: HAEMODYNAMIC AND ECHOCARDIOGRAPHY RESULTS

As shown in table 6.5, patients with diabetes alone or in combination with CKD have a significantly increased diastolic blood pressure (dBP) and significantly reduced stroke index (SI) compared to normal patients. Patients with diabetes and CKD have a significantly lower cardiac index (CI) and left ventricular ejection fraction (LVEF) compared to normal patients.

Table 6.5: Resting Haemodynamic and Echocardiography Results of Normal, Diabetic, and Diabetic with CKD Patient Groups.

Parameter	Normal	Diabetic	Diabetic & CKD
HR ($b \cdot min^{-1}$)	71 ± 12	75 ± 12.6	75.8 ± 10.9
sBP (mmHg)	126 ± 17	131 ± 26.7	139 ± 19.3
dBP (mmHg)	83 ± 11	88.4 ± 16.3*	89 ± 13.6*
PP (mmHg)	53.1 ± 13	56.8 ± 15.2	57.2 ± 14.6
SI ($ml \cdot m^2$)	35.9 ± 6	31.1 ± 9.4*	30.3 ± 9*
CI ($L \cdot min^{-1} \cdot m^2$)	2.68 ± 0.7	2.3 ± 0.7	2.15 ± 0.5*
TPRI ($dyne \cdot s \cdot m^2 \cdot cm^5$)	3369.9 ± 1348	3627.4 ± 1261	3753.1 ± 1289
LVEF (%)	61.8 ± 2.2	59.3 ± 1.3	55.5 ± 4.8**

Note: HR = Heart rate; sBP = Systolic blood pressure; dBP = Diastolic blood pressure; PP = Pulse pressure; SI = Stroke index; CI = Cardiac index; TPRI = Total peripheral resistance index; LVEF = Left ventricular ejection fraction; * = $p < 0.05$; ** = $p < 0.001$ vs. Normal.

As shown in table 6.6, CKD patients have a significantly increased sBP and dBP and significantly reduced LVEF compared to normal patients. Moderate to severe CKD patients have a significantly lower SI and CI compared to normal patients.

Table 6.6: Resting Haemodynamic and Echocardiography Results of Normal and CKD Patient Groups

Parameter	Normal	Mild CKD	Moderate CKD	Severe CKD
HR ($b \cdot min^{-1}$)	71 ± 12	71.7 ± 14	72.8 ± 2.1	75.6 ± 3.6
sBP (mmHg)	126 ± 17	131.3 ± 24*	141.3 ± 3**	150.3 ± 6**
dBP (mmHg)	83 ± 11	86.1 ± 14*	87.5 ± 1.9*	89.9 ± 3.8*
PP (mmHg)	53.1 ± 13	55.7 ± 18	58.5 ± 2	60.4 ± 3.5
SI ($ml \cdot m^2$)	35.9 ± 6	33.8 ± 9	29.9 ± 0.9*	29 ± 2.6*
CI ($L \cdot min^{-1} \cdot m^2$)	2.68 ± 0.7	2.38 ± 0.8	2.16 ± 0.8*	2.09 ± 0.4*
TPRI ($dyne \cdot s \cdot m^2 \cdot cm^5$)	3369.9 ± 1348	3536.2 ± 1135	4025.5 ± 165.4*	4218 ± 502.6*
LVEF (%)	61.8 ± 2.2	56 ± 7.9**	52.5 ± 10**	51.9 ± 6.9**
LVESD	2.6 ± 0.7	2.7 ± 0.5	2.9 ± 0.9	3.3 ± 0.8*
LVEDD	4.3 ± 0.7	4.1 ± 0.3	5.1 ± 0.6*	5.6 ± 1.7*
Mitral E/E'	6.1 ± 1.2	8.4 ± 2.3	10.2 ± 1.7*	14.3 ± 4.2*
Maximum LVWT	0.8 ± 0.2	0.9 ± 0.4	1.2 ± 0.6*	1.4 ± 0.7*

Note: HR = Heart rate; sBP = Systolic blood pressure; dBP = Diastolic blood pressure; PP = Pulse pressure; SI = Stroke index; CI = Cardiac index; TPRI = Total peripheral resistance index; LVEF = Left ventricular ejection fraction; WMSI = Wall motion score index; LVESD = Left ventricular end systolic diameter; LVEDD = Left ventricular end diastolic diameter; Mitral E/E' = Early mitral annular velocity; LVWT = Left ventricular wall thickness; * = $p < 0.05$; ** = $p < 0.001$ vs. Normal.

6.3.3: HAEMOTOLOGICAL RESULTS

As shown in table 6.7, diabetic patients with and without CKD have significantly elevated levels of C-reactive protein (CRP) and significantly lower levels of haemoglobin compared to normal patients. Patients with diabetes and CKD have a significantly reduced eGFR.

Table 6.7: Haematological Results of Normal, Diabetic, and Diabetic and CKD Patient Groups

Parameter	Normal	Diabetic	Diabetic and CKD
C-Reactive Protein ($mg \cdot L^{-1}$)	4.1 ± 1	32.7 ± 12.7**	36.8 ± 15.5**
eGFR ($ml \cdot min^{-1} \cdot 1.73m^2$)	127 ± 24	104 ± 12	57.9 ± 23**

Note: CKD = Chronic kidney disease; eGFR = Estimated glomerular filtration rate; * = $p < 0.05$ vs. Normal; ** = $p < 0.001$ vs. Normal.

As shown in table 6.8, CKD disease patients have significantly elevated levels of CRP and significantly lower levels of haemoglobin, albumin, and eGFR compared to normal patients.

Table 6.8: Haematological Results of Normal and CKD Patient Groups

Parameter	Normal	Mild CKD	Moderate CKD	Severe CKD
C-Reactive Protein ($mg \cdot L^{-1}$)	4.1 ± 1	15.5 ± 9.8**	31.5 ± 18.4**	54 ± 17.5**
eGFR ($ml \cdot min^{-1} \cdot 1.73m^2$)	127 ± 24	75.8 ± 8.6**	48.1 ± 7.9**	20.7 ± 8.2**

Note: CKD = Chronic kidney disease; eGFR = Estimated glomerular filtration rate; * = $p < 0.05$ vs. Normal; ** = $p < 0.001$ vs. Normal.

As illustrated in figure 6.4 and 6.5, there is significant correlation with PSD and CRP in normal patients and those with diabetes and CKD ($r = -0.82$, $p < 0.001$ and $r = -0.80$, $p < 0.001$; respectively).

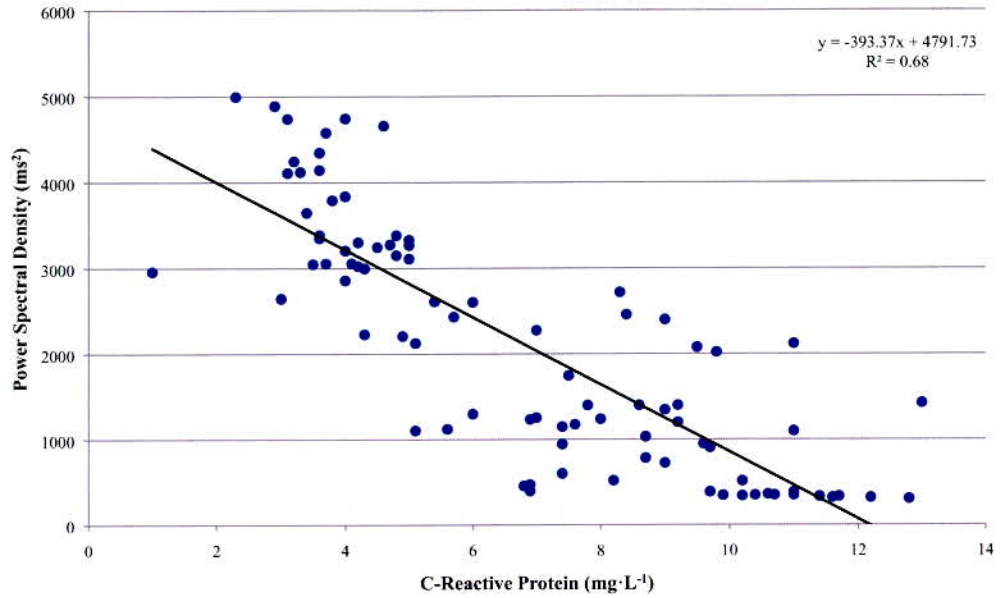

Figure 6.4: Relationship between power spectral density (ms^2) and C-reactive protein in normal and diabetic patients.

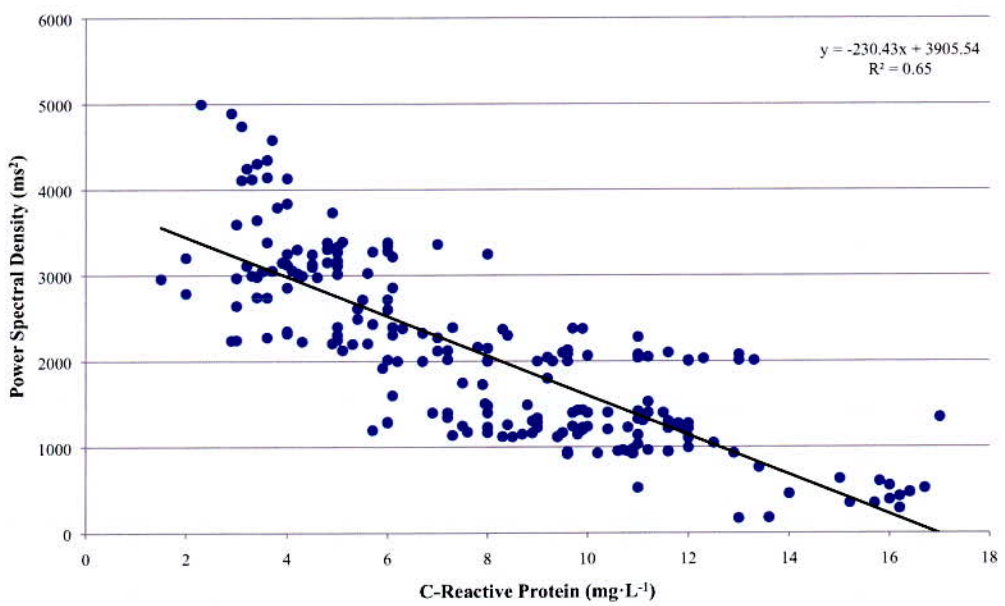

Figure 6.5: Relationship between power spectral density (ms^2) and C-reactive protein in normal and chronic kidney disease patients.

As depicted in figure 6.6, there is a significant correlation ($r = 0.7$, $p < 0.001$) between PSD and eGFR in patients with CKD.

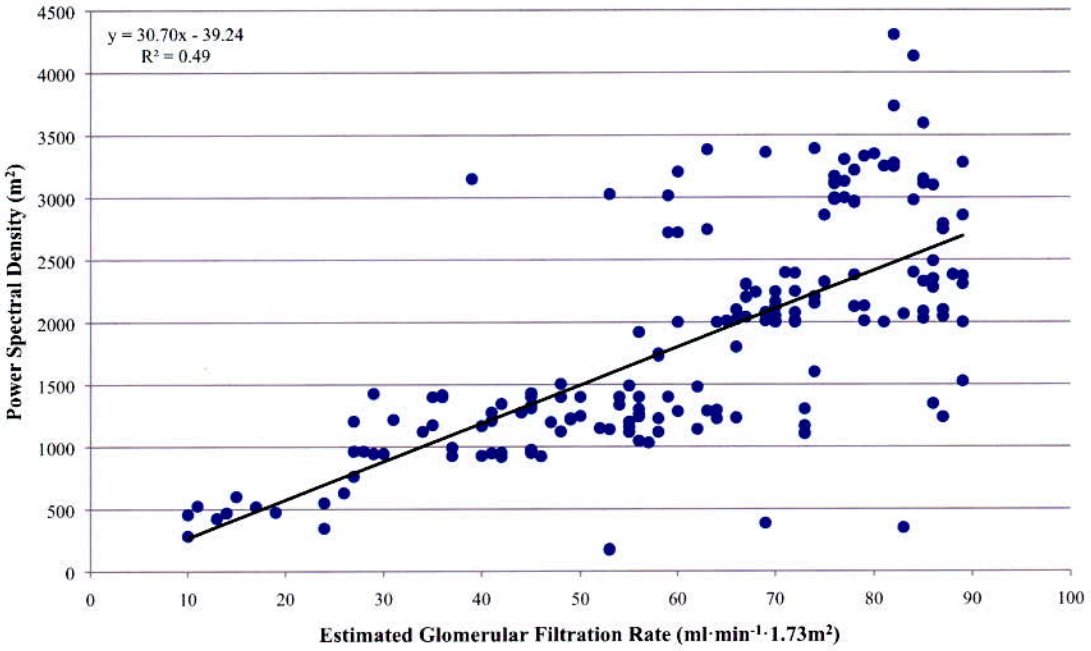

Figure 6.6: Relationship between power spectral density (ms^2) and estimated glomerular filtration rate (eGFR) in chronic kidney disease patients.

6.4: DISCUSSION

This series of investigations demonstrate that patients with diabetes and CKD have a significantly reduced PSD (HRV) compared to normal patients. This supports previous research. In diabetic and CKD patients, novel findings include a significant correlation with BRS and LF (ms^2) and a significant correlation with HRV and CRP. In patients with CKD, this is the first study to demonstrate a significant inverse relationship with PSD and eGFR. This study also demonstrated for the first time that in severe CKD patients no power spectral density was detectable in the low frequency component of HRV (figure 6.8 and 6.9).

