

**Investigating short-term blood pressure
regulation: peripheral baroreceptor
sensitivity**

PhD Thesis

2006

Susan Caroline Peirce

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CONTENTS

ACKNOWLEDGEMENTS.....	IV
DEDICATION.....	V
ABSTRACT	VI
ABBREVIATIONS	VII
CHAPTER 1 INTRODUCTION	1
Blood Pressure Regulation	1
Autonomic Control of Arterial Pressure.....	1
Measurement Methods	4
Analysis Methods	5
Summary and Organisation of the Thesis.....	6
CHAPTER 2 SHORT TERM BLOOD PRESSURE REGULATION.....	7
Physics of Vascular Resistance	7
Basic Physiology	8
Parasympathetic Innervation.....	9
Sympathetic Innervation	9
Sympathovagal Interactions	10
Failure of Blood Pressure Regulation.....	11
Response of Cardiovascular Variables to the Upright Position.....	12
Other Transient Investigation Methods.....	15
CHAPTER 3 VARIABILITY OF CARDIOVASCULAR PARAMETERS	16
Introduction	16
Spectral Power.....	16
Heart Rate or RR Interval?	17
Heart Rate and Blood Pressure Variability.....	18
Respiratory Frequency Variations	19
Arterial Pressure.....	19
RR Interval / Heart Rate.....	21
Cross Spectral Analysis.....	22
Low Frequency Variations	23
Arterial Pressure.....	23
RR Interval / Heart Rate.....	24
Cross Spectral Analysis.....	25
CHAPTER 4 CARDIAC BARORECEPTOR SENSITIVITY	26
Introduction	26
Further Baroreceptor Physiology	27
Estimation of Cardiac Baroreceptor Sensitivity	30
Imposed Ramp ('Oxford') Method	30
Neck Suction and Pressure.....	32
Estimates from Spontaneous Pressure Changes	32
Sequence Analysis	33
Spectral Analysis Methods.....	34
Transfer Function	36
Alpha Method	37
Comparison of the Methods	38
Diagnostic and Prognostic Use.....	41
Peripheral Baroreceptor Sensitivity	44

CHAPTER 5 MEASUREMENT AND ANALYSIS METHODS	46
Introduction	46
Finapres Blood Pressure Measurement	47
Method of Measurement	47
Reliability of Finapres Values	48
Peripheral Resistance	49
Spectral Analysis	52
FFT analysis for Power Spectral Density (PSD) Determination	52
Smoothing the Periodogram Estimates	53
Cross Spectra, Coherence and Transfer Functions	54
Analysis of the Coherence Function	55
Determination of Confidence Limits for Zero Coherence	55
Methods	56
Results	57
Determination of Confidence Limits for Non-Zero Coherence	59
Synchronisation of Beat-to-beat Events	62
Treatment of Ectopic Beats	64
Introduction	64
Identifying Ectopic or Missing Beats from the ECG	64
Results From Simulated Ectopics	65
Results From Real Data	65
Heart Rate Turbulence	66
The Effect of Supraventricular Ectopics on Beat-to-beat Cardiovascular Parameters	67
Methods	68
Results	68
Discussion	71
The Effect of Ventricular Ectopic Beats on Estimates of Cardiac BRS	72
Methods	74
Results	75
Ectopic-free Cardiac BRS	75
Comparison of Ectopic Treatment Options	76
Discussion	78
Signal Processing Methods Used in This Thesis	26
CHAPTER 6 PULSE CONTOUR DERIVATION OF STROKE VOLUME	80
From Stroke Volume to Peripheral Resistance	80
An Introduction to Pulse Contour Methods	80
Early Methods	82
The Corrected Impedance Method	82
Reliability of the Pulse Contour Method	83
Comparison of Finapres and Aortic Pressures as Input for Pulse Contour Method	84
Methods	84
Instrumentation	84
Analysis Programs	85
Custom vs. Beatscope Stroke Volume	86
Fidelity of the Aortic Pressure Recordings	86
Statistical Analysis	88
Results	88
Custom vs. Beatscope Stroke Volume	89
Fidelity of the Aortic Pressure Recordings	90
Finapres vs. Aortic Stroke Volume	92
Discussion	94
Custom vs. Beatscope Stroke Volume	94
Fidelity of the Aortic Pressure Recordings	95
Finapres vs. Aortic Stroke Volume	95
Transfer Function	96
CHAPTER 7 BEAT-TO-BEAT VALIDATION OF PULSE CONTOUR STROKE VOLUME	97
Introduction	97

Variability of Cardiovascular Parameters.....	97
Stroke Volume Variability	98
Doppler Measurement of Cardiac Output	98
Methods.....	100
Protocol.....	100
Instrumentation	101
Data Analysis	102
Results	104
Correlation of Beat-to-Beat Values.....	104
Power Spectra	105
Repeatability	110
Coherence.....	110
Beat-to-beat Peripheral Resistance.....	111
Discussion	113
 CHAPTER 8 PERIPHERAL BARORECEPTOR SENSITIVITY	116
Introduction	116
Peripheral Resistance as a Delayed Response.....	116
Methods.....	117
Data Analysis	117
Results.....	118
Discussion	121
Peripheral BRS Estimates.....	122
Methods.....	123
Subjects.....	123
Protocol.....	123
Instrumentation	123
Data Analysis	124
BRS Estimation.....	124
Alpha Method.....	125
Transfer Function Method.....	125
Sequence Method	125
Statistical Analysis.....	126
Results.....	127
Subjects.....	127
Rest Results - Cardiac BRS	127
Rest Results - Peripheral BRS	130
Tilt Results - Cardiac BRS.....	137
Tilt Results - Peripheral BRS.....	140
Peripheral versus Cardiac BRS.....	143
Discussion	144
Outcomes	144
Methods	146
Statistical Analysis.....	147
Conclusion	147
 CHAPTER 9 DISCUSSION.....	149
Thesis Summary	149
Sympathetic and Peripheral Baroreceptor Sensitivity	151
Study Limitations	154
Future Work	156
Statement of Contribution	157
 REFERENCES	159

ACKNOWLEDGEMENTS

University of Leicester, Cardiovascular Sciences Department:

My supervisors: Prof Ronney Panerai and Prof John Potter for their patience and erudite advice, and for reading this thesis in admirably short order.

Dr David Simpson, for providing several MATLAB functions and advice on programming, signal processing and analysis.

Dr Brian Carey and Dr Penny Eames for help and advice regarding physiology and pathophysiology, and for the subject data used in Chapter 8.

Other organisations:

Prof Karel Wesseling (Academic Medical Centre, University of Amsterdam) and Ben de Wit (FMS BV) for detailed information regarding the ‘corrected impedance’ pulse contour method and the Beatfast and Beatscope applications (Chapter 6).

Staff in the Cardiac Investigations Department (UHL NHS Trust) for assistance during the measurements in Chapter 6, and advice and learning material regarding Doppler and echocardiographic measurements.

Members of the Medical Physics Department (University of Leicester and UHL NHS Trust) for help with MATLAB programming, physiological measurements, analysis methods, and anything else vaguely scientific, and for agreeing to be subjects for the protocols in Chapter 7 and Chapter 8.

My new employers at Cardiff and Vale NHS Trust for the time and facilities used during the writing of this thesis, and the support and encouragement to use them.

Personal:

My friends and family, for their encouragement, forbearance, support and enjoyable company throughout the long journey. For coffee-room conversations and sympathetic ears.

Veronica, without whom I would definitely be somewhere else, doing something else and probably a lot worse off.

DEDICATION

Mum.....(who else?)

“Young fool.....Only now, at the end, do you understand.” – Emperor Palpatine

ABSTRACT

Investigating short-term blood pressure regulation: peripheral baroreceptor sensitivity

Susan C Peirce

Autonomically-mediated baroreflex control of heart rate has been extensively studied (cardiac BRS, cBRS), but peripheral control of blood pressure is less well known. Total peripheral resistance (TPR) was derived from pulse contour stroke volume (SV_{PC}) using non-invasive blood pressure (Finapres). The beat-to-beat variability of Finapres SV_{PC} was evaluated in cardiac catheterisation patients against aortic blood pressure SV_{PC} . Correlations were generally good (mean $R = 0.75$), but regression slopes tended to be less than unity and several aortic recordings were significantly affected by the dynamic response of the measurement system.

Finapres SV_{PC} was also compared to Doppler stroke distance (SD) in healthy volunteers. Both measures followed respiratory movements well, although SV_{PC} had higher coherence with respiration. Discrepancies between the results were considered to be mainly due to errors in the Doppler method.

Coherence thresholds for spectral cBRS were determined as a function of the number of subrecords available for ensemble averaging and the effect of ventricular ectopics on cBRS estimates was investigated. Pulse contour TPR data was then used to determine peripheral BRS (pBRS) in healthy controls and neurocardiogenic syncope patients (NCS) using methods adapted from cardiac BRS analysis.

Diastolic pressure produced greater pBRS estimates than systolic pressure and pBRS was generally higher in patients and fainters than in controls and non-fainters. Tilt did not have a consistent effect on pBRS and it was not linearly related to age or resting blood pressure, although it may be increased in hypertension and the elderly. pBRS was able to discriminate between the subject groups when cBRS methods showed no difference.

ABBREVIATIONS

ACh	acetylcholine
ANS	autonomic nervous system
ARMA	autoregressive moving average (parametric modelling)
AV	atrioventricular
BR	baroreceptor or baroreflex
BRS	baroreceptor sensitivity
CBF	cerebral blood flow
cBRS	cardiac baroreceptor sensitivity
CHF	chronic / congestive heart failure
CO	cardiac output
CP	cardiopulmonary
CVR	cerebrovascular resistance
DAP	diastolic arterial pressure
DAT	digital audio tape
dof	degrees of freedom
FFT	fast Fourier transform
FT	Fourier Transform
γ	ordinary coherence function
HF	high frequency (generally respiratory frequency)
HR	heart rate
HRV	heart rate variability
IQR	interquartile range
IRF	impulse response function
ITP	intrathoracic pressure
IWMF	intensity weighted mean frequency
LBNP	lower body negative pressure
LF	low frequency
LVSV	(left ventricular) stroke volume
MAP	mean arterial pressure
MI	myocardial infarct(ion)
MSA	muscle sympathetic (nerve) activity
NCS	neurocardiogenic syncope

OH	orthostatic hypotension
P_x	autospectrum of x
P_{xy}	cross-spectrum of x and y
PAF	pure autonomic failure
pBRS	peripheral baroreceptor sensitivity
PE	phenylephrine
PR	peripheral resistance
PSA	pulsatile systolic area
PSD	power spectral density
RAP	right atrial pressure
RR	interval between two consecutive R waves in the ECG, inverse of heart rate
RSA	respiratory sinus arrhythmia
SA	sinoatrial
SAP	systolic arterial pressure
SD	stroke distance
SNR	signal to noise ratio
SV_{PC}	pulse contour stroke volume
SVV	stroke volume variability
SVE	supraventricular ectopic
TF	transfer function
TPR	total peripheral resistance (also PR)
VE	ventricular ectopic
VPB	ventricular premature beats
VTI	velocity time integral
VVS	vasovagal syncope

CHAPTER 1 INTRODUCTION

Blood Pressure Regulation

“...after some 5-10 million years in the erect position..., *homo sapiens* is still occasionally troubled by the fact that more than two-thirds of the blood volume and vascular circuits are thereby placed below the level of the heart. This is almost unique among mammals, and for hydrostatic reasons, it presents a serious challenge to our pressure-controlling devices, with the baroreflex in the very centre.”

B. Folkow (Eckberg and Sleight, 1992)

Arterial blood pressure is a parameter ruled by the concept of ‘homeostasis’. In the face of continuous, and occasionally dramatic, changes in external conditions or internal requirements, several detection and feedback systems combine to maintain the systemic driving pressure within a range suitable for local flow regulation mechanisms. To effect this, several mechanisms exist that act over different timescales. The renal system regulates blood volume, and hence pressure, by the retention of water, affecting the long term blood pressure level (hours to days). In the medium term (minutes to hours), fluid transfers between the extravascular and intravascular volumes and hormones affect the resistance vessels. Also, anything which affects the composition of the blood (such as water retention) will also affect the viscosity and density, which will contribute to the resistance to flow (Chapter 2), and hence pressure. However, the autonomic nervous system is the only mechanism that can respond sufficiently quickly when variations act over a few seconds or less (‘short-term’ regulation). It is this system which responds maximally to perturbations such as changes in posture and sudden variations in activity, and the failure of which can produce a dramatic loss of arterial pressure in the upright position and large swings in pressure during normal activities. The scope of this thesis is limited to the development of methods to explore the effectiveness of this cardiovascular-neural feedback system.

Autonomic Control of Arterial Pressure

The baroreflex/autonomic nervous system (ANS) control loop is the fastest-responding arterial pressure regulation mechanism. The efferent signals affect the entire cardiovascular system but the most widely-known and well-researched component of these regulation mechanisms is the baroreceptor-mediated changes in heart rate. Research methods to

characterise this system can be separated into two categories: examination of long-term variability or measurement of the response to a transient input.

The variability of, and interactions between, blood pressure and heart rate have been extensively investigated and applied to a wide range of research fields. The clinical importance of heart rate variations was realised in 1965 by researchers investigating foetal distress during delivery (Hon and Lee, 1965). The advent of fast computational Fourier methods for spectral analysis there was a proliferation of research in the 1980's into the periodic variations in heart rate, and then into blood pressure variations and their mutual interactions.

In 1969 Smyth *et al* (1969) measured the change in heart rate produced in response to a pharmacologically-induced change in arterial pressure. The magnitude of this was termed baroreceptor sensitivity (BRS) and is most often measured in ms/mmHg (as the change in RR interval for a change in blood pressure). Despite a considerable accumulation of research since the 1970's this parameter has not yet become widely accepted as a clinical diagnostic test, probably largely due to the wide variety of methods of calculating it, and the qualitative and quantitative differences between them. However, cardiac BRS has found use as both a diagnostic and prognostic indicator in several disciplines. For example, it has discriminatory capability in diabetes, even where there are no other neurological symptoms (Weston *et al*, 1996a), and can improve risk stratification following myocardial infarction and stroke (Schwartz and La Rovere, 1998; Robinson *et al*, 2003).

It has long been a relatively simple matter to record high-fidelity ECG and arterial pressure data for anything up to 24hrs. However, in addition to heart rate there are at least two feedback mechanisms that respond rapidly to changes in arterial pressure and whose role in the maintenance of arterial pressure warrant investigation: myocardial contractility (inotropic cardiac changes) and peripheral resistance (SV and PR in Figure 1-1). In order to completely characterise the short-term blood pressure control system and quantify their relative contributions, all branches of the feedback loop should be investigated. This might also assist in identifying the parts of the system that fail in individual pathologies, as similar symptoms might be produced by faults at different points in the system.

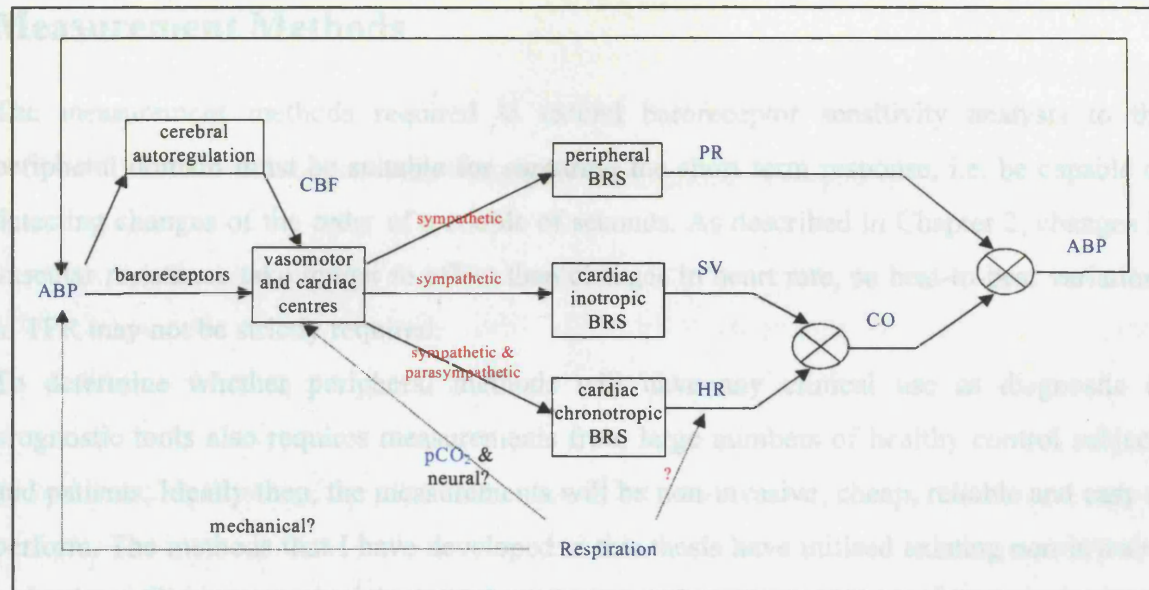


Figure 1-1: Three-loop model of baroreceptor/ANS-mediated arterial pressure control

Sympathetically-mediated total peripheral resistance (TPR) has been postulated to be more important to the control of blood pressure than heart rate (Sato *et al*, 1999; Mukkamala *et al*, 2003). For example, patients with heart transplants are not more susceptible to orthostatic hypotension (Wieling and Wesseling, 1993) and prevention of bradycardia does not prevent syncope in subjects prone to vasovagal episodes (van Lieshout *et al*, 1997). Also, Ogoh *et al* (2002) determined that cardiac effects (cardiac output) did not contribute as much as vascular effects (total vascular conductance) to the changes effected in mean arterial pressure in response to neck pressure/suction. Cardiac BRS is reduced in patients who have undergone neck irradiation, but this does not affect the heart rate and blood pressure responses to stand or Valsalva's manoeuvre (Timmers *et al*, 2004). Therefore, although cardiac chronotropic BRS has been demonstrated to have many useful applications, it does not provide all the answers and may not be the most appropriate diagnostic or prognostic indicator in pathologies that primarily affect blood pressure control.

However, until recently there has been no suitable measure of TPR which could enable the same level of detailed analysis to be applied to this feedback mechanism. This thesis seeks to help fill this gap in the model by examining the feasibility of measuring the peripheral baroreceptor sensitivity (pBRS) of individuals in a similar manner to cardiac BRS (cBRS). This would be defined similarly as the change in peripheral resistance for a given change in blood pressure.

Measurement Methods

The measurement methods required to extend baroreceptor sensitivity analysis to the peripheral domain must be suitable for capturing the short term response, i.e. be capable of detecting changes of the order of a couple of seconds. As described in Chapter 2, changes in vascular resistance take longer to effect than changes in heart rate, so beat-to-beat variations in TPR may not be strictly required.

To determine whether peripheral methods will have any clinical use as diagnostic or prognostic tools also requires measurements from large numbers of healthy control subjects and patients. Ideally then, the measurements will be non-invasive, cheap, reliable and easy to perform. The methods that I have developed in this thesis have utilised existing non-invasive technology (Finapres) and methods (pulse contour stroke volume, SV_{PC}) which have a proven record in cardiovascular research.

The major difficulty with TPR as a physiological parameter is that it cannot be measured directly. Local changes in limb musculoskeletal vascular resistance can be estimated from plethysmography (although not beat-to-beat values) and from variations in the quotient of mean arterial pressure to Doppler blood flow/velocity, e.g. in the brachial or femoral arteries (Lipsitz *et al*, 1991; Convertino, 1998; Imadojemu *et al*, 2001; Stewart and Weldon, 2003). However, sympathetic control of the vascular response is not systemically uniform and non-baroreflex mechanisms also contribute to local regulation (Edouard *et al*, 1994; Grassi and Esler, 1999; Shoemaker *et al*, 2000). The effect on the arterial blood pressure will be a result of the overall systemic resistance to the total blood flow (cardiac output). There is no gold standard method to measure TPR directly, so we must rely on the ability to quantify the ratio of systemic blood flow and pressure. From this, the total peripheral resistance can be derived on a beat-to-beat basis.

Therefore to measure beat-to-beat TPR requires the measurement of beat-to-beat cardiac output. Indicator dilution methods are generally accepted as the reference method for cardiac output, most commonly thermodilution. However, this is highly invasive, prevents subject movement and only provides mean values calculated over several seconds. There are three techniques that allow the non-invasive estimation of beat-to-beat blood flow in the aorta: Doppler ultrasound, pulse contour analysis and thoracic impedance. The use of pulse contour methods utilising Finapres blood pressure data allows the calculation of this parameter without the need for equipment supplementary to common research or clinical methods, or for continual operator/technician involvement.

Analysis Methods

To analyse the feedback processes shown in Figure 1-1 a system identification approach can be used. Inputs and outputs are defined and analysed to characterise the system that transforms one into the other (Xiao *et al*, 2005). In this way we can treat cardiovascular regulation in the same way as any other engineering system involving sensing and feedback. Where the purpose of the system is to maintain the input within a limited range of values then the feedback will be negative, i.e. it will oppose the change in the input.

There are many difficulties associated with human physiological research. Access to high fidelity, reliable data is often effectively impossible and substitutes must be found for those variables that cannot be accessed without a highly invasive procedure. For example, the direct measurement of efferent cardiac neural activity would be replaced with methods that measure the end-organ response (heart rate) or secondary effects (cardiac noradrenaline spillover). Also, in normal human physiology, homeostatic control systems are 'closed' (Figure 1-1). The loop needs to be broken to be able to fully separate cause from effect. If the negative feedback controls are operational, then the changes in pressure used for the stimulus will always be less than in an open loop system.

Therefore the processes of interest tend to be remote from the stimuli that can be applied and the measurements that can be acquired. The inputs and outputs are connected by a 'black box' process which is the object of the investigation but whose properties cannot be accessed directly. The physiological processes within the black box are considered to be acting as a filter, transforming the input signal to the output. Filter analysis techniques can be applied to each limb of the model in Figure 1-1 to determine their individual characteristics, however, anatomically separate parts of each limb must be lumped together in this analysis. For example, by characterising the filter between arterial pressure (input) and heart rate (output) the baroreceptor afferent response to the pressure changes, central processing of the afferent activity and the transmission between the sympathovagal efferents and the sinoatrial node have been lumped together.

Filter properties can be described and therefore investigated in either the time or frequency domains – the approaches are analytically equivalent. In the time domain the filter is fully characterised by the unit impulse response function, and in the frequency domain, by the transfer function. One function can be converted into the other by Fourier transformations. These approaches are valid assuming that the system is linear. When applying these techniques to blood pressure control analysis, we can impose an impulse or step change

transient in the input (Panerai *et al*, 2001, Ogoh *et al*, 2002), or have long sections of stationary data which are suitable for spectral analysis (Malliani *et al*, 1991). This thesis concentrates on methods applied to spontaneous variations. Data from tilt and standing were used in this thesis, but were limited to the use of stationary sections of the resulting supine and upright recordings.

Summary and Organisation of the Thesis

This thesis modifies methods used for investigating the heart rate response to pressure changes so that they can be applied to measuring the peripheral mechanism. The branches of the control loop can be studied independently as separate black boxes, but a complete understanding of the physiology involved can only be achieved by investigating the interactions between all of them. New tools for these investigations are required and must be developed and tested before this can occur.

The following three chapters comprise the literature review. Chapter 2 describes human baroreflex anatomy and physiology and the response of the cardiovascular system to the upright posture. Chapter 3 assesses the information available on short-term blood pressure regulation from studies utilising the variability of cardiovascular parameters. Chapter 4 reviews established methods of calculating cardiac chronotropic BRS and the use of this parameter as a diagnostic and prognostic indicator.

Chapter 5 discusses several methodological details that require consideration when measuring and analysing beat-to-beat cardiovascular data. It describes several empirical details that are common to the subsequent chapters and thus forms part of their Methods sections. The treatment of ventricular ectopic beats, synchronisation of beat-to-beat variables and spectral analysis methods are discussed, along with a review of the Finapres device and the nature of peripheral resistance as a steady-state parameter.

Chapters 6 and 7 introduce the pulse contour method and its use in quantifying stroke volume. Results of the algorithm using aortic pressure are compared with those using non-invasive finger pressure. The stroke volume determined by this latter method is compared to Doppler ultrasound measurements to assess the reliability of the beat-to-beat variation. Pulse contour stroke volume is used to derive beat-to-beat peripheral resistance and modified cBRS estimation methods are applied to this in Chapter 8. Chapter 9 assesses the success of extending these existing cardiac BRS techniques to a novel parameter and postulates possibilities for further testing and improving them.

CHAPTER 2 SHORT TERM BLOOD PRESSURE

REGULATION

Physics of Vascular Resistance

In a regime of steady state flow of a Newtonian liquid through a rigid cylinder, the rate of flow is determined by the pressure difference across a resistance to that flow.

$$\text{flow} = \frac{\text{pressure}}{\text{resistance}} \quad \text{Equation 2-1}$$

The resistance to flow, R , is determined by the Poiseuille equation:

$$R = \frac{8\eta L \rho}{\pi r^4} \quad \text{Equation 2-2}$$

Where: η - viscosity of the fluid

ρ - density of the fluid

r - radius of the cylinder

L - length of the cylinder

There are no perfect equations to describe the pulsatile flow of blood (a suspension of cells in a fluid) through vessels which are flexible and change calibre, both by passive elasticity and active musculature. Over the physiological time scales considered here, the viscosity and density of blood is assumed to be constant, as is the length of the cylinder. Resistance to blood flow is therefore only dependent upon the fourth power of the vessel radius.

The total resistance to blood flow as seen by the left ventricle can be determined from Equation 2-1 as the ratio of the mean pressure difference across the vascular system to the mean flow through that system:

$$TPR = \frac{MAP - RAP}{CO}$$

Equation 2-3

Where: *TPR* - total peripheral resistance

MAP - mean arterial pressure

RAP - mean right atrial pressure

CO - cardiac output

As right atrial pressure is very small compare to MAP, invasive to measure and varies little from beat-to-beat, this parameter is usually omitted from the equation and TPR is measured as the ratio of mean pressure to cardiac output.

Basic Physiology

The autonomic neural system (ANS) comprises two divisions: sympathetic and parasympathetic. Sympathetic activation tends to be associated with increases in activity and a general state of increased alertness, whereas parasympathetic activation is concerned with background restorative mechanisms and is associated with a relaxed state. The sinoatrial (SA) and atrioventricular (AV) nodes and ventricular conduction system are innervated by both divisions of the ANS – in the case of the parasympathetic system, by the vagus nerve (and hence ‘vagal’ is used synonymously with the term ‘parasympathetic’ in this context). The myocardium and vascular smooth muscle are only innervated by the sympathetic branch.

Changes in arterial blood pressure are detected as changes in the dimensions of special areas of the major arteries (aorta, carotid and pulmonary) and heart chambers, known as baroreceptors. An increase in blood pressure produces distension of the vessels, stimulating the baroreceptors and increasing the afferent signals to the nucleus tractus solitarius in the medulla oblongata where the signals affect both the vasomotor centre and the cardiac centre.

An increase in the incoming baroreceptor signals will activate the cardio-inhibitory and vasodilation centres. The former acts to decrease the heart rate and contractility via an increase in vagal and decrease in sympathetic efferent activity to the heart, and the latter to reduce the peripheral vascular resistance by a decrease in sympathetic activity to the arterioles. A decrease in arterial pressure will stimulate the vasoconstrictor and the cardio-stimulatory areas to produce the opposite effect on the efferent signals and end organs.

The major arterial baroreceptors are those in the carotid sinus and aorta. There are also stretch receptors in the low pressure blood circuit between the right and left sides of the heart (the

cardiopulmonary baroreceptors, CP, in the right atria and pulmonary artery) which respond to changes in central venous pressure and venous return (Wieling and Wesseling, 1993). The role of these and their interaction with the arterial pressure baroreceptors is not well established, as yet.

Parasympathetic Innervation

The cardiac vagal nerves originate in the medulla oblongata and pass down through the neck next to the carotid arteries as the cervical vagus. Acetylcholine (ACh) is released at the synapses and thus these are termed cholinergic fibres. Acetylcholine acts on muscarinic receptors which are directly coupled to potassium channels in the cell membrane, ensuring a rapid response from the end-organ. The nodes are also rich in the enzyme cholinesterase which can quickly hydrolyse the ACh and inactivate it. As a result the cardiac response to activation and inhibition of the efferent parasympathetic activity is very swift, allowing beat-to-beat modulation of the heart rate. The SA node can respond to vagal input within around 150ms (Berne and Levy, 1997; Levy, 1997), although the maximum change in heart rate takes around one and a half seconds (Cooke *et al*, 1999).

Acetylcholine hyperpolarises the pacing cells by increasing the potassium conductance across the cell membrane. This slows the automatic depolarisation of the SA node and tends to slow AV conduction, thus reducing heart rate. It also inhibits atrial and ventricular contraction due to a shortened action potential and reduced calcium conductance, but these effects are less well studied than those on heart rate. The relationship between stimulation frequency and beat interval is linear (Berntson *et al*, 1993; Hainsworth, 1995; Little *et al*, 1999) although the timing of the impulse relative to the cardiac cycle will affect the absolute amount of RR lengthening (Levy, 1997).

Sympathetic Innervation

Sympathetic fibres exit the spinal cord at the lower cervical and upper thoracic level. They affect heart rate, myocardial contractility and peripheral resistance. The post-ganglionic synapses release noradrenaline and are therefore referred to as adrenergic fibres, but also utilise neuropeptide Y and adrenaline as neurotransmitters. There are two types of adrenergic receptor: alpha and beta. Those in the nodes and myocardium are mostly beta type (specifically β_1), whereas alpha-type are abundant in all vascular smooth muscle and β_2 in the arterioles of skeletal and cardiac muscle. The onset and decay of sympathetic effects is much slower than those due to parasympathetic activity:

- postganglionic fibres are unmyelinated so that conduction velocities are lower
- the effect on the SA node is brought about by the build-up of 'second messengers' in the pacing cells
- removal of noradrenaline takes place by the relatively slow processes of reabsorption into the nerve ending and cardiac cells and diffusion into the bloodstream
- the release of noradrenaline is much slower than that of ACh.

Whereas vagal control of the heart rate can react at frequencies up to 1Hz, the sympathetic nervous system can only follow changes up to around 0.15Hz (Saul *et al*, 1991; Parati *et al*, 1995).

Sympathetic activation increases heart rate by accelerating the slow depolarisation of the pacemakers cells in the SA node. The reduction in systolic duration is proportionately greater than the effect on the total cycle length which, along with the reduction in AV conduction time, enables the diastolic filling time and end-diastolic volume to be preserved. Atrial and ventricular contractility is enhanced, and vasoconstriction produced, by increasing the membrane calcium conductance.

Changes in the diameter of the arterioles and capacitance vessels are vital to the regulation of systemic blood pressure. Vessels supplying skeletal muscle and skin are not innervated by the parasympathetic system. The sympathetic innervation of skeletal blood vessels includes both vasodilator and vasoconstrictor fibres: the former are mostly activated by emotional stress (they do not respond for example to changes in posture) and the latter by pressure receptors (Appenzeller and Atkinson, 1985).