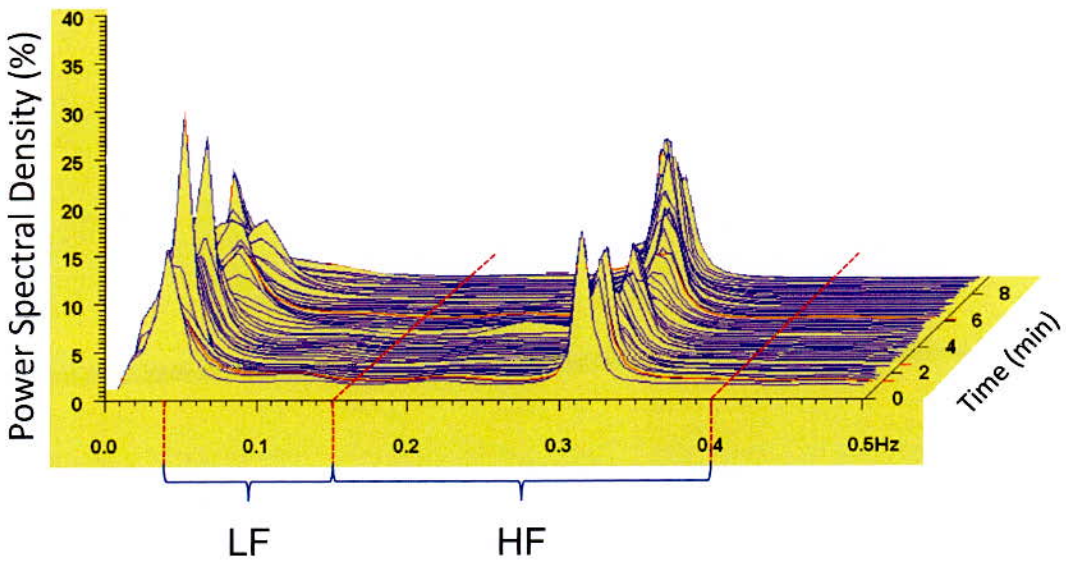

Figure 6.7: Power spectral analysis of a normal patient. Note: LF = Low frequency; HF = High frequency.

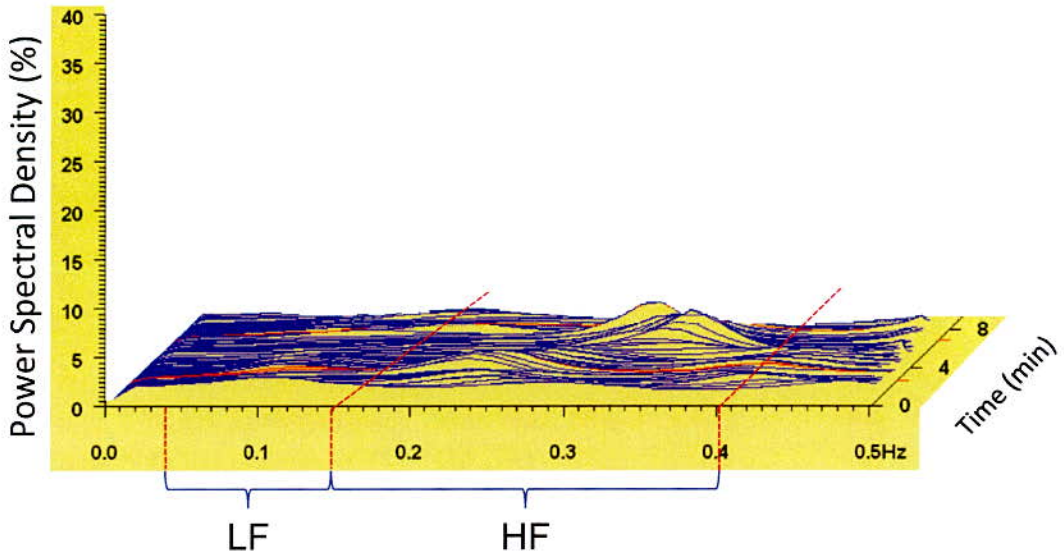

Figure 6.8: Power spectral analysis of a patient with severe chronic kidney disease: Note this patient had a normal left ventricular ejection fraction.

6.4.1: DIABETES

In patients with diabetes, the significant decrease in HRV is demonstrated in the LF (ms^2) and HF (ms^2) oscillations of HRV. This supports the concept that diabetes is associated with autonomic dysfunction involving both the sympathetic and parasympathetic pathways (Bellavere et al. 1992). When displayed in normalised units, patients exhibited a greater percentage of overall power in the HF distribution compared to LF distribution. This suggests that a greater percentage of their HRV is through parasympathetic modulation. The significant decrease in HRV is exacerbated when combined with CKD, which has been previously described (Cashion et al. 2000).

There was a significant correlation ($r = 0.89$, $p < 0.001$) with BRS and the LF (ms^2) power (sympathetic) in patients with diabetes. Diabetic patients had a significantly reduced BRS and LF (ms^2) power compared to normal patients. A reduced BRS is a marker of depressed vagal reflexes and associated with an increased risk of mortality (La Rovere et al. 1988). There is conflicting data concerning the significance of LF power, since recent research demonstrated LF power to reflect BRS function and not cardiac sympathetic innervation (Moak et al. 2009). Moak et al., (2009) assessed cardiac sympathetic activity by 6-[^{18}F] fluorodopamine scanning and reported that patients with a reduced BRS had a reduced LF power and patients with a normal BRS had normal LF power, regardless of cardiac sympathetic innervation. Furthermore, a dissociation of LF power with cardiac noradrenaline spillover, directly recorded muscle sympathetic nerve activity, and plasma noradrenaline levels have also been reported (Kingwell et al. 1994; Notarius et al. 1999; Saul et al. 1990). The results in this study support this concept.

In diabetic patients, a significant reduction in HRV and BRS has been projected to predict SCD (Cygankiewicz et al. 2004; La Rovere et al. 1998; La Rovere et al. 2001). Changes in autonomic control have been associated with changes in the electrophysiology of myocytes, which has been associated with susceptibility to ventricular fibrillation (Zhou et al. 2009). Indeed, in canine models with CHF, a significantly reduced HRV and BRS was correlated with ventricular fibrillation threshold (Zhou et al. 2009). Exercise training is consistently associated with improved HRV and outcome in patients with high CVD risk, such as a previous MI. In diabetic patients, exercise training was associated with improved HRV and BRS (Loimaala et al.

2003). Therefore, exercise training in combination with strict glycaemic control is a potential therapeutic method of improving prognosis in patients with diabetes.

There was a significant inverse correlation ($r = 0.89$, $p < 0.001$) with HRV and CRP. C-reactive protein is elevated in patients with a high CVD risk, yet the significance of this is unclear. Verma et al. (2005) detail that CRP actively contributes to all stages of atherogenesis. However, Elliott et al. (2009) demonstrated no causal association of CRP with coronary artery disease. Patients with diabetes have a two to 4-fold greater risk of developing atherosclerosis (Liao et al. 2002; Renard et al. 2004) and a decreased HRV has also been associated with an increased level of inflammation (Borovikova et al. 2000; Tracy 2002) and progression of atherosclerosis (Huikuri et al. 1999). Indeed, research has demonstrated that HRV is inversely correlated with inflammatory markers in healthy individuals as well as those with CVD (Haensel et al. 2008; Janszky et al. 2004; Lampert et al. 2008; Nolan et al. 2007). In patients with diabetes, inflammation has been identified as a strong independent predictor of cardiovascular mortality (Soinio et al. 2006). These findings support the concept that inflammation mirrors the pathogenesis of cardiovascular autonomic decline. However, ascertaining the cause of the increased cardiovascular mortality in patients with diabetes requires further research.

Animal and human studies demonstrate that the ANS plays a key role in regulating the magnitude of immune response to inflammatory stimuli. Parasympathetic signalling has been shown to inhibit the activation of macrophages and the release of cytokines and thus decrease local and systemic inflammation (Borovikova et al. 2000; Marsland et al.

2007; Tracy 2002). This is of clinical importance since an excessive inflammatory response increases morbidity and mortality in disease states such as diabetes and CKD (Tracy 2002; Zimmermann et al. 1999). In patients with CHF, cholinergic stimulation increased HRV and reduced ventricular arrhythmias (Behling et al. 2003); however, markers of inflammation were not documented.

6.4.2: CHRONIC KIDNEY DISEASE

Patients with CKD have an increased CVD risk and the reasons for this remain unclear. Possible mechanisms include coronary artery disease, left ventricular dysfunction, left ventricular hypertrophy (LVH), cardiac micro infarctions, arrhythmia, and autonomic dysfunction (Sharma et al. 2006a). This research demonstrated that patients with CKD have a significantly decreased HRV compared to normal patients. The significant decrease is demonstrated in LF (ms^2) and HF (ms^2) power. This illustrates that the significant autonomic dysfunction occurs in both the sympathetic and parasympathetic arms of the ANS. When displayed in normalised units, patients exhibited a greater percentage of overall power in the HF (nu) distribution compared to LF (nu) distribution, i.e., a greater percentage of their HRV is through parasympathetic modulation.

This may initially appear contradictory to present work, since CKD has been associated with increased sympathetic activity and reduced parasympathetic modulation (Hathaway et al. 1998; Rump et al. 2000; Vita et al. 1999). The LF component of HRV, although

under debate, is generally regarded to reflect sympathetic innervations (Montano et al. 1994; Pomeranz et al. 1985). Therefore, adhering to the traditional paradigm used to explain LF oscillations in HRV, it would be logical to assume that patients with CKD would exhibit marked increases in the LF component of cardiovascular variability. However, in this investigation patients with CKD had a significantly reduced LF power in both absolute and normalised units. Thus the association between the tonic and phasic characteristics of the LF contributions to autonomic control, which are evident in normal subjects (Pagani et al. 1997) are lost in patients with CKD. A reduced LF component of HRV has been previously reported in patients with CKD (Vita et al. 1999), however, this is the first study to demonstrate an undetectable LF power in patients with severe CKD. Similar findings have been documented in patients with chronic heart failure (CHF) (Guzzetti et al. 1995; van de Borne et al. 1997). In patients with CHF, the greater the reduction in LF power the higher the level of sympathetic activation and the greater the risk of mortality (van de Borne et al. 1997). Our work has suggested a similar potential for increased mortality in CKD.

This study demonstrated for the first time a significant positive correlation ($r = 0.7$, $p < 0.001$) between HRV and eGFR. This may indicate that the degree of autonomic dysfunction is related to the severity of CKD insufficiency or atherosclerosis or cardiomyopathy (Sharma et al. 2007; Sharma et al. 2006a; Sharma et al. 2006b; Sharma et al. 2006c). This may add important information to the literature since the presence and severity of autonomic neuropathy do not seem to be related with either the duration of CKD or with the duration of dialysis (Vita et al. 1999). Furthermore, research using

intravascular ultrasonography (IVUS) demonstrated that the lower the creatinine clearance the greater the severity of atherosclerotic plaque (Gruberg et al. 2005). However, only patients on dialysis treatment demonstrated statistically significant increases in plaque burden. This decline cannot be explained by a reduced LVEF.

As illustrated in diabetic patients, CKD patients demonstrated a significant correlation ($r = 0.84$, $p < 0.001$) with BRS and LF (ms^2) power. This may have clinical significance since a reduced BRS, LF power, and worsening renal function are associated with increased risk of all cause and cardiovascular morbidity and mortality. Exercise training has been shown to improve BRS and functional capacity in patients with CKD (Petraki et al. 2008). Therefore, exercise training may be a potential therapeutic method of improving prognosis in patients with CKD.

Chronic kidney disease patients had a significant correlation ($r = 0.84$, $p < 0.001$) with HRV and CRP. Previous research has documented that inflammation is a strong and independent predictor of cardiovascular mortality in CKD patients (Stenvinkel et al. 1999; Zimmermann et al. 1999). Patients within the highest quartile had a 4.6 and 5.5 fold higher relative risk for all-cause and cardiovascular mortality, respectively, compared to patients in the lowest quartile (Zimmermann et al. 1999). In addition, research has demonstrated a strong association between atherosclerosis and an elevated CRP in patients with CKD not on dialysis (Stenvinkel et al. 1999). The effect cholinergic stimulation has on inflammation, parasympathetic modulation, and prognosis in patients with CKD requires further research.

6.5: SUMMARY

Diabetes and CKD is associated with an increased risk of CVD risk. Possible mechanisms include coronary artery disease, left ventricular dysfunction, LVH, cardiac micro infarctions, inflammation, arrhythmia, and autonomic dysfunction. This work supports previous research, which demonstrates that significant autonomic dysfunction exists in patients with diabetes and CKD.

This study has shown that LF (ms^2) oscillations of HRV and BRS function are significantly reduced in diabetic and CKD patients. Therefore the research hypothesis, which stated that LF oscillations of HRV will be significantly elevated in patients with diabetes and CKD is rejected, and the hypothesis, which stated that BRS will be significantly attenuated in patients with diabetes and CKD is accepted. Analysis of BRS in patients with diabetes and CKD may provide a simple non-invasive assessment of cardiovascular autonomic decline and may contribute to more accurate identification of individuals at higher risk of mortality. The association with elevated CRP and autonomic decline in diabetic and CKD patients enables acceptance of the research hypothesis, which stated that HRV will be significantly associated with CRP in patients with diabetes and CKD. The effects interventions such as exercise training and cholinergic stimulation may have on HRV, inflammation, and BRS in diabetic and CKD patient's needs to be further studied.