Controlling the calibre of the capacitance vessels alters the volume of the peripheral circulation rather than the resistance to flow. Sympathetic stimulation increases the tone of capacitance vessels (venoconstriction) via direct activation of the alpha receptors by noradrenaline. Reflex activity is entirely due to alpha-adrenergic receptors so that venodilation under these circumstances is caused by a reduction in sympathetic activity.

Sympathovagal Interactions

Sympathetic and parasympathetic innervation of the heart is antagonistic: parasympathetic activity tends to reduce heart rate and stroke volume, and sympathetic activity to increase them and the ratio of efferent activity (sympathovagal balance) is controlled by the nucleus tractus solitarius. The activity in the two branches also tends to change in reciprocal

directions, such that physiological effects are brought about by simultaneous increases in one and decreases in the other, although concordant and independent changes are also possible (Berntson *et al*, 1993).

Failure of Blood Pressure Regulation

If the baroreflex loop is interrupted, the loss of negative feedback will result in excessive short-term pressure responses to both intrinsic and extrinsic stimuli. The afferent and central parts of the control mechanism may be disturbed or destroyed by trauma (such as accidental damage during neck surgery or stroke), irradiation damage or tumour growth. The acute phase of baroreflex failure is characterised by severe hypertension and tachycardia, to be replaced in the chronic stage by increased cardiovascular variability and bradycardia (Ketch *et al*, 2002; Timmers *et al*, 2004).

Orthostatic hypotension may also be experienced as a result of this pathology (Cooke *et al*, 1999). This is the inability to maintain arterial blood pressure when upright and is defined as a reduction in systolic and/or diastolic pressure of at least 20mmHg and 10mmHg respectively, within three minutes of standing or 60° head-up tilt (Consensus Committee of the American Autonomic Society and the American Academy of Neurology, 1996). It may be idiopathic or attributable to any of the causes listed in Table 2-1 in addition to the causes of baroreflex failure mentioned above. Orthostatic (or postural) hypotension may or may not result in presyncopal symptoms and the eventual loss of consciousness (orthostatic intolerance), depending on the subject's ability to regulate cerebral blood flow in the face of reduced pressure.

Table 2-1: Causes of orthostatic hypotension

Type	Example
neurological pathology	vasovagal syncope, pure autonomic failure, diabetic neuropathy
cardiovascular pathology	heart failure, myocardial infarction
low blood volume	haemorrhage, dehydration, anaemia, venous pooling
drug side effects	anti-psychotics, anti-hypertensives, anti-depressants
pregnancy	
deconditioning	extended bed-rest, space flight

The rapid changes in arterial blood pressure with which this thesis is concerned can be due to a large number of phenomena, but the most common laboratory (and clinical) event is the movement to the upright position. In the laboratory and clinic the active stand manoeuvre is often replaced by passive upright tilt, which is a more reproducible event and removes the variable effects of muscle contraction and breath holding. Other challenges which can have

similarly fast and dramatic effects on blood pressure are Valsalva's manoeuvre, the rapid application and release of thigh cuffs or lower body negative pressure (LBNP), the onset of exercise and respiratory movements.

Response of Cardiovascular Variables to the Upright Position¹

The temporal record of the physiological response to the upright position is usually separated into three phases: the immediate response covers the first minute after the manoeuvre, the early steady-state (stabilised) condition describes the state at around 1-3min, and by 3-5min all variables should have settled into their long-term response and be either stable or undergo only slow changes in normal, non-fainting subjects (Wieling and Wesseling, 1993; Smith *et al*, 1994). A generic illustration of the response to stand of several cardiovascular parameters is presented in Figure 2-1.

1. All changes are presented as mean differences from the control values before the manoeuvre. This baseline will be supine in most cases, but sitting values are used where the stand has been from this position.

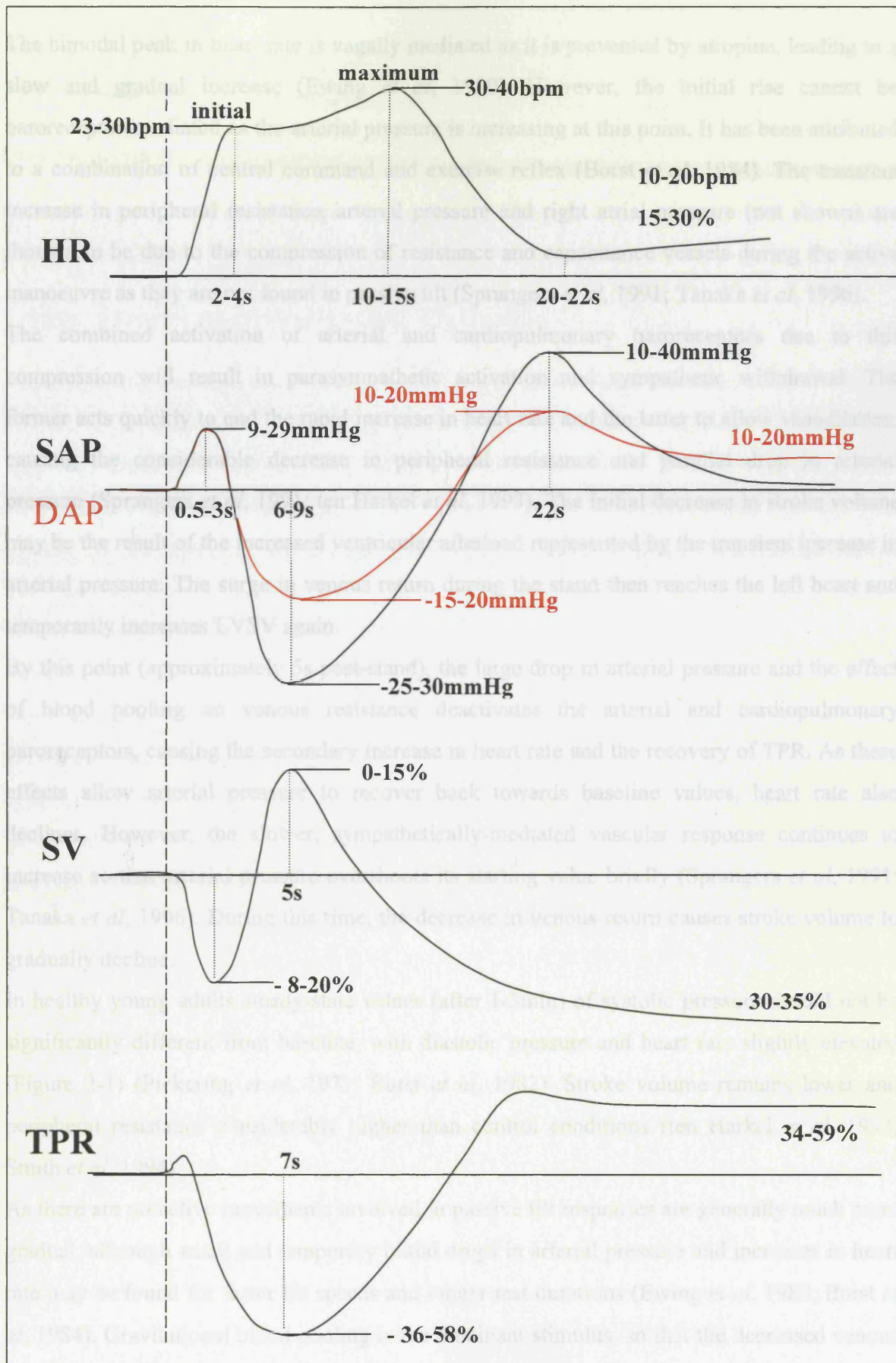


Figure 2-1: Normal cardiovascular responses to active standing. Values given are differences from baseline.

The bimodal peak in heart rate is vagally mediated as it is prevented by atropine, leading to a slow and gradual increase (Ewing *et al*, 1980). However, the initial rise cannot be baroreceptor-mediated as the arterial pressure is increasing at this point. It has been attributed to a combination of central command and exercise reflex (Borst *et al*, 1984). The transient increase in peripheral resistance, arterial pressure and right atrial pressure (not shown) are thought to be due to the compression of resistance and capacitance vessels during the active manoeuvre as they are not found in passive tilt (Sprangers *et al*, 1991; Tanaka *et al*, 1996).

The combined activation of arterial and cardiopulmonary baroreceptors due to this compression will result in parasympathetic activation and sympathetic withdrawal. The former acts quickly to end the rapid increase in heart rate and the latter to allow vasodilation, causing the considerable decrease in peripheral resistance and parallel drop in arterial pressure (Sprangers *et al*, 1991; ten Harkel *et al*, 1993). The initial decrease in stroke volume may be the result of the increased ventricular afterload represented by the transient increase in arterial pressure. The surge in venous return during the stand then reaches the left heart and temporarily increases LVSV again.

By this point (approximately 5s post-stand), the large drop in arterial pressure and the effect of blood pooling on venous resistance deactivates the arterial and cardiopulmonary baroreceptors, causing the secondary increase in heart rate and the recovery of TPR. As these effects allow arterial pressure to recover back towards baseline values, heart rate also declines. However, the slower, sympathetically-mediated vascular response continues to increase so that arterial pressure overshoots its starting value briefly (Sprangers *et al*, 1991; Tanaka *et al*, 1996). During this time, the decrease in venous return causes stroke volume to gradually decline.

In healthy young adults steady-state values (after 1-3min) of systolic pressure should not be significantly different from baseline, with diastolic pressure and heart rate slightly elevated (Figure 2-1) (Pickering *et al*, 1971; Borst *et al*, 1982). Stroke volume remains lower and peripheral resistance considerably higher than control conditions (ten Harkel *et al*, 1993; Smith *et al*, 1994).

As there are no active movements involved in passive tilt responses are generally much more gradual, although small and temporary initial drops in arterial pressure and increases in heart rate may be found for faster tilt speeds and longer rest durations (Ewing *et al*, 1980; Borst *et al*, 1984). Gravitational blood pooling is the dominant stimulus, so that the decreased venous return slowly reduces stroke volume. Peripheral resistance and heart rate increase steadily to oppose this and maintain arterial pressure (Imholz *et al*, 1990; Sprangers *et al*, 1991). Steady-

state values should be comparable to those following active stand (Dambrink and Wieling, 1987; Wieling and Wesseling, 1993).

Muscle sympathetic activity (MSA) and other measures of sympathetic efferent activation (e.g. plasma noradrenaline) are increased in the upright posture and thoracic blood volume (measured as thoracic impedance) decreases significantly in the first couple of minutes (Wieling *et al*, 1998).

Other Transient Investigation Methods

Any perturbing measure that creates a suitably rapid change in cardiovascular status can be utilised to investigate the functioning of the pressure-regulating aspect of the autonomic nervous system (Panerai *et al*, 2001). Other methods that induce slower responses or a steady-state change can also provide information on various parts of the control system. For example, the use of low level (up to -20mmHg) LBNP to selectively affect the cardiopulmonary baroreceptors. However, the longer a non-resting state is maintained, or the slower the effect produced, the more likely it is that non-neural compensating mechanisms will be involved.

Advances in methods such as MSA, Doppler ultrasound, pulse contour and thoracic impedance to measure beat-to-beat changes in parameters additional to heart rate and blood pressure should allow the fullest examination of the rapid-response of the autonomic nervous system. The utilisation of pulse contour methods for the determination of stroke volume additionally will enable the re-analysis of many previous studies where beat-to-beat heart rate and blood pressure have been measured.

CHAPTER 3 VARIABILITY OF CARDIOVASCULAR PARAMETERS

Introduction

The principle of homeostasis requires that changes in blood pressure be counteracted by negative feedback mechanisms to keep it within a narrow range of suitable values. Constant perturbations by internal activity and reactions to the external environment therefore result in periodic variations in cardiovascular parameters: in a resting state heart rate varies by about 10-20 beats per minute and systolic pressure by about 5-15mmHg. An analysis of these periodicities can provide information supplementary to the static values, but it must be remembered that power spectral analysis reflects the variability of autonomic efferent activity, not its tonic level.

Similar variations have been found in other cardiovascular parameters (for example, stroke volume and muscle sympathetic activity), suggesting that the variations are transmitted throughout the cardiovascular system via reflex mechanisms, and/or that they are the effect of intrinsic frequencies in central command affecting all parameters simultaneously.

Spectral Power

Due to the limitations of spectral analysis methods, periodic oscillations can only be reliably analysed if the sampling rate is at least twice the maximum frequency (Nyquist limit). Beat-to-beat parameters are sampled once per heartbeat (Sayers, 1973) and therefore the maximum frequency of variation that can be determined is around 0.5Hz for normal heart rates (approximately 60bpm). Slower pulse rates will reduce the maximum frequency that can be reliably reproduced (e.g. 48bpm has a Nyquist limit of 0.4Hz).

The spectral peaks of heart rate and systolic blood pressure variability were described initially by Sayers (1973) and are usually defined as high or respiratory frequency (HF), low frequency (LF) and very low frequency (VLF) (Figure 3-1), although the ranges, and sometimes the names, of these bands differ slightly between research groups (Robbe *et al*, 1987). The high frequency band usually ranges from around 0.15Hz or 0.2Hz up to 0.3-0.4Hz and the low frequency (occasionally called the mid-frequency, MF, band) from 0.04Hz or 0.05Hz up to 0.15Hz (Akselrod *et al*, 1981; Parati *et al*, 1995; European Society of Cardiology and North American Society of Pacing and Electrophysiology, 1996; Taylor and

Eckberg, 1996; Cooke *et al*, 1999). The very low frequencies, below around 0.05Hz, require long data recordings for reliable estimates and are not thought to be related to the baroreflex, so they are not considered here.

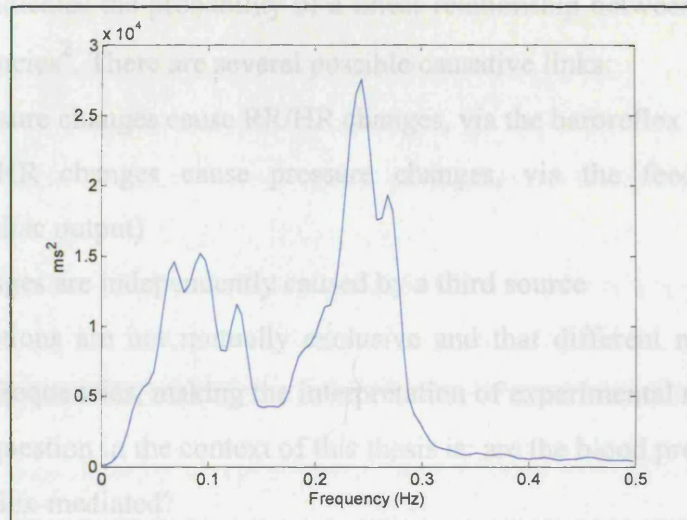


Figure 3-1: Low and respiratory frequency peaks in an RR interval autospectrum in a healthy, supine subject. High-pass filtering has removed the VLF power.

Spectral power is often quoted in absolute and/or normalised units, whereby the power in each band is divided by the total power (usually minus the dc or VLF component) (Pagani *et al*, 1992; European Society of Cardiology and North American Society of Pacing and Electrophysiology, 1996). This allows inter-subject comparisons and group means to be used and also takes account of any changes in total power (variance) with different conditions so that the distribution of the available spectral power can be easily determined. However, the use of only normalised powers may mask changes in absolute power and can indicate significant peaks where there is actually very little variability (Taylor and Eckberg, 1996). Ideally, both absolute and normalised values should be reported.

Heart Rate or RR Interval?

There is a large body of research related to the subject of heart rate variability (HRV), especially since its introduction as a risk stratifier in post-myocardial infarct patients (La Rovere *et al*, 1998; Stein and Kleiger, 1999). The relationship between heart rate and RR interval is non-linear but the normalised powers are virtually interchangeable. Absolute powers may be different for the two quantities though, especially where changes in mean value occur (Castiglioni, 1995).

Heart Rate and Blood Pressure Variability

High values of coherence exist between the LF and HF spectral peaks of arterial pressure and RR interval or heart rate, and tends towards zero elsewhere (de Boer *et al*, 1985b; Pagani *et al*, 1986), which indicates the probability of a linear relationship between pressure and heart rate at these frequencies². There are several possible causative links:

1. pressure changes cause RR/HR changes, via the baroreflex
2. RR/HR changes cause pressure changes, via the feedforward effect (cardiac output)
3. changes are independently caused by a third source

Note that these options are not mutually exclusive and that different mechanisms may be acting at the same frequencies, making the interpretation of experimental results a challenging task. The primary question in the context of this thesis is: are the blood pressure and heart rate oscillations baroreflex-mediated?

The general consensus of opinion is that the respiratory frequency variations in heart rate are mediated primarily by the parasympathetic system, whereas both branches of the autonomic system are considered to contribute to the LF variations. High frequency variations in blood pressure are considered to be mechanically driven by changes in intrathoracic pressure and the origin of the LF BP waves has been discussed at length, but no accord has been arrived at. The rest of this chapter will consider the evidence that has been published over the last few decades to illuminate these statements and the insight that this provides into short term regulation of arterial pressure.

In the supine position there are strong respiratory and low frequency variations in heart rate/RR interval and systolic blood pressure but the spectral powers are unequally distributed with the low frequency band just predominant³ and the LF:HF power ratio generally greater than one (Pagani *et al*, 1986). Respiratory variations in diastolic power may (Pagani *et al*, 1986) or may not (de Boer *et al*, 1985b) be significant. Manoeuvres that are thought to increase sympathetic efferent activity (orthostasis, reductions in arterial pressure and mental stress) redistribute the power so that the LF predominates (Pomeranz *et al*, 1985; Saul *et al*, 1990; Malliani *et al*, 1991; Pagani *et al*, 1992). The LF:HF ratio increases significantly in the

2. However, the claim of 'significant coherence' in many published reports must be treated with caution as most authors choose an arbitrary threshold of 0.5 (Pagani *et al*, 1986). See Chapter 5 for a fuller discussion.

3. Although the power may be more dispersed through the LF band than that in the HF band so that the peak power is actually lower.

upright posture, from around 1-4 up to approximately 20, reflecting the large increase in normalised LF power (Malliani *et al*, 1991).

In the upright posture, blood pressure total variance increases so that both LF and HF have increased absolute powers, but normalised power increases for LF and decreases for HF (Pagani *et al*, 1986; Saul *et al*, 1991; Cooke *et al*, 1999). Heart rate total variance also increases, although RR interval does not (Pagani *et al*, 1986; Lipsitz *et al*, 1990; Saul *et al*, 1991). The cardiac chronotropic variability response to orthostasis is generally described better by a redistribution of spectral power to the LF band than absolute increases in either of the frequency bands (Pagani *et al*, 1986; Moriguchi *et al*, 1993; Cooke *et al*, 1999).

The spectral power in the LF and HF bands appears to be relatively reproducible in subjects over time (Pagani *et al*, 1986; European Society of Cardiology and North American Society of Pacing and Electrophysiology, 1996).

Respiratory Frequency Variations

Arterial Pressure

Respiratory variations in arterial pressure are larger than the intrathoracic pressure changes producing volume changes and airflow in the lungs. Therefore, HF pressure oscillations cannot simply be the direct result of ventilatory pressure changes (Eckberg, 2000).

Inspiration is associated with falling blood pressure and increasing heart rate (Figure 3-2) at moderate breathing rates (around 0.15-0.3Hz, 9-18 br/min). However, the magnitudes of systolic pressure and heart rate variations decrease with increasing respiratory rate (Dornhorst *et al*, 1952; Angelone and Coulter, 1964; Saul *et al*, 1991; Sleight and Casadei, 1995) and spontaneous respiration at rates close to the LF peak are considered to entrain the existing LF oscillations.

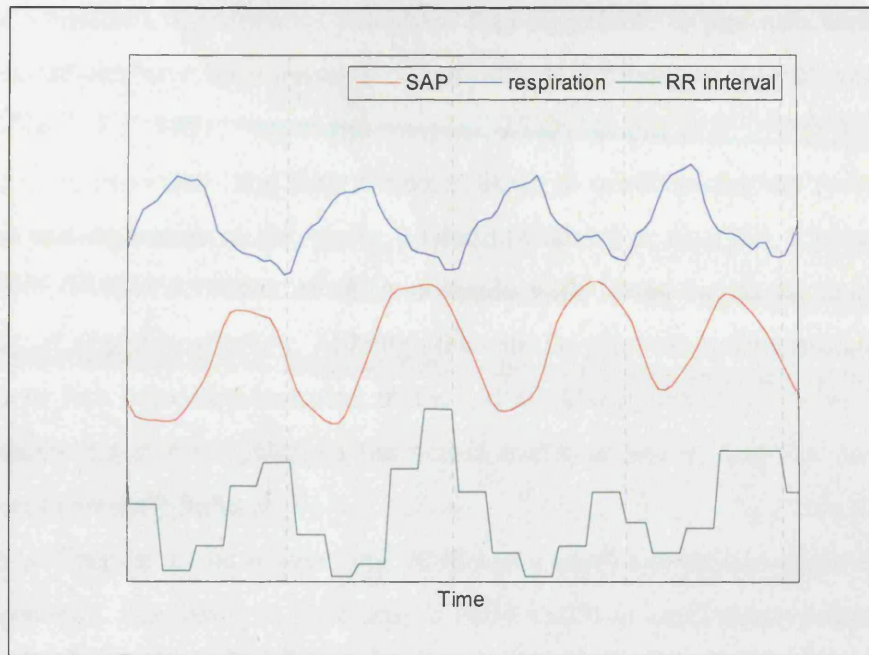


Figure 3-2: Respiratory changes during controlled breathing at 0.25Hz in the supine position. Inspiration is shown by an upwards deflection. Vertical lines indicate end-expiration.

The reduced intrathoracic pressure during inspiration enhances right heart filling by drawing venous blood in from the extra-thoracic vessels, but it reduces left ventricular stroke volume (LVS_V) due to an increased afterload and also due to competition for space in the pericardial sac by the two ventricles (Robotham *et al*, 1979; Saul *et al*, 1991; Blaber and Hughson, 1996; Wandt *et al*, 1998; Akselrod *et al*, 2000). The increased right heart output increases pulmonary pressure and therefore left heart filling after a suitable time lag. Respiratory modulation of LVS_V appears to cease immediately with end-expiratory breath-hold and the magnitude of the variability is independent of the breathing rate (Guz *et al*, 1987), indicating that direct mechanical effects of respiratory pressures on the left ventricle dominate the pulmonary time lag. In contrast, the decrease in systolic pressure has been shown to continue following an end-expiratory breath-hold, indicating that it is the delayed result of the preceding breath. This may be the effect of LF variations in SAP that continue in the absence of respiratory movement (Dornhorst *et al*, 1952).

The respiratory frequency blood pressure variations can be postulated to result from the combined variations in heart rate and stroke volume resulting in cardiac output changes. In the absence of opposing changes in vascular resistance this will produce arterial pressure changes. Cardiac output is known to vary at respiratory rates (Jansen *et al*, 1990; Toska and Eriksen, 1993), although the variable phase relationships found with this parameter are probably the result of measurements in highly pathological cardiovascular systems.

Additionally, variations in peripheral resistance may contribute to pressure variability. Both LF and HF variations have been detected in sympathetic efferents in animals and in MSA in humans (Malliani *et al*, 1991; Pagani and Malliani, 2000; Malpas *et al*, 2001). Bursts of MSA are gated by respiration such that they are more likely to occur during the period from end-inspiration to end-expiration in the supine position (Eckberg *et al*, 1984; Cooke *et al*, 1999; Eckberg, 2000). However, bursts of MSA coincide with lower values of arterial pressure, which also occur at end-inspiration. Although the cellular physiology for gating in the central nervous system has been demonstrated in the cat (Eckberg, 2000), whether synchronised bursts in humans are due to gating of the neural traffic or are in response to the pressure variations is not currently defined.

As described in Chapter 2, the sympathetic ANS has a much slower end-organ response than the parasympathetic. For example, increases in MSA result in maximal increases in diastolic pressure⁴ after about 5 seconds (Wallin and Nerhed, 1982) and transfer function analysis indicates that sympathetically-mediated RR interval changes have significant magnitude only up to around 0.1-0.15Hz (Saul *et al*, 1991). It therefore seems unlikely that sympathetically-mediated changes in vascular resistance would contribute to arterial pressure oscillations at typical respiratory frequencies.

RR Interval / Heart Rate

High frequency RR interval variations are almost abolished by the administration of atropine and virtually unaffected by intravenous propranolol (Pomeranz *et al*, 1985), indicating predominance of vagal control (Malliani *et al*, 1991). However, some respiratory frequency RR variability may be linked to mechanical effects on the sinus node itself, as a residual amount can be found in heart transplant patients and following combined blockade (Saul *et al*, 1991; Parati *et al*, 1995; Lord *et al*, 1997). Controlling breathing rate at a fixed frequency (metronome breathing) reduces the bandwidth of the HF RR peak and increases the fractional power under it compared to spontaneous breathing (Malliani *et al*, 1991).

The removal of respiratory frequency oscillations in RR interval by atrial pacing results in decreased HF power in SAP in the supine position (Taylor and Eckberg, 1996). Combined pharmacological blockade also decreases the lung volume-SAP transfer function magnitude up to around 0.3Hz (Saul *et al*, 1991). Both effects are minimal for diastolic pressure. This

4. Diastolic and mean pressures have been shown to be more strongly related to MSA than systolic pressure (Sundlöf and Wallin, 1978; Sanders and Ferguson, 1989).

suggests that HF RR interval augments pressure variations rather than buffering them, as would be expected from a baroreflex (BR) response. There are two rationalisations for this. Firstly, the lack of mitigating effect of RR interval on SAP does not necessarily mean that the former is not an attempt to moderate the latter by the baroreflex. It may indicate merely that this mechanism is ineffective under the experimental conditions. Sleight *et al* (1995) applied BR stimulus with neck suction at 0.2Hz whilst subjects breathed at 0.25Hz. Despite an overwhelming peak in the RR spectrum at 0.2Hz there was almost no power in the systolic pressure spectra at the applied frequency. This suggests an ineffectiveness of the RR interval feedforward division of the baroreflex loop in the supine position.

Secondly, several authors have suggested that the baroreflex may be selectively engaged by the upright posture (Saul *et al*, 1991; Taylor and Eckberg, 1996). Absolute pressure variabilities are increased during orthostasis (Dornhorst *et al*, 1952; Pagani *et al*, 1986; Saul *et al*, 1991; Taylor and Eckberg, 1996; Cooke *et al*, 1999) and there is evidence that the relationship between pressure and heart rate is altered in this position. The phase between systolic pressure and RR interval becomes more negative during tilt and exercise (Pagani *et al*, 1987; Taylor and Eckberg, 1996; Cooke *et al*, 1999) and the respiratory gating of MSA disappears with orthostatic stress and during intense carotid stimulation (Cooke *et al*, 1999; Eckberg, 2000). Atrial pacing results in an increase in HF arterial pressure oscillations only in the upright position (Taylor and Eckberg, 1996). In de Boer's beat-to-beat model of the cardiovascular system the spectral features of pressure and RR interval measured in seated subjects were reproduced only by treating high frequency variations as a BR-mediated effect of pressure on interval (de Boer *et al*, 1987). Dornhorst *et al* (1952) claimed that stroke volume is also more variable in the upright posture and that this is due to the greater sensitivity of the right atrium to changes in filling pressure when this is lower.

Cross Spectral Analysis

The phase between RR interval and systolic pressure in the HF band is around zero, but tends to increase linearly with frequency from negative to positive (pressure lead to pressure lag) (de Boer *et al*, 1985; Baselli *et al*, 1986; Pagani *et al*, 1986). This has been cited as evidence that the respiratory variations in RR interval cannot be baroreceptor mediated (Eckberg, 2000). However, these values of phase result when systolic pressure and RR interval are synchronised in the time series, e.g. when the tachogram and systogram are used without the interpolation of a uniform time base. Therefore a phase of zero indicates that the changes occur in the same direction in the same beat. At slow heart rates the short latency of the vagal

BR response is considered to be able to influence the interval in which the stimulus (systolic pressure) occurs (Chapter 1 and Chapter 4). Keyl *et al* (2000) calculated the phase between SAP and RR interval using the method mentioned above and after correcting the phase for the mean latency between systolic pressure and the following R-wave. They determined that the commonly-quoted value for phase of approximately zero at normal respiratory frequencies was then translated into a pressure lead of around 55°. In addition to this, when arterial pressure is measured peripherally the time delay between the pressure stimulus at the aortic and carotid baroreceptors and the measurement site should be taken into account. In this case the phase lead for pressure in Keyl's results would be slightly greater.

The issue of time series synchronisation is discussed further in Chapter 5. Briefly, RR interval (or heart rate) is sampled once per beat at the R-waves. However, it is a value with a finite duration and does not occur as a discrete time sample, unlike systolic pressure. The second R-wave does define the end of the interval, but it requires two R-waves to define the duration. I propose that neither R-wave has an *a priori* preference as a temporal synchronisation point and that the error in determining phases involving RR interval or heart rate is approximately half a beat. Therefore, the small phase values found at respiratory frequencies do not provide evidence that these cardiac oscillations cannot be baroreflex-mediated.

All this information suggests that mechanical LVSV modulation is the primary source of the systolic pressure oscillations. The decrease in SV during inspiration at normal breathing rates is associated with a decrease in systolic and pulse pressures (Sleight and Casadei, 1995). Also, the coupling mechanism between arterial pressure and heart rate at this frequency is dependent on the posture of the subject, so that baroreflex is most effective during orthostasis. It is well accepted that the afferent response of the arterial baroreceptors depends on the magnitude and rate of pressure change (Eckberg and Sleight, 1992, p136-138). Possibly, it requires larger pressure variations than are commonly found in the supine position to effect a significant cardiac chronotropic response.

Low Frequency Variations

Arterial Pressure

Increases in MSA produce maximal increases in diastolic pressure after about 5 seconds and the latency from pressure stimulus to change in MSA activity is about 1s (Wallin *et al*, 1975; Wallin and Nerhed, 1982; Rea and Eckberg, 1987). Therefore the closed MSA/pressure loop has a natural period of around 12s. This slow stimulus-response resonance in the sympathetic

baroreflex pressure loop has been shown to be sufficient cause to produce typical LF variations in a beat-to-beat cardiovascular model (de Boer *et al*, 1987; Whittam *et al*, 2000). Phase values from the model were also consistent with measured values.

However, a physical resonant system still requires a perturbing input to enable the oscillations to perpetuate. Alpha-adrenergic blockade both reduces, and prevents increases in, LF pressure variations (Pagani *et al*, 1992; Parati *et al*, 1995), indicating that these must be considerably influenced by sympathetic vasomotor changes in peripheral resistance. Also, the removal of RR variability by atrial pacing or combined blockade does not affect LF pressure variability in the supine position (Malliani *et al*, 1991; Parati *et al*, 1995; Taylor and Eckberg, 1996) so they are therefore sustained without HR feedforward effects.

Some authors have hypothesised a central oscillator (Malpas, 2002). Blood pressure variations at 0.1Hz persist in the absence of respiratory influences due to breath-holding for up to 30s. Dornhorst *et al* (1952) ascribed these to a central 'Traube oscillator' producing changes in vasomotor tone. Alternatively, spontaneous and intrinsic variations in arteriole vessel diameter occur around the frequencies in the LF band (termed 'vasomotion') and are related to local blood flow regulation mechanisms (Levick, 2003, p151, p203). This perpetual change in local resistance might interact with a resonant baroreflex system to produce continual arterial pressure oscillations.

RR Interval / Heart Rate

The origin of the low frequency RR interval oscillations is more complex than that of the respiratory frequency band as both sympathetic and vagal efferents have been shown to have a role in their generation. Atropine reduces the absolute LF RR power in both the supine and upright positions, whereas propranolol only reduces it in the latter (Pomeranz *et al*, 1985; Pagani *et al*, 1986). Orthostasis increases the tonic activity of the sympathetic system (Burke *et al*, 1977; Zhang *et al*, 1998), and so the lack of consistent effect of β -blockade in supine subjects is taken as an indicator that there is little cardiac sympathetic drive in the resting state (Pomeranz *et al*, 1985).

Other conditions that are thought to increase the level of sympathetic activity are also associated with increases in normalised LF RR interval power, e.g. mental stress, mild exercise and various pathological conditions, whereas metronome breathing and sleep have the opposite effect (Malliani *et al*, 1991; Pagani *et al*, 1992; Parati *et al*, 1995). Metronome breathing reduces the RR interval LF:HF ratio to below one, indicating the predominance of the HF power.

Cross Spectral Analysis

The phase between systolic pressure and RR interval is between 60-90° (pressure leads RR interval by about 1.5-3s (but see discussion above about phase values) and has been found to be unaffected by tilt (Baselli *et al*, 1986; Pagani *et al*, 1986; Cooke *et al*, 1999; Keyl *et al*, 2000). This is consistent with the delays between baroreceptor stimulation and vagal cardiac modulation and therefore lead to speculation that LF RR oscillations are baroreflex-mediated (Eckberg, 2000). Sleight *et al* (1995) demonstrated a lack of response in the RR interval to 0.1Hz sinusoidal neck suction in supine CHF patients with poor BRS values, whereas a control subject and patients with intact baroreflexes were able to generate RR interval oscillations at the appropriate frequency. From this they concluded that an intact baroreflex (indicated by normal BRS values) is necessary for LF RR interval variability.

There is evidence that the relationship between low frequency pressure and RR intervals is also altered by a change in posture. Atrial pacing during tilt augments LF diastolic power (Taylor and Eckberg, 1996). However, the LF phase between pressure and RR interval is unaffected by tilt (Saul *et al*, 1991; Cooke *et al*, 1999). There is still no real consensus regarding the pressure/cardiac relationship in the low frequency band (Eckberg, 2000; Lanfranchi and Somers, 2002; Malpas, 2002).

CHAPTER 4 CARDIAC BARORECEPTOR

SENSITIVITY

Introduction

As briefly described in Chapter 1, the term baroreceptor sensitivity (BRS), sometimes also called baroreflex gain, is commonly used to describe the magnitude of the heart rate response to changes in arterial pressure. Specifically, it is the change in RR (or pulse) interval for a given change in systolic pressure (in ms/mmHg). In fact, baroreceptor gain would be a more accurate description of the parameter, as the use of the term 'sensitivity' implies the size of the change in pressure required to bring about a change in heart rate. However, the terminology is now in relatively common usage.

Until recently there has been no need to further qualify the term 'baroreceptor sensitivity' as the above definition was the only one in use. However, as the introduction of new types of BRS is gathering pace, these parameter names require further refinement (Rudas *et al*, 1999; Mukkamala *et al*, 2003; Ogoh *et al*, 2003). If we consider that the baroreceptors also affect efferent autonomic activity to the myocardium (Figure 1-1), then we could define a second parameter, cardiac inotropic BRS, which might be measured as the change in stroke volume per mmHg change in systolic pressure (Casadei *et al*, 1992). As stroke volume is strongly dependent upon both preload and afterload, and as cardiac output is limited by venous return, it appears that this parameter would be subject to significant influence from non-baroreceptor inputs and will not be considered further in this thesis.

Cardiac chronotropic BRS (cBRS) seems ideally suited to assist with the diagnosis or prognosis of pathologies that are specifically related to, or heavily dependent on, the function of the heart, and also for pathologies that could affect the baroreceptors themselves or the central processing. In this way cardiac BRS has previously been applied to patients with pathologies such as myocardial infarction, congestive heart disease, hypertension and stroke (La Rovere *et al*, 1995; Patton *et al*, 1996; Mortara *et al*, 1997; Robinson *et al*, 2003). It has also found application to conditions that primarily affect the nervous system, such as diabetes and the neurally-mediated syncope (Eckberg *et al*, 1986; El-Sayed and Hainsworth, 1995; Weston *et al*, 1996b; Benditt *et al*, 1998). These uses will be expanded upon later.

However, the heart has a limited role in the determination of cardiac output and hence arterial blood pressure. Subjects whose cardiac baroreceptor loop has been interrupted (by

pharmacological or pathological means), for example, are not predisposed to orthostatic hypotension (Wieling and Wesseling, 1993). The role of the peripheral vasculature to reflexly alter systemic resistance is likely to be more central to pathologies that are particularly related to the short-term control of blood pressure, such as orthostatic hypotension. This parameter would be termed peripheral BRS (pBRS) and would quantify the change in peripheral resistance per mmHg change in pressure.

This chapter discusses the detailed physiology of the baroreceptor control of heart rate. It then reviews some of the methods that have previously been evaluated for characterising the cardiac baroreceptor magnitude and the useful information that has resulted from their use⁵.

Further Baroreceptor Physiology

In Chapter 2 I described the basic anatomy and physiology of the system that comprises the baroreceptors and autonomic nervous control of cardiac and peripheral effects. Additional details of the physiology are required to inform critical examination of the methods used to quantify it. Eckberg and Sleight (1992) have produced a comprehensive review of this subject, some aspects of which will be reproduced here.

Afferent baroreceptor nerve activity is pulse synchronous and is caused by changes in the dimensions of the arteries. Thus it is determined by the transmural pressure rather than absolute arterial blood pressure. As a consequence of this, the use of neck chambers to alter external neck pressure has been able to provide a significant proportion of the information currently available on human baroreceptor physiology.

The response of the arterial baroreceptors to changes in pressure is highly non-linear and complex. Baroreceptors exhibit a sigmoidal response to pressure, characterised by threshold and saturation features linked by a linear region (Figure 4-1) (Eckberg and Sleight, 1992, p86-87). It has been demonstrated in animals and *in vitro* that this response shape exists between the arterial baroreflex and the afferent neural activity and that individual nerve fibres have different threshold pressure levels partly determined by whether they are myelinated. A linear

5. The onset of heart rate turbulence following ventricular premature beats has been used to characterise cardiac BRS, but this does not result in exactly the same parameter, as changes in RR interval are not related to changes in blood pressure. Its use is described briefly in the section on ectopic beats in Chapter 5.

relationship exists between efferent vagal stimulation frequency and pulse interval⁶ (Hainsworth, 1995).

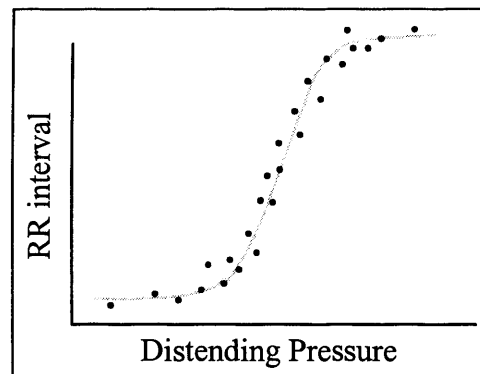


Figure 4-1: Sigmoid pressure response of the arterial baroreceptors

It is difficult to estimate at what point on the sigmoid curve human baroreceptors are operating without applying relatively large changes in pressure in order to characterise the entire range of the response. Normal physiological variations during resting conditions may exist in the linear region or close to the threshold. It is thought that healthy adults probably have an operational point at the threshold, cardiovascular patients below this and trained athletes may instead occupy positions on the saturation region (Eckberg, 1980; Eckberg and Sleight, 1992, p88; Mortara *et al*, 1997).

Cardiac chronotropic BRS estimates the ability of the heart to respond to changes in pressure. If the estimate is calculated from a region close to threshold or saturation then a much smaller value will be obtained than if it were determined from the linear region, which represents the maximal response (Figure 4-2). Without characterisation of the whole curve it is impossible to determine whether intra and inter-subject differences in BRS values are the result of repositioning of the operational point along similar curves or differences in the slope of the curve (Farquhar *et al*, 2000). It has been demonstrated that the human sigmoid response exhibits circadian shifts both horizontally and vertically (Eckberg and Sleight, 1992, p142-143).

6. The linearity of the vagus/sinus node relationship is a strong argument for the use of RR interval rather than heart rate as the end-organ variable used in analysis.

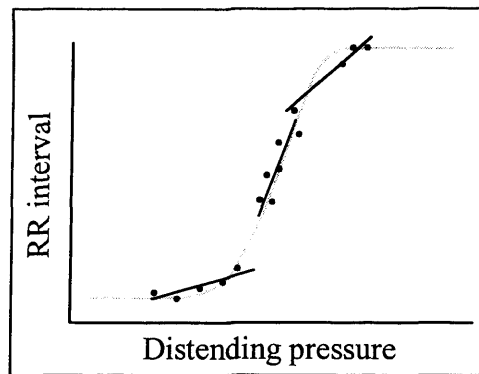


Figure 4-2: Effect of operational point on the BRS slope

The response activity in afferent baroreceptor fibres is dependent on the rate of pressure changes as well as the absolute difference. Steeper pressure gradients provoke a higher frequency of neural activity and at lower threshold pressures than slow changes. Hysteresis also exists, whereby the neural impulse frequency at a particular pressure is lower when the pressure is falling than when it is rising. This may affect comparisons between BRS methods that predominantly use unidirectional changes and those that are non-discriminatory.

Baroreceptors also exhibit adaptation and resetting to a step pressure change. Adaptation occurs very quickly: following an increase in activity in response to a step up in pressure, substantial drops in activity can occur within 0.1s and then continue to decline in an exponential fashion. Resetting occurs with chronic changes in pressure levels and appears to result in the sigmoid response being shifted to the right. Therefore similar changes in neural afferent activity may take place in hypertensive as in normal subjects, but at higher absolute pressures (Eckberg and Sleight, 1992, p140-141).

The response of the sinus node to a particular change in pressure is also dependent on the relative timing of the stimulus to the cardiac cycle. Minimum latencies (i.e. the period during which no effect is seen at the sinus node) are of the order of 0.2-0.5s, but the maximal first response has been found to occur when the pressure stimulus occurs approximately 0.8s before the ECG P-wave. RR interval prolongation increases up to a maximum at around 1.5s after the pressure stimulus, but RR interval reduction after pressure decrease does not reach a maximum until around 3-4s (Eckberg, 1980). The rapidity of the chronotropic response and the fact that it is effectively abolished following atropine but augmented after propranolol indicates that this is a vagally-mediated effect.

As a single-value, cBRS can only quantify the response of the baroreflex system between arterial pressure and sinus node as a whole. It cannot distinguish between the various stages: the baroreceptor transduction of arterial pressure changes, central processing of the baroreceptor afferents to produce changes in sympathovagal efferent balance, and the

response of the sinus node to those inputs. Changes in value between, or within, subjects do not allow us to identify where in the chain such changes may be operating.

Estimation of Cardiac Baroreceptor Sensitivity

Imposed Ramp ('Oxford') Method

The first quantifications of baroreceptor sensitivity, published by Smyth *et al* (1969), were determined from the increase in RR interval following small intravenous boluses of angiotensin-II. Originally, only intervals occurring during expiration were used, but this was found to have little effect on the values determined. However, angiotensin also affects the vagus nerve and thus was soon replaced as a pressor agent by phenylephrine (PE). This only stimulates α -adrenoreceptors and thus has no direct effect on cardiac function.

Variations in this method include the pharmacological agent used, direction of pressure change and the nature and duration of the administration of the agent. The drug may be given as a bolus injection, a series of boluses or an infusion of variable duration. Pressure changes are most commonly around 20-30mmHg over a time period of 10-30s, but may take longer (Panerai *et al*, 1997b; Lord *et al*, 1998). Sodium nitroprusside (SNP) is often used as a depressor agent and may be used in a protocol of alternating pressor and depressor effects (Eckberg, 1980; Lipman *et al*, 2003).

RR intervals are plotted against the change in systolic pressure resulting from these interventions and linear regression slopes are determined. Minimum correlation coefficients or *p*-values for these relations are defined to ensure a reasonably strong relationship exists (Robbe *et al*, 1987; Lord *et al*, 1998; Lipman *et al*, 2003), and usually at least three statistically suitable slopes are required to be averaged before an estimate is deemed reliable. Arterial pressures were previously determined from intra-arterial catheters in the radial or brachial arteries, but more recently are often measured non-invasively using Finapres or Portapres devices (Lord *et al*, 1998; Pinna *et al*, 2000; Davies *et al*, 2001b).

The advantage of this method is that the subject should be unaware of the stimulus, i.e. the pressure change, although they may be aware of the injection/infusion. As mental state is known to have an effect on sympatho-vagal balance (Robbe *et al*, 1987; Malliani *et al*, 1991) the subject's psychological condition may affect the values obtained. However, as the stimulus is initiated by alterations in peripheral resistance this method is unsuitable for investigating peripheral BRS and also cannot be used to determine the effect of the change in RR interval on the arterial pressure.

The moderate pressure changes elicited by the use of a single vasoactive agent are unlikely to be able to fully characterise the entire sigmoid response. For subjects whose operational point lies at the extremes of the linear region the relationship described during the pressure change may actually be curvilinear, although this would not necessarily prevent a statistically significant linear relationship being found for each individual ramp. The use of longer ramps from slow drug infusions are more likely to exhibit non-linearity and this may be exacerbated by the feedforward regulating effect of RR interval on arterial pressure. Also, although cBRS is considered to be a vagally-mediated effect, longer ramps will allow the slower sympathetic branch to affect the heart via both the sinus node and the myocardial contractility. As the speed of the pressure ramp will be an important determinant of the value obtained, long slow changes in pressure may also include adaptation effects. There is no standardisation of method currently available and all or any of these effects may lead to reduced BRS values being calculated.

The use of positive and negative changes in pressure may allow a fuller characterisation of the sigmoid response in which case the model to which the data is fitted need not be a simple linear one (Farquhar *et al*, 2000; Lipman *et al*, 2003). Reports indicate that BRS values determined following nitroprusside administration are generally slightly lower than those found using phenylephrine (Panerai *et al*, 1997b; Porta *et al*, 1999; Lipman *et al*, 2003). This could be a result of the lower pressures being located on or near the threshold region and/or of the hysteresis effect.

The length of delay between the SAP and RR interval values plotted is determined by the latency between stimulus and response. The parasympathetic latencies are very short, so most authors have used a one beat delay, i.e. systolic pressure is considered to influence the following RR interval. However, given that the times involved are a few hundred milliseconds, it is possible that a systolic peak could affect the length of the interval in which it occurs (zero delay). This is more appropriate for longer mean RR intervals. Colombo *et al* (1999) used a ramp technique in which they chose the slope with the best correlation coefficient out of zero or one beat delays. Correlation coefficients for regression slopes are highest for zero and single beat delays, and it is feasible to consider adapting the method to each subject's base heart rate (Eckberg and Sleight, 1992, p90-91; Lipman *et al*, 2003). However, the choice between zero and one beat's delay appears to be more academic than clinical, as the difference between cBRS values calculated with each choice are small for both real data (Eckberg and Sleight, 1992, p91-92) and modelled data (de Boer *et al*, 1987). The choice of delay is also an issue for the sequence method (see below).

Neck Suction and Pressure

Pharmacological agents produce a stimulus that is difficult to control. The pressure response to a particular dose of agent is susceptible to both inter and intra-subject variability (Rudas *et al*, 1999). An infusion may be continued until a particular pressure change has been effected (Lord *et al*, 1998) or alternatively the magnitude of a subject's response may be determined before calculation of the BRS begins (Colombo *et al*, 1999). However, it is not certain that a sufficiently large pressure change can be elicited from every subject or that the slope from each repetition will reach the statistical standards required.

For the application of changes in external pressure on the carotid artery a chamber or collar is strapped around the neck and delivers suction or positive air pressure to either the entire neck surface or just the anterior and lateral surfaces. Depending on the quality of the air seal on the skin and the frequency response of the servomechanism the type, magnitude and form of the pressure stimulus can be highly variable and can be controlled to a very fine degree (Eckberg and Sleight, 1992, p108-109). Cardiac BRS is calculated in a similar manner as for the ramp method, i.e. the gradient of RR interval against systolic carotid distending pressure (arterial minus external pressure).

When applied in this way, with otherwise constant conditions, the stimulus to the baroreceptors is limited to the carotid sinuses and does not affect the aortic receptors. Thus the differential contributions of carotid and aortic baroreceptors can be studied using this method. Another advantage is that, as arterial pressure is not perturbed by the stimulus, the baroreceptor control of pressure itself can also be investigated. However, even modern equipment is relatively cumbersome and the subject is highly aware of the stimulus, so that mental and emotional factors might contribute to the responses. Also, due to the highly controllable nature of the stimulus and the variety of procedures and dynamic capabilities of the equipment, it is difficult to compare different studies. There may also be incomplete transmission of the external pressure through the neck tissues, requiring the application of a correction factor (Eckberg and Sleight, 1992, p100). This mitigates the advantage of the stimulus controllability somewhat.

Estimates from Spontaneous Pressure Changes

The advantage of all spontaneous methods is that they require no interventional inputs and the changes are physiological in nature, so they are suitable for analysing long data recordings during a variety of conditions. However, there is obviously no control over the magnitude,

rate or direction of the pressure stimulus and these methods are unlikely to encompass a large enough range of pressures to characterise the whole response curve.

Sequence Analysis

The analysis of spontaneously occurring sequences can be regarded as the ramp method applied to spontaneous ramps in arterial pressure that are accompanied by a parallel change in RR interval (Davies *et al*, 2001b; Di Rienzo *et al*, 2001). A sequence is usually defined as any continuously and monotonically increasing or decreasing series of three or more successive systolic pressure values. As is the case for the ramp method above, a delay of one beat is usually employed between the pressure and RR interval changes, although zero or two may also be used (Blaber *et al*, 1995; Panerai *et al*, 1997a; Di Rienzo *et al*, 2001).

These sequences have been shown to be baroreceptor-mediated in cats and to possibly represent an open-loop method of quantification, as the surgical opening of the baroreceptor afferent limb did not affect the occurrence of the pressure sequences but did prevent the RR interval slope responses (Eckberg and Sleight, 1992, p115; Di Rienzo *et al*, 2001). The frequency of such slopes varies greatly between subjects but has been estimated to be around 100-500 per hour for healthy controls, although only 10-30% of systolic pressure increases are accompanied by parallel increases in RR interval (James *et al*, 1998; Di Rienzo *et al*, 2001). They are less common at night than during the day, and when supine rather than in the upright posture (Farquhar *et al*, 2000). As the slopes tend to be short in comparison with the pharmacological ramp method, the regression slopes are subject to greater random error and thus it is more important that several estimates be averaged.

The sequence method differs from the ramp method by having many more, shorter events in a typical length recording. Therefore the sequence method requires automated detection of suitable beat series. This involves the consideration of several criteria which are not immediately apparent. For example, the constraint of strict monotonicity can severely limit the availability of appropriate sequences. Small contrary variations within an otherwise continuous slope may be the result of measurement and rounding errors. Also, where the precision of the measurement is a significant proportion of the beat-to-beat changes, there may be two or three successive beats with differences of zero. This leads to decisions along the lines of 'Should a long parallel sequence that appears to reflect baroreceptor control be discarded because of a single small inflection in the middle?' and 'Should a beat-to-beat difference of zero be included in a sequence?'

Such fine detail of the method is not generally reported in published studies. Personal experience with this method on the data in Chapter 8 concurs with published results indicating that such ‘tweaking’ of the technique affects the gradients much less than it affects the number of suitable parallel sequences (Davies *et al*, 2001b). This is supported by the strong correlation found between sequence methods employed by different groups (Laude *et al*, 2004) and by the small differences in slope found when using lags of different durations (Blaber *et al*, 1995; Davies *et al*, 2001b). Some groups have included a minimum beat-to-beat pressure and/or RR interval change in their criteria for suitable sequences (Kim and Euler, 1997; James *et al*, 1998; Laude *et al*, 2004). Davies *et al* (2001) have shown that both the number of sequences and the cBRS values are particularly sensitive to thresholds for systolic pressure; both parameters decrease as the threshold is increased. Typically, minimum correlation and/or *p*-values are defined (Weston *et al*, 1996b; Kim and Euler, 1997; James *et al*, 1998; Laude *et al*, 2004) and an average of at least three slopes is required, as for the ramp method.

Where bi-directional sequences have been compared the up and down sequence values are usually comparable (Weston *et al*, 1996b; James *et al*, 1998). This is in contrast to cBRS values determined by the ramp method, where decreasing slopes produce consistently lower estimates than those from increasing ramps (Rudas *et al*, 1999; Lipman *et al*, 2003).

Spectral Analysis Methods

In Chapter 3 I described spectral analysis of the relationship between naturally occurring variations in systolic pressure and RR interval. The generally high coherence values between these variables in bands below 0.5Hz indicate a strong linear relationship at these frequencies. Two methods in particular have gained popularity in the published literature: the alpha (α) and transfer function (TF) methods.

A common requirement for spectral methods is that the data be stationary for the duration of the analysis. Some statistical test must also be performed to ensure that the estimate is reliable. In the case of the alpha and transfer function methods this test is that the ordinary coherence is significant at the frequencies used. In most published papers a value of 0.5 has been adopted (Robbe *et al*, 1987; Lipman *et al*, 2003) without any testing of the threshold limit or examination of the effect of the analysis method on this level⁷ (Panerai *et al*, 1997b). The use of a coherence threshold may consist of the mean coherence over the band(s) of

7. The empirical determination of coherence limits using surrogate data are described in detail in Chapter 5.

interest achieving this threshold (Lin *et al*, 2002), or alternatively only frequency components with sufficiently high coherence values within that band may contribute to alpha or the TF value (Kim and Euler, 1997). The importance of this criteria is illustrated by the finding that most spontaneous pressure sequences are not accompanied by parallel ramps in RR interval (see above). These non-baroreceptor ramps will contribute to the spectral power of the signals, so only where a statistically significant linear relationship exists between SAP and RR interval should these powers be used to estimate the magnitude of cBRS. However, the application of an untested threshold results in the preferential exclusion of subjects with low BRS values – specifically the groups in which cardiac BRS is proposed to be a useful diagnostic indicator (Panerai *et al*, 1997b; Pinna *et al*, 2002).

Spectral analysis can be carried out using parametric (causal and non-causal) modelling methods, or non-parametric (non-causal only) techniques. In most reports these choices reduce to ARMA modelling (Patton *et al*, 1996; Mainardi *et al*, 1997; Barbieri *et al*, 2001; Cevesse *et al*, 2001) or Fourier methods (Kim and Euler, 1997; Robinson *et al*, 1997; Lord *et al*, 1998; Davies *et al*, 2001a; Head *et al*, 2001). The latter cannot separate the feedforward effects (RR interval on SAP) from the baroreflex feedback effects (SAP on RR interval) and thus treats the whole loop as a black-box (Panerai *et al*, 1997b; Barbieri *et al*, 2001), i.e. Fourier methods treat a closed loop system as if it were open-loop (Figure 4-3). However, these approaches are generally faster and less labour-intensive than parametric methods, which require user choices about model-types, orders and validation methods. Clayton *et al* (1995) showed little difference in the cBRS values determined for healthy subjects using four different spectral analysis methods.

The feedforward effect of RR interval on SAP has been estimated as 0.05-0.07mmHg/ms using ventricular pacing (Patton *et al*, 1996) and reverse impulse response analysis (Panerai *et al*, 1997b).

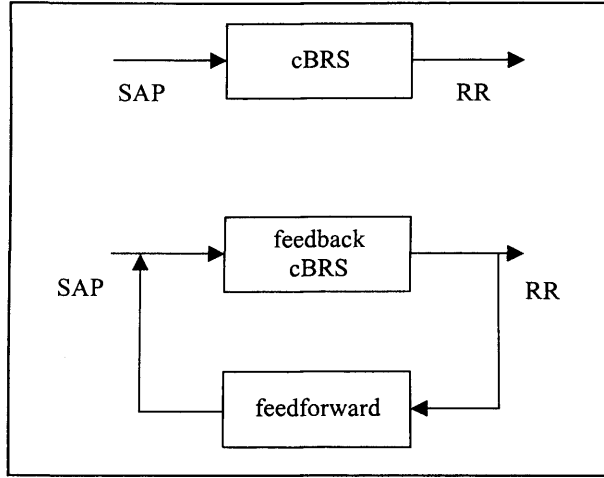


Figure 4-3: Simple open-loop (top) and closed-loop (bottom) models of the cardiac chronotropic baroreflex⁸

Transfer Function

The transfer function is a staple analysis tool for system characterisation. Technically, in engineering terminology, the transfer function is the Laplace transform of the unit impulse (weighting) function, h , (Bendat and Piersol, 1986, p29). The Fourier transform of h is the frequency response function, H . However, it is this latter quantity that has been used for cBRS estimation and referred to as the ‘transfer function method’ in the papers quoted below. In the ‘black box’ model, the transfer function is the frequency domain description of how the output is obtained from the input (Bendat and Piersol, 1986, p30):

$$Y_k = H_k \times X_k \quad \text{Equation 4-1}$$

Where X_k and Y_k are the Fourier transforms of the input and output respectively, and k is the index of the discrete frequency component. The transfer function, H , is complex-valued so that, for each k , there is a gain ($|H_k|$) and a phase (ϕ):

$$H_k = |H_k| \exp(-j\phi_k) \quad \text{Equation 4-2}$$

8. As pressure is the regulated parameter, it would traditionally appear on the right side of the system diagram, in which case the terms feedforward and feedback would seem more appropriate. The diagram is depicted in this way to maintain consistency with the idea of pressure as the input parameter.

The gain of the transfer function can be estimated as the ratio of the cross-spectrum between the input and output, P_{xy} , to the autospectrum of the input, P_x (Bendat and Piersol, 1986, p303):

$$|H| = \frac{|P_{xy}|}{P_x} \quad \text{Equation 4-3}$$

Which in terms of the cBRS estimate becomes:

$$|H| = \frac{|P_{SAP-RR}|}{P_{SAP}} \quad \text{Equation 4-4}$$

The gain of the transfer function over either or both the low and high frequency bands has been reported as cardiac BRS (Robbe *et al*, 1987; Munakata *et al*, 1994; Kim and Euler, 1997; Lipman *et al*, 2003; Laude *et al*, 2004).

Alpha Method

The transfer function gain can also be determined from the power spectra of the input and output (Bendat and Piersol, 1986, p167):

$$|H|^2 = \frac{P_y}{P_x} \quad \text{Equation 4-5}$$

Therefore the alpha estimate of the baroreflex gain is the square root of the ratio of the power contained in the RR interval and systolic pressure spectra (Pagani *et al*, 1987).

$$\alpha = \sqrt{\frac{P_{RR}}{P_{SAP}}} \quad \text{Equation 4-6}$$

As spectral power should be determined in $units^2/Hz$ (where ‘unit’ is appropriate for the variable in question), the dimensions of alpha are ms/mmHg. Alpha is determined for either or both the low and high frequency bands separately (Rudas *et al*, 1999; Lin *et al*, 2002; Laude *et al*, 2004), and/or the mean of these is used as the estimate for cBRS (Robinson *et al*, 1997; Colombo *et al*, 1999; Blake *et al*, 2000; Lipman *et al*, 2003). Note that the upper and lower limits of these bands vary between different authors (Chapter 3).

Comparison of the Methods

The effects of rate, magnitude, timing and direction of pressure change should all be taken into account when comparing cBRS results and will contribute to the scatter of repeated measurements even within the same method. Reproducibility is not impressive. Standard deviations of repeated measures are related to the magnitude of the measurement so that larger BRS values have larger variances (Lord *et al*, 1998; Pinna *et al*, 2000). However, when using normalised values, coefficients of variation for short-term reproducibility were found to be much worse for CHF patients with low cBRS values (around 30-50% for controls and 30-100% for patients), although correlation coefficients for repeated estimates are around 0.7-0.8 (Davies *et al*, 1999). The gain of the cardiac chronotropic baroreflex is likely to vary with frequency (de Boer *et al*, 1987; Patton *et al*, 1996). Also, the 'true value' of the baroreflex gain may vary significantly over a short timescale (Eckberg, 1980).

All the methods have their own procedural or conceptual advantages and disadvantages. Rarely is a technique fully described in the published literature in such a way that all significant criteria have been defined. For each method there is no guarantee that suitable data can be found for every subject. Statistically significant slopes are not guaranteed for either pharmacological ramps or spontaneous sequences (Robbe *et al*, 1987; Munakata *et al*, 1994; Rudas *et al*, 1999) or pressure may not increase sufficiently (Lord *et al*, 1998). Significant coherence may not exist across a large portion of the required frequency bands, especially where thresholds have not been experimentally determined (Panerai *et al*, 1997b; Colombo *et al*, 1999; Rudas *et al*, 1999). Non-stationarities, trends and cardiac arrhythmias may prevent the use of spectral analysis. Although careful attention to experimental and analysis techniques can reduce the number of subjects for whom estimates cannot be obtained, there is no method that provides 100% estimation. Also, it is often the subjects with low cBRS values who cannot be quantified, i.e. the groups for whom this parameter should be particularly useful (Panerai *et al*, 1997b; Davies *et al*, 1999; Pinna and Maestri, 2002; Pinna *et al*, 2002).

Inevitably, there are differences between the cBRS values determined by disparate methods. There are obviously greater conceptual similarities between the ramp and sequence and between the alpha and transfer function methods than between other combinations and it could be expected that there would be greater agreement between estimates from these.

Ideally, when using a variety of methods to determine the same quantity, we hope to obtain identical values for identical conditions, i.e. we wish to assess how well the methods 'agree'. Bland and Altman (1986) have pointed out that many authors compare different methods only

by the use of the correlation coefficient. This is a useful and necessary statistic for determining whether two estimates are linearly (proportionally) related, but is not sufficient for defining the degree of agreement. Linear regression provides an estimate of the gradient, and how close this is to unity tells us whether the difference between the methods is independent of the value. This is also easily identified in the plots of difference versus mean as described in the above reference ('Bland-Altman' plots). However, when assessing agreement we need to consider whether we are really comparing like with like and also whether a lack of agreement between two methods means that one is unusable.

Fourier methods for spectral quantification require strict stationarity of the data and effectively produce an average value for cBRS over the duration used for the analysis (Di Rienzo *et al*, 2001). The sequence method can provide an estimate with a much finer, if unevenly-spaced, temporal resolution, although averaging of several slopes should ideally be carried out to provide a reliable estimate. Parametric spectral methods can be used to separate the feedforward and feedback parts of the system. Barbieri *et al* (2001) used parametric modelling to compare open and closed loop estimates of transfer function gain and the alpha estimate (which they termed 'cross-spectral' and 'ratio' respectively). In their qualitative analysis they found that there were close correlations between the closed and open loop transfer function gains, but that closed loop values tended to underestimate open loop ones. They stated that there was 'no fixed relationship' between the two.