Autonomic dysfunction is related to the severity of CKD insufficiency. Therefore the research hypothesis, which stated that HRV is significantly attenuated as kidney function declines as measured using eGFR is accepted. Furthermore, in severe CKD an undetectable LF (ms^2) power is similar to that demonstrated in patients with severe CHF. Invasive measures of sympathetic activity in combination with HRV assessment may provide a deeper understanding in the progressive decline in the LF oscillatory component of HRV in CKD. Comparisons with findings described in patients with CHF may further facilitate understanding.

Improving HRV, BRS, and inflammation are potential therapeutic targets. The effect pharmacological optimisation, exercise training, glycaemic control and time of dialysis merit further research. This chapter demonstrated that LF oscillations of HRV significantly decline as metabolic disease worsens; therefore LF oscillations may not accurately reflect sympathetic activity at rest in diabetic and CKD patients. The functional cardio-dynamics of patients with hypertension are impaired and as a consequence sympathetic activity is elevated. Previous research documented significantly elevated LF oscillations at rest in hypertensive patients; however, due to the findings in this chapter future research is needed. In addition, the vascular compliance of patients with hypertension is reduced and the HRV response to haemodynamic stress is unclear and requires future research.

CHAPTER 7: IMPACT OF DOBUTAMINE INFUSION ON HAEMODYNAMIC AND AUTONOMIC CONTROL IN PATIENTS WITH AND WITHOUT HYPERTENSION

7.0: ABSTRACT

BACKGROUND: Autonomic dysfunction is an established risk factor for adverse cardiovascular events in patients with hypertension. This investigation aimed to compare changes in autonomic modulation measured non-invasively using HRV during dobutamine stress in hypertensive and non-hypertensive patient groups.

METHODS: 314 consecutive patients (mean age 64 ± 11 years, 164 male) referred for dobutamine stress echocardiography. On the basis of three successive blood pressure (BP) recordings, patients were characterised as hypertensive (HTN) (n = 114) or non-hypertensive (non-HTN) (n = 200). Dobutamine infusion was given in a stepwise manner up to a dose of 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Haemodynamic and autonomic data were obtained from beat-to-beat analysis of heart rate and blood pressure using a plethysmographic device, the Task Force® Monitor. We thereby determined total power spectral density (PSD) and associated low frequency (LF) and high frequency (HF) power spectral components in absolute (ms^2) and normalised units (nu).

RESULTS: At rest, HTN patients had a significantly increased heart rate ($p < 0.05$), systolic BP ($p < 0.001$), diastolic BP ($p < 0.001$), stroke index ($p < 0.05$), total peripheral resistance index ($p < 0.001$), and significantly reduced RR interval ($p < 0.05$), PSD ($p < 0.05$), LF (ms^2) power ($p < 0.05$), and HF (ms^2) power ($p < 0.05$) compared to non-HTN patients. The normal response to dobutamine infusion was a rise in LF (nu) power followed by a sharp drop at peak dose dobutamine. However, in patients with hypertension this initial rise in LF (nu) power is significantly exaggerated compared to non-hypertensive patients.

CONCLUSION: Haemodynamic responses to dobutamine infusion are influenced by the stability of the autonomic nervous system in patients with and without hypertension. Hypertensive patients exhibit significantly exaggerated HRV responses to dobutamine infusion with an indication of increased sympathetic activity. This may explain the increased risk of cardiovascular events seen in patients with hypertension.

7.1: INTRODUCTION

The previous chapter demonstrated that LF oscillations of heart rate variability (HRV) are significantly reduced in patients with diabetes and chronic kidney disease (CKD). Therefore non-invasive autonomic assessment using HRV methodology may not accurately detect elevated sympathetic activity at rest. Significantly elevated LF oscillations of HRV have been previously reported in patients with hypertension (HTN). However, due to the results documented in chapter 6 future research is needed. In addition, the vascular compliance of patients with hypertension is reduced, which may account for the increased morbidity and mortality in HTN patients, and the HRV response to haemodynamic stress is unclear.

Arterial HTN affects approximately 1-billion individuals worldwide (Kearney et al. 2005; Smith et al. 2006). In England it is the most prevalent cardiovascular disorder (Pagani and Lucini 2001) and significantly increases the risk of mortality, coronary artery disease, heart failure, chronic kidney disease, stroke and peripheral vascular disease (Fagard et al. 1996; Huikuri et al. 1999; Smith et al. 2006). Blood pressure is controlled by a complex mechanism involving haemodynamic, neural and hormonal factors, with cardiac output (CO) and peripheral vascular resistance (PVR) as its main determinants (Grassi 2003). Systolic blood pressure is primarily related to factors that influence ventricular function; including contractility, ventricular after load, blood volume, and heart rate (Salles et al. 2006).

The haemodynamic changes associated with HTN are characterised by enhanced sympathetic activity and reduced vagal tone (Esler 2003; Grassi and Esler 1999; Guzzetti et al. 1988). Chronic imbalance of the autonomic nervous system is an established risk factor for adverse cardiovascular events (Curtis and O'Keefe 2002). The sympathetic over-activity appears to have adverse metabolic and other consequences beyond initiating and maintaining blood pressure elevation (Esler 1998, 2003; Grassi et al. 1998; Julius 1991, 1998; Mancia et al. 1983; Somers 2002). These include trophic effects, which contribute to cardiovascular growth and the development of left ventricular hypertrophy (Laks et al. 1973; Ostman-Smith 1981; Simpson 1983) and structural changes in blood vessels (growth of vascular muscle), which increases vascular resistance (Esler 1998; Somers 2002). Furthermore, the neural vasoconstriction due to enhanced sympathetic activation is associated with impaired glucose delivery to muscle, insulin resistance, and hyperinsulinaemia and reduced postprandial clearing of lipids, contributing to hyperlipidaemia (Esler 1998). Other adverse effects associated with increased sympathetic activity include sodium retention and renin release (DiBona 1992; Somers 2002).

Exercise causes an increase in blood pressure, HR, and CO with a decrease in peripheral vascular resistance (Dewey et al. 2007; Fagard et al. 1996; O'Sullivan and Bell 2000). A decreased heart rate variability (HRV) at rest and during exercise is associated with increased cardiovascular risk and worse prognosis (Leino et al. 2009). In addition, greater systolic blood pressure and reduced systemic vascular resistance at peak exercise in hypertensive men were positively related to increased mortality (Fagard et al. 1996)

and future cardiovascular events (Laukkanen et al. 2004). During the early stages of exercise in sedentary and athletic subjects, research has reported a decrease in high frequency (HF) power (a reflection of parasympathetic modulation) and increase in low frequency (LF) power (a reflection of sympathetic activity). At peak exercise intensity HF increased and LF decreased and during recovery LF increased and HF decreased (Bernardi et al. 1990; Pichon et al. 2004). However, recent research produced conflicting results and detailed that greater HRV, increased HF power and a reduced LF/HF ratio were associated with increased risk of cardiovascular death (Dewey et al. 2007). The autonomic changes during exercise may be related to cardiac protection by reducing the possibility of exercise induced ventricular arrhythmias, although the causality and mechanisms have not been established (Malik et al. 1996). However, autonomic changes during dobutamine infusion are less well described.

Research in canine models demonstrated that the autonomic nervous system plays an important role in modulating the cardiovascular effects of dobutamine, buffering the chronotropic and pressor responses, causing vasodilation and increasing skeletal muscle blood flow (Liang and Hood 1979). Human studies have suggested dobutamine infusion initiates similar effects to exercise, with significant increases in blood pressure, heart rate and cardiac output and a decreased peripheral vascular resistance (Binkley et al. 1995; Hogue et al. 1995; Liang and Hood 1979; Schobel et al. 1991; van de Borne et al. 1999). However, Houge et al., (1995) reported variations in blood pressure responses to dobutamine infusion. This group suggested the responses were in part explained by changes in autonomic tone, but the significance of this is unclear.

Potential mechanisms for different blood pressure response during dobutamine infusion include baroreflex intervention, (Binkley et al. 1995; Liang and Hood 1979) changes in central command (Raven et al. 2006), mechanoreceptor influences (Hogue et al. 1995) and dynamic left ventricular outflow tract obstruction (Sharma et al. 2006c). Baroreflex resetting is an important mechanism that allows increased sympathetic outflow and arterial blood pressure (Chapleau 1993). During exercise the baroreflex adjusts its operating point in order to optimally counteract hypertensive stimuli (Raven et al. 2006). However, this adjustment reduces the sensitivity of the baroreflex and occurs in direct relation to the exercise intensity (Ogoh et al. 2005; Raven et al. 2006). During dobutamine infusion the chronotropic effects of dobutamine prevent a reflex bradycardia in response to increasing blood pressure and the baroreflex sensitivity decreases as the infusion of dobutamine increases (van de Borne et al. 1999).

7.1.1: AIM

To date, no study has compared haemodynamic and autonomic changes measured non-invasively using HRV during dobutamine infusion in patients with and without HTN. Such a comparison may provide useful information regarding the significance of an elevated blood pressure and resting sympathetic predominance in HTN patients, and the control mechanisms employed to buffer the haemodynamic challenge induced by dobutamine between HTN and non-HTN patient groups. Changes in autonomic control may in part be responsible for the observed increased mortality and morbidity related to HTN. Therefore the aim of the study was to assess the impact of dobutamine infusion

during stress echocardiography on the frequency domains of HRV in patients with and without HTN.

7.1.2: HYPOTHESIS

1. H_1: Significant differences in heart rate variability exist at rest between hypertensive and non-hypertensive patients.

2. H_1: Patients with hypertension have significantly elevated sympathetic drive, represented by low frequency heart rate variability oscillations compared to non-hypertensive patients at rest.

3. H_1: Autonomic modulation, represented by frequency oscillations of heart rate variability is significantly different between hypertensive and non-hypertensive patients during dobutamine-induced stress.

7.2: METHOD

7.2.1: PARTICIPANTS

As detailed in Chapter 3: General Methods, the number of patients studied was 314. Patient selection and the collection of demographic data are described in Chapter 3: General Methods, and the baseline characteristics of these patients are described in Chapter 4: Baseline Population. The patients were divided into two groups, hypertensive-group (HTN) and non-hypertensive-group (non-HTN). Patients were divided into these groups according to the British Hypertension Society's classification of hypertension with the diagnosis made by three successive blood pressure readings (blood pressure >140/90 mmHg) (Williams et al. 2004). Table 7.1 details the baseline physical characteristics of both patient groups.

Table 7.1: Baseline Characteristics of Hypertensive and Non-Hypertensive Groups

Parameter	Hypertensive Patients (n = 114)	Non-Hypertensive Patients (n = 200)
Age (Years)	64.7 ± 10.6	64.6 ± 12
Gender	♂ 62 (54.4%)	♂ 102 (51%)
Ethnicity	Asian = 72 (63.2%)	Asian = 111 (55.5%)
	Caucasian = 24 (21.1%)	Caucasian = 77 (38.5%)
	Black = 17 (14.9%)	Black = 10 (5%)
	Other = 1 (0.9%)	Other = 2 (1%)
Height (cm)	164.5 ± 9.3	164.4 ± 10
Weight (kg)	80.2 ± 16.8	76.4 ± 18.4
BSA (m^2)	1.86 ± 0.21	1.83 ± 0.23

Note: BSA = Body surface area.

7.2.2: AUTONOMIC ASSESSMENT

The Task Force® Monitor (TFM) (CNSystems, Graz, Austria) was used for the continuous non-invasive beat-to-beat monitoring and real time calculation of all cardiovascular haemodynamic and autonomic parameters. Parameters included; continuous electrocardiography, continuous blood pressure, stroke index, cardiac index, total peripheral vascular resistance index, baroreceptor reflex sensitivity, and HRV as outlined in Chapter 3: General Methods. After 30-minutes of supine rest, 15-minutes of resting haemodynamic and autonomic data was recorded. Intervention marks separated the stages of testing for automated statistical analysis and these were set at baseline, at each incremental dose of dobutamine infusion (10, 20, 30, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$), and in recovery. Ten seconds of data was sampled at the end of each intervention mark, and the mean of this interval data was calculated and subsequently used for statistical analysis.

7.2.3: DOBUTAMINE STRESS ECHOCARDIOGRAPHY

This was performed using a General Electric Vingmed System 7 ultrasound machine. Images were acquired in standard parasternal long- and short-axis and apical 2-, 3-, 4-chamber views at baseline and during stepwise infusion of dobutamine. This was given according to a protocol based on 3-minute stages of 10, 20, 30, 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$ as outlined in Chapter 3: General Methods.

7.2.4: STATISTICAL ANALYSIS

Once the data was collated onto a spreadsheet, it was analysed using the statistical package for social sciences (SPSS 17 release version of SPSS for Windows; SPSS Inc., Chicago IL, USA). Continuous variables were expressed as mean ± SD. Differences between and within groups were determined by 2-way repeated measures ANOVA. Independent samples t-test was used to test the differences between patient group medication. An alpha level of 0.05 was considered indicative of a statistically significant difference ($p < 0.05$).

7.3: RESULTS

As shown in table 7.2, 7.3, and 7.4 significant differences at baseline between HTN and non-HTN patients are seen in heart rate (HR), systolic blood pressure (sBP), diastolic blood pressure (dBP), stroke index (SI), total peripheral resistance index (TPRI), RR interval (RRI), power spectral density (PSD), LF (ms^2) power, and HF (ms^2) power.

Table 7.2: Baseline Haemodynamic Characteristics

Parameter	Hypertensive Patients	Non-Hypertensive Patients
Heart Rate ($b \cdot min^{-1}$)	76 ± 15	70 ± 14*
sBP (mmHg)	151 ± 29	126 ± 13**
dBP (mmHg)	92 ± 17	79 ± 17**
PP (mmHg)	59 ± 18	47 ± 8**
SI ($ml \cdot m^2$)	31.7 ± 8.7	34.9 ± 10*
CI ($L \cdot min^{-1} \cdot m^2$)	2.39 ± 0.77	2.41 ± 0.68
TPRI ($dyne \cdot s \cdot m^2 \cdot cm^5$)	3783.2 ± 114	3152.3 ± 81**

Note: sBP = Systolic blood pressure; dBP = Diastolic blood pressure; PP = Pulse pressure; SI = Stroke index; CI = Cardiac index; TRRI= Total peripheral resistance index. Note: * = $p < 0.05$; ** = $p < 0.001$.

Table 7.3: Baseline Autonomic Modulation

Parameter	Hypertensive Patients	Non-Hypertensive Patients
RRI (ms)	819.1 ± 144.6	887.5 ± 165.2*
PSD (ms^2)	2074.2 ± 352.1	2757.4 ± 324*
LF (nu) (%)	47.5 ± 23.2	45.5 ± 22.3
HF (nu) (%)	52.5 ± 23.2	54.5 ± 22.3
LF (ms^2)	687.9 ± 91.7	1031.9 ± 104*
HF (ms^2)	760.3 ± 229.9	1270.5 ± 413*
BRS $ms \cdot mmHg^{-1}$	11.3 ± 1.79	13.2 ± 1.1

Note: RRI = RR interval; PSD = Power spectral density; LF (nu) = Normalised units low frequency; HF (nu) = Normalised units high frequency; LF (ms^2) = Low frequency; HF (ms^2) = High frequency; BRS = Baroreceptor reflex sensitivity; * = $p < 0.05$.

Table 7.4: Baseline Echocardiographic Characteristics

Parameter	Hypertensive Patients	Non-Hypertensive Patients
LVESD (cm)	2.6 ± 1.0	3.0 ± 0.7*
LVEDD (cm)	4.8 ± 1.4	5.3 ± 0.9*
LVFS (%)	28 ± 15	25 ± 11
LVEF (%)	65 ± 12	59 ± 11
LA (cm)	3.9 ± 1.6	3.2 ± 1.4*
LVMI ($g \cdot m^2$)	122 ± 16	108 ± 17*
LV wall thickness	1.3 ± 0.6	1.1 ± 0.5
Mitral E/E'	13 ± 6	9 ± 4*
WMSI	1.1 ± 0.4	1.1 ± 0.6

Note: LVESD = Left ventricular end systolic diameter; LVEDD = Left ventricular end diastolic diameter; LVFS = Left ventricular fractional shortening; LVEF = Left ventricular ejection fraction; LA = Left atrium; LVMI = Left ventricular mass index; E/E' = Early mitral annular velocity; WMSI = Wall motion score index; * = $p < 0.05$.

As shown in table 7.5, significant differences in medication between HTN and non-HTN patients are seen in calcium channel blockers, diuretics, angiotensin receptor blockers, doxazosin, and digoxin.

Table 7.5: Medication differences between Hypertensive and Non-Hypertensive Patients

Parameter	Hypertensive Patients	Non-Hypertensive Patients
Beta Blocker	59	50
ACEI	50	47
CCBs	61	43*
Aspirin	87	85
Clopidogrel	25	26
Statin	95	93
Diuretic	51	36*
ARB	32	19*
Nitrate	26	32
Nicorandil	15	11
Ezetimibe	7	7
Doxazosin	18	7*
Digoxin	0	4*
Amiodarone	2	5
Warfarin	6	5

Note: ACEI = Angiotensin converting enzyme inhibitors; CCB = Calcium channel blockers; ARB = Angiotensin receptor blockers. Note: * = $p < 0.05$.

7.3.1: HAEMODYNAMIC RESPONSES

As shown in table 7.6, the HTN group had a significantly higher HR, sBP, dBP, TPRI, and a significantly lower SI compared to the non-HTN group at baseline. From baseline to peak dose dobutamine, HR significantly increased in both groups ($p < 0.001$), sBP significantly increased in the HTN group only ($p < 0.05$), CI significantly increased in the non-HTN group only ($p < 0.05$) and SI ($p < 0.001$ and $p < 0.05$) and TPRI ($p < 0.001$ and $p < 0.05$) significantly decreased in HTN and non-HTN patient groups respectively. At peak dose dobutamine the HTN group had a significantly higher sBP, dBP, and TPRI. In recovery, HR ($p < 0.001$) and CI ($p < 0.001$ and $p < 0.05$; HTN and non-HTN group respectively) significantly decreased and TPRI significantly increased ($p < 0.001$). At end recovery the HTN group had a significantly higher HR, sBP, dBP and TPRI.

Table 7.6: Haemodynamic Responses to Dobutamine Infusion in Hypertensive and Non-Hypertensive Patients.

Parameter	Baseline		10 $\mu g \cdot kg^{-1} \cdot min^{-1}$		Peak		3-min Recovery		10-min Recovery	
	HTN	Non-HTN	HTN	Non-HTN	HTN	Non-HTN	HTN	Non-HTN	HTN	Non-HTN
HR ($b \cdot min^{-1}$)	75.8 ± 14.9	70.1 ± 14.2*	83.3 ± 17.2	77.3 ± 16.4*	144.5 ± 16.8$^{\#\#}$	140.4 ± 18.7$^{\#\#}$	130.1 ± 16.7	126.3 ± 19	100 ± 10.7^{++}	96.3 ± 12.3$^{*/++}$
RRI (ms)	819.1 ± 144.6	887.5 ± 165.2*	749.1 ± 146.9	809.8 ± 167.7*	421.7 ± 57.6$^{\#\#}$	437 ± 75.7$^{\#\#}$	470.1 ± 71.4	489.6 ± 102	607.5 ± 70.2^{++}	634.9 ± 96.7$^{*/++}$
sBP (mmHg)	150.9 ± 28.6	125.9 ± 12.6**	155.4 ± 32	131.8 ± 23.9**	160. 4 ± 37$^{\#}$	140.6 ± 29.7**	169.9 ± 32	152.8 ± 27.3**	154 ± 27.3	137.7 ± 19.6**
dBP (mmHg)	91.5 ± 17.2	79.2 ± 10.4**	92.2 ± 20.3	78.9 ± 17.1**	96 ± 22.8	87.5 ± 20.2**	95.7 ± 19.9	88.3 ± 16.7**	91.6 ± 18.3	84.8 ± 13.2**
SI ($ml \cdot m^{2}$)	31.8 ± 8.7	34.9 ± 9.7*	31.4 ± 9.4	33.4 ± 10.2	26.6 ± 6.4$^{\#\#}$	28 ± 8$^{\#}$	28.3 ± 10.5	28.5 ± 7.6	26.9 ± 9	28 ± 7.9
CI ($L \cdot min^{-1} \cdot m^{2}$)	2.39 ± 0.76	2.41 ± 0.68	2.6 ± 0.89	2.54 ± 0.8	3.84 ± 0.96	3.91 ± 1.31$^{\#}$	3.65 ± 1.23	3.57 ± 0.98	2.69 ± 0.89^{++}	2.69 ± 0.85^{+}
TPRI ($dyne \cdot s \cdot m^{2} \cdot cm^{5}$)	3783 ± 1218	3152 ± 898.2**	3558 ± 1326	3088 ± 1035*	2372 ± 786$^{\#\#}$	2115 ± 632$^{\#}$	2624 ± 811	2435 ± 719*	3396 ± 1024^{++}	3102 ± 917$^{*/++}$

Table 7.6: Haemodynamic responses to dobutamine infusion in hypertensive and non-hypertensive patient groups. Note: HR = Heart rate; RRI = RR interval; sBP = Systolic blood pressure; dBP = Diastolic blood pressure; SI = Stroke index; CI = Cardiac index; TPRI = Total peripheral resistance index; * = $p < 0.05$; ** $p < 0.001$ for between groups; # = $p < 0.05$; ## $p < 0.001$ for within groups from baseline to peak dose dobutamine; + = $p < 0.05$, ++ = $p < 0.001$ from peak dose dobutamine to recovery.

7.3.2: DIFFERENCES IN ECHOCARDIOGRAPHY PARAMETERS

Patients with HTN have significantly (p <0.05) smaller left ventricular (LV) cavity, increased left atrial (LA) size, increased wall thickness, and increased LV mass index compared to non-HTN patients. These patients had similar left ventricular ejection fractions (LVEF) but significantly (p <0.05) higher estimated LV filling pressures (mitral E/E') compared to non-HTN patients.

7.3.3: AUTONOMIC RESPONSES

As shown in figure 7.1 and table 7.7 there were significant differences in PSD (HRV) and HF (ms^2) power between HTN and non-HTN groups at baseline, peak dose dobutamine, and in recovery. There were also significant differences between groups in LF (ms^2) power at baseline and peak dose dobutamine.

From baseline to peak dose dobutamine, PSD, LF (ms^2) and HF (ms^2) power and BRS significantly decreased ($p < 0.001$) in both groups. In the non-HTN group, there were no significant changes in the early stages of dobutamine infusion. However, at peak dose dobutamine there is a significant decrease ($p < 0.05$) in LF (nu) power and significant increase ($p < 0.05$) in HF (nu) power, which continues into recovery. In the HTN group, LF (nu) power significantly increased ($p < 0.05$) from baseline to low dose dobutamine and then significantly decreased ($p < 0.05$) to peak dose dobutamine and continues to decrease into recovery as seen with the non-HTN group. The reverse is seen in HF (nu)

power as shown in figure 7.1. Interestingly, there was a significant difference in LF (nu) power and HF (nu) power between HTN and non-HTN groups at low dose dobutamine and the significance of this will be addressed. From peak dose dobutamine to recovery, LF (ms^2) and HF (ms^2) power significantly decreased ($p < 0.05$) in both groups.

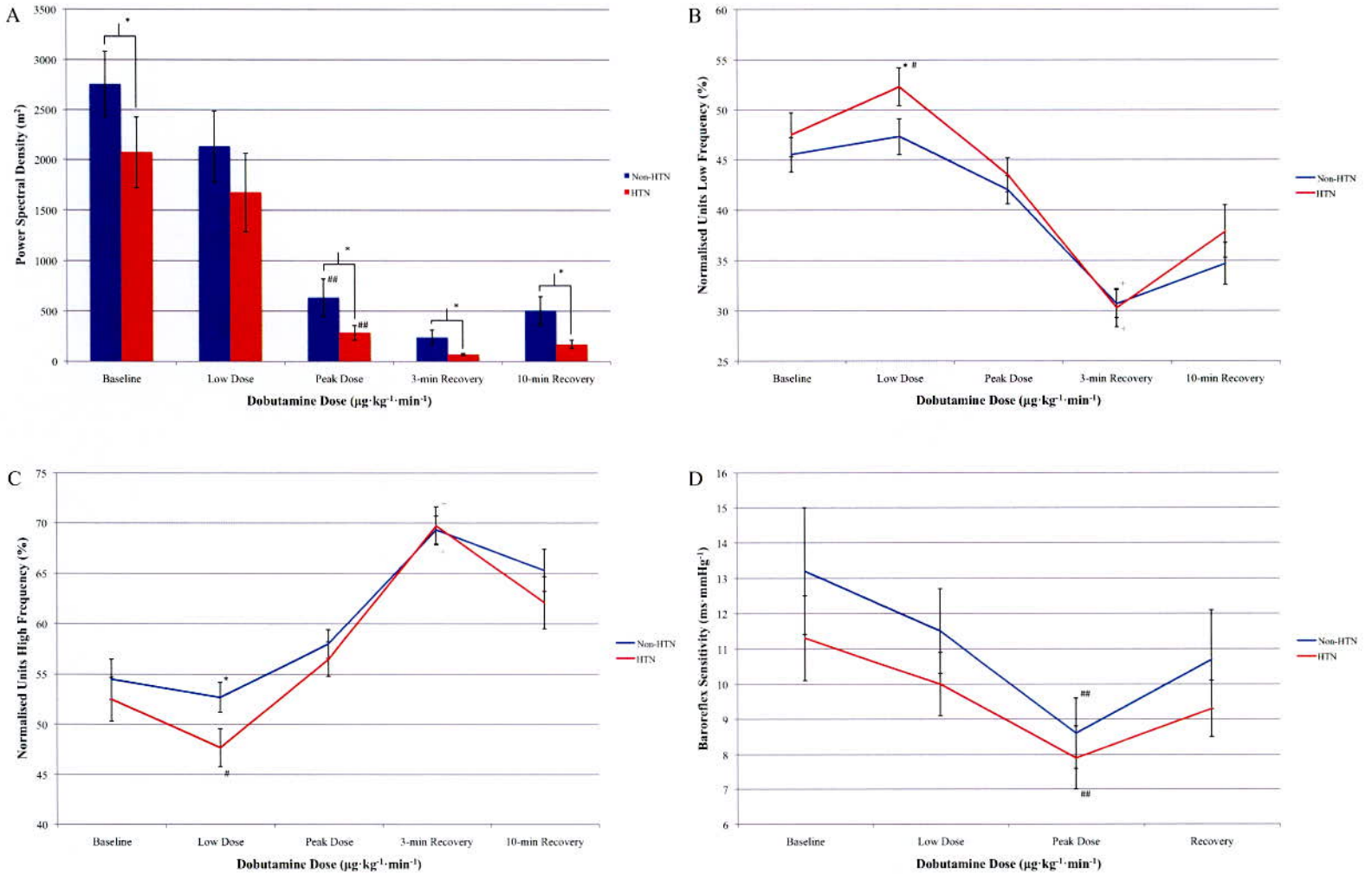

Figure 7.1: Autonomic response to dobutamine stress in hypertensive and non-hypertensive patient groups. Note: A = Power spectral density (HRV) response; B = Normalised units low frequency response; C = Normalised units high frequency response; D = Baroreceptor reflex sensitivity response; * = $p < 0.05$ for between groups; # = $p < 0.05$ and ## = $p < 0.001$ for within groups from baseline to peak dose dobutamine; + = $p < 0.05$ for within groups from peak dose dobutamine to recovery.