In the same paper they also point out that, analytically, transfer function estimates should always be less than alpha estimates. From the definitions:

$$|H|^2 = \frac{|P_{SAP-RR}|^2}{P_{SAP}^2} \quad \text{Equation 4-7}$$

$$\alpha^2 = \frac{P_{RR}}{P_{SAP}} \bullet \frac{P_{SAP}}{P_{SAP}} = \frac{P_{RR} P_{SAP}}{P_{SAP}^2} \quad \text{Equation 4-8}$$

However, the cross-spectral inequality states (Bendat and Piersol, 1986, p136-137):

$$|P_{SAP-RR}|^2 \leq P_{RR} P_{SAP} \quad \text{Equation 4-9}$$

Therefore $|H|^2 \leq \alpha^2$. Barbieri *et al* (2001) also confirmed this experimentally.

Lipman *et al* (2003) compared a modified Oxford ramp method with spontaneous methods, across a mixed sample of subjects with and without coronary artery disease. They refer to the ramp technique as the “gold standard” method which represents “true baroreflex gain” and maintain that the spontaneous techniques do not necessarily quantify the gain of the arterial baroreflex effect on heart rate. However, for all the reasons mentioned above (differing rates and magnitudes of pressure change, variable latencies, duration of pressure change, inclusion of temporal information, etc) different methods should not be expected to produce identical values. The authors indicate that phenylephrine and nitroprusside ramps produced different slopes and that the pressor agent often induced a rise into the saturation region, so that the entire sigmoid response was characterised. However, better agreement might have been found with spontaneous methods if they had selected portions of the ramps that had comparable absolute values of, and changes in, pressure. They do not describe how they determine a linear regression from a sigmoid response. Three of the spontaneous methods that they compared (TF, sequence and alpha) overestimated cBRS with respect to the ramp method. By selecting the maximum slope produced by the pharmacological ramps better agreement may have been achieved.

The authors suggested that the discrepancy might exist because carotid compliance did not appear to be related to the spontaneous estimates, as it was for the Oxford ramp method. This apparent difference can probably also be attributed to the different stimuli provided. Larger pressure changes are more likely to be affected by the distensibility of the arteries. Therefore these results are unsurprising and do not provide grounds for dismissing the spontaneous techniques.

Although the Oxford ramp method is the original and has been most widely reported, that does not mean that it is the most suitable for all situations or that it is the best estimate of the ‘true value’ to which all other methods should be compared. In a research environment, the worth of a physiological parameter resides in the information it can provide about human physiology and pathology. In a clinical environment its worth can be judged by its diagnostic and prognostic discrimination power. Therefore, although there is a large amount of information regarding the use of the ramp method in pathological conditions, the absence of good agreement with another technique (when appropriately compared) does not preclude the use of the other method. It simply requires that we are specific about the method used and do not conclude that identical units mean identical quantities. With increasing dissemination of the newer methods it may transpire that some are found to have particular relevance and usefulness to particular conditions. By utilising the normal physiological perturbations that

occur around each subject's operating point, the spontaneous methods may provide the most suitable estimates for assessing subjects under conditions where pressure changes are small.

There have been numerous published reports comparing the different methods. Many compare group means, some provide correlation coefficients and a few provide bias and regression estimates. Due to the lack of detailed information regarding the estimation methods and the limited statistical efforts to determine the degree of agreement it is difficult to compare the individual published results. Neither is a comprehensive review of the comparisons a particularly useful exercise in this thesis, as the results in most cases are not sufficiently unambiguous that a decision can be made to discard one method in favour of another or determine that two techniques are interchangeable. Also small differences in the detailed methodology may significantly alter the degree of agreement. For example, higher pressure thresholds resulted in improved correlation coefficients between the sequence and the phenylephrine methods for normal subjects, but for CHF patients the opposite was true (Davies *et al*, 2001a).

Different methods demonstrate similar changes with posture (Kim and Euler, 1997) and may show greater agreement when standing (Laude *et al*, 2004), although this may be an artifact of the lower values in this position. Similarly, smaller differences in elderly subjects may be a product of the reduced range of values as cBRS decreases with age (Panerai *et al*, 1997b; James *et al*, 1998; Rudas *et al*, 1999).

Values for healthy control subjects in the supine position vary from around 5 to 20 ms/mmHg (Kim and Euler, 1997; Rudas *et al*, 1999; Lipman *et al*, 2003). Correlation coefficients between the methods are usually between 0.5 and 0.9 (Patton *et al*, 1996; Panerai *et al*, 1997b; Davies *et al*, 1999; Rudas *et al*, 1999; Lipman *et al*, 2003; Laude *et al*, 2004) whereas systematic bias can be of a similar order to the values obtained (Patton *et al*, 1996; Rudas *et al*, 1999). There are a few consistencies in published reports. Sodium nitroprusside ramps produce lower cBRS values than phenylephrine ones (Panerai *et al*, 1997b; Rudas *et al*, 1999). The alpha and sequence methods tend to produce higher values (Panerai *et al*, 1997b; Rudas *et al*, 1999; Lipman *et al*, 2003). Spectral estimates limited to the high frequency band tend to be comparable to or lower than those from the LF band (Munakata *et al*, 1994; Tank *et al*, 2001; Laude *et al*, 2004).

Diagnostic and Prognostic Use

Cardiac chronotropic BRS measures the ability of the sinus node to reflexly respond to changes in systolic pressure and has therefore found clinical use in conditions that affect the

heart and the autonomic nervous system. Conditions which are associated with activation of the sympathetic branch (e.g. mental stress, the upright posture and exercise) tend to produce lower values of cBRS (Pickering *et al*, 1971; Robbe *et al*, 1987; Weston *et al*, 1996b; Kim and Euler, 1997; Blake *et al*, 2000; Barbieri *et al*, 2001). This is most likely due to the restraining influence exerted by sympathetic efferents on cardiac vagal activity (Vanoli and Adamson, 1994). Muscarinic blockade, which prevents vagal effects, also reduces cBRS (Vanoli and Adamson, 1994; Lin *et al*, 2002), and it is lower during the day than at night (La Rovere *et al*, 1995).

Patients with heart failure, hypertension or previous myocardial infarcts (MI) are also considered to have increased sympathetic tone (Munakata *et al*, 1994; La Rovere *et al*, 1995, 1998; Mortara *et al*, 1997; Rudas *et al*, 1999). Patients with various forms of heart disease have been found to have significantly reduced cBRS values (3.7 vs. 16 ms/mmHg, La Rovere *et al*, 1995). In the acute post-MI phase, cBRS values are lowered, but often recover somewhat after about a month. However, patients who continue to have very low values after this period (<3 ms/mmHg) are at greater risk of sudden cardiac death (Vanoli and Adamson, 1994; La Rovere *et al*, 1995). The same threshold has been found to be an effective risk stratifier for patients with chronic heart failure (Mortara *et al*, 1997). Results from canine models show that a low cBRS is indicative of a disposition towards malignant arrhythmias following MI (Vanoli and Adamson, 1994; La Rovere *et al*, 1995).

The ATRAMI study (Autonomic Tone and Reflexes After Myocardial Infarction) was a large, international, multi-centre prospective trial that defined the cBRS limits for increased risk following an MI (Vanoli and Adamson, 1994; La Rovere *et al*, 1995, 1998). It also investigated other methods of quantifying autonomic tone, including various heart rate variability (HRV) measures. HRV and cBRS were found to be weakly correlated, which is unsurprising as HRV is an approximate indicator of cardiac sympathovagal tonic activity⁹, whereas cBRS estimates the ability for increases in vagal tone to effect changes in the sinus node. Subjects who had cBRS values >6.1 ms/mmHg (by phenylephrine ramp) had 2% 2yr mortality compared to 9% for subjects with cBRS <3.0 ms/mmHg (the 15th percentile). Phenylephrine ramp cBRS was shown to provide a very strong independent risk stratifier which should be used to compliment left ventricular ejection fraction (an existing risk stratifier) and HRV.

9. Considering that HRV reflects the variability in cardiac efferent activity, not the mean level.

Heart failure patients have hypertrophied myocardium and oedematous lungs and body tissues due to the inability of the heart to pump the blood effectively. Thickened and damaged heart muscle is likely to have inferior mechanical qualities (e.g. reduced compliance) and this may also affect the response of the ventricular mechanoreceptors. Such patients have smaller increases in RR intervals following pressure rises. Neck pressure experiments have shown that they have shallower sigmoid functions, i.e. the linear portion has a reduced slope and saturation occurs at a lower RR interval value (Eckberg, 1997).

Using the sequence method, patients with diabetes were found to have both fewer baroreflex sequences and lower cBRS slopes (11 vs. 20 ms/mmHg) than control subjects (Weston *et al*, 1996b). An inverse relationship between duration of diabetes and cBRS was determined despite the finding that other measures of autonomic function could not discriminate between the two groups. The Oxford technique however, showed that the effect of falling pressure on RR interval was not different between diabetic patients and control subjects, but the effect of increasing pressure was much lower for patients (Eckberg *et al*, 1986).

Various methods have demonstrated that patients with hypertension also have significantly reduced cBRS values. In fact, although the absolute values varied between the methods, within each method the estimates for older hypertensive patients were found to be approximately half the value for age-matched controls (Panerai *et al*, 1997b; James *et al*, 1998). For example, 4.9 vs. 9.4 ms/mmHg for the alpha method and 3.0 vs. 8.6 ms/mmHg for the phenylephrine ramp. This indicates that, although estimates from different methods are not interchangeable, they may provide similar discriminatory power and thus it is important to always provide the method when quoting a value.

Combined and HF alpha estimates of cardiac BRS are reduced in acute (<72hrs) stroke compared to matched control subjects (Robinson *et al*, 1997, 2003). This difference appears to be due to larger normalised HF SAP power for patients, as coherence values and pulse interval spectra were similar between the groups. Combined alpha BRS has recently been found to have a similar prognostic value to that for the phenylephrine method for post-MI mortality risk. Although not significantly prognostic for short term outcomes (1 month), patients with cBRS less than 5ms/mmHg had an overall mortality of 28% compared to 8% for those with higher values (Robinson *et al*, 2003).

Other conditions also affect the baroreflex control of heart rate. For example, alpha cBRS determined in the supine position declines during pregnancy, from early to mid gestation, and returns to normal levels within weeks of delivery (Blake *et al*, 2000). Idiopathic orthostatic intolerance (i.e. syncope which is not accompanied by a drop in arterial pressure) is

characterised by a shallower sigmoid response and lower cBRS using a modified ramp method (approx 12 vs. 24 ms/mmHg, Farquhar *et al*, 2000).

It can be seen therefore that cardiac chronotropic BRS has both a wide variety of applications and a choice of methods, which may be influenced by factors such as convenience and degree of invasiveness as well as concerns about physiological appropriateness. There are currently no agreed standards for any of the methods of estimating cBRS. There is no consistent definition of what constitutes a 'slope' in terms of length, delay, minimum change, statistical significance or number. Spectral methods vary as to duration, frequency band and details particular to the analysis method, (Lord *et al*, 1998). Potentially, where a subject has a high frequency of baroreflex slopes, the variation of cBRS over time can be documented. For example, during the onset of orthostatic stress.

Other methods have been used in addition to those described here, particularly using the impulse response function and the Valsalva manoeuvre (Patton *et al*, 1996; Kim and Euler, 1997; Panerai *et al*, 1997b; Lord *et al*, 1998; Davies *et al*, 1999), and most undergo repeated revision and update as new information is discovered and techniques become available (Colombo *et al*, 1999; Lucini *et al*, 1999; Avolio *et al*, 2001). However, this variety and state of renewal also prevents the common acceptance of cBRS as a clinical diagnostic and prognostic indicator and it still remains largely the province of research.

Peripheral Baroreceptor Sensitivity

The contribution of peripheral control to arterial pressure regulation has been the subject of several recent investigations. The sympathetic response to baroreceptor stimulation has been characterised as having a similar sigmoid shape as that for the RR interval or heart rate (Sato *et al*, 1999; Ogoh *et al*, 2002; Cooper *et al*, 2004). Most investigations involved the MSA response to changes in pressure (Rea and Eckberg, 1987; Jacobsen *et al*, 1993; Rudas *et al*, 1999; O'Leary *et al*, 2003), but this does not necessarily indicate the effectiveness of the neural output in producing an end-organ response (Shoemaker *et al*, 2000). Mukkamala *et al* (2003) determined the static gain of the peripheral baroreflex using the peripheral resistance step response from an ARMA model and normal subject data. This gave a dimensionless quantity that could represent the percentage change in PR for a percentage change in pressure. This was found to increase non-significantly from supine to 30° tilt. Direct estimation of beat-to-beat TPR values (MAP/CO) resulted in an erroneous step response, however their values of TPR were smoothed over 11 beats.

Individual estimates of forearm peripheral BRS in humans have recently been published (Cooper *et al*, 2004). Forearm resistance was averaged over the respiratory cycle and the sigmoid response was determined from the maximum resistance response (after 10-15s) to steps in neck pressure/suction. Peripheral BRS was taken as the maximum slope of the arm resistance sigmoid function and calculated as percentage change from the value before the neck stimulus was applied. However, sympathetic outflow is known to be highly differentiated and the forearm response cannot be extrapolated to the systemic vasculature (Esler, 1993; Grassi and Esler, 1999; Malpas *et al*, 2001).

In this thesis I shall define peripheral baroreceptor sensitivity as the change in total peripheral resistance for a given change in arterial blood pressure. As described later, the measurement of TPR will be uncalibrated and the input pressure variable (SAP, DAP, MAP) has yet to be decided. The units of such a parameter will therefore be in %/mmHg. This will therefore represent the gain of the peripheral branch of the autonomic control loop (Figure 1-1). Quantitative assessment of peripheral baroreceptor sensitivity (pBRS) has not previously been reported for different subject populations. Neither have different methods of estimating this parameter been compared. This thesis investigates a non-invasive method for estimating beat-to-beat TPR and then establishes different algorithms for the estimation of pBRS in subject groups with disorders of blood pressure control.

CHAPTER 5 MEASUREMENT AND ANALYSIS

METHODS

Introduction

In order to quantify the peripheral response to changes in baroreceptor input it is necessary to establish a measurement method which can determine total peripheral resistance with sufficient temporal resolution. The chosen method is to use pulse contour derivation of stroke volume (using a non-invasive arterial pressure waveform) from which cardiac output, and hence peripheral resistance, can be determined¹⁰.

$$TPR = \frac{MAP - RAP}{CO} \approx \frac{MAP}{SV \times HR} \quad \text{Equation 5-1}$$

By replacing RR interval with TPR, peripheral baroreceptor sensitivity (pBRS) can be calculated using spontaneous variability methods similar to those detailed in the previous chapter for cardiac BRS. Pulse contour methods have not previously been used for the detailed analysis of stroke volume, cardiac output or peripheral resistance variability so Chapter 6 and Chapter 7 describe methods to verify this variability measurement.

This chapter will describe some of the methodological details that need consideration before this type of cardiovascular data can be analysed or the results interpreted. These include descriptions of parts of the investigative methods common to the following three experimental chapters, analytical concepts and some minor experimental investigations. These have been brought together here to prevent repetition and allow the experimental chapters to concentrate on the new techniques and results.

This chapter comprises:

- a review of Finapres non-invasive blood pressure measurement
- a discussion of peripheral resistance as a steady-state parameter
- the signal processing and spectral analysis methods used in the experimental chapters

10. See Chapter 6 for full details of the method.

- an examination of the synchronisation used for different cardiovascular parameters
- the different methods and impact of analysing cardiac ectopic beats on cardiac BRS

Finapres Blood Pressure Measurement

Finapres (FINger Arterial PRESSure) is a device for measuring non-invasive, phasic, beat-to-beat arterial pressure in the finger. It is based on the volume clamp method patented by Penaz in 1967 and has been in experimental and clinical use since the mid 1980's (Imholz *et al*, 1998). It has recently been superseded by the wearable version (Portapres) and a refined model (Finometer)¹¹ which is currently produced by FMS BV. Between 1986 and 1993 the devices were made under licence by Ohmeda for TNO BMI¹².

Method of Measurement

An inflatable cuff is placed around the finger. This contains an infra-red transmission photoplethysmograph (PPG) which measures the blood volume in the arteries of the fleshy pad of the middle phalanx. The set point is determined by inflating the cuff until the transmural pressure of the arteries is minimised, at which point the pressure in the cuff is equal to mean arterial pressure. Arterial flow is unimpeded so that the device can be worn for an extended period without restricting the blood supply to the finger tip. As pressure increases in the artery, the arterial walls distend, blood volume and therefore light absorption increase and the PPG signal starts to drop. The servo feedback mechanism maintains the PPG signal by altering the cuff pressure to keep the transmural pressure zero. Finger arterial pressure is measured indirectly as cuff pressure (Wesseling *et al*, 1985; Imholz *et al*, 1988; Wesseling, 1996). The setpoints are regularly adjusted to account for steady-state changes in physiology which might lead to drifting of the values over time ('Physiocal', Imholz *et al*, 1998). For all data acquisition procedures in this thesis this function was switched off for the recordings once steady-state conditions had been achieved.

11. The term 'Finapres' is used to represent all of these devices in the rest of this chapter.

12. Information from FMS website: <http://www.finapres.com>

Reliability of Finapres Values

It is well established that peripheral pressures, such as brachial and radial intra-arterial measurements, do not provide values identical to aortic or other central pressure measurements (Karamanoglu and Feneley, 1997). As the pressure pulse is transmitted to the periphery it encounters more reflections, producing an amplification of the pulse pressure, and an increased resistance which serves to impose a pressure gradient (Imholz *et al*, 1998). Peripheral diastolic and mean pressures are therefore generally lower than central ones, whereas systolic values tend to be augmented and these effects can be expected to be even greater in the finger (Bos *et al*, 1996; Wesseling, 1996).

Finapres (and Portapres) pressure values have been fairly extensively compared with both non-invasive and intra-arterial, brachial and radial pressure measurements under a variety of conditions and manoeuvres (Imholz *et al*, 1998; Silke and McAuley, 1998). There appears to be a reasonable amount of variation in the absolute mean differences between finger and intra-arterial measurements (Wesseling, 1996), however most authors have found the values to be reliable in comparison with other clinical devices (Pace and East, 1991) and with international standards (Eckert and Horstkotte, 2002).

The accuracy of this device has been tested during laboratory manoeuvres that are of interest in this thesis. For tilt and standing manoeuvres the difference between Finapres and intra-brachial mean and diastolic pressures did not change significantly during orthostatic stress, remaining within ± 6 mmHg (Imholz *et al*, 1990). Systolic pressures differences were more variable, ranging between -5 and $+11$ mmHg, although the authors concluded that the important pressure changes during the movements were well reproduced. Variability, as measured by the standard deviation of beat-to-beat changes, and cardiac baroreceptor sensitivity (ramp and neck pressure/suction methods) were found to be highly comparable with parameters determined from radial pressures (Parati *et al*, 1989). Finapres systolic spectral powers are considerably augmented in the VLF band, slightly increased in the LF band and slightly decreased in the HF band compared to peripheral intra-arterial measurements (Omboni *et al*, 1993; Pinna *et al*, 1996), although this will not affect intersubject comparisons of the relative values of LF and HF quantities.

Advice is often given to keep the hands warm during Finapres measurements to avoid complications of vasoconstriction adding to the pulse amplification (Wesseling, 1996; Imholz *et al*, 1998). Gradual vasoconstriction during surgery tended to increase the Finapres overestimation of systolic pressure and the pulse pressure (Wesseling *et al*, 1985), but more rapid changes in resistance (due to phenylephrine infusion) removed the bias completely

(Imholz *et al*, 1992). The difference between finger and aortic pressure increases with exercise intensity (Eckert and Horstkotte, 2002). However, the tracking of beat-to-beat changes in blood pressure is more important than absolute accuracy in the applications used here, and for this Finapres has been found to be comparable with intra-arterial methods for a variety of manoeuvres (Imholz *et al*, 1998).

Peripheral Resistance

The definition of resistance to blood flow is derived from Poiseuille's equation (Equation 2-2) and is calculated as the ratio of mean pressure to total flow (Equation 5-1). However, Poiseuille's equation applies to steady flow and so this definition is only applicable to the 'steady component' of blood flow. The concept of peripheral resistance is therefore inapplicable to transient cardiovascular conditions such as changes of position or the onset of exercise (Toorop *et al*, 1987; Nichols and O'Rourke, 1990, p26; Smith *et al*, 1994; McViegh *et al*, 1999) as arterial compliance will buffer the transmission of rapid beat-to-beat variations in the cardiac output due to the Windkessel effect (Wieling *et al*, 1998).

Toorop *et al* (1987) used an equation that corrected Equation 5-1 for transient conditions by taking into account the arterial compliance (C_a), the difference in pressure between the start and end of the cycle (ΔP) and the cycle length (T).

$$PR_{corr} = \frac{MAP}{CO - \left(\frac{\Delta P \times C_a}{T} \right)} \quad \text{Equation 5-2}$$

This appeared to correctly calculate peripheral resistance during rapid changes in heart rate in an electrical simulation and cat model. However, ΔP is typically only a couple of mmHg in a quietly resting individual and for other representative values ($T=1s$, $C_a=1ml/mmHg$), the correction to cardiac output would be of the order of 50-100ml/min. This is only around 10% of the beat-to-beat CO variance. Experimental trials indicate that this formula does not reduce (probably artifactual) respiratory frequency variations in calculated peripheral resistance.

When considering conditions other than steady flow we should ideally be using the term systemic impedance (which is a complex, frequency-dependent parameter). Total peripheral resistance is then strictly the dc or zero frequency term. But this would provide us with no information about changes in vascular resistance as produced by sympathetic activation. If the circulation is described in terms of the three-element Windkessel model (Toorop *et al*, 1987; Laskey *et al*, 1990; Wesseling *et al*, 1993) then the input impedance as seen by the left

ventricle, Z_{in} , is composed of the characteristic impedance of the aorta, Z_c , the total arterial compliance, C_a , and the peripheral resistance, R_p . Total peripheral resistance is then the sum of the aortic and peripheral resistances:

$$TPR = R_c + R_p \quad \text{Equation 5-3}$$

As R_c is much smaller than R_p the terms ‘peripheral resistance’ and ‘TPR’ are often used interchangeably. In impedance terms (Laskey *et al*, 1990):

$$Z_{in} = Z_c + Z_p \quad \text{Equation 5-4}$$

$$\text{where } Z_p = \frac{R_p}{1 + j\omega R_p C_a}$$

If typical physiological values are used for R_p and C_a then we can plot the modulus of Z_p with frequency (Figure 5-1). Typical values are: $R_p = 800\text{-}1500 \text{ dyne.s/cm}^5$, $C_a = 0.75\text{-}2 \times 10^{-3} \text{ cm}^5/\text{dyne}$ and $\tau = 1\text{-}2\text{s}$ (Toorop *et al*, 1987; Laskey *et al*, 1990; Nichols and O'Rourke, 1990, p28)¹³. The graph shows that the roll-off is greater for larger resistance and capacitance values. We can also use the concept of the time constant, $C_a R_p = \tau$. At the frequency $f_\tau = 1/2\pi\tau$, Z_p will be reduced to 71% of the dc value (R_p). Therefore (excepting combinations of large peripheral resistance with large compliance) f_τ will typically be around 0.1-0.2Hz as also seen in Figure 5-1.

13. Peripheral resistance is a parameter which has been quantified in a variety of units in the literature. Given the method of derivation it might be quoted in mmHg.min/L or mmHg.s/ml. Some authors use the Peripheral Resistance Unit (PRU), but care must be taken as this is variously defined as 1mmHg.min/L or 1mmHg.s/ml. The cgs SI unit is Pa.s/cm³, but a common medical unit is the dyne.s/cm⁵. Pascals, mmHg and dyne/cm² are units of pressure. Some conversions are given here (using ml in place of cm³):

$$1 \text{ mmHg} = 1333 \text{ dyne/cm}^2 = 133.3 \text{ Pa}$$

$$\begin{aligned} 1 \text{ mmHg s/ml} &= 16.7 \text{ mmHg min/L} = 1333 \text{ dyne s/cm}^5 = 133.3 \text{ Pa s/ml} \\ 0.06 \text{ mmHg s/ml} &= 1 \text{ mmHg min/L} = 79.9 \text{ dyne s/cm}^5 = 7.99 \text{ Pa s/ml} \end{aligned}$$

Thus the typical ranges of peripheral resistance given above convert to 80-150 Pa.s/ml or 1-1.87 mmHg min/L and for arterial compliance, 7.5-20 x10⁻³ ml/Pa or 1.0-2.7 ml/mmHg.

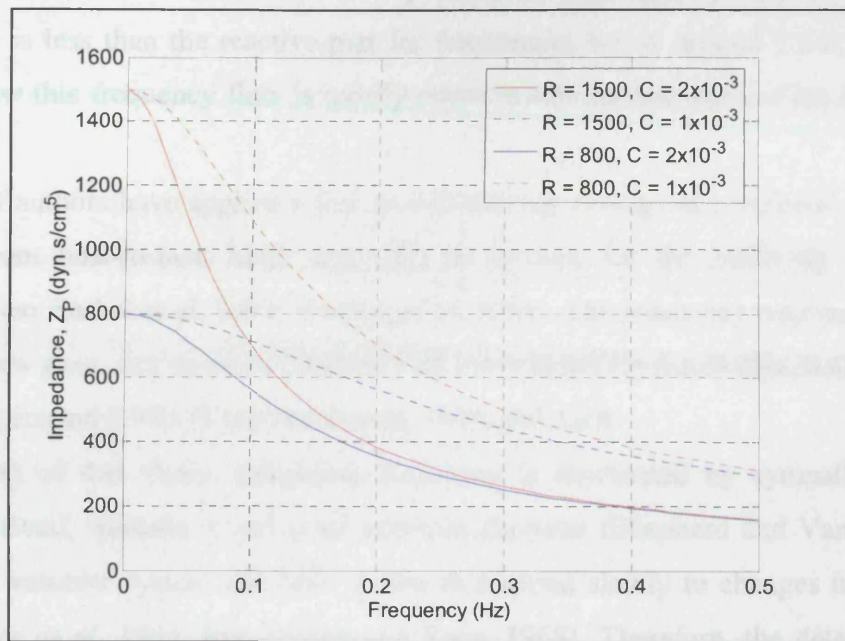


Figure 5-1: Modulus of peripheral impedance for typical values of compliance and peripheral resistance

We can also look at the individual components of the peripheral impedance and determine at what frequency the flow through the resistive part becomes dominated by the flow through the reactive (capacitive) part (the denominator of Equation 5-4).

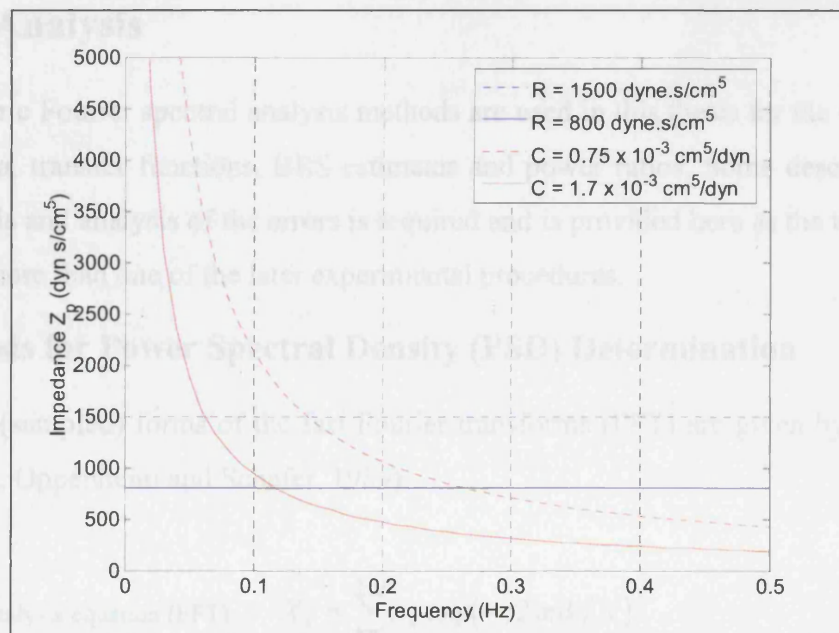


Figure 5-2: Modulus of separate resistive and reactive components of peripheral impedance

Figure 5-2 shows the variation of these impedances with frequency and indicates that the resistive part is less than the reactive part for frequencies below around 0.1-0.15Hz, i.e. for changes below this frequency flow is mostly resistive and the influence of the capacitance is reduced.

One group of authors have applied a five second moving average to peripheral resistance (as calculated from beat-to-beat MAP and CO) to account for the buffering effect of the Windkessel (ten Harkel *et al*, 1993; Wieling *et al*, 1998). The frequency response of this type of filter is a low pass, sinc-squared function with zeros at $n/5$ Hz (i.e. 0.2Hz, 0.4Hz, etc) and a -3dB point at around 0.9Hz (Lynn and Fuerst, 1989, p83,136).

In the context of this thesis, peripheral resistance is modulated by sympathetic nervous control of skeletal, splanchnic and renal arteriole diameter (Shepherd and Vanhoutte, 1975, p12) and the vascular system has been shown to respond slowly to changes in sympathetic activity (Scher *et al*, 1963; Rosenbaum and Race, 1968). Therefore, the detection of high frequency changes in the ratio of mean pressure and cardiac output should be viewed with scepticism and described as an artefact of the measurement and analysis process. The analysis of beat-to-beat peripheral resistance data in Chapter 8 will be restricted to slow responses and the low frequency band in agreement with other published reports of sympathetic system analysis (Wallin and Nerhed, 1982; Saul *et al*, 1991).

Spectral Analysis

Non-parametric Fourier spectral analysis methods are used in this thesis for the calculation of power spectra, transfer functions, BRS estimates and power ratios. Some description of the method details and analysis of the errors is required and is provided here as the techniques are common to more than one of the later experimental procedures.

FFT analysis for Power Spectral Density (PSD) Determination

The discrete (sampled) forms of the fast Fourier transforms (FFT) are given by (Bendat and Piersol, 1986; Oppenheim and Schafer, 1989):

$$\text{analysis equation (FFT): } X_k = \sum_{n=0}^{N-1} x_n \exp(-j2\pi nk/N) \quad \text{Equation 5-5}$$

$$\text{synthesis equation (inverse FFT): } x_n = \frac{1}{N} \sum_{k=0}^{N-1} X_k \exp(j2\pi nk/N) \quad \text{Equation 5-6}$$

Where N = number of samples

x_n = n th time domain value

X_k = k th frequency value (complex)

$j = \sqrt{-1}$

Parseval's Theorem states that the energy or power in the time domain is equal to the energy or power in the frequency domain (Press *et al*, 1990). I have used the mean squared amplitude estimate, which is equivalent to the time domain variance. The power spectral density (PSD, autospectrum) is then:

$$\text{mean squared amplitude} \quad \frac{1}{N} \sum_{n=0}^{N-1} |x_n|^2 = \frac{1}{N^2} \sum_k |X_k|^2 = P_x \quad \text{Equation 5-7}$$

For a single-sided spectrum.

$$\text{for } k = 0 \text{ and } k=N/2 \quad P_k = \frac{1}{N^2} |X_k|^2 \quad \text{Equation 5-8}$$

$$\text{for } k = 1, 2, \dots, N/2-1 \quad P_k = \frac{2}{N^2} |X_k|^2 \quad \text{Equation 5-9}$$

Each sample, P_k , is the average power over a frequency bin, centred on f_k . The units of this quantity are unit^2/Hz .

Smoothing the Periodogram Estimates

X_k is composed of two independent parts which have Gaussian distributions. Due to the linearity property of the FT, the square of the FT is the sum of the square of two independent Gaussian variables, the distribution of which is chi-squared, χ_n^2 with n degrees of freedom (dof), a mean of n and a variance of $2n$ (Bendat and Piersol, 1986, p78). The standard error of the estimate is:

$$\varepsilon_r = \frac{\sigma}{\mu} = \frac{\sqrt{2n}}{n} = \sqrt{\frac{2}{n}} \quad \text{Equation 5-10}$$

The PSD estimated from a single data record is therefore inconsistent as the error does not tend to zero with increasing data length. As there are two independent variables for each

frequency component $n = 2$ and the error is unity, i.e. the standard deviation of the estimate is as large as the estimate (Bendat and Piersol, 1986, p285). To reduce the random error the autospectrum is computed from n_d distinct subrecords and the results are averaged (ensemble averaging).

$$\hat{P}_k = \frac{2}{n_d N^2} \sum_{i=1}^{n_d} |X_{ki}|^2 \quad \text{Equation 5-11}$$

Each additional estimation adds two degrees of freedom so that the standard error is now:

$$\varepsilon_r = \frac{\sqrt{4n_d}}{2n_d} = \frac{1}{\sqrt{n_d}} \quad \text{Equation 5-12}$$

Where ensemble averaging is used, any data window is applied to each subrecord. In order to increase the number of subrecords available without reducing the window length, the ends can be overlapped. It is unlikely that the data length will be a whole multiple of subrecords so there will be unused data at the end of any recording. By adding one further window and spreading the unused samples evenly throughout the subrecords, this data can be included in the analysis. The power lost by data windowing can be compensated for by taking account of the window power which is given by:

$$P_w = \frac{1}{N} \sum_{n=0}^{N-1} |w_n|^2 \quad \text{Equation 5-13}$$

The number of degrees of freedom can also be increased by smoothing the frequency components. The additional degrees of freedom depend on the shape of the smoothing window - a square window adds its width, whereas a triangular one adds 3/4 of its width. When calculating the BRS using the α -index or transfer function, several harmonics (each with a χ_n^2 distribution and 2dof) are summed thus increasing the total number of degrees of freedom of the estimate.

Cross Spectra, Coherence and Transfer Functions

Where two synchronous time series, x and y , are being considered, as well as the individual power spectra, the frequencies common to both can be ascertained by the cross-spectrum. This is similar to the previous definition of the autospectrum:

$$\hat{P}_{xy} = \frac{2}{n_d N^2} \sum_{i=1}^{n_d} X_{ki}^* Y_{ki}$$
Equation 5-14

The results of this analysis are complex-valued and the cross spectra contains both power and phase information. It does not imply causality, just commonality. The existence of a linear relationship between the two variables can be examined using coherence and transfer function estimates. The ordinary coherence is the frequency domain equivalent of the correlation coefficient and measures the extent to which y can be predicted from x by an optimum linear least squares relationship (Bendat and Piersol, 1986, p409,534). It is defined as:

$$\hat{\gamma}_{xy}^2 = \frac{|\hat{P}_{xy}|^2}{\hat{P}_x \hat{P}_y}$$
Equation 5-15

Due to normalisation by the autospectra in the denominator it varies from 0 (no relationship) to 1 (perfect relationship). This is not a description of the postulated linear model, but estimates the likelihood of it existing at each frequency. The linear relationship is described in the frequency domain by the transfer function $H(f)$:

$$H_{xy}(f) = \frac{P_{xy}}{P_x}$$
Equation 5-16

This is also complex-valued and is usually decomposed into its constituent gain (modulus) and phase (argument) components (Chapter 4).

Analysis of the Coherence Function

The reliability of the transfer function estimate depends upon the value of the coherence function in the same way that a linear regression depends on the correlation. In order to be confident in the estimates of these quantities it is necessary to examine the errors involved.

Determination of Confidence Limits for Zero Coherence

As no real data or analysis process is free from noise or errors the estimation of coherence will produce non-zero values where no true coherence exists. Threshold confidence limits for ordinary coherence can be determined empirically by the use of simulated data which has a

true coherence of zero (Panerai *et al*, 1997b). By subjecting the simulated data to the same coherence estimation as the real data we can determine a threshold for the 95% confidence interval. As coherence only exists between 0 and 1, this will provide a lower threshold above which we can be 95% confident that a real coherence exists.

Methods

This simulation was carried out to determine the spectral analysis parameters to be used for the stroke volume data in Chapter 7, but the method is applicable to the longer data records in Chapter 8.

One thousand beat-to-beat RR interval and systolic pressure records were created by sampling a random number generator with a Gaussian distribution. The mean and variance of the simulated data is immaterial as the mean is removed before the spectra are calculated and the variance will cancel out in the coherence calculations¹⁴. However, the simulations were carried out using values appropriate to the real variables. Surrogate RR interval used a mean value of 1s and a standard deviation of 0.05s, whereas systolic pressure had a mean of 120mmHg and a standard deviation of 5mmHg. A 300 sample data series was used to simulate a five minute recording. These beat-to-beat time series were interpolated using a cubic spline and resampled at 5Hz. Spectral analysis was carried out using a 512-point FFT and a 20% cosine tapered data window (i.e. 10% of the data length at each end). The effect of different subrecord overlaps and frequency smoothing on the threshold was investigated. Overlaps ranged from 0 to 25% in 5% intervals. A non-causal, triangular moving-average filter was applied to the auto- and cross-spectra using 3, 5, 7 and 9-point lengths before calculating the coherence (Otnes and Enochson, 1978, p356; Carter, 1993, p31). Zero-padding of up to 20% of the window length was allowed if this increased the number of subrecords by one.

14. Having removed the mean, the variance can be altered by multiplying the data by a scalar. The linearity property of the Fourier transform shows that this also applies in the frequency domain e.g.:

$$l_n = ax_n \Leftrightarrow L_k = aX_k \quad m_n = by_n \Leftrightarrow M_k = bY_k$$

Then calculating the coherence gives:

$$\gamma_{lm}^2 = \frac{|\sum L_k^* M_k|}{\sum |L_k|^2 \cdot \sum |M_k|^2} = \frac{|ab \sum X_k^* Y_k|}{a \sum |X_k|^2 \cdot b \sum |Y_k|^2} = \gamma_{xy}^2$$

i.e. the increased variance cancels out.

As coherence does not have a normal distribution the threshold at specific frequencies was determined as follows. The distribution of the coherence values were plotted on a histogram and smoothed with a four-point moving-average filter. These were used to plot the normalised cumulative probability from which the coherence at 0.95 was determined. Three runs of 1000 data pairs were conducted at each frequency and the coherence limits averaged.

Results

For these data lengths, a 10% window overlap allowed three subrecords to be generated, and a 15% overlap produced four. For overlaps of up to 25% there was no further improvement in the confidence limit as this still only allowed four subrecords. Figure 5-3 shows the results of the simulation for 0.1Hz. It was found that the difference in the 95% limit found between 10% and 15% overlap diminished with the increase in smoothing filter length. Also, the absolute improvement in the limit for each additional 2 samples in the filter length diminished with increasing filter length.

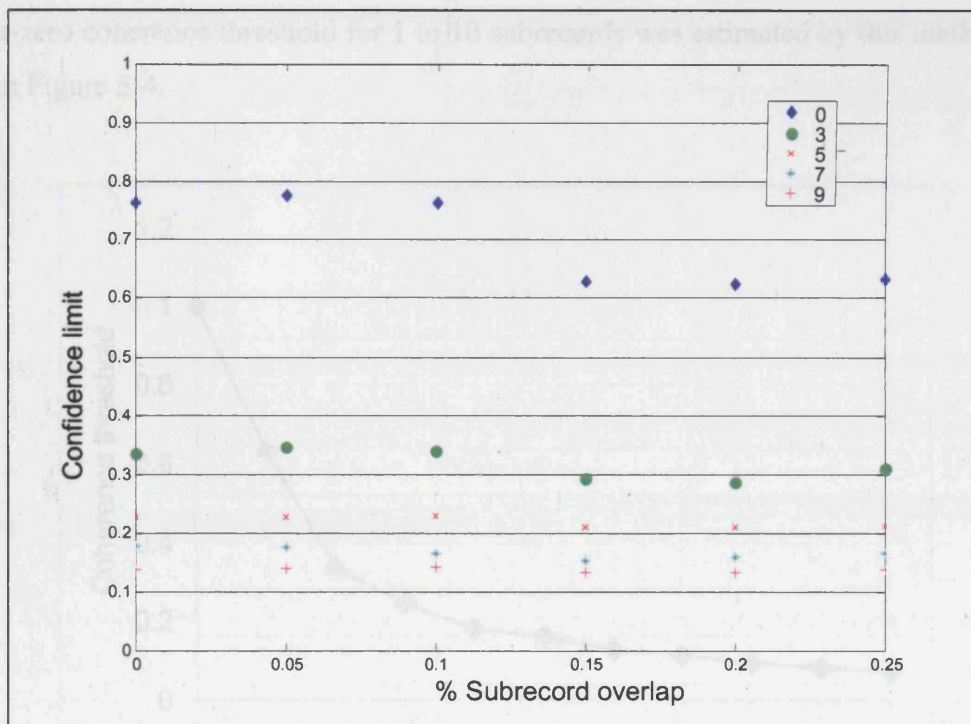


Figure 5-3: 95% confidence limits for non-zero coherence at 0.1Hz for 300 beat simulation

A confidence limit of greater than 0.6 is unacceptable, indicating that some frequency smoothing is required. A 15% subrecord overlap was chosen for this data length as a compromise between a larger overlap (which ensures a greater number of ensembles and thus

lower confidence limits and a smaller random error) and a smaller overlap (which maintains the independence of the subrecords and reduces computational time).

The frequency sample spacing (resolution bandwidth) before smoothing is:

$$B_e = \frac{1}{NT} = \frac{1}{512 \times 0.2} = 0.0098\text{Hz} \quad \text{Equation 5-17}$$

A 7-point non-causal filter will not smooth the first and last three frequency estimates, so that the smoothed spectrum will start from 0.039Hz. The lower limit of the low frequency band is taken as 0.04Hz so that this limit would be acceptable, but an additional smoothed estimate at the lower limit would be preferable. Therefore, a 5-point smoothing filter was chosen, and an average of five runs of 1000 data pair series was used to determine the 95% confidence limit of 0.20 (SD 0.006) at 0.1Hz and 0.2Hz. Therefore, any real Gaussian data, which is processed in the same manner and results in a coherence of greater than 0.2 is 95% likely to have a non-zero coherence.

The non-zero coherence threshold for 1 to 10 subrecords was estimated by this method and is shown in Figure 5-4.

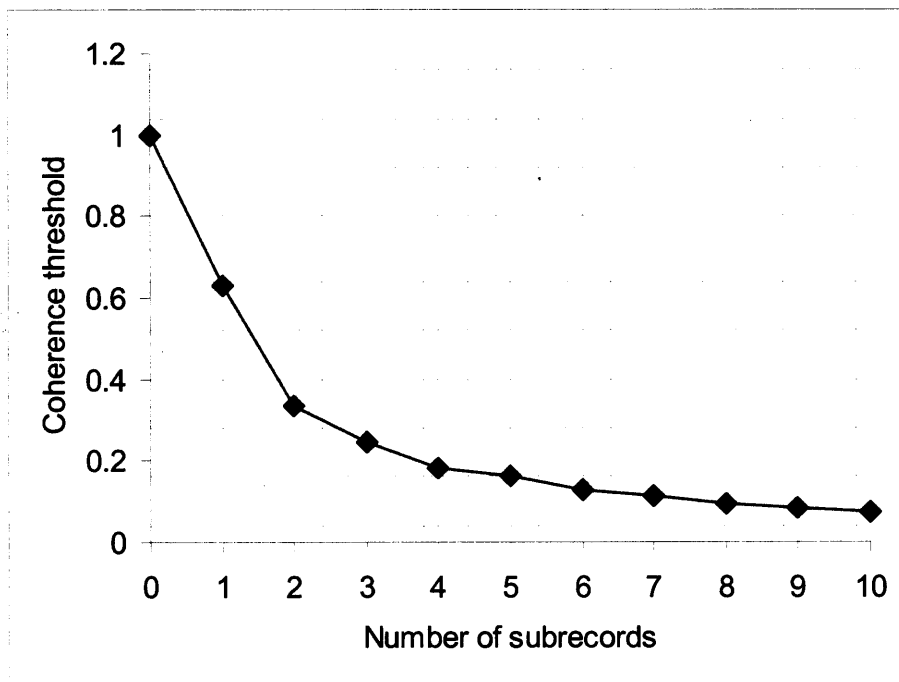


Figure 5-4: Non-zero coherence threshold from random surrogate data for different numbers of subrecords

Determination of Confidence Limits for Non-Zero Coherence

An estimate for the bias error in coherence function estimates can be determined analytically for Gaussian data with no overlap and ideal windowing (Carter *et al*, 1973; Bendat and Piersol, 1986, p309). An approximation for this for large values of n_d (>32 subrecords) is:

$$b[\hat{\gamma}_{xy}]^2 \approx \frac{1}{n_d} (1 - |\hat{\gamma}_{xy}|^2)^2 \quad \text{Equation 5-18}$$

This is plotted in Figure 5-5 as the blue line for the case where $n_d = 4$. The other lines in the same graph show increasingly accurate approximations of the true bias expression.

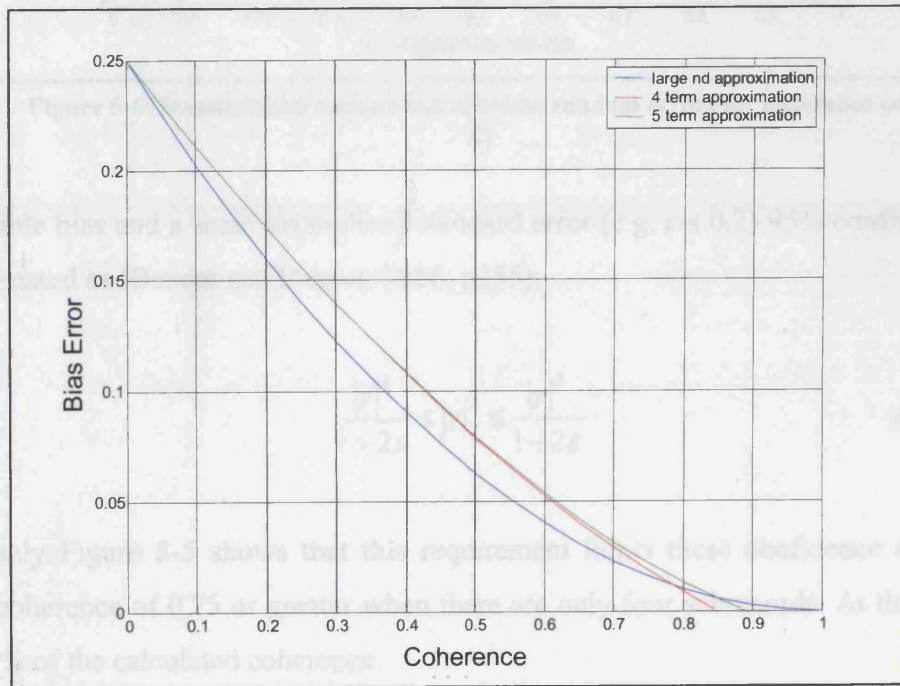


Figure 5-5: Approximations to the coherence bias error ($n_d = 4$)

The equivalent large n_d approximation for the normalised standard random error for coherence is:

$$\varepsilon[\hat{\gamma}_{xy}]^2 = \frac{\sigma[\hat{\gamma}_{xy}]^2}{|\hat{\gamma}_{xy}|^2} = \frac{\sqrt{2}(1 - |\gamma_{xy}|^2)}{|\gamma_{xy}|\sqrt{n_d}} = \frac{1 - |\gamma_{xy}|^2}{\sqrt{2}|\gamma_{xy}|} \quad \text{Equation 5-19}$$

This has been plotted in Figure 5-6 along with the absolute random error ($\varepsilon \cdot \gamma_{xy}^2$).

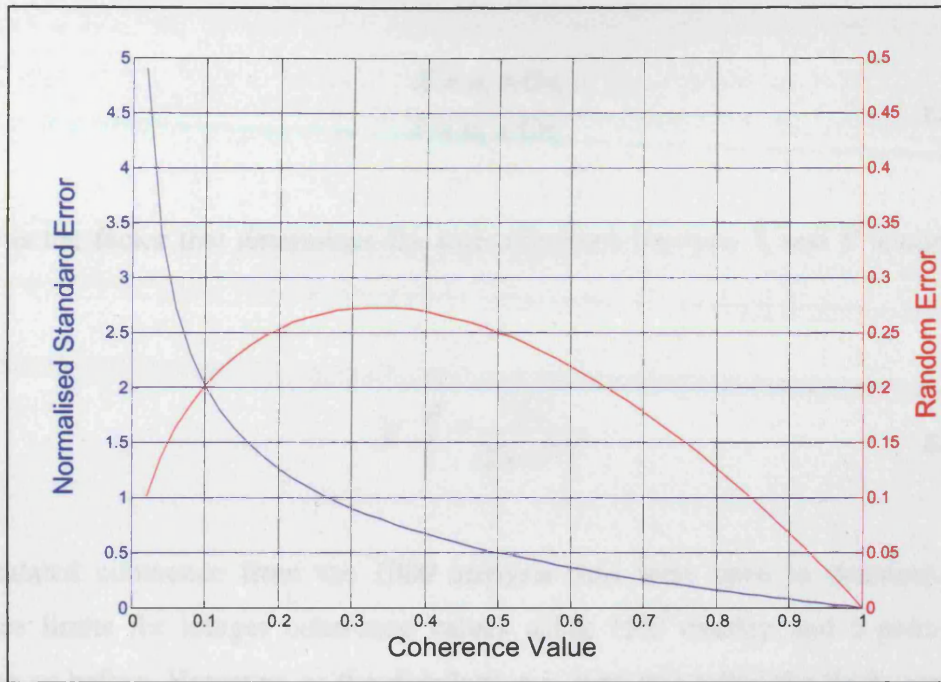


Figure 5-6: Standardised normal and absolute random errors for coherence ($n_d = 4$)

For negligible bias and a small normalised standard error (e.g. $\varepsilon \leq 0.2$) 95% confidence limits can be estimated as (Bendat and Piersol, 1986, p255):

$$\frac{|\gamma|^2}{1+2\varepsilon} \leq |\gamma|^2 \leq \frac{|\gamma|^2}{1-2\varepsilon}$$

Equation 5-20

Unfortunately Figure 5-5 shows that this requirement limits these confidence estimates to values of coherence of 0.75 or greater when there are only four subrecords. At this value $b = 0.015$ or 2% of the calculated coherence.

Carter *et al* (1973) determined a full description for the confidence limits using the sampling distribution of the coherence. However, as noted above these analytical results apply to the case for non-overlapping records and ideal data windowing. For the real situation the analytical approach becomes inapplicable and an empirical method is required to determine the accuracy and precision of the estimate being used.

The confidence limits can be determined for any coherence value by generating random data record pairs as for the zero-coherence case, but with a pre-determined coherence. Di Rienzo *et al* (1996) used the method derived by Carter *et al* to determine empirical bias and variance estimates for their wide-band spectral analysis. One thousand pairs of data records obtained from a Gaussian random number generator were used as inputs n_1 and n_2 to the equations:

$$X = n_1 + Gn_2$$

$$Y = n_2 + Gn_1$$

Equation 5-21

where G is the factor that determines the true coherence between X and Y according to the relation:

$$|\gamma_{xy}|^2 = \frac{4G^2}{(1+G^2)^2}$$

Equation 5-22

The calculated coherence from the 1000 analysis runs were used to determine the 95% confidence limits for integer coherence values using 15% overlap and 5-point triangular smoothing as before. However, as the distribution is now two-tailed the limits were taken at 2.5% and 97.5% of the population distribution. The results of this simulation are plotted in Figure 5-7.



Figure 5-7: 95% CI for coherence by surrogate random data analysis

If the mean calculated coherence is used as an estimate of the expected value then the difference between this and the true coherence is an estimate of the bias. Figure 5-8 shows a plot of the bias calculated in this way and the theoretical bias determined from Equation 5-18.

The empirical bias can be seen to be much smaller than the theoretical one up to coherence values of around 0.8. This is probably due to overlapping subrecords reducing the bias and variance of the estimate (Carter *et al*, 1973).

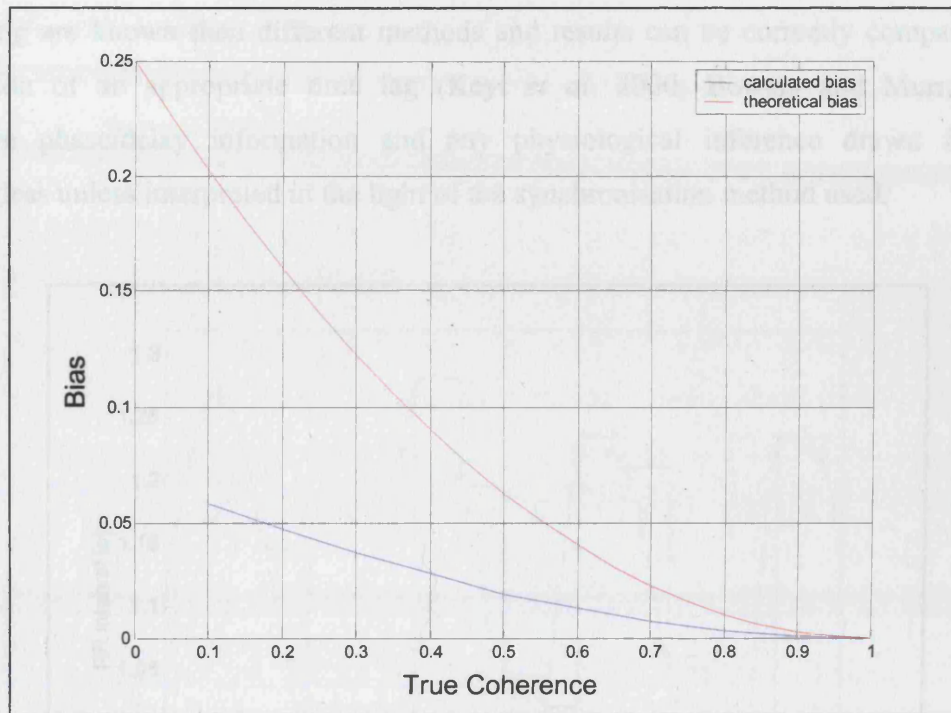


Figure 5-8: Empirical and theoretical estimates of coherence bias

Synchronisation of Beat-to-beat Events

Interbeat intervals do not occur as point events – they exist for a finite duration and require two temporally spaced events to define them. Therefore there is a choice as to whether RR interval or HR values should be timed as occurring at the first R-wave, second R-wave, or not related to real time at all (i.e. tachogram) (de Boer *et al*, 1985a; European Society of Cardiology and North American Society of Pacing and Electrophysiology, 1996, p1048). The temporal relationship with samples of other parameters (pressure, respiration, blood flow, etc) also requires definition. RR interval, SAP and DAP may be treated as occurring synchronously, DAP may be associated with the interval and systole preceding it or with those following (de Boer *et al*, 1985b; Eichinger *et al*, 1994; Baselli *et al*, 1986; Di Rienzo *et al*, 1996).

Also, as beat-to-beat data are sampled irregularly, time series are usually interpolated to a uniform time base, allowing the determination of absolute frequency. Cubic and linear

methods are most popular, but constant-value (zero-order hold) has also been used (Eichinger *et al*, 1994; Laude *et al*, 1995; Di Rienzo *et al*, 1996; Zhang *et al*, 1998; Cooke *et al*, 1999).

All of these details will affect primarily any phase or time delay calculations obtained¹⁵, but often this information is absent or ambiguous in published reports. If the details of data processing are known then different methods and results can be correctly compared by the application of an appropriate time lag (Keyl *et al*, 2000; Bowers and Murray, 2004). Therefore phase/delay information and any physiological inference drawn from it is meaningless unless interpreted in the light of the synchronisation method used.

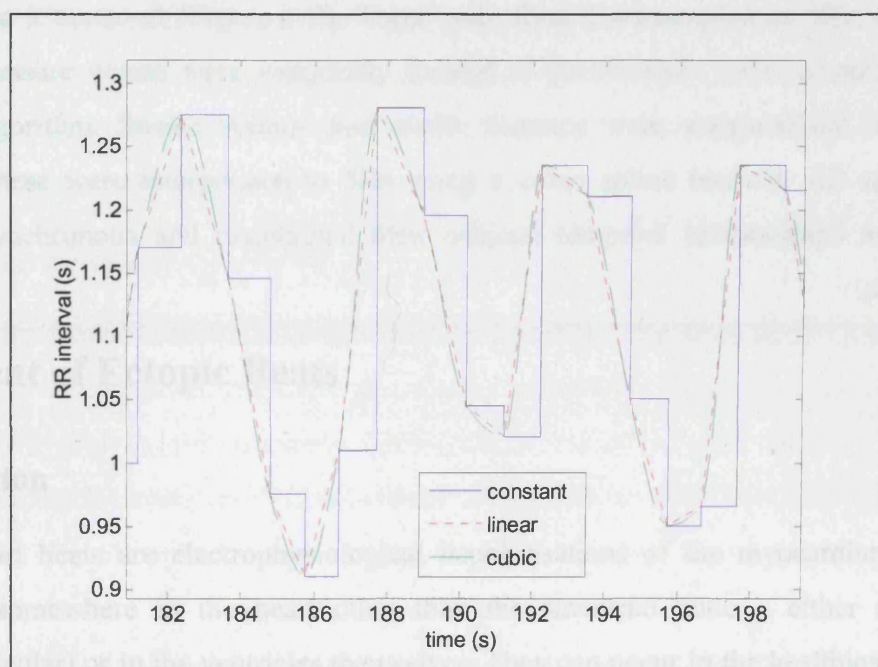


Figure 5-9: Example of the effect of different plotting methods on RR interval. RR intervals for linear and cubic methods are plotted at the first R-wave

Constant value interpolation effectively circumvents the issue of choosing a single time point for the interval values. However, it does not result in a smoothly 'continuous' signal (Figure 5-9). The discontinuities between values contain high frequencies which may be aliased into the bandwidth of physiological interest when the signal is resampled (Berger *et al*, 1986). Applying constant value interpolation at 100Hz to RR interval data shows that this value is around 1-3% of the total power. Therefore the problem of aliasing is unlikely to produce noticeable effects in the power spectra.

15. Although power spectra may also be altered (Berger *et al*, 1986).

There seems to be no *a priori* reason to prefer plotting the interbeat interval at either the first or second R-wave, except that the latter allows online data processing. Also, synchronising events that do not occur simultaneously imposes a time lag on the relationship that is rarely accounted for in the results and will vary slightly with the location of the measurement. It seems most appropriate to me to alter the natural temporal relationship between cardiovascular variables as little as possible. Therefore, in the experimental work in Chapter 7 and Chapter 8 the beat-to-beat events were all located on an absolute time scale as they occurred. Non-point event parameters (HR, RR interval, MAP, CO and TPR) were treated with constant value interpolation at 100Hz, so that each RR interval was plotted for the duration that it occurred (Figure 5-9). These were then downsampled to 5Hz. Systolic and diastolic pressure values were temporally located at the absolute times determined by the marking algorithm. Stroke volume and stroke distance were synchronised with systolic pressure. These were interpolated to 5Hz using a cubic spline method. All samples were therefore synchronous and maintained their original temporal relationships as closely as possible.

Treatment of Ectopic Beats

Introduction

Ectopic heart beats are electrophysiological depolarisations of the myocardium that have originated somewhere in the heart other than the sinoatrial node – either in the atria (supraventricular) or in the ventricles themselves. They can occur in the healthiest person but are usually both uncommon and benign (Kamath and Fallen, 1995). The prevalence increases with age and can be increased by stimulants (caffeine, nicotine), alcohol, exercise and cardiac disease.

Identifying Ectopic or Missing Beats from the ECG

Detection of the QRS complex is usually automated, thus any high peak (threshold detection method) or sharp spike (template matching) in the ECG is likely to be identified and marked as a beat. Premature and late or missing beats are normally easily identified from the RR-interval tachogram as they produce a large spike in the signal which can easily be distinguished visually or automatically and rectified.

It is likely that ECG signals collected for variability analysis will be recorded using a basic three or four-electrode system which will be incapable of filtering significant movement artifacts and generates only the lead II signal. Therefore any effort (Valsalva, handgrip) or

movement (tilt, stand) by the subject may result in noisy signals and decreased accuracy of the QRS detecting algorithm. Ventricular ectopics (VEs) are most often large with very different morphology to a normal beat, and are therefore easily identifiable in all but the noisiest recordings. However, identification is more difficult in the case of supraventricular ectopics (SVEs), which originate in the atrium and can appear very similar to normal beats. Additional leads may be required to allow positive identification (Acar *et al*, 2000). If the signal is noisy it can be impossible to detect changes in P-wave morphology, thus denying the ability to discriminate between early sinus-beats and supraventricular ectopics.

There are several approaches that can be taken to handle ectopic beats in heart rate signal processing, the choice of which is likely to depend at least partly on the amount of ectopy present in the data. They may be ignored/accepted, deleted, replaced by interpolated intervals or ectopic-containing data segments may be removed from the analysis entirely (Lippman *et al*, 1994; Kamath and Fallen, 1995).

Results From Simulated Ectopics

Several authors have investigated the effects of ectopics on various parameters derived from the ECG. This can be done by inserting simulated ectopic beats into otherwise ectopic-free recordings. The advantage of this method is that the ‘true’, ectopic-free value of the parameter of interest can be compared to that derived from the same recording with differing levels of ectopy added. It is assumed that the simulated ectopic beats are identical to those occurring normally.

Storck *et al* (2001) stated that increases in time-domain variance and spectral power were statistically significant for levels of simulated ectopy of only 0.5%. Deletion and linear interpolation of the non-normal intervals diminished, but did not eliminate, the effect on the spectral power, with linear interpolation performing slightly better than deletion.

Lippman *et al* (1994) carried out a similar study and also found that several measures of heart rate variability increased with the degree of ectopy. Deletion was found to be at least, if not more, effective than linear or cubic spline interpolation at reducing the effects of the ectopic beats on the spectral power.

Results From Real Data

Unsurprisingly the incidence of ectopics varies depending on the length of the ECG recording used in the study. For a standard diagnostic ECG lasting around 45-50s, the frequency of one or more ventricular premature beats (VPB) is less than 1% in a healthy population (Birkett *et al*, 1992). For a 2min recording this rises to around 5% (Crow *et al*, 1975) and 33% for a 1hr

duration (Bikkina *et al*, 1992). Cardiac pathologies can increase the level of ectopy significantly. For example, subjects with coronary heart disease have much higher prevalence; 58% of men and 49% of women have at least one VPB and 17-19% have frequent VPBs in 1hr (Bikkina *et al*, 1992).