Table 7.7: Autonomic Responses to Dobutamine Infusion in Hypertensive and Non-Hypertensive Patients.

Parameter	Baseline		10 $\mu g \cdot kg^{-1} \cdot min^{-1}$		Peak		3-min Recovery		10-min Recovery	
	HTN	Non-HTN	HTN	Non-HTN	HTN	Non-HTN	HTN	Non-HTN	HTN	Non-HTN
LF (ms^2)	687.9 ± 91.7	1031.9 ± 104*	354.2 ± 51.3$^{\#}$	697.4 ± 75$^{\#}$	63.1 ± 19.7$^{\#\#}$	173.6 ± 40.2$^{*/\#\#}$	16.2 ± 3.5^{+}	64 ± 23.7^{+}	56.6 ± 12.2	159.1 ± 52.2
HF (ms^2)	760.3 ± 229.9	1270.5 ± 413*	323.1 ± 212.7$^{\#}$	787.3 ± 275.7$^{\#}$	81.9 ± 24.1$^{\#\#}$	237.3 ± 62.1$^{*/\#\#}$	37.3 ± 7.4^{+}	144.6 ± 35.4$^{*/+}$	92.7 ± 54	331.8 ± 119.6*
BRS ($ms \cdot mmHg^{-1}$)	11.3 ± 1.79	13.2 ± 1.16	10 ± 1.18	11.5 ± 0.91	7.9 ± 1.01$^{\#\#}$	8.55 ± 1.38$^{\#\#}$	-	-	9.28 ± 1.38	10.7 ± 1.16

Table 7.7: Autonomic responses to dobutamine infusion in hypertensive and non-hypertensive patient groups. Note: LF (ms^2) = Low frequency; HF (ms^2) = High frequency; BRS = Baroreceptor reflex sensitivity; $^{*} = p < 0.05$ for between groups; $^{\#} = p < 0.05$; $^{\#\#} p < 0.001$ for within groups from baseline to peak dose dobutamine; $^{+} = p < 0.05$ for within groups from peak to recovery.

7.4: DISCUSSION

This study demonstrates that HTN patients have a significantly reduced HRV compared to non-HTN patients at rest, which supports previous research. However, this study failed to demonstrate that HTN patients have a significantly elevated sympathetic drive, represented by LF oscillations of HRV compared to non-HTN patients at rest. This investigation further demonstrates that haemodynamic responses to dobutamine administration are similar in HTN and non-HTN patient groups. However, dobutamine produces significantly different responses in frequency oscillations of HRV in HTN patients compared to non-HTN patients.

At rest, the HTN group had a higher LF (nu) power (sympathetic drive), lower HF (nu) power (parasympathetic modulation), a significantly higher HR, sBP, dBP, TPRI and significantly lower HRV, LF (ms^2) power, HF (ms^2), and SI compared to the non-HTN group. These resting haemodynamics and autonomic function are consistent with established HTN (Esler 2003; Grassi and Esler 1999; Guzzetti et al. 1988; Julius 1988, 1991; Julius and Nesbitt 1996) and contribute to the complexity of maintaining homeostatic balance through autonomic control mechanisms during dobutamine stress.

Baseline echocardiography data demonstrated that hypertensive patients have significantly smaller LV cavity size and increased LV mass index. Markers of LV systolic function were similar between groups, but markers of diastolic function

suggested significantly higher LV filling pressure in HTN compared to non-HTN patients. These results are consistent with previous reports.

Dobutamine is predominately a β_1 adrenoreceptor agonist with weaker β_2 and α_1 properties and as a result HR and CI significantly increased from baseline to peak dose dobutamine in both groups. The augmented HR reduces left ventricular filling time and as a result SI significantly decreased from baseline to peak dose dobutamine in both groups. In order to maintain efficient homeostasis and minimise arterial blood pressure elevation, TPRI significantly decreased from baseline to peak dose dobutamine in both groups.

With incremental doses of dobutamine, LF (nu) power initially increased, with a parallel decrease in HF (nu) power in both groups. The augmented LF (nu) power attenuates at low dose dobutamine infusion with a coexisting increase in HF (nu) power to peak dose dobutamine, which continues into recovery in both groups. This autonomic adjustment is governed by increased vagal control mechanisms and reduced sympathetic activity directed on the myocardium (Akselrod et al. 1981; Binkley et al. 1991b; Binkley et al. 1995; Pomeranz et al. 1985; Salo et al. 2007). The reason for this autonomic change is thought to arise due to the increased ventricular contraction produced by dobutamine. An augmentation in myocardial contractility increases the rate and rise of ventricular and systemic arterial blood pressure that would serve as a stimulus to the baroreceptors and activation of myocardial mechanoreceptors, which is thought to cause sympathetic withdrawal and enhanced parasympathetic modulation (the von Bezold-Jarisch reflex)

(Hogue et al. 1995; Salo et al. 2007). The autonomic shift produces vasorelaxation to minimise arterial blood pressure elevation (Liang and Hood 1979; Salo et al. 2007). Research in canine models demonstrated the importance of autonomic control mechanisms to cause vasodilatation and increasing skeletal muscle blood flow in order to buffer the inotropic and chronotropic effects of dobutamine, similar to the exercise response (Liang and Hood 1979). Indeed, this autonomic reflex is more preserved in the non-HTN group, with a significantly lower LF (nu) power (sympathetic drive) at low dose dobutamine and this has been previously determined to play a cardio protective role by reducing the workload of the heart (Hainsworth 1991; Mark 1983; Salo et al. 2007; Schultz 2001).

In addition, the frequency oscillations of HRV changes between baseline and peak dose dobutamine were significant in the HTN group only. This non-invasive index of autonomic modulation indicates that the HTN patients' autonomic control mechanisms are exaggerated and hyper responsive compared to non-HTN patients. It appears that the HTN patients may have a reduced ability to buffer the haemodynamic stress induced by dobutamine as adequately as the non-HTN patients and may be a reason for this excessive autonomic cardiovascular control response, which could be related to their increased TPRI, reduced baroreflex sensitivity, and/or other neural control mechanisms (Pagani 2003).

The chronotropic effect of dobutamine prevents the baroreceptors initiating a reflex bradycardia (van de Borne et al. 1999). However, an impaired or reduced BRS is a

major restraining mechanism of the parasympathetic and sympathetic autonomic control mechanisms (Grassi 2003; Mancia 1997), which was an observed finding between HTN and non-HTN patients in this study. In addition, cardiopulmonary receptor pathway activation appears to be reduced in hypertension, thereby reducing sympathoinhibitory influences in controlling circulating blood volume and release of vasoactive substances (Grassi 2003; Mancia 1997). Furthermore, it has been documented that in patients with hypertension, adrenaline may be released from extra-adrenal areas such as the heart as well as from the adrenal medulla and may act as an amplifier of sympathetic activity at both central and peripheral levels (Floras 1992; Rumantir et al. 2000). Moreover, conclusive evidence demonstrates that sympathetic activation promotes cardiac and vascular alterations including left ventricular hypertrophy and arteriolar wall hypertrophy or remodelling as well as decreased arterial distensibility, which increases the hypertension-related morbidity and mortality independent of blood pressure elevation (Bernini et al. 1993; Folkow 1978; Heagerty 1997; Somers 2002). The summation of these physiological alterations induced by HTN may explain the significant difference in autonomic regulation during dobutamine infusion.

This study illustrates that the autonomic control mechanisms measured non-invasively using frequency oscillations of HRV, implemented to buffer the haemodynamic effects of dobutamine infusion are exaggerated in HTN patients compared to non-HTN patients. This study suggests that the cause of this hyper-responsive autonomic control mechanism is complex and multifaceted in its nature, but in part influenced by the greater TPRI and reduced BRS seen in the HTN patient group.

The exaggerated HRV response to dobutamine infusion may negatively impact upon morbidity and mortality of HTN patients and have numerous clinical implications. These include a reduced level of cardio-protection with an increased workload of the heart (Hainsworth 1991; Mark 1983; Salo et al. 2007; Schultz 2001), a reduced arrhythmogenic threshold due to an increased heart rate, reduced coronary perfusion triggered by sympathetic activation and parasympathetic inhibition, and acceleration of the progression of atherosclerosis and end organ damage (Bernini et al. 1993; Grassi 1998, 2003; Mancia et al. 1999).

7.5: SUMMARY

At rest, this study demonstrates that HTN patients have a significantly reduced HRV compared to non-HTN patients. This supports previous research and suggests that HTN patients have reduced cardiac autonomic modulation compared to non-HTN patients. Therefore the research hypothesis, which stated that significant differences in HRV exist at rest between HTN and non-HTN patients is accepted. However, this study failed to demonstrate that HTN patients had significantly elevated LF oscillations compared to non-HTN patients, therefore the research hypothesis, which stated that patients with HTN have significantly elevated sympathetic drive, represented by LF HRV oscillations compared to non-HTN patients at rest is rejected.

This study indicates that the haemodynamic responses to dobutamine infusion are similar between HTN and non-HTN patients. However, the stability and control of the

autonomic nervous system measured non-invasively using HRV in response to a haemodynamic challenge are significantly different between patients with and without HTN. Therefore, the hypothesis, which stated that autonomic modulation, represented by frequency oscillations of HRV is significantly different between HTN and non-HTN patients during dobutamine-induce stress is accepted.

When faced with circulatory instability, this study indicates that the autonomic nervous system has a fundamental role in assuring a new state of equilibrium in patient haemodynamics due to the changing frequency oscillations of HRV. The significantly different LF oscillations of HRV in response to dobutamine administration demonstrated in the HTN patient group may have numerous clinical implications, which may directly impact upon morbidity and mortality in HTN patients and may in part explain the increased risk of cardiovascular events in this patient group. Ischaemic heart disease significantly increases mortality and the frequency oscillations of HRV during transient myocardial ischaemia compared to non-ischaemic patients may also produce significantly different HRV responses and requires future research.

CHAPTER 8: DIFFERING AUTONOMIC RESPONSES TO DOBUTAMINE STRESS IN THE PRESENCE AND ABSENCE OF MYOCARDIAL ISCHAEMIA

8.0: ABSTRACT

BACKGROUND: Autonomic dysfunction is known to have prognostic significance in patients with coronary artery disease. This investigation aimed to assess changes in autonomic balance measured non-invasively using HRV during dobutamine stress in the presence and absence of myocardial ischaemia.

METHOD: 314 consecutive patients (mean age 64 ± 11 years, 164 male) referred for dobutamine stress echocardiography. Dobutamine infusion was given in a stepwise manner up to a dose of 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$. On the basis of a deterioration in wall motion score in one or more left ventricular segments, patients were categorised as non-ischaemic (NI, n = 252) or ischaemic (IS, n = 62) responders. Haemodynamic and autonomic data were obtained from beat-to-beat analysis of heart rate and blood pressure (BP) using a plethysmographic device, the Task Force® Monitor. We thereby determined total power spectral density (PSD) and associated low frequency (LF) and high frequency (HF) power spectral components in absolute (ms^2) and normalised units (nu).

RESULTS: At rest, diastolic blood pressure (BP) [89 ± 16 mmHg vs. 84 ± 13 mmHg for NI vs. IS, $p = 0.017$] and left ventricular ejection fraction (LVEF) [64 ± 17 vs. 55 ± 15 % for NI vs. IS, $p = 0.02$] discriminated between the groups. However, at peak stress, NI patients showed greater responses with respect to heart rate (143 ± 18 $b \cdot min^{-1}$ vs. 124 ± 16 $b \cdot min^{-1}$, $p = 0.001$); systolic BP 156 ± 34 mmHg vs. 138 ± 37 mmHg, $p = 0.03$); diastolic BP (94 ± 22 mmHg vs. 86 ± 18 mmHg, $p = 0.004$) and LVEF (76 ± 33 vs. 62 ± 18, $p = 0.002$). With respect to autonomic parameters, dobutamine stress produced a decrease in LF (nu) in NI (43 ± 13 % to 40 ± 12 %, 2-way ANOVA, $p = 0.03$) but an increase in LF (nu) in IS (39.5 ± 21 % to 56 ± 15 %, $p = 0.03$). In contrast, there was an increase in HF (nu) in NI (57 ± 13 % to 60 ± 19 %, $p = 0.03$) but a decrease in HF in IS (60.5 ± 21 % to 44 ± 15 %, $p = 0.04$). Consequently LF/HF ratio decreased in NI (from 1.6 ± 0.12 to 1.3 ± 0.09, $p = 0.02$), but rose in IS (from 1.32 ± 0.21 to 1.7 ± 0.09, $p = 0.01$).

CONCLUSION: In the absence of myocardial ischaemia, this study indicates that dobutamine stress is associated with a significant increase in parasympathetic tone and reduced sympathetic activity. Under conditions of ischaemia, there is a sharp alteration of this autonomic balance with suggestion of sympathetic activation and parasympathetic withdrawal. The autonomic differences demonstrated may contribute to the propensity to arrhythmia of the ischaemic myocardium.

8.1: INTRODUCTION

The previous chapter demonstrated that hypertensive (HTN) patients had significantly different frequency oscillations of heart rate variability (HRV) compared to non-HTN patients during dobutamine stress. Ischaemic heart disease increases mortality and the incidence of malignant ventricular dysrhythmias, therefore frequency oscillations of HRV during transient myocardial ischaemia may be significantly different compared to non-ischaemic patients and merits further research.