Storck *et al* (2001) also classified sections of data from both healthy and post-MI subjects by frequency of ectopics (up to 10%) and found that increases in ectopy were accompanied by larger increases in spectral power for post-MI subjects than for normal subjects. The application of linear interpolation to remove the non-normal beats significantly attenuated this increase in both groups, but did not remove it completely. The implicit assumption in this analysis that the 'true' spectral components for the ectopic-containing data segments should be the same as for the ectopic-free sections, which may have come from different subjects.

Heart Rate Turbulence

Birkett *et al* (1992) identified that, in CHF patients, RR interval oscillates for 2-3 beats after the compensatory pause following a VPB. Later this phenomenon of increased variability was termed 'heart rate turbulence' and was found to occur especially in patients with a low risk of MI and healthy controls (Schmidt *et al*, 1999). Two parameters in particular have been defined over the fifteen to twenty beats following a VPB: turbulence onset and turbulence slope (Davies *et al*, 2001a). These are believed to contain significant information in regard to cardiac risk stratification and have been found to be strongly related to measures of baroreceptor sensitivity (Mwrowka *et al*, 2000; Lin *et al*, 2002).

As might be expected, the decrease in pressure following the compensatory pause elicits a brief increase in muscle sympathetic activity (Welch *et al*, 1989). This effect is dependent on the coupling interval between the premature beat and the preceding one, with shorter intervals being associated with larger increases in MSA. Approximately 5-10 beats after the VPB, systolic pressure undergoes a significant increase (Figure 5-10), consistent with a sympathetically-mediated increase in peripheral resistance resulting from the MSA response. Five to fifteen beats post-ectopic the RR interval also increases. As this is significantly diminished following atropine administration (Lin *et al*, 2002) this would appear to be a mainly parasympathetically-mediated response to the pressure increase.

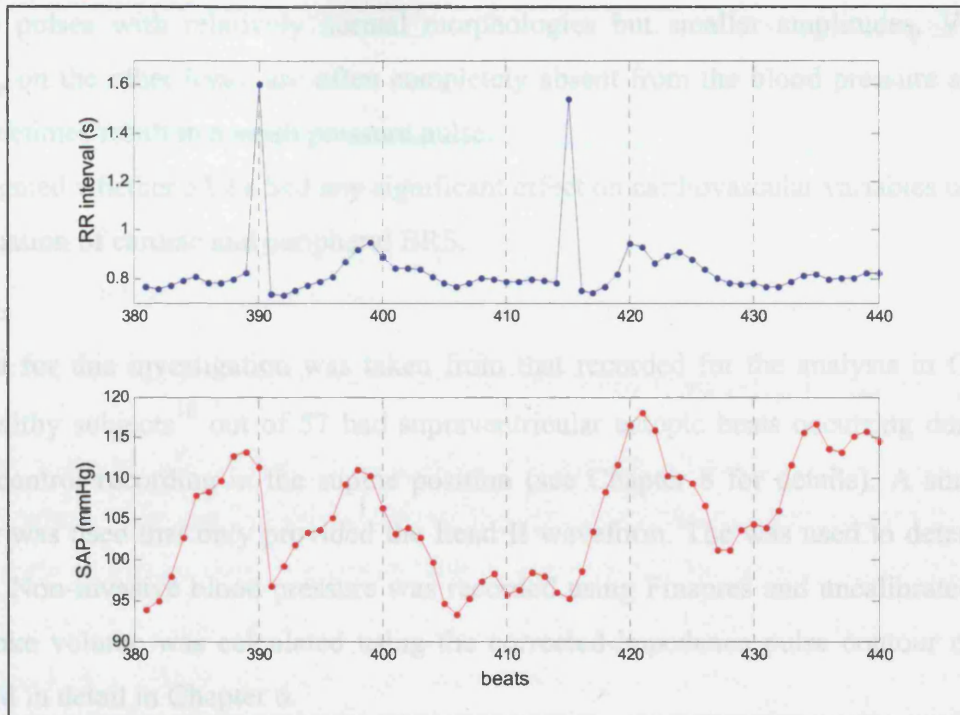


Figure 5-10: Effect of ventricular ectopics on beat-to-beat RR interval and SAP. (The ectopics have not been marked on the ECG and therefore the large RR intervals indicate the duration from the R-wave preceding the ectopic to that following it.)

We should therefore expect that deletion or interpolation of non-normal intervals due to ventricular ectopics will still result in data segments containing more spectral power than segments without ectopics. If heart rate turbulence is simply another manifestation of the baroreflex control of blood pressure, as it appears to be, then measures of BRS calculated from ectopic-containing data segments might actually be more valuable than ectopic-free segments. Segments of data containing ventricular ectopics should not therefore be removed from baroreceptor sensitivity analysis.

The Effect of Supraventricular Ectopics on Beat-to-beat Cardiovascular Parameters

The effect of a premature beat on the blood pressure and other cardiovascular parameters will depend on the haemodynamic effectiveness of the contraction. A supraventricular ectopic is most like a normal beat except that the depolarisation originates somewhere in the atrium other than the sinus node. The conduction of the impulse to the ventricles will still pass through the AV node and the His-Purkinje system, so the ventricular contraction will be normal. The systolic pressure and stroke volume achieved will depend on the amount of blood within the ventricle at the time, which will depend, in turn, on the effectiveness of the atrial contraction and the previous diastolic duration. Supraventricular ectopics tend to produce

pressure pulses with relatively normal morphologies but smaller amplitudes. Ventricular ectopics, on the other hand, are often completely absent from the blood pressure signal, but may sometimes result in a small pressure pulse.

I investigated whether SVEs had any significant effect on cardiovascular variables used in the determination of cardiac and peripheral BRS.

Methods

The data for this investigation was taken from that recorded for the analysis in Chapter 8. Nine healthy subjects¹⁶ out of 57 had supraventricular ectopic beats occurring during a ten minute control recording in the supine position (see Chapter 8 for details). A simple ECG recorder was used that only provided the Lead II waveform. This was used to determine RR interval. Non-invasive blood pressure was recorded using Finapres and uncalibrated beat-to-beat stroke volume was calculated using the corrected-impedance pulse contour method as described in detail in Chapter 6.

RR interval data was examined visually and any large deviations from the mean value were examined on the ECG signal. Ventricular ectopics were identified as large, sharp spikes in the ECG without a preceding P-wave. If a normal-looking premature beat occurred with a following compensatory pause then this was labelled as a supraventricular ectopic whether or not the P-wave morphology was altered. This was due to the relatively poor signal to noise ratio in some of the recordings and to the lack of complimentary ECG waveforms to enable better discrimination of changes in P-waves.

The mean values of RR interval, diastolic and systolic pressure and pulse contour stroke volume were calculated for ten beats prior to the beat before the premature contraction and the ten beats following the beat after the compensatory pause (i.e. a beat before and after the two non-normal intervals were excluded). Student's paired t-test was used to determine if the difference between before and after values were statistically significant.

Results

Twenty-five individual SVEs were located. A recording from one subject contained ten SVEs and the others contained between one and four SVEs. The number of beats before and following each SVE was limited by the minimum separation between ectopic beats, therefore data segments comprised sixteen beats before and fourteen following the premature contraction. The segments were synchronised with the SVE as the seventeenth beat, i.e. the

16. From both the fainting and non-fainting groups.

sixteenth RR interval. The SVEs are characterised by a shortened interval followed by a pause. The compensatory nature of the longer interval is confirmed by a strong negative linear relationship between the length of the shortened (sixteenth) and the lengthened (seventeenth) RR intervals (correlation coefficient, $R = 0.90$, $p < 0.001$). There was no discernable difference in the mean values of all four parameters whether the non-normal intervals were included or excluded.

Figure 5-11 to Figure 5-14 show how the beat-to-beat RR interval, systolic and diastolic pressures and stroke volume vary around the individual SVEs. The line colours indicate individual subjects. The ectopic beat can be clearly seen on all parameters except for systolic pressure, where only one out of the twenty-five examples has any significant effect on this parameter. In two other cases (dark blue lines in Figure 5-12) the mean pressure following the ectopic is larger than that preceding it, although it appears that SAP may be increasing before the ectopic beat occurred. For the other twenty-two segments there appears to be very little effect on the beat-to-beat systolic pressure. There is no evidence of heart rate turbulence following these SVEs (Figure 5-11).

Diastolic pressure is elevated just prior to the ectopic beat and decreased just following it. This is due to the reduced and then increased duration of the diastolic run-off. Following a reduced diastolic filling period, systolic pressure for the premature beat could also be expected to be reduced, but this is not a common feature (Figure 5-12). It would appear that the pulse pressure is reduced for the premature beat, but added to an increased diastolic pressure, this results in almost no effect on the systolic record. As would be expected, stroke volume is reduced for the premature beat and increased for the following beat (Figure 5-14).

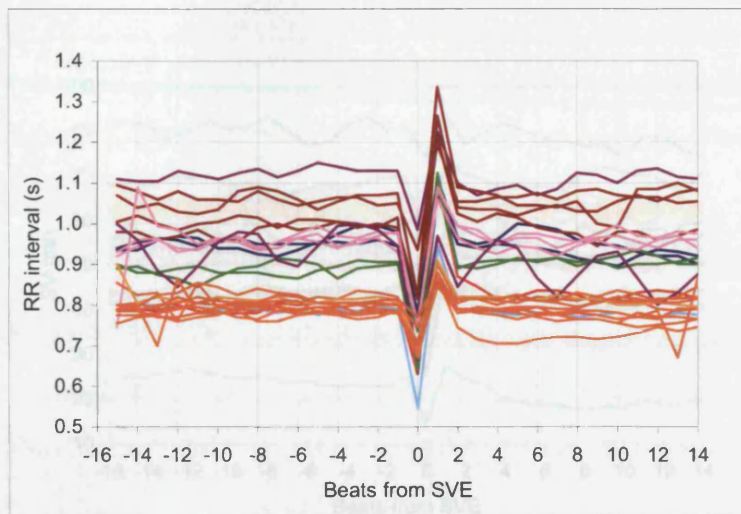


Figure 5-11: Beat-to-beat RR interval before, and following, an isolated SVE

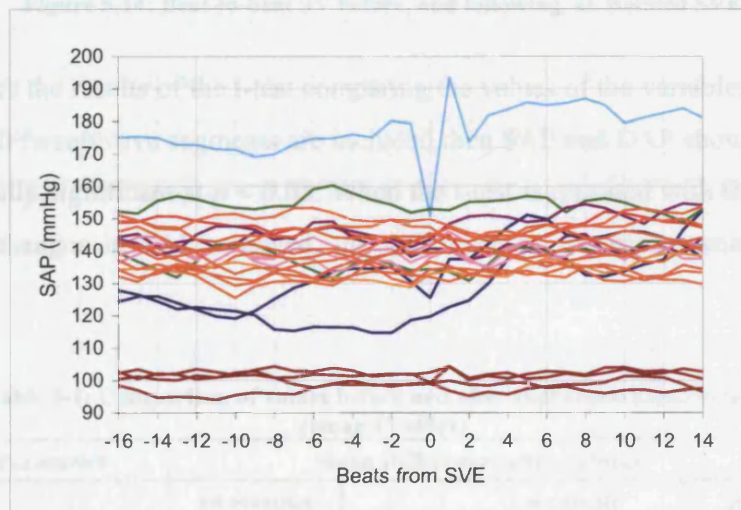


Figure 5-12: Beat-to-beat SAP before, and following, an isolated SVE

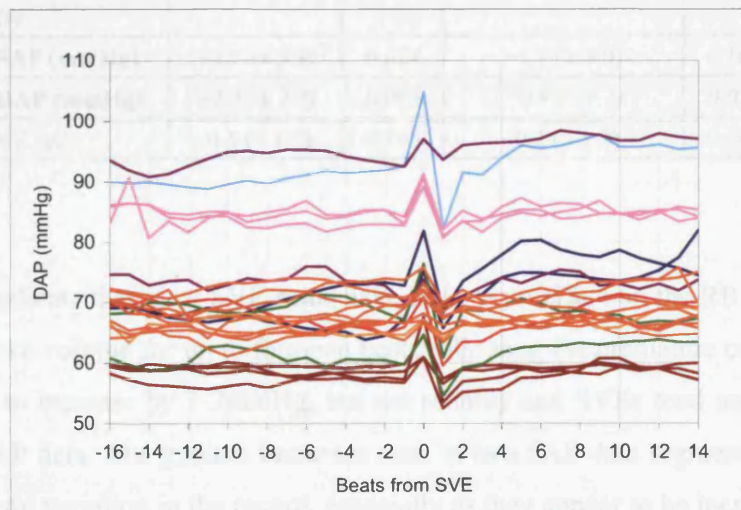


Figure 5-13: Beat-to-beat DAP before, and following, an isolated SVE

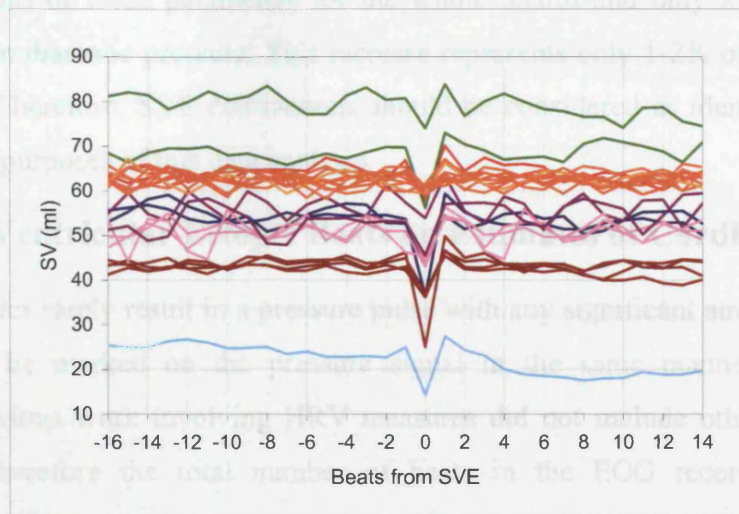


Figure 5-14: Beat-to-beat SV before, and following, an isolated SVE

Table 5-1 shows the results of the t-test comparing the values of the variables before and after each SVE. If all twenty-five segments are included then SAP and DAP show a small increase that is statistically significant at $p < 0.05$. When the t-test is repeated with the three segments identified by changes in SAP removed, the difference in systolic pressure becomes non-significant.

Table 5-1: Comparison of values before and after supraventricular ectopics (mean \pm 1 stdev)

Parameter	Mean Difference (after-before)			
	all ectopics	<i>p</i> value	3 segments removed	<i>p</i> value
RR Interval (s)	-0.01 (\pm 0.03)	0.154	-0.01 (\pm 0.03)	0.295
SAP (mmHg)	+2.7 (\pm 5.9)	0.024	+1.1 (\pm 3.0)	0.167
DAP (mmHg)	+1.6 (\pm 2.4)	0.003	+0.9 (\pm 1.1)	0.010
SV (ml)	-0.5 (\pm 1.5)	0.105	-0.2 (\pm 1.2)	0.064

Discussion

These results indicate that most SVE beats have little or no effect on the RR interval, systolic pressure or stroke volume for up to fourteen beats following the premature contraction. Blood pressure tends to increase by 1-2mmHg, but not reliably and SVEs tend not to be visible in beat-to-beat SAP data. The gradual increases seen in two SAP data segments may simply be part of the natural variation in the record, especially as they appear to be increasing before the premature beat occurs. There is no evidence of turbulence following atrial premature beats.

Including or discarding the premature and compensatory beat values has no discernable effect on the mean values of these parameters for the whole record and only a very small post-ectopic increase in diastolic pressure. This increase represents only 1-2% of typical diastolic pressure values. Therefore, SVE contractions should be considered as identical to a normal sinus beat for the purposes of this data analysis.

The Effect of Ventricular Ectopic Beats on Estimates of Cardiac BRS

Ventricular ectopics rarely result in a pressure pulse with any significant amplitude, therefore the beat cannot be marked on the pressure signal in the same manner as for normal contractions. Previous work involving HRV measures did not include other cardiovascular parameter and therefore the total number of beats in the ECG record was relatively immaterial. Ventricular ectopic beats were either deleted and the subsequent intervals shifted down in the sequence, or the duration between the normal beats either side of the ectopic was filled with an appropriate number of beats determined by the interpolation method (Lippman *et al*, 1994; Storck *et al*, 2001). However, for BRS calculations it is important that the temporal relation between electrical events and pressure events remains unaffected.

The ECG signal processing options then are:

1. to mark the VE R-wave and leave the intervals unaltered – there will be one additional RR-interval for each VE, which will be marked by a short and long interval pair
2. not to mark the VE R-wave and leave the intervals unaltered - there will be equal numbers of electrical and pressure events in the record and a single long interval replacing the VE
3. not to mark the VE R-wave and then replace the long interval, or to mark the VE R-wave and then replace each pair of non-normal intervals, in each case with a single normal interval

In Figure 5-15, the blue line shows the intervals produced by option 1, the red line shows the result of option 2 and the green line shows the result of option 3. If the VE is interpolated or deleted then the timings of all the parameters subsequent to the event will be shifted forward by the difference between the non-normal interval(s) and the resulting one. In this case the difference between the non-normal interval(s) and the interpolated one is 1s, so all subsequent intervals are shifted forward by this amount.

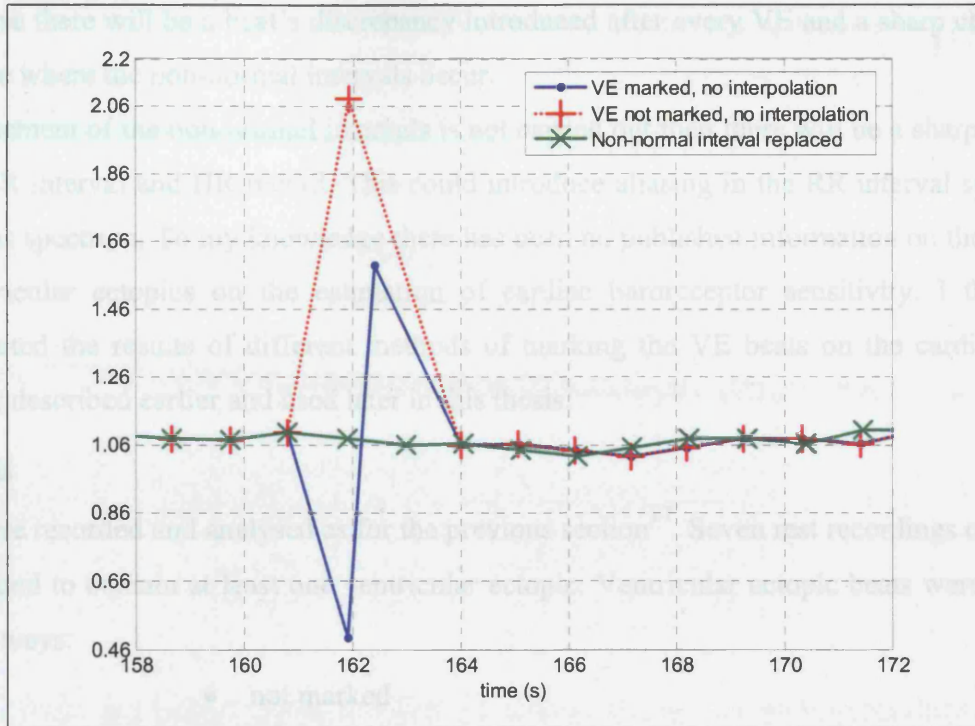


Figure 5-15: RR intervals around a ventricular ectopic for the three processing options listed above. For the green line the single long interval at 162s (red) has been replaced with a normalised interval and the subsequent interval shifted forward by the difference between the two.

The number of RR intervals and pressure pulses must be the same in order to use the sequence method of cardiac BRS calculation. This is because the method uses beat-to-beat data (e.g. a tachogram) rather than data located on an absolute temporal scale. Thus any disparity between the number of events will result in sequences following the ectopic beat undergoing a relative shift. Therefore previous, 'real' baroreflex sequences will be destroyed and 'new' artifactual sequences will be created. For this method, option 1 is therefore not suitable unless the contraction is 'haemodynamically effective' enough to produce a near-normal pressure event, when both signals should be marked as a normal beat. A VE interval is unlikely to be included in a parallel increasing sequence due to the large and sudden change in RR value.

For all spectral BRS methods the beat-to-beat data is interpolated onto a temporal scale with uniform intervals using the original absolute event times. Therefore, record lengths will be the same for all parameters, regardless of whether the VE is marked. The pressure parameters will be identical in both cases but the RR intervals and heart rate will differ.

Stroke volume is calculated from the pressure pulse but is corrected using the heart rate. This is calculated on a beat-to-beat basis before interpolation, therefore the HR values used for this must be from the ECG where the VE is not marked or has been interpolated or deleted.

Otherwise there will be a beat's discrepancy introduced after every VE and a sharp change in SV value where the non-normal intervals occur.

If replacement of the non-normal intervals is not carried out then there will be a sharp change in the RR interval and HR record. This could introduce aliasing in the RR interval spectrum and cross spectrum. To my knowledge there has been no published information on the effects of ventricular ectopics on the estimation of cardiac baroreceptor sensitivity. I therefore investigated the results of different methods of marking the VE beats on the cardiac BRS methods described earlier and used later in this thesis.

Methods

Data were recorded and analysed as for the previous section¹⁷. Seven rest recordings out of 88 were found to contain at least one ventricular ectopic. Ventricular ectopic beats were treated in three ways:

- not marked
- marked and untreated
- not marked and the non-normal interval replaced

The longest segment of the ten minute recording that did not contain any ectopic beats was also analysed and was considered to provide the 'true' values of cardiac BRS. Beat-to-beat RR interval and SAP from the records where VEs were not marked were used for the sequence BRS method (seqBRS)¹⁸. Replacement of single and double VEs were performed on the beat-to-beat data. A double VE occurs when two VEs are separated by a single normal beat. This produces two consecutive long RR intervals. Sequences of more than two non-normal intervals were excluded from the analysis. For single VEs each long interval was replaced by a shorter one that was determined by linearly interpolating a normal interval between the interval immediately preceding and immediately following it. For double VEs, two intervals were interpolated. The difference between the original and replacement interval(s) was determined and subtracted from the subsequent values of the temporal variables (e.g. R-wave times, systoles and beat starts; see the green line in Figure 5-15).

Details of synchronisation and interpolation of beat-to-beat data and the spectral analysis applied are given in the following section. Cardiac BRS was also determined using the alpha (α BRS) and transfer function (tfBRS) methods over both the LF and HF bands. Mean

17. All eighty-eight subjects from the patient and control groups in Chapter 8 were examined as there were insufficient VEs in the control group alone.

18. Details of the BRS methods are given in Chapter 4 and Chapter 8.

coherence was determined over the frequency bands used for the spectral estimates (0.04-0.4Hz). The BRS values were compared between ectopic-free segments and the segments including VEs treated using the three method identified above.

Results

The distribution of VEs is shown in Table 5-2.

Table 5-2: Characteristics of rest recordings containing ventricular ectopic beats

Subject	No. of VEs	Comments
A	1	1 SVE
B	1	3 SVEs
C	18	2 double VEs
D	1	1 SVE
E	2	1 double VE, 6 SVEs
F	1	
G	9	1 double VE

SeqBRS could not be calculated for three recordings due to insufficient numbers of BRS sequences. None of the sequences identified in any record included ectopic beats. The presence of frequent VEs was found to be beneficial to the sequence method as the periods of heart rate turbulence following each premature contraction provided many BRS sequences used in the calculation. This can be seen in Table 5-2 and Table 5-3 whereby the two subjects with the most VEs (C and G) also contained the most numerous suitable sequences.

Table 5-3: Estimates of 'true' BRS from ectopic-free segments (BRS in ms/mmHg)

Subject	No. of subrecords	Mean coherence	α BRS	tfBRS	seqBRS	No. of sequences
A	5	0.50	5.1	4.5		
B	4	0.55	6.4	4.8		
C	1		9.2	11.6	11.0	25
D	3	0.54	4.2	3.5	2.5	5
E	5	0.38	2.8	1.7		
F	7	0.17	13.0	6.9	5.9	3
G	1		5.2	6.9	6.1	12

Ectopic-free Cardiac BRS

Each complete ten-minute rest recording provides seven 512-point subrecords for averaging the FFT-derived spectra. The ectopic-free segments of data ranged from 441s to 2925s (1539 ± 898 s). As a result, the two subjects who had the most VEs (C and G) had spectra which were derived from a single subrecord. This increases the variance of the estimate and results

in a coherence of unity. The other recordings provided between 3 and 7 subrecords (Table 5-3).

Table 5-3 shows the spectral BRS estimates from these segments compared to the available sequence estimates. It can be seen that these estimates of ‘true’ BRS show reasonable agreement, even for the two recordings with only one subrecord. Only subject F shows a large discrepancy between α BRS and other two methods. This contained only one VE near the beginning of the recording so that there was only a small section of data unused for the ectopic-free estimate. This difference was entirely due to a very large gain in the high frequency band and is possibly a result of the low level of mean coherence. This record was excluded from the rest of the analysis.

Comparison of Ectopic Treatment Options

BRS estimates determined from the three methods of treating VEs were compared with the ectopic-free values in six recording. The mean values for α BRS and tfBRS for each method are shown in Table 5-4. It can be seen that not marking the VEs produces a particularly profound increase in BRS estimates which is greatest for the alpha method. Marking the VEs produces a smaller increase in estimates, but is of a magnitude which is clinically important. Interval replacement produces estimates which are much closer to the ‘true’ value. These effects were clearly seen in all six individual records.

Table 5-4: Mean BRS values for VE treatment methods (ms/mmHg)

	Ectopic-free	Not marked	Marked	Replacement
α BRS	5.47	25.49	10.55	6.66†
tfBRS	5.48	18.43	9.13	4.80

† Difference from ectopic-free estimate significant ($p < 0.05$)

Student’s paired t-test was used to test whether the ectopic-free and replacement estimates were statistically different for these six subjects. Only α BRS was significant at $p < 0.05$. Figure 5-16 and Figure 5-17 show the regressions for α BRS and tfBRS. The BRS values determined from records where the VEs were not marked are plotted on a separate axis due to their large difference. Figure 5-16 clearly shows that there is a very strong linear relation between the estimates from the replacement data series and those from the ectopic-free segments (pink line, correlation coefficient $R = 0.97$). This regression slope is very close to the line of identity ($y = 1.17x + 0.28$), but the replacement estimates are slightly but uniformly larger. The mean bias between the ectopic-free and interpolated estimates for alpha BRS is

1.19ms/mmHg. In contrast, Figure 5-17 shows a much poorer regression between tfBRS from the replacement data and that from the ectopic-free segments ($R = 0.67$, $y = 0.52x + 1.97$).

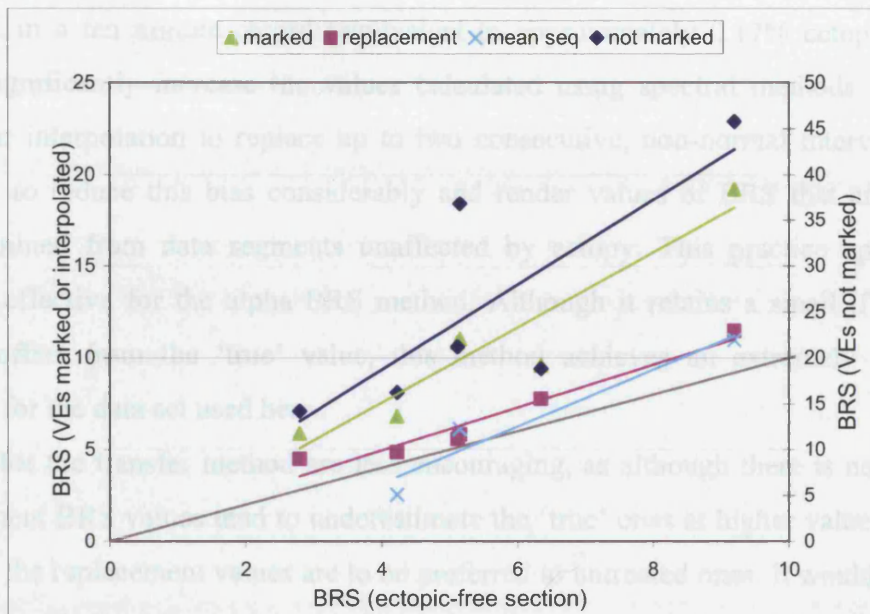


Figure 5-16: Regressions for alpha BRS between treated ventricular ectopic beats and ectopic-free segments

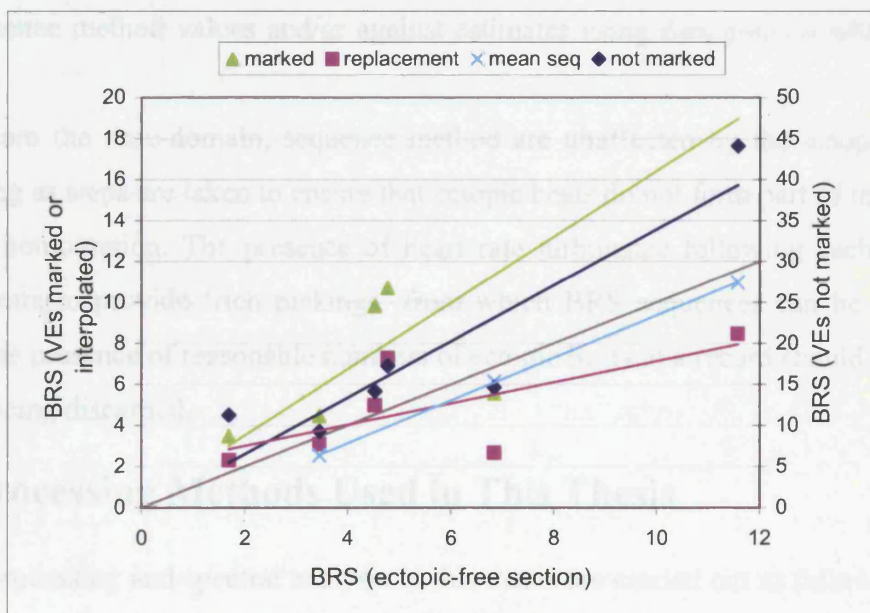


Figure 5-17: Regressions for transfer function BRS between treated ventricular ectopic beats and ectopic-free segments

Discussion

Despite the small group size the results of this investigation clearly indicate that the presence of ventricular ectopics in cardiovascular recording must not be ignored when determining baroreceptor sensitivity. Whether the R-waves of the VEs are marked or not, even a single ectopic beat in a ten minute record (equivalent to approximately 0.17% ectopy) has been shown to significantly increase the values calculated using spectral methods. The use of simple linear interpolation to replace up to two consecutive, non-normal intervals has also been shown to reduce this bias considerably and render values of BRS that are similar to those determined from data segments unaffected by ectopy. This practice appears to be particularly effective for the alpha BRS method. Although it retains a small if statistically significant offset from the 'true' value, this method achieves an extremely good linear relationship for the data set used here.

The results for the transfer method are less encouraging, as although there is no mean bias, the replacement BRS values tend to underestimate the 'true' ones at higher values. However, despite this, the replacement values are to be preferred to untreated ones. It would appear that utilising this method of processing ventricular ectopic events renders reliable BRS estimates when using the alpha method. However, due to the small sample used here and the imperfect effect on the transfer function estimates, it is advisable to check spectral BRS estimates against sequence method values and/or against estimates using data periods without ectopic activity.