Coronary artery disease (CAD) is the leading cause of mortality in the United Kingdom (UK), accounting for approximately 94,000 deaths annually (Allender 2007). Mechanisms of death include myocardial infarction, heart failure, and arrhythmia. Numerous studies have demonstrated that a reduced HRV predicts morbidity and mortality in apparently healthy populations (Dekker et al. 1997; Tsuji et al. 1994) and in patients with CAD (Hayano et al. 1990; van Boven et al. 1998).

Under conditions of myocardial ischaemia there is an increased vulnerability to malignant ventricular dysrhythmias and poor outcome. Sympathetic activation is considered one of the factors implicated in life-threatening dysrhythmias (Guzzetti et al. 2005) and it has been suggested that reflex vagal activity provides protection against this vulnerability (De Ferrari et al. 1991; Hohnloser et al. 1994b; Schwartz et al. 1992). Indeed, previous research has demonstrated that during transient myocardial ischaemia (Bernardi et al. 1988), dipyridamole induced myocardial ischaemia (Petrucci et al.

1996), and in patients with variant angina (Yoshio et al. 1993), LF oscillations of HRV increase. However, balloon occlusion during coronary angiography produced conflicting autonomic responses (Airaksinen et al. 1993). This illustrates the complexity of the interaction between myocardial ischaemia and autonomic modulation and the physiological response, which may be a balance between autonomic inhibition and activation in response to ischaemia and which may also be anatomically determined (Lombardi et al. 1984; Malliani 1982; Malliani et al. 1969; Zipes 1990).

Dobutamine is a positive inotropic and chronotropic agent, which increases myocardial oxygen demand by increasing heart rate and force of contraction. Changes in autonomic modulation have been suggested to play an important role in stabilising the haemodynamic responses to dobutamine administration (Liang and Hood 1979). Previous work has suggested that vasodilation is derived in part from direct β-2 stimulation of the vasculature, but may also result from a reflex withdrawal of sympathetic drive mediated via the baroreceptor reflex and/or myocardial mechanoreceptor activation due to increased myocardial contractility (Binkley et al. 1991a; Binkley et al. 1995; Liang and Hood 1979).

Changes in frequency oscillations of HRV may reflect alterations in autonomic modulation during transient myocardial ischaemia and this research aimed to study this using the functional test, dobutamine stress echocardiography (DSE). Dobutamine stress echocardiography is a validated technique for the diagnosis of CAD and differentiating low and high-risk patients according to the presence or absence of myocardial ischaemia

(Marwick et al. 2001; Poldermans et al. 1999; Sharma et al. 2007; Sharma et al. 2006a; Sicari et al. 2003). The analysis is based on the visual interpretation of endocardial motion and wall thickening during dobutamine infusion. A detailed non-invasive analysis of autonomic modulation in patients with and without myocardial ischaemia during DSE has not previously been reported.

Autonomic reflex modulation measured non-invasively using frequency oscillations of HRV in the presence of myocardial ischaemia may produce different responses compared to non-ischaemic responders due to greater haemodynamic instability. Understanding autonomic control mechanisms during dynamic stress may provide important clinical information for outcome and SCD risk in patients with CAD.

8.1.1: AIM

The aim of this study is to evaluate changes in frequency oscillations of HRV during dobutamine stress echocardiography (DSE) in ischaemic and non-ischaemic responders. The results may provide useful clinical information of autonomic control and further insight into the mechanisms responsible for the increased risk of mortality seen in patients with CAD.

8.1.2: HYPOTHESIS

1. H_1: Significant differences in heart rate variability exist at rest between ischaemic and non-ischaemic patients.

2. H_1: Patients with ischaemia have significantly elevated sympathetic drive, represented by low frequency heart rate variability oscillations compared to non-ischaemic patients at rest.

3. H_1: Autonomic modulation, represented by frequency oscillations of heart rate variability is significantly different between ischaemic and non-ischaemic patients during dobutamine-induced stress.

8.2: METHOD

8.2.1: PARTICIPANTS

All patients studied were referred for a DSE on clinical grounds. Patient management was determined only by the results of the DSE. As detailed in Chapter 2: Methods, the number of patients studied was 314. Patient selection and the collection of demographic data are described in Chapter 3: General Methods, and the baseline characteristics of these patients are described in Chapter 4: Baseline Population. On the basis of a deterioration in wall motion score in one or more left ventricular segments, patients were categorised as non-ischaemic or ischaemic responders. Table 8.1 details the baseline physical characteristics of both patient groups.

Table 8.1: Baseline Characteristics of Ischaemic and Non-Ischaemic Patient Groups

Parameter	Non-Ischaemic (n = 252)	Ischaemic (n = 62)
Age (Years)	64 ± 11.2	66.9 ± 11.9
Gender	♂ = 128 (50.8%)	♂ = 36 (58.1%)
Ethnicity	Asian = 149 (59.1%)	Asian = 34 (54.8%)
	CAU = 77 (30.6%)	CAU = 24 (38.7%)
	Black = 24 (9.5 %)	Black = 3 (4.8 %)
	Other = 2 (0.8 %)	Other = 1 (1.6 %)
Height (cm)	164.4 ± 9.9	164.8 ± 8.8
Weight (kg)	78.6 ± 18.5	75.2 ± 14.5
BSA (m^2)	1.85 ± 0.23	1.85 ± 0.18

Note: CAU = Caucasian; BSA = Body surface area.

8.2.2: AUTONOMIC ASSESSMENT

The Task Force® Monitor (TFM) (CNSystems, Graz, Austria) was used for the continuous non-invasive beat-to-beat monitoring and real time calculation of all cardiovascular haemodynamic and autonomic parameters. Parameters included; continuous electrocardiography, continuous blood pressure, stroke index, cardiac index, total peripheral vascular resistance index, baroreceptor reflex sensitivity, and HRV as outlined in Chapter 3: General Methods. After 30-minutes of supine rest, 15-minutes of resting haemodynamic and autonomic data was recorded. Intervention marks separated the stages of testing for automated statistical analysis and these were set at baseline, at each incremental dose of dobutamine infusion (10, 20, 30, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$), and in recovery. Ten seconds of data was sampled at the end of each intervention mark, and the mean of this interval data was calculated and subsequently used for statistical analysis.

8.2.3: DOBUTAMINE STRESS ECHOCARDIOGRAPHY

This was performed using a General Electric Vingmed System 7 ultrasound machine. Images were acquired in standard parasternal long- and short-axis and apical 2-, 3-, 4-chamber views at baseline and during stepwise infusion of dobutamine. This was given according to a protocol based on 3-minute stages of 10, 20, 30, 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$ as outlined in Chapter 3: General Methods. In the normal response, a segment is normokinetic or hypokinetic at rest and normal or hypokinetic during stress. In the ischaemic response, a segment worsens its function during stress from normokinesis to

hypokinesis, akinesis, or dyskinesis. In this way patients were categorised as non-ischaemic or ischaemic responders.

8.2.4: STATISTICAL ANALYSIS

Once the data was collated onto a spreadsheet, it was analysed using the statistical package for social sciences (SPSS 17 release version of SPSS for Windows; SPSS Inc., Chicago IL, USA). Continuous variables were expressed as mean ± SD. Differences between and within groups were determined by 2-way repeated measures ANOVA. An alpha level of 0.05 was considered indicative of a statistically significant difference ($p < 0.05$).

8.3: RESULTS

8.3.1: HAEMODYNAMIC RESULTS

As shown in table 8.2, significant differences between ischaemic and non-ischaemic responders were seen in diastolic blood pressure (dBP) at baseline, peak dose dobutamine, and in recovery, systolic blood pressure (sBP) at low dose and peak dose dobutamine, heart rate (HR) at peak dose dobutamine, cardiac index (CI) at peak dose dobutamine and in recovery and stroke index (SI) in recovery.

From baseline to peak dose dobutamine, HR significantly increased in the ischaemic ($p < 0.05$) and non-ischaemic ($p < 0.001$) groups, sBP and CI significantly increased in the non-ischaemic group only ($p < 0.05$), and SI ($p < 0.05$) and TPRI ($p < 0.001$) significantly decreased in the ischaemic and non-ischaemic patient groups. At peak dose dobutamine to recovery the ischaemic group had a significant ($p < 0.05$) increase in sBP. In recovery, HR ($p < 0.001$ and $p < 0.05$, non-ischaemic and ischaemic group respectively) and CI ($p < 0.05$) significantly decreased and TPRI significantly increased ($p < 0.001$) in both groups.

Table 8.2: Comparison of Haemodynamic Parameters during Dobutamine Stress between Non-Ischaemic and Ischaemic Responders.

Parameter	Baseline		10 $\mu g \cdot kg^{-1} \cdot min^{-1}$		Peak		3-min Recovery		10-min Recovery	
	Non-Ischaemic	Ischaemic	Non-Ischaemic	Ischaemic	Non-Ischaemic	Ischaemic	Non-Ischaemic	Ischaemic	Non-Ischaemic	Ischaemic
HR ($b \cdot min^{-1}$)	74 ± 15	72 ± 11	82 ± 18	79 ± 14	143 ± 18$^{\#\#}$	124 ± 16$^{**/\#}$	128 ± 19	129 ± 16	98 ± 13$^{\#\#}$	97 ± 11$^{\#}$
sBP (mmHg)	129 ± 26	136 ± 25	148 ± 32	141 ± 28*	156 ± 34$^{\#}$	138 ± 37*	166 ± 31	161 ± 34	150 ± 26	145 ± 25
dBP (mmHg)	89 ± 16	84 ± 13*	89 ± 20	84 ± 20	94 ± 22	86 ± 18*	95 ± 18	89 ± 20*	90 ± 16	85 ± 18*
PP (mmHg)	56 ± 17	57 ± 20	62 ± 21	57 ± 19	56 ± 28	58 ± 29	71 ± 21	72 ± 21	60 ± 17	60 ± 19
SI ($ml \cdot m^{2}$)	33 ± 10	34 ± 11	32 ± 10	32 ± 10	28 ± 7$^{\#}$	27 ± 8$^{\#}$	29 ± 9	26 ± 6.6*	28 ± 9	26 ± 7
CI ($L \cdot min^{-1} \cdot m^{2}$)	2.4 ± 0.8	2.4 ± 0.4	2.6 ± 0.9	2.5 ± 0.7	3.9 ± 1.2$^{\#}$	3.7 ± 1.2*	3.6 ± 1.1	3.4 ± 0.88*	2.7 ± 0.9$^{\#}$	2.5 ± 0.7$^{\#}$
TPRI ($dyne \cdot s \cdot m^{2} \cdot cm^{5}$)	3604 ± 1270	3493 ± 1063	3378 ± 1215	3328 ± 1285	2304 ± 728$^{\#\#}$	2194 ± 820$^{\#\#}$	2603 ± 802	2618 ± 916	3305 ± 1029$^{\#\#}$	3333 ± 914$^{\#\#}$

Table 8.2: Haemodynamic responses to dobutamine infusion in non-ischaemic and ischaemic patient groups. Note: HR = Heart rate; sBP = Systolic blood pressure; dBP = Diastolic blood pressure; PP; Pulse pressure; SI = Stroke index; CI = Cardiac index; TRPI = Total peripheral resistance index; * = $p < 0.05$ between groups; # = $p < 0.05$; ## = $p < 0.001$ within groups from baseline to peak dose dobutamine; + = $p < 0.05$; ++ = $p < 0.001$ within groups from peak dose dobutamine to recovery.

8.3.2: AUTONOMIC FUNCTION RESULTS

As shown in figure 8.1 and table 8.2, significant differences ($p < 0.05$) between ischaemic and non-ischaemic responders were seen in power spectral density (PSD) at low dose dobutamine and in recovery, normalised units low frequency (LFnu) and high frequency (HFnu) power at peak dose dobutamine, LF/HF ratio at peak dose dobutamine and in recovery, LF (ms^2) at baseline, low dose dobutamine, peak dose dobutamine, and in recovery, HF (ms^2) at peak dose dobutamine and in recovery, and baroreceptor reflex sensitivity in recovery.

From baseline to peak dose dobutamine, PSD, LF (ms^2), and HF (ms^2) significantly decreased ($p < 0.001$) in both groups. Normalised units LF significantly increased and HFnu significantly decreased ($p < 0.05$) from baseline to peak dose dobutamine in the ischaemic patients only. At peak dose dobutamine to recovery, PSD, LFnu, and LF/HF ratio significantly decreased ($p < 0.05$), and the HFnu significantly increased ($p < 0.05$) in ischaemic patients only. The LF (ms^2) significantly decreased in the ischaemic ($p < 0.001$) and non-ischaemic ($p < 0.05$) responders and the LF/HF ratio significantly increased ($p < 0.05$) in the non-ischaemic group from peak dose dobutamine to end recovery.