Estimates from the time-domain, sequence method are unaffected by the ectopic treatment option as long as steps are taken to ensure that ectopic beats do not form part of the sequences used in the computation. The presence of heart rate turbulence following each ventricular ectopic appears to provide 'rich pickings' from which BRS sequences can be determined. Therefore, the presence of reasonable numbers of ectopic beats in a record should not result in that record being discarded.

Signal Processing Methods Used in This Thesis

The signal processing and spectral analysis in this thesis are carried out as follows. ECG and Finapres signals are digitised from DAT onto PC at 200Hz. The Finapres signal is calibrated at 0 and 100mmHg. Both signals are visually inspected and truncated to remove the calibration signal. The diastolic and systolic pressures, systolic upstroke and dicrotic notch are marked on the Finapres signal and the beat-to-beat systolic and diastolic pressures are determined (details of this program are given in Chapter 6).

The R-waves are detected on the ECG using a threshold algorithm and any non-normal intervals are examined. Supraventricular ectopic beats are treated as normal beats and marked accordingly. Ventricular ectopics are not marked to maintain beat-to-beat synchrony with the pressure data. Mean arterial pressure is determined as the numerical integral of the pressure between systolic upstrokes (beat starts). Uncalibrated stroke volume is calculated using the pulse contour method described in Chapter 6. Cardiac output is the beat-to-beat product of heart rate and stroke volume and peripheral resistance is the quotient of MAP and CO.

RR interval and heart rate are timed at the first R-wave of the interval; MAP, CO and PR at the upstrokes of the first pressure pulse. RR interval, heart rate, mean arterial pressure, cardiac output and peripheral resistance are interpolated using a constant value method at 100Hz and downsampled to 5Hz. Systolic pressure and stroke volume are synchronised to the time that systolic pressure occurred and diastolic pressure to its occurrence. These three variables are interpolated using a cubic spline to 5Hz.

Therefore all variables are rendered at a 5Hz sampling rate with their temporal relationships maintained. As R-waves precede the pressure upstrokes, the beginning of all the 5Hz variables must be truncated to remove leading zeros from the interpolated pressure parameters. Also, as there are one more beat start, systole and diastole than beat-to-beat stroke volume, MAP, CO and PR the end of the data is also truncated.

Any non-normal RR intervals due to ventricular ectopic beats are replaced by linearly interpolated ones in the beat-to-beat series. The 5Hz variables are re-interpolated to the 5Hz time-base using the revised beat timings. Therefore events following the ectopics are effectively shifted earlier in the time series by the difference between the replacement interval(s) and the original long interval(s).

Spectral analysis is carried out using a 512-point FFT, a 20% cosine tapered data window and 15% overlap. Linear trends are removed from the total data length. The number of complete subrecords is determined and the effect of adding a further subrecord and redistributing the data evenly between all the subrecords is calculated. If this produces subrecords with at least 80% data occupancy (i.e. a maximum of 20% zero-padding) then the extra subrecord is included. The Fourier terms (X_k) are corrected for the data windowing using the square of the norm of the window (Equation 5-13) and the auto and cross-spectra are determined using Equation 5-11 and Equation 5-14. A 5-point triangular smoothing window is applied to the frequency components and the coherence and transfer functions are determined using the smoothed estimates (Equation 5-15, Equation 5-16). Any deviations from this procedure are described in the relevant Methods section.

CHAPTER 6 PULSE CONTOUR DERIVATION OF

STROKE VOLUME

From Stroke Volume to Peripheral Resistance

There is no method of measuring the peripheral resistance of the whole arterial tree other than by determining mean arterial pressure and cardiac output simultaneously, and computing their ratio (Equation 5-1). Local changes in resistance can be estimated by measuring Doppler blood flow and mean arterial pressure, or by using plethysmography, although these techniques are limited to individual limbs (Delius *et al*, 1972; Imadojemu *et al*, 2001). As practical beat-to-beat measurement of cardiac output is essentially based on stroke volume measurements, validation of beat-to-beat changes in total peripheral resistance is thus carried out by validating estimates of stroke volume. In this and the following chapter the reliability of a pulse contour method for estimating stroke volume changes is investigated.

An Introduction to Pulse Contour Methods

The relationship between pressure and flow is the basis of pulse contour algorithms. In a rigid tube containing an incompressible fluid, changes in pressure will be proportional to changes in flow. If the complex impedance of a non-rigid tube is known then changes in pressure can again be related precisely to changes in flow.

Consider a basic model of the arterial system where the aorta is assumed to be of uniform cross-sectional area, wall thickness and elasticity, length L and terminated by a lumped peripheral resistance, R_p (Wesseling *et al*, 1983). The characteristic impedance of the aorta, Z_c , is the ratio of the pressure pulse amplitude to the flow pulse amplitude.

$$\frac{\text{pressure amplitude}}{\text{flow amplitude}} = Z_c \quad \text{Equation 6-1}$$

The value of this is determined by the cross-sectional area, density of the blood and the compliance of the tube (per unit length) and will also therefore be uniform along the length of the aorta. The compliance of the rest of the arterial system is insignificant in comparison to that of the aorta and the resistance of the aorta is negligible in comparison to that of the peripheral vessels.

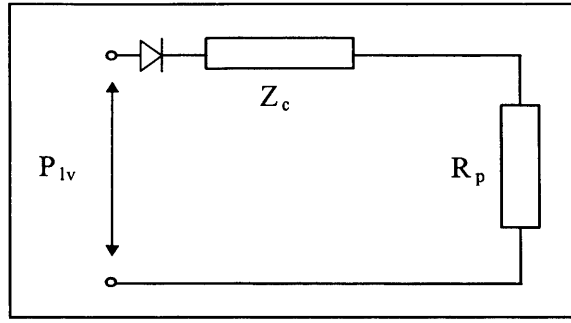


Figure 6-1: Basic electrical analogue of the circulation

We can use an electrical analogue to obtain the representation in Figure 6-1. The pressure pulse from the left ventricle (P_{lv}) travels the length of the aorta and is partially reflected and partially absorbed by R_p . The reflected wave returns to the aortic root after $2L/v_p$ seconds (where v_p is the transmission velocity of the pressure pulse). If this is longer than the ejection time then the peripheral resistance is not ‘seen’ by the input pressure, i.e. it does not contribute to the ventricular afterload. This means the input pressure waveform is not distorted and the relationship between pressure and flow at the aortic root is independent of R_p . Therefore, knowing the pressure and characteristic impedance, the stroke volume can be calculated as the integrated flow during systole. The flow in the aorta due to the current systole is determined by the increase in aortic pressure due to systolic contraction, so the diastolic pressure, P_d , is subtracted (Figure 6-2):

$$SV = \frac{\int_{\text{ejection time}} P(t) - P_d}{Z_c} \quad \text{Equation 6-2}$$

The numerator of Equation 6-2 is often referred to as the pulsatile systolic area (PSA).

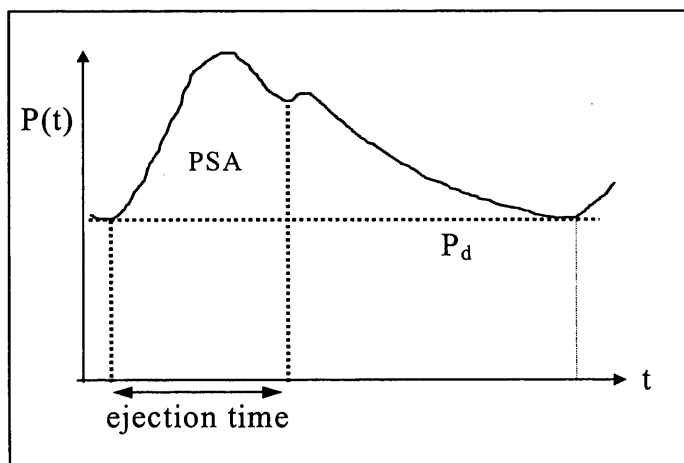


Figure 6-2: Determination of pulsatile systolic area

Early Methods

The relationship between stroke volume and pressure pulse was noted in 1904, but pulse contour techniques have still to gain clinical acceptance today (van Lieshout and Wesseling, 2001). Early methods assumed a constant value for aortic compliance, and hence for aortic impedance. These could produce reasonable results in young, healthy subjects at rest, but compliance and aortic diameter both alter with pressure. Compliance also changes with age (Langewouters *et al*, 1984) and decreases towards the periphery (Sprangers *et al*, 1991). Other problems with simple methods are that the peripheral resistance (R_p) is distributed along the length of the aorta as arteries branch off it, and the aorta itself tapers in diameter, wall-thickness and elasticity. Therefore the impedance increases distally and reflections are produced gradually.

The Corrected Impedance Method

Wesseling *et al* (1983) derived a 28 compartment model for the arterial system and modelled ventricular pressure input to generate arterial pressure and flow waveforms at various points in the arterial tree. Stroke volume was calculated as the flow in the 'ascending aorta' compartment during systole. The basic pulse contour method (Equation 6-2) was used to determine stroke volume from the modelled aortic pressure and Z_c was altered to obtain equivalent SV values for the two methods. The characteristic impedance was found to be:

- virtually independent of peripheral resistance
- strongly affected by arterial compliance
- inversely related to heart rate

The characteristic impedance is higher for lower heart rates because, with a longer ejection time, more reflections will return to the aortic root during systole. This leads to overestimation of the stroke volume. This effect is more apparent for stiffer aortas, presumably because the pulse wave velocity is greater, or the increased pulse pressure results in larger reflections.

Using (modelled) peripheral pressures as input to the pulse contour equation (rather than aortic pressure) did not affect the stroke volume values significantly. The use of 'brachial' compartment pressures introduced errors of less than 10% for heart rates below 140bpm. Pressure modelled as measured using a catheter-manometer system was found to have an insignificant effect on pulse contour SV unless it contained an air bubble (system resonated at 4Hz rather than 18Hz).

Corrections were therefore considered necessary for compliance and heart rate. As compliance in an individual (which is difficult to measure) is related to changes in mean arterial pressure (which is easy to measure), a correction for compliance was based on mean pressure. Wesseling *et al* (1983) compared the initial and corrected formulae, using brachial and radial pressures, to dye dilution measurements in young subjects. The corrected formula produced better agreement for cardiac output with the reference method (dye dilution) than the uncorrected one and has been tested by various groups in different conditions (Stok *et al*, 1993; Wesseling *et al*, 1993; Godje *et al*, 1998). However, the impedance corrected method is linear and does not account properly for the pressure-dependence of the compliance and the characteristic impedance. It will still tend to overestimate stroke volume at low heart rates (below around 80bpm) and for lower compliances (higher mean pressures), i.e. during pressor conditions.

Reliability of the Pulse Contour Method

This pulse contour method has been compared with other methods of measuring cardiac output: thermodilution, inert-gas rebreathing, dye dilution and Modelflow (Wesseling *et al*, 1983; Jansen *et al*, 1990; Stok *et al*, 1993; Wesseling *et al*, 1993; Godje *et al*, 1998; Jellema *et al*, 1999). All except the last have therefore only been compared using values averaged over several beats. Measurements have been made under various physiological conditions: surgery, stand and head-up tilt, head-down tilt, LBNP, 24hr variability determination and in critically ill patients (Jansen *et al*, 1990; Sprangers *et al*, 1991; Stok *et al*, 1993; Veerman *et al*, 1995; Godje *et al*, 1998; Wieling *et al*, 1998; Jellema *et al*, 1996). Correlations ranged from 0.78 (Wesseling *et al*, 1983) to 0.96 (Stok *et al*, 1993) and regression slopes are generally close to unity. Differences between the methods do not appear to be affected by significant changes in haemodynamic conditions (Jansen *et al*, 1990; Stok *et al*, 1993; Godje *et al*, 1998).

Most of these studies have utilised either radial or brachial intra-arterial pressure, although the methods were originally designed for use with intra-aortic pressure. Cardiac output and stroke volume results using Finapres pressure have been compared to those from peripheral intra-arterial, but not aortic pressure (Imholz *et al*, 1992; Wieling *et al*, 1992; Jellema *et al*, 1996). These results showed excellent agreement during conditions of tilt and phenylephrine-induced vasoconstriction, even when differences in mean pressure were changing (Jellema *et al*, 1996). The use of a pulse contour method utilising non-invasive finger pressure would provide a

method of measuring stroke volume and derived parameters that was suitable for widespread dissemination in both clinical and research use.

Comparison of Finapres and Aortic Pressures as Input for Pulse Contour Method

Aortic blood pressure is obviously a highly invasive parameter to acquire and is not often measured in the clinical setting, even during surgery or intensive care. However, it is routinely measured during cardiac catheterisation for angiography or angioplasty. To my knowledge, no results have previously been published describing the relationship between pulse contour SV derived from aortic and non-invasive finger blood pressures. Several reports have been published comparing intra-aortic pressure and invasive, or non-invasive, peripherally-measured blood pressure (Giannattasio, 2003), however virtually all of these reports focus on comparisons of the basic pressure parameters (systolic, diastolic and mean pressures). Therefore it was decided to compare stroke volumes determined at finger and aortic locations using the pulse contour method.

Methods

Instrumentation

Simultaneous aortic and Finapres pressure data were collected from 23 patients undergoing routine cardiac catheterisation in the Cardiac Catheter Suite at Glenfield General Hospital. The aortic pressure was recorded using the catheter-manometer system employed as standard during angiography procedures. Patients were supine and sedated. Catheters were inserted into the right femoral artery under local anaesthetic and advanced into the aorta under radiographic guidance. The catheters were then attached to a Novatrans pressure transducer by fluid-filled pressure lines (Medex Medical). This was fixed at approximately the same height as the left ventricle, and flushed to remove air bubbles. Finapres pressure was measured from the left hand, which rested on a support raised to a similar level as the heart.

The pressure transducer output signal was connected to a Midas polygraph (PPG Biomedical Systems). The aortic pressure and ECG Lead II signal from the analogue output of this device, along with the Finapres pressure, were recorded onto DAT using a Sony PC-108M Recorder (sampling bandwidth 5kHz)¹⁹. The aortic pressure channel was zeroed and

19. The sampling frequency is not specified in the manual. The 'highest frequency' is 5 or 10kHz depending on the configuration of the channels.

calibrated at 100mmHg for each patient. Seventy seconds of continuous signals were recorded for analysis, during which no interventions took place and the patient remained still.

Analysis Programs

The blood pressure and ECG signals were resampled from DAT onto computer at 200Hz using software produced in the Medical Physics Department (PHYSIDAS Data Editor). Further in-house software (PEDIT15) was used to calibrate the pressure channels and extract the seventy seconds of quiet, simultaneous pressure recording.

An analysis program was written in MATLAB (The Mathworks, Inc) to calculate pulsatile systolic area (PSA, Equation 6-2) from the calibrated pressure recordings. Pressure pulse marking began with detection of the systolic peaks. The signal was band pass filtered around the mean heart rate to produce an approximation of a sine wave. The peak pressure on the unfiltered pressure signal within a quarter of a beat of the sine wave maximum was marked as the systolic pressure. The upstroke of the pressure pulse (beat start) was taken to be the point at which the slope up to the systolic peak reached 20% of its maximum value. This was done to avoid variability in marking noisy signals in the region immediately surrounding the diastolic pressure. Diastolic pressure was determined from the minimum pressure value in the 10 samples (50ms) preceding the beat start. PSA was defined as the region bounded by the pressure curve between the upstroke of the pressure pulse, the dicrotic notch and the line between the previous and subsequent diastolic pressures (Figure 6-2).

The PSA program was written to conform as closely as possible to the corrected impedance pulse contour method described by Wesseling *et al* (1983) using additional information from private correspondence. However, not all details were available, specifically the algorithm employed to mark the end of systolic ejection was unknown. In the custom version, search limits for the incisura were defined graphically by the user on a representative beat. If a true minimum was present, end-systole was determined as the minimum pressure within that. If no minimum was found then the second derivative of the pressure signal was examined to find the shallowest part of the systolic downslope, i.e. the 'shoulder'. To remove problems with noisy signals the data could be smoothed with a median filter of variable width before marking. All beat marks (diastolic and systolic pressure, beat start and incisura) and the determined pulsatile systolic area could be visually inspected and manually corrected, if necessary.

The corrected impedance pulse contour method by Wesseling *et al* (1983) was implemented in MATLAB based on Equation 6-2:

$$SV = \frac{PSA}{Zao_{ini}} \text{ (ml)} \quad \text{Equation 6-3}$$

Where an initial estimate of characteristic aortic impedance is (Stok *et al*, 1993; Wesseling *et al*, 1993):

$$Zao_{ini} = \frac{90 + Age}{1000} \text{ (mmHg s/cm}^3\text{)} \quad \text{Equation 6-4}$$

The impedance correction factor is applied to the initial estimate of stroke volume:

$$SV_{corr} = [0.66 + 0.005.HR - 0.01Age(0.014.MAP - 0.8)].SV \quad \text{Equation 6-5}$$

The R-wave detection algorithm was based on a simple threshold value and allowed visual inspection and manual correction of the R-wave marks. Heart rate was determined from the reciprocal of RR interval and mean arterial pressure for each beat was the numerical integral of the blood pressure signal between beat starts (pressure upstroke).

Custom vs. Beatscope Stroke Volume

Results from the custom-built program (SV_{PC} , SAP_{PC} , etc) were compared to those from a commercially available implementation (Beatscope 1.1, FMS BV, SV_{BS} , SAP_{BS} , etc) to assess the replication of the original algorithm. Pulsatile systolic area is not accessible as a separate parameter from this commercial software so this could not be compared between applications. Systolic, diastolic and mean pressures, beat start, ejection time and stroke volume can all be extracted on a beat-to-beat basis. Pressure results are quoted in multiples of 0.25mmHg and stroke volume in multiples of 0.25ml. Beat-to-beat results from the custom application are more easily accessible in a format suitable for further analysis than those from the commercial software.

Fidelity of the Aortic Pressure Recordings

All fluid-filled manometer systems have a dynamic response which can affect the fidelity of the recorded pressure (Gardner, 1981; Yeomanson and Evans, 1983). The linearity of the fluid-filled catheter-manometer system in the Catheter Suite was checked and found to be excellent for all three scales (60/100/200mmHg full scale deflection). The resonant frequency of the Catheter Suite system was measured by both the swept frequency and ringing methods for the two most common cardiac catheter types used. The ringing method estimated the resonant frequency at approximately 6.5-8Hz. The swept frequency method showed a peak

response at 5Hz, although measurements below 3Hz could not be conducted due to noisy signals. The augmentation of the catheter-manometer system at 4Hz, 5Hz and 6Hz was respectively 1.28, 1.48 and 1.46 times that at 3Hz.

A first order, low-pass Butterworth filter with a cut-off at 6Hz was used as an anti-resonance filter to reduce the effect of the fluid-filled measurement system on the recorded signal. Applying the filter twice (forward and reverse filtering, resulting in zero phase shift), the attenuation at 4-5Hz is sufficient to correct the effect of the resonance and produce an almost flat response between 3-5Hz (Table 6-1). Normalised amplitude was obtained by multiplying the augmentation relative to 3Hz by the amplitude reduction.

Table 6-1: Effects of 1st order Butterworth filter on the catheter-manometer resonant response

	3Hz	4Hz	5Hz	6Hz
Attenuation (dB)	-0.52	-3.18	-4.58	-6.02
Amplitude reduction (%)	94	69	59	50
Normalised amplitude (%)	94	88	87	73

Higher frequencies would be expected to alter the shape of maxima and minima, and as such would affect absolute values of systolic and diastolic pressure. However, they would be unlikely to affect the area under the pressure pulse significantly, although alterations in the positions of the marks used could affect the area calculation.

Therefore it was necessary to establish whether the aortic pressure, as measured by the catheter-manometer system, was a reliable reference against which to compare the Finapres values. If aortic SV is unaffected by the application of this anti-resonance filter, then it can be concluded that the dynamic response of the measurement system has not influenced the pulse contour calculations and the aortic values can be considered as a suitable standard.

To assess this, SV values were calculated from the raw aortic signal and from the same signal after low-pass filtering with the above Butterworth filter. The proportion of the spectral power of the raw aortic pressure signal contained in frequencies below 3Hz and 5Hz was determined. Stroke volume values were compared using correlation coefficients (R), linear regression coefficients (slope of the regression line, b) and Bland-Altman agreement plots (Bland and Altman, 1986). If the slope of the linear regression between the raw and filtered aortic SV is not statistically different from unity, then the changes in SV can be said to be unaffected by the measurement system and any bias will be independent of magnitude. This will be true when the 95% confidence interval for the regression slope includes unity.

The purpose was to determine reliable aortic pressure data against which to compare Finapres-derived pulse contour results. Therefore the recordings were edited to improve the agreement between raw and filtered values. The recordings were shortened to remove end sections which contained, for example, sudden changes in pressure or ectopic beats, and the residual segments of continuous data were used for comparison. When comparing Finapres with aortic SV values, beats that were considered to be affected by finger movement were also deleted. Results are presented as ranges and as mean \pm one standard deviation (stdev).

The gain of the transfer function between aortic and Finapres pressure was determined by cross-spectral analysis. The original phasic blood pressure signals for the data used for aortic and Finapres comparisons were used for this.

Statistical Analysis

The absolute values of stroke volume calculated from pulse contour algorithms are not accurate without additional calibration of the aortic impedance. Therefore, the differences between two sets of results, as displayed in Bland-Altman agreement analysis, can be skewed by unusually large or small uncalibrated values. In practice, stroke volume and cardiac output calculated in this way are usually reported as changes from the mean or from an initial value, or they are calibrated against an independent measurement method. As such, any constant bias between the two methods is irrelevant. The stroke volume results have therefore only been analysed using correlation and linear regression statistics (except for the fidelity of the aortic pressure).

Results

Four recordings were rejected due to technical difficulties during recording, so that nineteen recordings were available for the comparisons. Age range for these subjects was 39-77yrs (mean 60 ± 13 yrs) and the gender ratio was 15:4 (male:female).

The random error in PSA and SV_{PC} calculations for the custom application was estimated by manually changing the diastolic pressure, beat start and dicrotic notch markings on three beats from separate recordings. The random error in pulse markings was considered to be \pm one sample (5ms). The maximum PSA was deemed to be achieved by moving the diastolic pressure and beat start marks earlier by one sample and the incisura later by one sample, and vice versa for the smallest value. Errors for PSA were 1.3% to 1.7% and those for SV_{PC} were 1.6% to 2.2%.

Custom vs. Beatscope Stroke Volume

Including sections of data with finger movement artifacts and ectopics did not introduce any significant differences between the custom-built pulse contour and Beatscope results, except in one case. In this instance two ventricular ectopics and a separate single pressure spike occurred in the second half of the aortic and finger pressure recording. The Beatscope program marked these beats erroneously and, in addition, an extra beat was mistakenly marked on the Finapres signal. The removal of the ectopic beats improved the comparisons for systolic pressure and stroke volume in this recording. Table 6-2 shows the improvements for the aortic pressure parameters as an example.

Table 6-2: Improvement in aortic comparisons between custom and commercial algorithms for one subject after removal of ectopic beats (R, correlation coefficient)

	SAP		SV	
	R	Slope (95% CI)	R	Slope (95% CI)
With ectopic beats	0.70	0.54 - 0.89	0.48	0.14 - 0.36
Without ectopic beats	1.00	1.00 - 1.01	0.89	0.95 - 1.22

The beat-to-beat values for systolic and diastolic pressure and the beat start were virtually indistinguishable between the two programs. For example, systolic pressure had a mean correlation coefficient and regression slope of 0.999 for both pressure signals, and a mean bias of $0.25 \pm 0.04\text{mmHg}$ for Finapres and $0.26 \pm 0.07\text{mmHg}$ for aortic pressure²⁰ (SAP_{PC} overestimated SAP_{BS}). The comparison between SV_{PC} and SV_{BS} is summarised in Table 6-3.

Table 6-3: Comparisons for SV between custom and commercial programs for 19 recordings

	R (mean \pm stdev)	Slope (mean 95% CI)
Aortic	0.93 ± 0.06	1.06 - 1.26
Finapres	0.94 ± 0.05	1.06 - 1.24

For both of the pressure signals there were five records with regression slopes close to unity. Two of these records were the same for both pressure signals. Both pressures had one record with a slope less than unity (although not the same record) and thirteen that were greater than unity, indicating that SV_{PC} tended to overestimate SV_{BS} at larger values, and/or underestimate it at smaller values.

20. Note that the commercial program has a precision of 0.25mmHg for the output data.

Fidelity of the Aortic Pressure Recordings

The percentage of spectral power in the raw aortic signal contained in frequencies up to 3Hz was between 94.6% and 99.0% for eighteen of the subjects, the mean for this subgroup was 97.0% and for 5Hz the results were 96.2% to 99.5% (mean 98.4%). For one subject, only 76.7% of the power was contained below 3Hz and 94.3% was below 5Hz. However, this subject had a mean resting heart rate of 102bpm and the spectral power would therefore be expected to shift to slightly higher frequencies.

Figure 6-3 shows three examples of beat-to-beat SV_{PC} for three subjects. Subject 15 has the highest correlation and a slope close to unity and therefore represents one of the closest matches for beat-to-beat changes. Similarly, subject 23 has the lowest correlation and a slope significantly lower than unity. Subject 19 represents a typical subject.

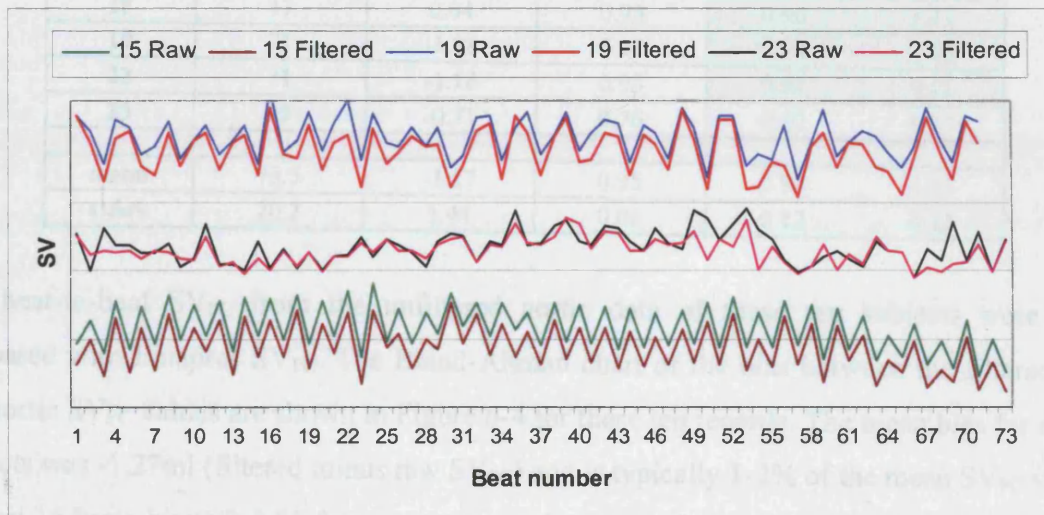


Figure 6-3: Beat-to-beat comparison of raw and filtered aortic SV_{PC} for three subjects

Table 6-4 lists the comparison results for raw and filtered aortic SV_{PC} for all nineteen records. The correlation coefficient ranged from 0.76 to 0.99, and the regression slope of filtered SV_{PC} on raw aortic SV_{PC} from 0.63 to 1.10. The 95% confidence intervals included unity in ten records out of nineteen (shaded cells).

Table 6-4: Correlations and regression slopes for raw vs. filtered aortic SV. Shaded cells indicate records with slopes close to unity used for Finapres comparison. Bias is filtered aortic SV_{PC} minus raw aortic SV_{PC}.

Record	No. beats	Bias	R	Slope (95% CI)	
02	67	-1.43	0.98	0.79	0.88
03	79	-1.06	0.96	0.95	1.09
04	46	0.21	0.98	0.96	1.08
06	78	-0.37	0.99	0.91	0.98
07	71	1.02	0.97	0.86	0.97
08	68	0.00	0.95	0.94	1.10
09	50	-3.24	0.88	0.67	0.92
10	69	-3.71	0.97	0.87	0.99
11	71	-0.82	0.95	0.99	1.15
12	70	-1.66	0.96	0.88	1.03
13	63	-4.05	0.98	0.73	0.81
14	76	-0.08	0.98	1.05	1.15
15	136	-2.85	0.99	0.95	1.01
16	69	0.10	0.98	0.98	1.09
17	96	-2.44	0.97	0.84	0.93
18	47	0.04	0.95	0.90	1.11
19	71	-1.98	0.92	0.94	1.16
22	71	-1.18	0.93	0.91	1.11
23	98	-0.71	0.76	0.52	0.75
mean	73.5	-1.27	0.95	0.88	1.02
stdev	20.2	1.44	0.05	0.12	0.12

The beat-to-beat SV_{PC} from the unfiltered aortic data of these ten subjects were then compared with Finapres SV_{PC}. The Bland-Altman chart of the bias between the filtered and raw aortic SV_{PC} values are shown in Figure 6-4 for these ten records. The mean bias for all 19 subjects was -1.27ml (filtered minus raw SV_{PC}) and is typically 1-2% of the mean SV_{PC} value. Subject 15 has a bias of -2.85 for mean values of approximately 30-40ml, which is around an 8% difference. However, this recording has an excellent correlation coefficient and regression slope and therefore the resonance has not noticeably affected the beat-to-beat changes in SV_{PC}.

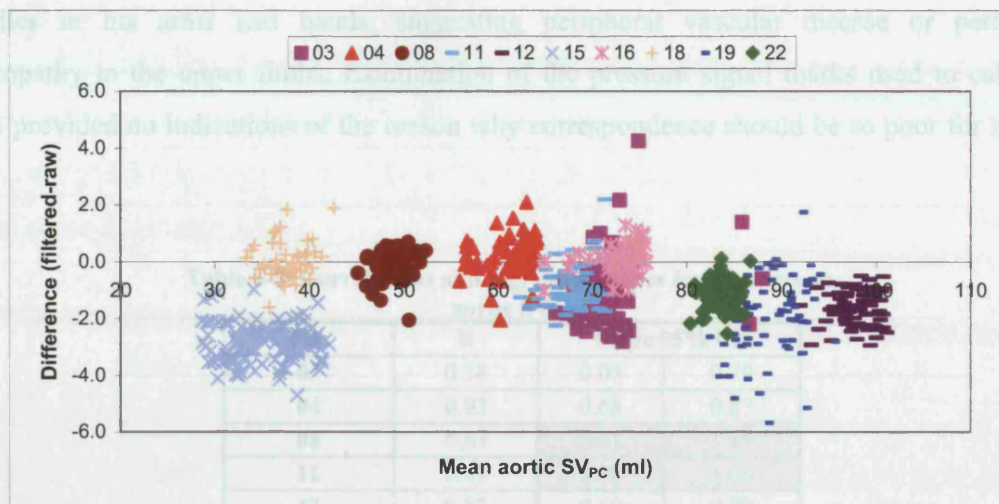


Figure 6-4: Bland-Altman chart of the bias between filtered and raw aortic SV_{PC} for the ten recordings identified in Table 6-4 used for the Finapres comparison

Finapres vs. Aortic Stroke Volume

Three examples of the beat-to-beat variations in SV_{PC} using the unfiltered aortic and Finapres blood pressure signal are shown in Figure 6-5. As previously, these represent subjects with the best (subject 15), worst (subject 03) and typical (subject 11) correspondence between the values.