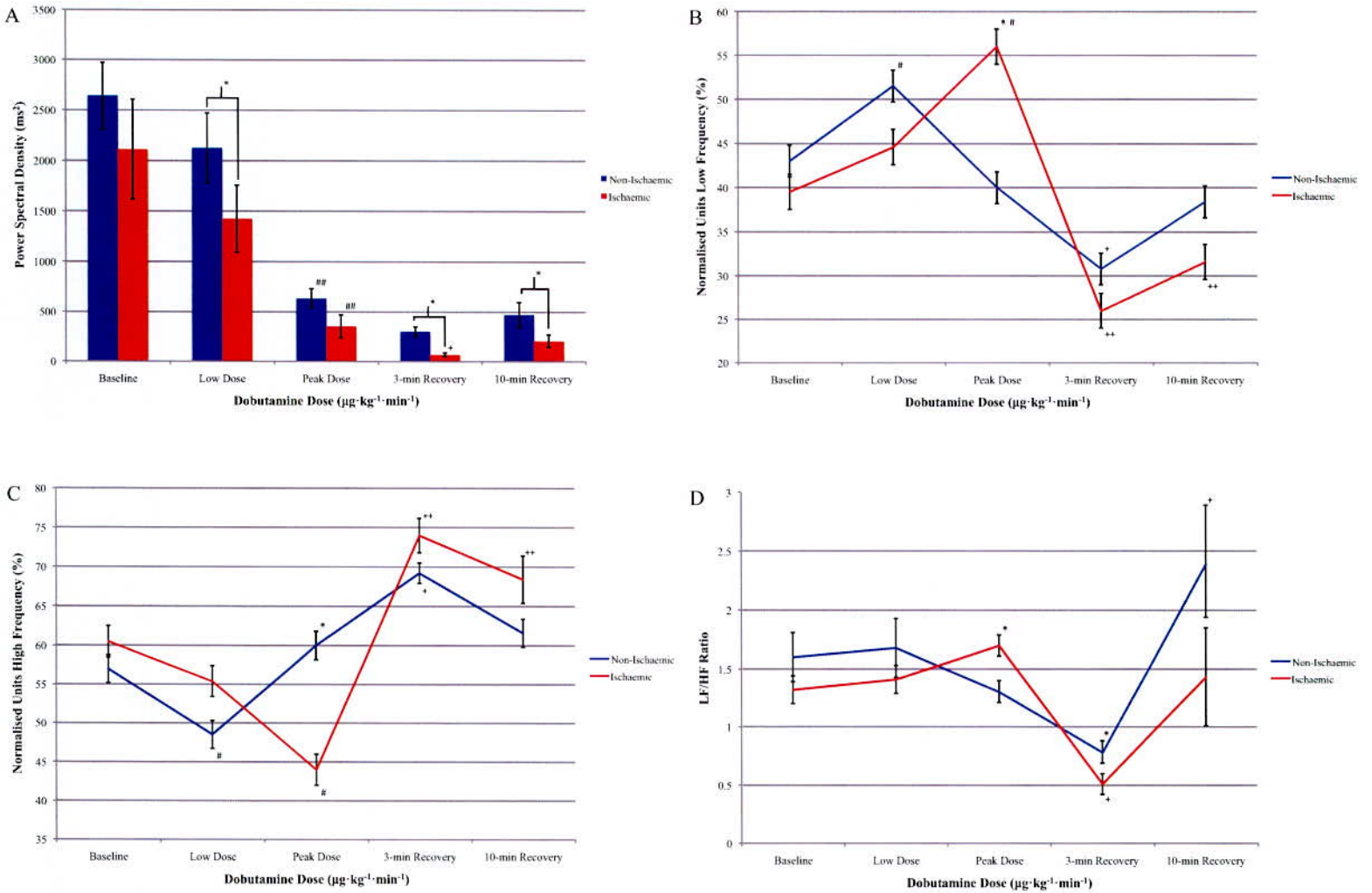

Figure 8.1: Autonomic response to dobutamine stress in ischaemic and non-ischaemic patient groups. Note: A = Power spectral density (HRV) response; B = Normalised units low frequency response; C = Normalised units high frequency response; D = LF/HF Ratio; * = $p < 0.05$ between groups; # = $p < 0.05$; ## = $p < 0.001$ within groups from baseline to peak dose dobutamine; + = $p < 0.05$; ++ = $p < 0.001$ within groups from peak dose dobutamine to recovery.

Table 8.3: Comparison of Autonomic Parameters during Dobutamine Stress between Ischaemic and Non-Ischaemic Responders.

Parameter	Baseline		10 $\mu g \cdot kg^{-1} \cdot min^{-1}$		Peak		3-min Recovery		10-min Recovery	
	Non-Ischaemic	Ischaemic	Non-Ischaemic	Ischaemic	Non-Ischaemic	Ischaemic	Non-Ischaemic	Ischaemic	Non-Ischaemic	Ischaemic
LF (ms^2)	981 ± 86	639 ± 116*	926 ± 59	518 ± 87*	213 ± 31$^{\#\#}$	110 ± 33$^{*/\#\#}$	84 ± 18^{+}	15 ± 4$^{*/++}$	160 ± 30	61 ± 15*
HF (ms^2)	1301 ± 249	979 ± 293	872 ± 228	644 ± 236	319 ± 48$^{\#\#}$	87 ± 41$^{*/\#\#}$	189 ± 28	43 ± 13*	257 ± 94	131 ± 47*
BRS ($ms \cdot mmHg^{-1}$)	12.73 ± 1.16	11.18 ± 1.38	12.31 ± 1.24	9.67 ± 1.09	8.11 ± 0.88	7.78 ± 0.92	-	-	10.52 ± 0.88	7.02 ± 0.77*

Table 8.3: Autonomic responses to dobutamine infusion in ischaemic and non-ischaemic patient groups. Note: LF (ms^2) = Low frequency; HF (ms^2) = High frequency; BRS = Baroreceptor reflex sensitivity; $^{*} = p < 0.05$ for between groups; $^{\#\#} p < 0.001$ for within groups from baseline to peak dose dobutamine; $^{+} = p < 0.05$; $^{++} = p < 0.001$ for within groups from peak dose dobutamine to recovery.

8.3.3: ECHOCARDIOGRAPHY RESULTS

As shown in table 8.4 and 8.5, significant differences ($p < 0.05$) between ischaemic and non-ischaemic responders were seen in left ventricular ejection fraction (LVEF), wall motion score index (WMSI), left ventricular end systolic volume (LVESV), left ventricular end diastolic volume (LVEDV) at baseline and peak dose dobutamine and with mitral E/E' at baseline.

Table 8.4: Comparison of Echocardiography Results at Baseline in Ischaemic and Non-Ischaemic Patient Groups

Parameter	Non-Ischaemic	Ischaemic
LVEF (%)	64 ± 17	55 ± 15*
WMSI	1.0 ± 0.2	1.2 ± 0.5*
LVESV	32 ± 12	41 ± 16*
LVEDV	64 ± 33	79 ± 22*
Mitral E/E'	8 ± 3	12 ± 5*
Maximum LVWT	1.2 ± 0.3	1.1 ± 0.7

Note: LVEF = Left ventricular ejection fraction; WMSI = Wall motion score index; LVESV = Left ventricular end systolic volume; LVEDV = Left ventricular end diastolic function; Mitral E/E' = Early mitral annular velocity; LVWT = Left ventricular wall thickness; * = $p < 0.05$.

Table 8.5: Comparison of Echocardiography Results at Peak Dose Dobutamine Stress in Ischaemic and Non-Ischaemic Patient Groups

Parameter	Non-Ischaemic	Ischaemic
LVEF (%)	76 ± 33	62 ± 18*
WMSI	1.0 ± 0.1	1.4 ± 0.2*
LVESV	21 ± 12	34 ± 16*
LVEDV	48 ± 26	73 ± 36*

Note: LVEF = Left ventricular ejection fraction; WMSI = Wall motion score index; LVESV = Left ventricular end systolic volume; LVEDV = Left ventricular end diastolic function; * = $p < 0.05$.

8.4: DISCUSSION

This study demonstrated that autonomic modulation during dobutamine infusion is significantly different between ischaemic and non-ischaemic responders. Importantly although there were significant differences in cardiac structure at rest, there were no significant autonomic differences at rest and this emphasises the need for functional testing to risk stratify patients.

In the absence of myocardial ischaemia, non-invasive autonomic assessment using HRV suggests dobutamine stress is associated with a significant increase in parasympathetic tone and reduced sympathetic activity. Under conditions of ischaemia, there is a sharp alteration of this autonomic balance with indications of sympathetic activation and parasympathetic withdrawal. This supports the concept that the autonomic nervous system influences the stability of cardiovascular haemodynamic control. Figure 8.2 and 8.3 illustrates the power spectral density of a normal response and ischaemic response during dobutamine infusion. Non-ischaemic responders (Figure 8.2) demonstrate a balance between low and high frequency (sympathetic and parasympathetic cardiac modulation respectively) power, whereas ischaemic responders (figure 8.3) show a greater proportion of total power within the low frequency (sympathetic modulation) component of HRV.

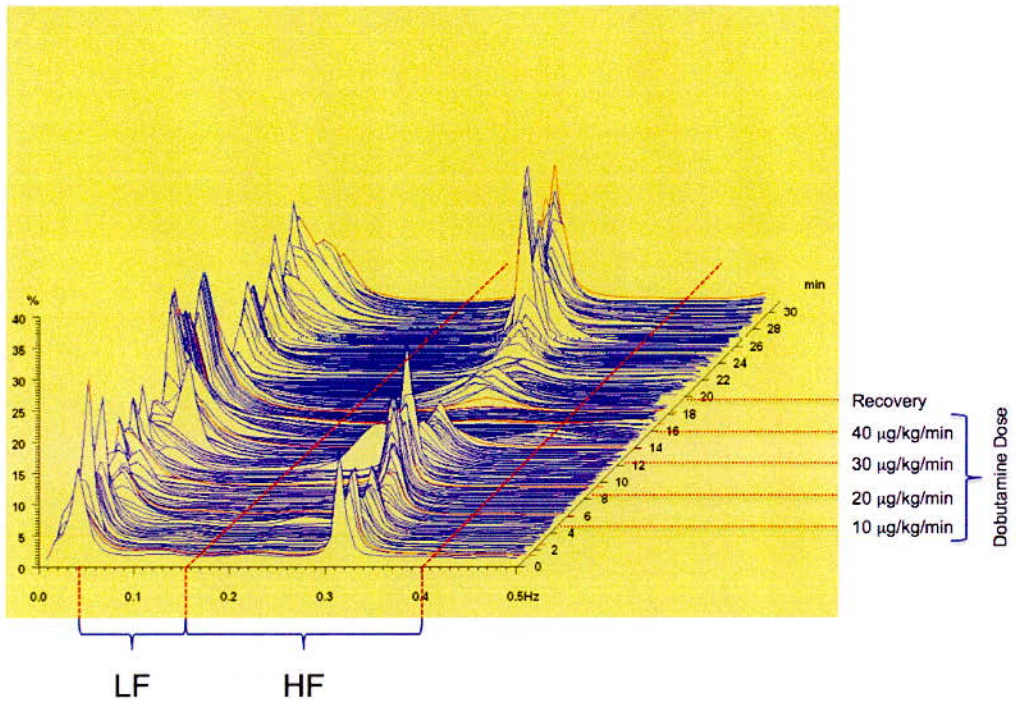

Figure 8.2: Power spectral analysis of a normal patient during dobutamine stress echocardiography. Note: LF = Low frequency; HF = High frequency.

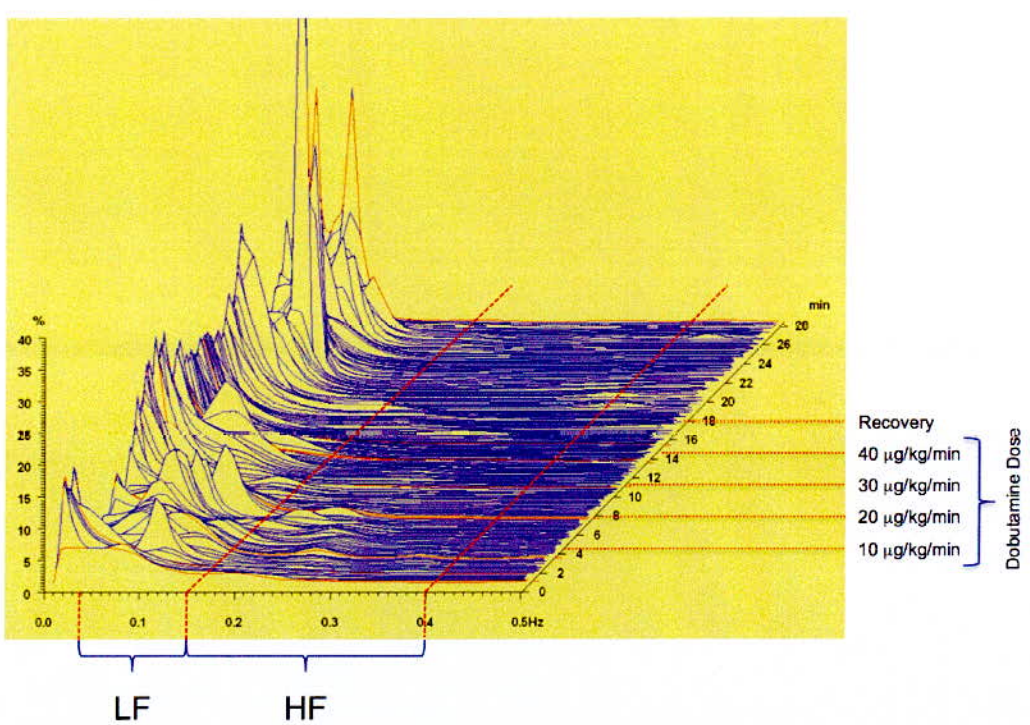

Figure 8.3: Power spectral analysis of an ischaemic patient during dobutamine stress echocardiography. Note: LF = Low frequency; HF = High frequency.

During dobutamine infusion, both groups initially exhibited an augmentation in LF (nu) power and attenuation of HF (nu) power, which reflects a relative increase in sympathetic drive and reduced parasympathetic modulation from baseline to low dose dobutamine. In the presence of ischaemia this sympathetic drive continued to increase with a further decrease in parasympathetic modulation into peak dose dobutamine.

However, non-ischaemic responders had directionally opposite autonomic changes, with a withdrawal in sympathetic drive and augmentation in parasympathetic modulation. This was also illustrated in the LF/HF ratio, where from low dose to peak dose dobutamine the ischaemic and non-ischaemic response intersect each other, with the former representing greater sympathetic drive and the later demonstrating sympathetic withdrawal.

These intergroup HRV response differences cannot be completely explained by BRS function since there is no significant difference in BRS during dobutamine infusion. However, other important influences include mechanoreceptor stimulation due to stretch, chemoreceptor stimulation due to acidosis and reactive oxygen species (Huang et al. 1995; Kumar 2009; Longhurst et al. 2001) and functional neural impairment (Zipes 1990) particularly in the ischaemic myocardium. Indeed, a cardiac sympathetic reflex response is activated by metabolic mediators (Longhurst et al. 2001; Malliani et al. 1983; Wang et al. 2006) and this suggests a biochemical stimulus in the ischaemic cascade. In non-ischaemic responders, the Bezold-Jarish reflex (Mark 1983) may explain the HRV response seen, where stimulation of inhibitory cardiac receptors by

stretch, chemical stimuli, or drugs, augments parasympathetic modulation and inhibits sympathetic activity, governed by vagal reflexes. Indeed, the non-ischaemic responders had a significantly increased LVEF, CI, and sBP, which support this concept.

The coronary circulation is dynamic, responding to changes in metabolic tissue demand via arterial vasodilatation and vasoconstriction and therefore controlling blood flow and oxygen delivery to the myocardium. Neurohormonal stimuli including cardiac adrenergic signals are important mediators in regulating myocardial blood flow (Di Carli et al. 1997). An increased sympathetic drive causes coronary artery dilatation and thus increases myocardial blood flow (Zeiher et al. 1989), and atherosclerosis is associated with an impairment in the capability of the coronary arteries to dilate (Zeiher and Drexler 1991; Zeiher et al. 1991). In addition, ischaemia and the metabolic process itself may directly promote an increase in sympathetic drive as described above. Furthermore, sympathetic drive and parasympathetic withdrawal reflected by changes in HRV has been demonstrated in ambulatory human subjects with ischaemic episodes (Bernardi et al. 1988) and before the onset of major arrhythmic events in patients with implantable cardioverter defibrillators (Guzzetti et al. 2005). This may explain the increased LF oscillations of HRV, which is suggestive of sympathetic activity at peak dose dobutamine in ischaemic patients.

The autonomic nervous system plays an important role in modulating the cardiovascular effects of dobutamine (Liang and Hood 1979) and the ischaemic responders demonstrated a drop in systolic blood pressure from low dose to peak dose dobutamine.

This may be due to greater sympathetic stimulation at peak dose dobutamine primarily to increase blood supply to the myocardium, but which will also result in peripheral arterial dilatation. Furthermore, the ischaemic responders had a significantly reduced heart rate, principally due to termination of the DSE protocol due to a new wall motion abnormality; however, total peripheral vascular resistance index was similar between groups. This indicates a relative mismatch in the degree of peripheral vascular resistance and heart rate response between groups. The above may explain the sBP drop in the ischaemic responders. However, myocardial ischaemia and deterioration in wall motion function results in significantly reduced LVEF and CI, which also contributes to the sBP drop.

The results of this study may have many clinical implications for patients with ischaemic heart disease and explain, in part, a potential cause of the increased incidence of malignant ventricular dysrhythmias and mortality seen in this group. This assumption is supported by previous research that reported an increase in LF oscillations of HRV before implantable cardioverter defibrillator discharge. Furthermore, the results in this study supports previous research with indications of sympathetic activation in ischaemic responders. This response reinforces the use of coronary intervention, revascularisation, and pharmacological optimisation to protect against premature mortality.

8.5: SUMMARY

At rest, this study failed to demonstrate any significant differences in HRV between ischaemic and non-ischaemic patients. Therefore, the hypothesis, which stated that significant differences exist at rest in measures of HRV between ischaemic and non-ischaemic patients is rejected. Furthermore, this study failed to demonstrate any significant differences in LF oscillations of HRV. Therefore, the hypothesis, which stated that patients with ischaemic heart disease have significantly elevated sympathetic drive, represented by LF oscillations of HRV compared to non-ischaemic patients at rest is rejected.

This study has demonstrated that autonomic modulation measured non-invasively using HRV is significantly different between ischaemic and non-ischaemic responders during dobutamine stress echocardiography. Therefore, the hypothesis, which stated that autonomic modulation, represented by frequency oscillations of HRV is significantly different between ischaemic and non-ischaemic responders during dobutamine-induced stress is accepted. In the absence of myocardial ischaemia, dobutamine stress is associated with a significant increase in parasympathetic modulation and sympathetic withdrawal. However, under conditions of ischaemia, there is a directionally opposite autonomic response with sympathetic activation and reduced parasympathetic modulation.

Assuming that these changes in HRV are associated with autonomic control of the cardiopulmonary circulation, these methods could be used to non-invasively monitor autonomic variations associated with myocardial ischemia in humans. The indication of adrenergic activation seen in ischaemic responders may contribute to the propensity to arrhythmia and haemodynamic instability of the ischaemic myocardium and reinforces coronary intervention procedures.

Finally, non-invasive autonomic assessment using HRV methodology is a potential tool with the ability to discriminate between ischaemic and non-ischaemic responses to dobutamine stress. Future research could verify these findings with simultaneous invasive methods of autonomic function and further assess the potential clinical application of HRV.

CHAPTER 9: DISCUSSION AND CONCLUSIONS

This thesis set out to non-invasively measure autonomic and haemodynamic function in a cohort of patients characterised as high and low cardiovascular disease risk. In addition, this thesis set out to combine heart rate variability analysis with other risk stratification tools, including resting and stress echocardiography and haematological parameters. The empirical work within this thesis is deliberately diverse in their methods of data treatment, analysis, and most of all in terms of the populations they represent. Three hundred and fifty consecutive patients who were referred for dobutamine stress echocardiography were recruited for the study.

Assessing cardiovascular disease risk is important and remains a problem for many physicians, especially those who care for patients with known risk factors such as heart failure, diabetes, chronic kidney disease (CKD), hypertension, and coronary artery disease (CAD). A variety of invasive and non-invasive tools exist including electrocardiography (ECG), echocardiography, coronary angiography, computed tomography (CT), and magnetic resonance imaging (MRI), all of which have strengths and limitations. Assessment of autonomic function is an important additional tool, but this has been poorly implemented and studied in well-characterised cardiovascular disease risk patients. Autonomic function can be studied invasively and non-invasively. Early non-invasive technology had many limitations. However, more modern equipment, such as the Task Force® Monitor (TFM) can reliably record continuous haemodynamic and autonomic data. This thesis sought to implement this technology in high and low cardiovascular disease risk patients further.

One component of autonomic function, which has been extensively researched, is heart rate variability (HRV). However, it is only in the last 20-years with the arrival of readily accessible computer hardware and software with built in algorithms capable of fast and reliable calculation of autonomic and haemodynamic variables that the utility of cardiovascular variability has become to be appreciated. Indeed, at present and an advantage to this research, the TFM is the only commercially available system capable of performing continuous, uninterrupted haemodynamic and autonomic assessment.

In Chapters 5, 6, and 7, patients with impaired left ventricular systolic and diastolic function, diabetes, CKD, and hypertension have a significantly lower HRV compared to normal patients at rest. These findings support previous research and indicate that total power spectral density is a reliable indicator of patients at increased risk of cardiovascular disease. In addition, an important observation was that there are significant resting autonomic differences in the normalised frequency components of HRV in patients with diabetes, CKD, and declining left ventricular function compared to normal patients. However, at rest these differences do not exist in patients with hypertension and CAD. This supports the concept that stress is required to risk stratify patients with ischaemic heart disease.

Chapter 5 is the first study to systematically analyse echocardiography parameters of congestive cardiac failure with autonomic function. Peak systolic velocity (PSV) and not left ventricular ejection fraction (LVEF) was independently associated with HRV. In addition, this study demonstrated that declining LVEF was associated with declining

HRV. This again mirrors increased mortality with declining LVEF. Furthermore, this study demonstrated for the first time that diastolic function was the strongest independent associate of a reduced HRV.

In chapter 6, the degree of autonomic dysfunction declines as the severity of kidney disease increases. This mirrors increasing mortality with declining renal function. Indeed, HRV significantly correlated with the estimated glomerular filtration rate (eGFR). When patients present with both diabetes and CKD, autonomic dysfunction is exacerbated. This suggests a clear role of autonomic function in contributing to the increased mortality in this patient group. Moreover, in both disease states HRV significantly correlated with C-reactive protein, a marker of inflammation.

Chapters 7 and 8 presented empirical work analysing changes in frequency oscillations of HRV during dynamic pharmacological stress using dobutamine. Frequency domain analysis of HRV was able to demonstrate significant differences in autonomic modulation in hypertensive and ischaemic patients compared to normal patients. Hypertensive patients demonstrated a significantly greater sympathetic (low frequency) activation in an attempt to buffer the inotropic and chronotropic effects of dobutamine and this adrenergic response may in part explain the increased risk of cardiovascular events in hypertensive patients. Ischaemic responders demonstrated directionally opposite autonomic responses at peak dose dobutamine compared to non-ischaemic responders and this may contribute to the propensity to arrhythmia and haemodynamic instability of the ischaemic myocardium. These autonomic differences that were not

seen at rest in ischaemic responders emphases the need for functional testing to risk stratify these patients.

Previous research has proposed that predominance in the low frequency (LF) power band is a sign of sympathetic activation, a characteristic seen in populations within this thesis. However, through analysing frequency components of HRV at rest, the research within this thesis failed to demonstrate that these disease states are characterised with sympathetic activation, i.e., a significant increase in the LF power component of HRV. In fact, the reverse is seen. Therefore, this thesis has provided evidence that the use of resting frequency domain analysis of HRV is not a reliable indicator of sympathetic activation. Indeed, no research has demonstrated a positive correlation with LF power and invasive measures of sympathetic activity, such as muscle sympathetic nerve activity (MSNA) or cardiac noradrenaline spillover. The opposite appears to be true, where the lower the LF power the higher the MSNA and cardiac noradrenaline spillover and the higher risk of cardiac death.

Based on this research, clinical intervention aimed at improving autonomic modulation in high-risk cardiovascular disease patients should be explored further. A 5-year follow up analysis will be performed on the patients within this thesis in order to record outcome and the predictive power of a reduction in autonomic modulation and death from cardiovascular cause. Thus far we have recorded 6-deaths from cardiac cause in groups at higher risk of mortality.

Future research could assess the benefits cholinergic stimulation, exercise training, and optimal pharmacological intervention will have on autonomic control in high-risk cardiovascular disease states. In addition, further work could assess the change, if any, in cardiovascular variability post coronary intervention or surgery due to the directionally opposite autonomic responses seen in ischaemic compared to non-ischaemic patient groups. An improvement in autonomic modulation would further support the use of such interventions. Furthermore, sampling more sensitive cytokines as measures of inflammation, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFα) with HRV as well as comparing invasive measures of autonomic modulation with non-invasive measures will advance our findings and may provide further insight into the mechanisms causing a reduction in HRV and increased mortality.

9.1: LIMITATIONS

A limitation of this thesis is the nature of the normal patient population studied. All patients were recruited on clinical grounds and as such our normal patients were being investigated due to suspected chest pain and therefore cannot be truly characterised as healthy. However, a fair comparison has been made since these patients had no cardiovascular disease risk factors, were on no pharmacological treatment, and had a normal dobutamine stress echocardiogram (DSE). In addition, a normal DSE is associated with a < 1% yearly mortality rate and it would have been unethical to subject normal age matched individuals to undergo a DSE due to the underlying risks involved.

A further limitation is that respiratory rate was not controlled at rest or during dobutamine infusion. However, metronome-guided respiration is not necessarily required for HRV measurement if subjects avoid irregular respiration (Kobayashi 2009). Furthermore, previous research has demonstrated that dobutamine infusion does not significantly alter breathing rate (van de Borne et al. 1999).

Furthermore, HRV is a non-invasive index of the autonomic nervous system. Its measurements reflect the ability with which post-junctional sino atrial node receptors react to oscillations in sympathetic and parasympathetic nerve discharge, and do not provide an absolute magnitude of neurotransmitter release. Therefore, HRV is unable to quantify the intensity of the stimulus and perhaps explains why frequency analysis of HRV is unable to determine sympathetic activation at rest. However, HRV is dynamic and demonstrated significantly different frequency domain alterations during dobutamine infusion in hypertensive and ischaemic patient groups compared to non-hypertensive and non-ischaemic patients and in this essence the addition of HRV analysis as an additional clinical tool requires further research.

Finally, it may also be debated that it is impossible to differentiate endogenous autonomic modulation from direct myocardial stimulation from dobutamine on HRV. However, it has been previously proposed that persistent beta stimulation produced by a steady-state infusion of dobutamine may not itself be expected to directly alter the dynamic phasic changes associated with HRV (Binkley et al. 1995; Malik and Camm 1993). Therefore, it appears likely that the differences observed in HRV in Chapters 7

and 8 are induced by reflexive autonomic modulation in response to the chemical and mechanical milieu of the myocardium with dobutamine administration.

9.2: CONCLUSION

Non-invasive analysis of autonomic function using HRV methodology is simple and provides great potential to identify risk in a number of populations. This measure is also dynamic and can be acutely altered by pharmacological stimuli. Important differences exist at rest with cardiac dysfunction, diabetes, CKD, and hypertension. However, no such differences exist with ischaemic heart disease at rest but there is an unfavourable altered pattern of autonomic modulation with dobutamine stress. Although the exact physiological meanings of certain HRV measures remain unclear, the fact that they predict risk makes them invaluable due to their strong associations and the simplicity and non-invasive nature of their acquisition. Further work is needed to determine whether commercially available software adds to existing diagnostic tools, but the results within this thesis are promising in this respect.

CHAPTER 10: REFERENCES

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