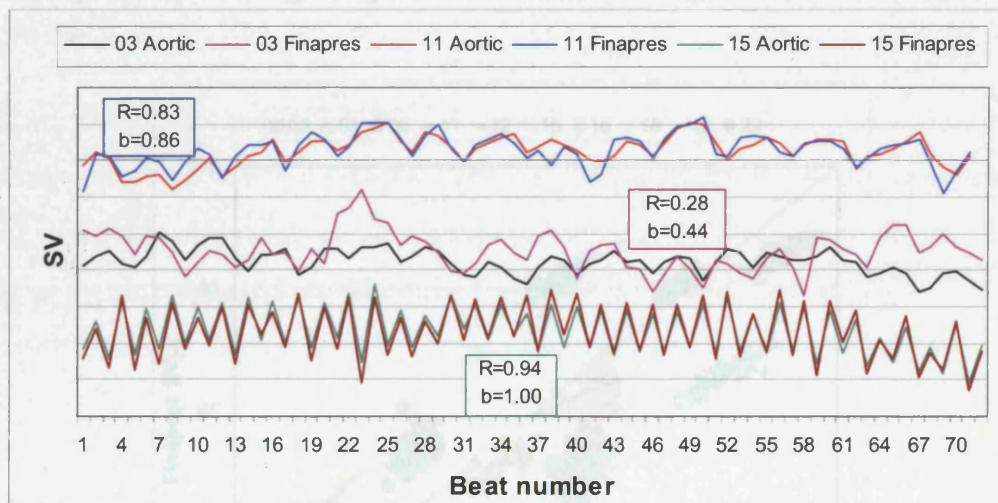


Figure 6-5: Beat-to-beat comparison of Finapres and aortic SV_{PC} for three subjects

The correlation coefficients between aortic and Finapres stroke volume for these ten records are displayed in Table 6-5. Three records had slopes close to unity and the other six had slopes less than unity. Two subjects (03 and 16) had particularly poor correlations and regression slopes. Subject 16, suffered from diabetes and complained of occasional pins and

needles in his arms and hands, suggesting peripheral vascular disease or peripheral neuropathy in the upper limbs. Examination of the pressure signal marks used to calculate PSA provided no indications of the reason why correspondence should be so poor for subject 03.

Table 6-5: Correlations and regression slopes for Finapres vs. aortic SV_{PC}

File	R	Slope 95% CI	
03	0.28	0.08	0.79
04	0.93	0.68	0.87
08	0.63	0.61	1.19
11	0.83	0.72	1.00
12	0.87	0.60	0.79
15	0.94	0.93	1.06
16	0.40	0.13	0.57
18	0.89	1.04	1.43
19	0.87	0.57	0.75
22	0.88	0.76	0.99
mean	0.75	0.61	0.94
stdev	0.23	0.30	0.25

The linear regression slopes are shown in Figure 6-6 along with the line of identity. The large amount of scatter for subjects 03 and 16 are clearly visible, as are the well-defined linear relationships for the other eight subjects over a reasonable range of individual SV_{PC} values.

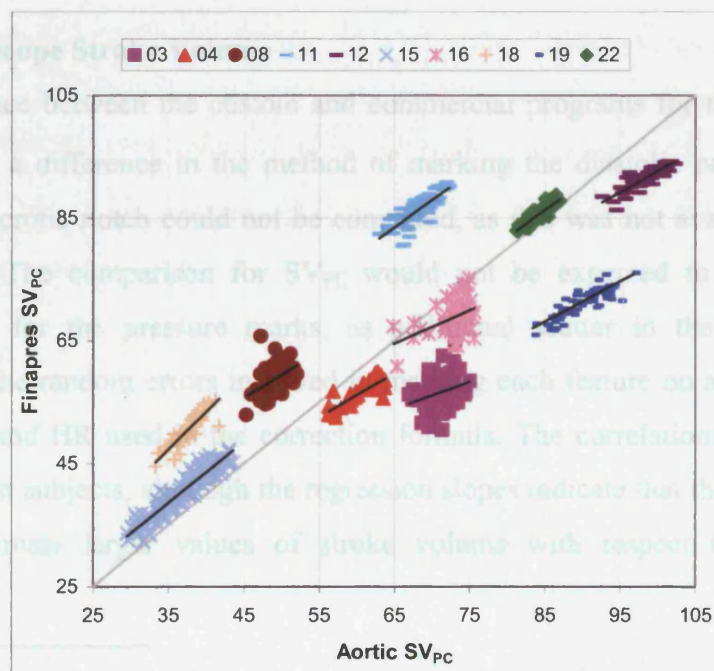
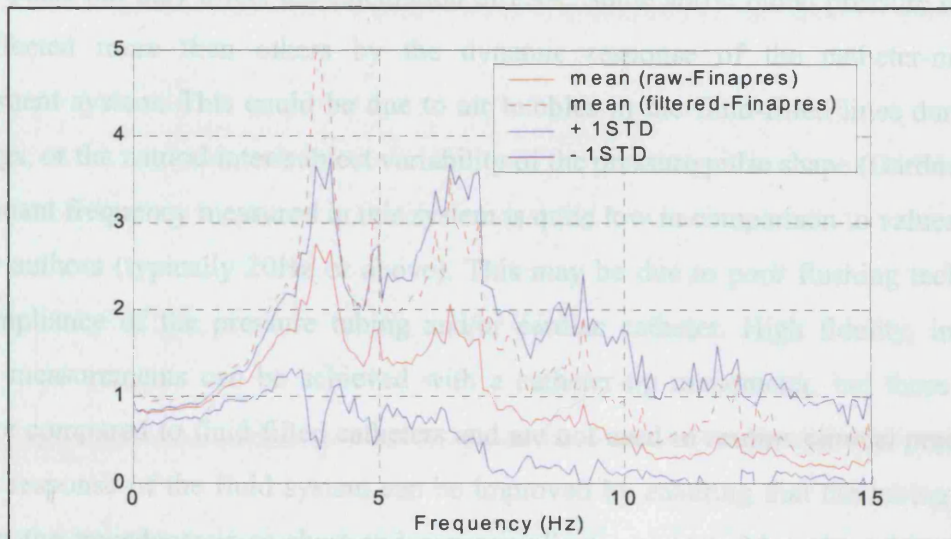


Figure 6-6: Linear regression of Finapres vs. aortic SV_{PC}

The mean and standard deviation of the transfer function gain from raw aortic to Finapres blood pressure is shown in Figure 6-7. The figure also shows the mean transfer function from filtered aortic to Finapres pressure. There is a significant gain in the transmission of the blood pressure pulse at around 3-4Hz.

**Figure 6-7: Transfer function between aortic and Finapres blood pressure signals**

Discussion

Custom vs. Beatscope Stroke Volume

The correspondence between the custom and commercial programs for the beat marks was excellent, despite a difference in the method of marking the diastolic pressure²¹. Only the marking of the diastolic notch could not be compared, as this was not available as an output from Beatscope. The comparison for SV_{PC} would not be expected to achieve the same accuracy as that for the pressure marks, as additional scatter in the results would be introduced from the random errors involved in marking each feature on a beat and from the values for MAP and HR used in the correction formula. The correlations for SV_{PC} are still very good for most subjects, although the regression slopes indicate that the custom algorithm tends to overestimate larger values of stroke volume with respect to Beatscope. The

21. In the MATLAB version diastolic pressure is marked as the lowest pressure within ten samples of the beat start, whereas in Beatscope it is the pressure just before the beat start.

differences between the two sets of values is most likely due to differences in the diastolic pressure marks, which could not be compared.

Fidelity of the Aortic Pressure Recordings

Ninety-seven percent of the spectral power in the aortic pressure signal was contained in frequencies below 3Hz, and 98% in frequencies below 5Hz. The tiny proportion of residual power contained in higher frequencies will not significantly affect the true area under the pressure pulse but may affect the calculation of PSA. Some aortic blood pressure recordings were affected more than others by the dynamic response of the catheter-manometer measurement system. This could be due to air bubbles in the fluid-filled lines during those recordings, or the natural inter-subject variability of the pressure pulse shape (Gardner, 1981). The resonant frequency measured in this system is quite low in comparison to values reported by other authors (typically 20Hz or above). This may be due to poor flushing technique or high compliance of the pressure tubing and/or cardiac catheter. High fidelity, intra-aortic pressure measurements can be achieved with a catheter-tip manometer, but these are very expensive compared to fluid-filled catheters and are not used in routine clinical practice. The dynamic response of the fluid system can be improved by ensuring that the tubing from the patient to the transducer is as short and non-compliant as reasonably achievable. The fluid transmission medium must also be free from air-bubbles which can be achieved by careful flushing, but is difficult to check visually.

In order to minimise the impact of this study on the normal routine of the Catheter Suite, the standard equipment and technical practice of that department was maintained and only seventy seconds of synchronous data were recorded. This duration was sufficient to provide enough individual beats in each recording to ensure suitably small errors in the statistical tests for correlation and regression.

Finapres vs. Aortic Stroke Volume

Discrepancies in SV_{PC} may be the result of the pulse-marking algorithm employed. As the transmission from aorta to non-invasive finger blood pressure amplifies at 3-4Hz, we can expect that the positions of the pulse markings will be different between the two signals. In general, mean aortic pressure exceeded Finapres pressure, so that the stroke volume correction factor applied to the same beat in the two signals will not be the same (Equation 6-5).

It should be noted that the presence of significant vascular disease may impair the correspondence between SV_{PC} values calculated from aortic and peripheral pressure sites. This type of pathology is expected to be relatively prevalent in patients requiring cardiac

catheterisation. Possibly better comparisons would have been achieved in a healthy subject sample. The poor correspondence between aortic and finger pulse contour stroke volume for subject 16 indicate that Finapres may not be a suitable method for estimating this parameter in patients with symptomatic vascular disease in the upper limbs.

Transfer Function

Several of the individual records contained sharp spikes in the transfer function gain and these are still evident in the group mean. The autospectra of the aortic and Finapres blood pressure signals contain most of their power in peaks at the mean heart rate and its harmonics. The Finapres signal contains more power in the higher harmonics than the aortic signal does and these lead to the spikes in the transfer function. A smoother function could have been achieved by whitening the pressure signal with variable heart rates, but the underlying shape can be seen to be consistent with functions previously relating peripheral and central blood pressures (Karamanoglu, *et al*, 1993; Chen *et al*, 1997).

The results from this study do not allow a definitive acceptance of the corrected impedance pulse contour method as implemented in the custom program. In the following chapter, non-invasive pulse contour stroke volume is compared with an independent method.

CHAPTER 7 BEAT-TO-BEAT VALIDATION OF PULSE CONTOUR STROKE VOLUME

Introduction

As the results of the comparison between pulse contour stroke volume from aortic and Finapres arterial pressures were not unequivocal I compared pulse contour stroke volume (as described in Chapter 6) with Doppler measurements of stroke distance, to determine the reliability of beat-to-beat variations in the pulse contour data. I am not aware of any published results comparing the corrected impedance pulse contour method (Wesseling *et al*, 1983) with any independent beat-to-beat measure of stroke volume or cardiac output other than the conference paper based on this work²² (Peirce *et al*, 2001).

Variability of Cardiovascular Parameters

As described in Chapter 3, beat-to-beat variations in heart rate/RR interval and arterial blood pressure have been known for many years and investigated widely. As techniques and knowledge have progressed, so other parameters have been included in these investigations and models: e.g. respiration (often in terms of instantaneous lung volume) and, more recently, stroke volume and peripheral sympathetic effects (as MSA and peripheral resistance).

The role of changes in stroke volume in the relationship between arterial pressure and heart rate is not yet fully defined. The beat-to-beat variability of stroke volume has been investigated by a few authors previously (Guz *et al*, 1987; Toska and Eriksen, 1993; Blaber and Hughson, 1996; Siebert *et al*, 1999; Liu *et al*, 2004), but the data have not yet been fully integrated into quantitative models of the cardiovascular system.

Electromagnetic flowmeters are currently accepted as the gold standard for real-time blood flow measurement, but require direct access to the vessel concerned. Thus there is no simple method against which comparisons can be made for accuracy and precision. Where this is the case, comparing two methods based on disparate physical principles provides the next best evaluation. Pulse contour cardiac output has been compared to other methods of measurement

22. The method has been compared to the Modelflow method, but this is not an independent method as it is based on the same pressure signal (Wesseling *et al*, 1993; Jellema *et al*, 1999).

for comparing mean values and tracking changes (Jansen *et al*, 1990; Stok *et al*, 1993; Rauch *et al*, 2002). These studies do not indicate the true accuracy or precision though, as these methods are also subject to significant variation in repeated measurements (Jansen *et al*, 1990; Espersen *et al*, 1995).

Doppler ultrasound provides a suitable non-invasive, beat-to-beat measure of left ventricular output that is sufficiently disparate in principle to provide an independent methods for comparison with pulse contour. Agreement between these methods would provide good evidence that the result reflects the true stroke volume variability (SVV) and not measurement noise or interference from other processes.

Stroke Volume Variability

Left ventricular stroke volume (LVSV) is known to vary with respiration (Chapter 3). It decreases during inspiration, whereas right ventricular stroke volume increases and the ventricular septum moves to the left (Guz *et al*, 1987; Sleight and Casadei, 1995). Previous measurements on the variability of left ventricular stroke volume in humans have used Doppler, thoracic impedance and echocardiographic imaging methods and have been mostly restricted to the respiratory frequency effects (Guz *et al*, 1987; Toska and Eriksen, 1993; Siebert *et al*, 1999; Liu *et al*, 2004). To my knowledge, the same information has not been demonstrated using a pulse contour algorithm, and the presence or absence of LF variations was not established prior to 2001 (Peirce *et al*, 2001; Liu *et al*, 2004), although power spectra and phase information have been published for the Doppler and echocardiographic methods (Blaber and Hughson, 1996; Liu *et al*, 2004).

In an informative series of experiments on a small number of subjects, Guz *et al* (1987) used Doppler ultrasound to demonstrate the temporal relation between stroke volume and heart rate changes with respiration. The magnitude of the LVSV changes increased with tidal volume and the frequency changed to match the breathing rate. Stroke volume variability continued during cardiac pacing and occluded breathing, and it was absent during breath-holding and in two subjects with pericardectomies. These results indicate that stroke volume changes are mechanically driven by respiratory changes in intrathoracic pressure (Chapter 3).

Doppler Measurement of Cardiac Output

Cardiac output is investigated more widely than stroke volume, probably because it is a more useful clinical indicator of a patient's physiological condition. In Doppler ultrasound, the

frequency shift, Δf , of a reflected ultrasonic beam is proportional to the velocity of the reflectors (in this case red blood cells, RBCs).

$$\Delta f = \frac{2fv\cos\theta}{c} \quad \text{Equation 7-1}$$

Where f is the transmitted frequency (usually 2-10MHz), v is the velocity of the reflectors, θ is the angle between the beam and the direction of flow and c is the speed of sound in the medium (Evans and McDicken, 2000, p2). Pulsed wave (PW) Doppler devices transmit discrete bursts of ultrasound and the duration of the pause between transmission and reception at the transducer determines the depth at which the measurement is made.

In healthy adults, without valve or heart pathologies, the velocity profile in the ascending aorta is relatively flat and becomes more rounded with distance from the aortic valve (Huntsman *et al*, 1983; Evans and McDicken, 2000, p20-21). For a flat profile the maximum velocity will be similar to the mean velocity, although the boundary layer near the wall of the aorta (a couple of millimetres) will contain a steep velocity gradient. Maximum velocities in the ascending aorta are generally not more than about 150cm/s (Huntsman *et al*, 1983; Coats, 1990). The majority of RBCs will be travelling in a narrow range of velocities, so there will be a narrow band of Doppler frequencies in the received signal (Figure 7-1). This profile will be distorted by properties of the measurement, such as non-uniform insonation and intrinsic spectral broadening (Evans and McDicken, 2000, p142-146).

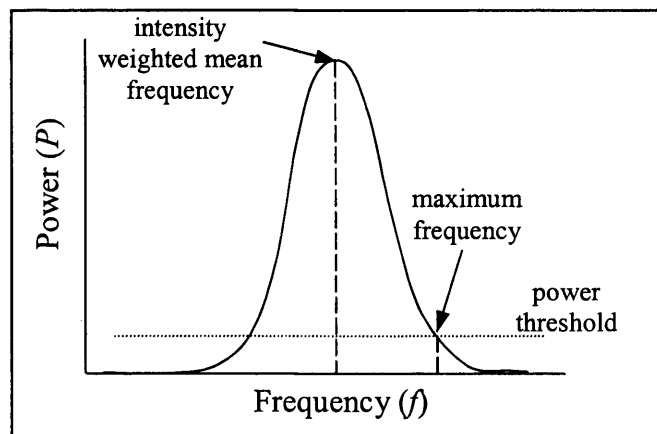


Figure 7-1: Use of a threshold to determine the maximum frequency envelope

The intensity weighted mean frequency (IWMF) is proportional to the mean flow velocity (Evans and McDicken, 2000, p169) and is defined as:

$$f = \frac{\int P f df}{\int P df} \quad \text{Equation 7-2}$$

The envelope determined by this measure is less susceptible than the maximum frequency to the random variation in signal power associated with RBC reflectors (Evans and McDicken, 2000, p130, p151).

Stroke distance (SD) is the integral of blood velocity during systole. If an estimate of aortic cross-sectional area is available then stroke distance can be multiplied by this to determine stroke volume. However, the measurement of aortic diameter by echocardiography requires a skilled operator and is the greatest source of error in aortic flow measurements (Robson *et al*, 1988). Aortic root diameter is implicitly assumed to remain constant throughout the cardiac, respiratory and low frequency cycles, and has been found to remain unchanged when breathing against a resistance and during exercise (Coats, 1990; Blaber and Hughson, 1996). A Medline search was unable to determine whether aortic dimensions have been shown to vary at the frequencies of interest here (<0.5Hz). The difference between stroke distance and stroke volume is therefore considered to be a constant factor, the inclusion of which will have no effect on beat-to-beat variability or spectral analysis measures. Also, as the pulse contour method is not calibrated, comparison of the absolute levels of stroke volume is not appropriate. Aortic cross-section was therefore not measured in this study.

Methods

Protocol

Recordings were taken from seventeen healthy volunteers. Following instrumentation and time for adaptation to the position (approximately ten minutes), two five minute recordings were taken in the supine position with the subject breathing spontaneously (spontaneous, supine; SS1 and SS2). One five minute recording was conducted with the subject supine and controlling their breathing at a fixed rate of 0.2Hz or 12 breaths per minute (controlled, supine; CS). The subject was then either tilted upright to 70°, or stood leaning against a wall for support (see below). At 70° tilt the orthostatic stress is 94% of the full upright position. The subject was allowed to acclimatise to the upright position for several minutes so that a steady cardiovascular state was achieved before further recording. As indicated in Chapter 2, once the early steady state has been achieved (by around 1-3min) there is little difference

between the cardiovascular response to head-up tilt and active standing. These positions were therefore considered to be identical for the purpose of the study. Two further five minute recordings in the upright posture were made for spontaneous and controlled breathing respectively (spontaneous, upright and controlled, upright; SU and CU).

Instrumentation

Non-invasive finger blood pressure (Finapres), ECG, respiratory frequency (thoracic circumference, Respiband, Nims) and Doppler blood velocity in the ascending aorta (Digidop, SciMed) were recorded simultaneously. The Digidop is specifically designed to measure cardiac output from the suprasternal notch using a pen-shaped 2MHz pulsed Doppler transducer. The flow in the ascending aorta was detected by slowly tilting the applicator in lateral and anterior-posterior planes until the strongest forward-flow signal was determined. The angle between the ultrasound beam and the direction of flow was assumed to be zero in this orientation. The device displays the time averaged mean (TAM) velocity envelope, so the velocity signal was seen as a series of discrete pulses corresponding to each systolic expulsion.

Good positioning of the sample volume produced a large, clean systolic pulse with little or no flow during diastole (Huntsman *et al*, 1983; Robson *et al*, 1988). The audible Doppler signal was also used to ensure proper positioning of the probe as it was more sensitive to changes in the quality of the detected flow than the graphical display (Innes *et al*, 1986). The operator listened to this signal via headphones so that the subjects did not subconsciously entrain their respiration frequency to their pulse rate.

The depth of the measurement volume was altered to achieve the optimal signal at the maximum depth, to ensure that the sample volume was close to the aortic valve. The direction of the transducer was adjusted to maintain the maximum envelope on the display. Relative movement of the internal organs and major vessels is likely following the change from supine to upright, so the depth and direction of the Doppler signal was re-assessed after the change of position.

The operation of the Digidop required the application of an appreciable, but not uncomfortable, pressure to the subject's suprasternal notch. In order to minimise Doppler probe movement and maximise subject and operator comfort the operator's arm was supported during the recordings. During the supine measurements a pillow was placed upon the subject's chest for this purpose and the operator took care not to apply undue pressure to the thorax. For tilted subjects, a shelf was attached to the tilt table. This allowed the subject to

rest their left hand at the level of the heart, to avoid a hydrostatic pressure difference between aortic and Finapres arterial pressure, and also provided support for the operator's arm. The left hand of standing subjects was raised to the heart level by the use of a sling and the operator's arm was supported at an appropriate height. The standing subjects leant against a wall to prevent swaying and ensure that the anterior-posterior pressure from the Doppler probe did not unbalance them. Controlled breathing frequency was timed using a visual stimulus and was evenly cycled between inspiration and expiration.

The phase quadrature signal output from the Digidop was converted to a directional flow signal because phase errors in the recording heads of the DAT recorder could alter the reconstituted signal (Evans and McDicken, 2000, p406-407). The directional flow, blood pressure, respiration and surface ECG were recorded simultaneously onto DAT.

Data Analysis

The recordings were later resampled from DAT onto a PC at 200Hz using in-house software in real-time (FTNEW) and with a 5ms time resolution for the Doppler sonogram. Custom-built MATLAB software (STROKE.M) was used to inspect and edit the signals, calibrate the Finapres signal and mark the IWMF Doppler pulses (Figure 7-2). These were delineated using a simple zero-crossing detector and the marks were manually checked and corrected with the aid of plots of beat-to-beat ejection time, pulse interval and stroke distance. The stroke distance was calculated using the velocity-time integral (VTI) of the IWMF between the marks defined for each systole. The pulse contour analysis and derivation of beat-to-beat pressure values was carried out using a custom version of the corrected impedance pulse contour algorithm as described in Chapter 6 (Wesseling *et al*, 1983). RR intervals were determined using a threshold-crossing algorithm to detect the R-waves on the ECG signal.

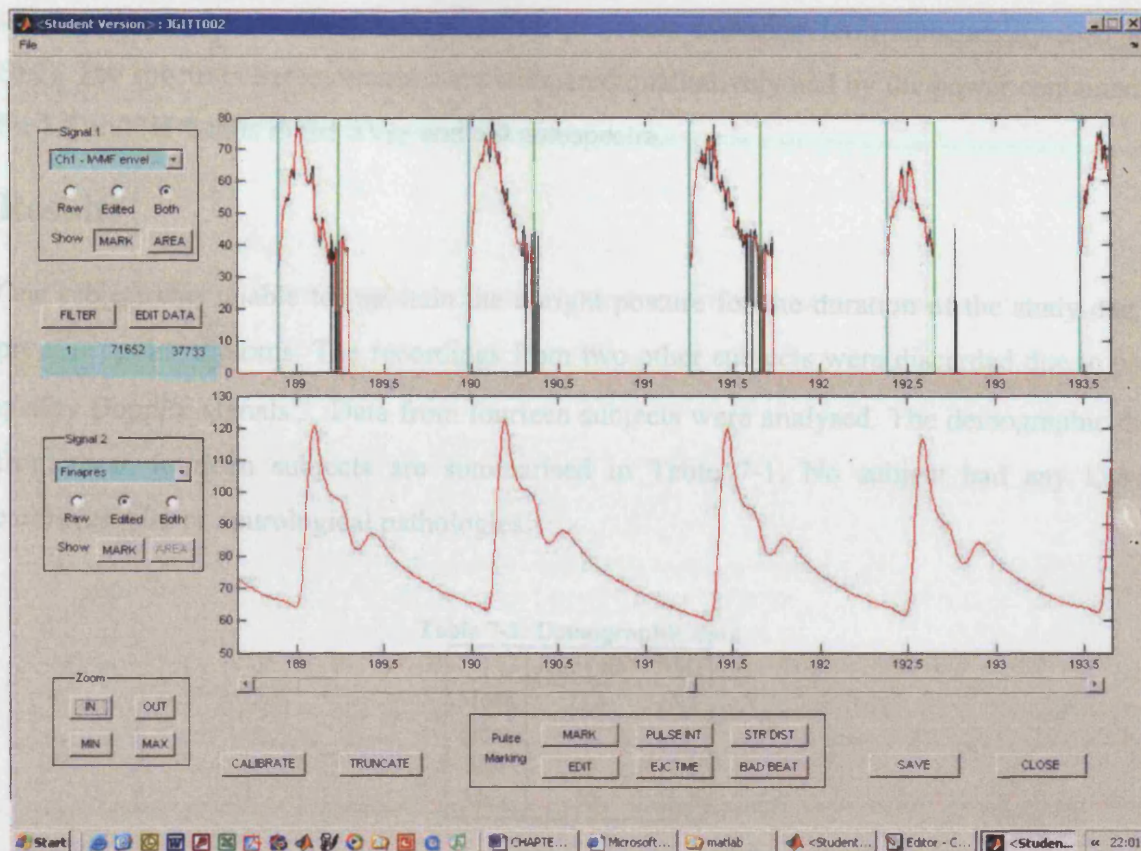


Figure 7-2: Custom program for data editing, Doppler pulse marking and stroke distance calculation (STROKE.M). Top trace shows a marked IWMF Doppler signal and bottom trace is the calibrated Finapres pressure.

Parameter interpolation and spectral analysis has been described in detail in Chapter 5. In brief, beat-to-beat values were interpolated to 5Hz. Linear trends were removed from the data before spectral analysis was performed using a 512-point FFT, 20% tapered cosine data window and 15% subrecord overlap. Power and cross spectra were averaged with a moving five-point triangular window. For most recordings this produced four subrecords to be averaged for the periodogram, providing spectral estimates with 30 degrees of freedom. Due to technical difficulties and non-stationarities seven recordings were shorter than five minutes and only provided three subrecords for averaging. The process for determining a threshold for non-zero coherence was described in Chapter 5. For four subrecords this limit was 0.2 and for three subrecords it was 0.25. The low frequency (LF) band was defined as 0.04-0.15Hz and high frequency (HF) as 0.15-0.4Hz (European Society of Cardiology and North American Society of Pacing and Electrophysiology, 1996). Spectra were normalised to allow for inter-subject comparisons.

Correlation analysis was used to investigate the correspondence between beat-to-beat variability in pulse contour stroke volume (SV_{PC}) and stroke distance (SD). Student's paired

t-tests were used to assess the effect of the protocols and short term repeatability (SS1 and SS2). The spectral characteristics were compared qualitatively and by the power contained in the LF and HF bands in the SV_{PC} and SD autospectra.

Results

One subject was unable to maintain the upright posture for the duration of the study due to pre-syncope symptoms. The recordings from two other subjects were discarded due to poor quality Doppler signals²³. Data from fourteen subjects were analysed. The demographic data from these fourteen subjects are summarised in Table 7-1. No subject had any known cardiovascular or neurological pathologies.

Table 7-1: Demographic data

	Age (yrs)	BMI
Mean	33.4	24.1
Stdev	8.0	2.8
Min	23	19.3
Max	50	28.7

Correlation of Beat-to-Beat Values

Correlation coefficients between pulse contour stroke volume and Doppler stroke distance are summarised in Table 7-2.

Table 7-2: Correlation coefficients between SV_{PC} and SD

Condition	Mean	Stdev	Range	
Overall	0.62	0.19	-0.07	0.90
SS1	0.53	0.25	-0.07	0.80
SS2	0.60	0.19	0.17	0.83
CS	0.70	0.17	0.32	0.90
SU	0.61	0.17	0.18	0.78
CU	0.67	0.15	0.32	0.84

SS – spontaneous, supine CS – controlled, supine
SU – spontaneous, upright CU – controlled, upright

The correlations were generally good, although there were isolated recordings with very low values. One subject had particularly poor correlations in the supine position (0.07, 0.17 and 0.37; SS1, SS2 and CS), but very good values in the upright position (0.78 and 0.73; SU and CU). In a second subject this pattern was reversed: 0.55, 0.65 and 0.83 supine (SS1, SS2 and CS), versus 0.18 and 0.32 upright (SU and CU).

23. The subject with presyncope and one of the poor quality recordings both had low BMI values (17-18).

Following the results of the spectral analysis (see below) the effect of removing the very low frequency (VLF, $<0.04\text{Hz}$) variations on the beat-to-beat correlations was assessed. As the pulse contour technique is being evaluated for the calculation of parameters using the LF and HF bands, the quality of the measurement in these bands is most important. Also, VLF variations require longer recordings to be reliably quantified, so in this case they were regarded as noise and removed. A 2nd order digital Butterworth high pass filter with a corner frequency of 0.02Hz was applied in the forward and reverse directions (zero phase shift) to the 5Hz interpolated SV_{PC} and SD data. The filtered signals were then re-interpolated to the original beat-to-beat time samples and the correlation analysis repeated.

Table 7-3: Correlation coefficients between SV_{PC} and SD following high pass filtering at 0.02Hz

Condition	Mean	Stdev	Range	
Overall	0.70	0.17	0.15	0.92
SS1	0.65	0.21	0.15	0.82
SS2	0.71	0.15	0.35	0.86
CS	0.78	0.13	0.47	0.92
SU	0.66	0.19	0.21	0.81
CU	0.72	0.16	0.38	0.86

SS – spontaneous, supine CS – controlled, supine
SU – spontaneous, upright CU – controlled, upright

The results following the high pass filtering are shown in Table 7-3. Several individual recordings with very low correlations were significantly improved by the filter (e.g. -0.07 became 0.40), although most results were improved only slightly. Four individual recordings in the upright position (two in each of the breathing regimes) were decreased slightly by the process, whereas all of the results from the supine recordings were improved. The postural differences for the two subjects described above were still considerable (e.g. 0.69 , 0.68 , 0.84 – supine; 0.21 , 0.38 – upright), although the two worst values in the supine position were improved significantly (0.40 , 0.35 , 0.47 – supine; 0.81 , 0.77 upright). Correlations were slightly better for controlled rather than spontaneous breathing in both positions, although this was only significant in the supine position ($p < 0.05$).

Power Spectra

Some subjects demonstrated spontaneous breathing rates that fell within the LF band during both supine and upright recordings. Both the SV_{PC} and SD power spectra demonstrated peaks that mirrored these spontaneous respiratory frequencies over a broad range (approximately 0.1 - 0.37Hz). Figure 7-3 contains several SD and SV_{PC} power spectra that show the ability of

the stroke volume to follow respiratory movements at different breathing rates. The SD spectra contained more power in the VLF band than the SV_{PC} spectra.

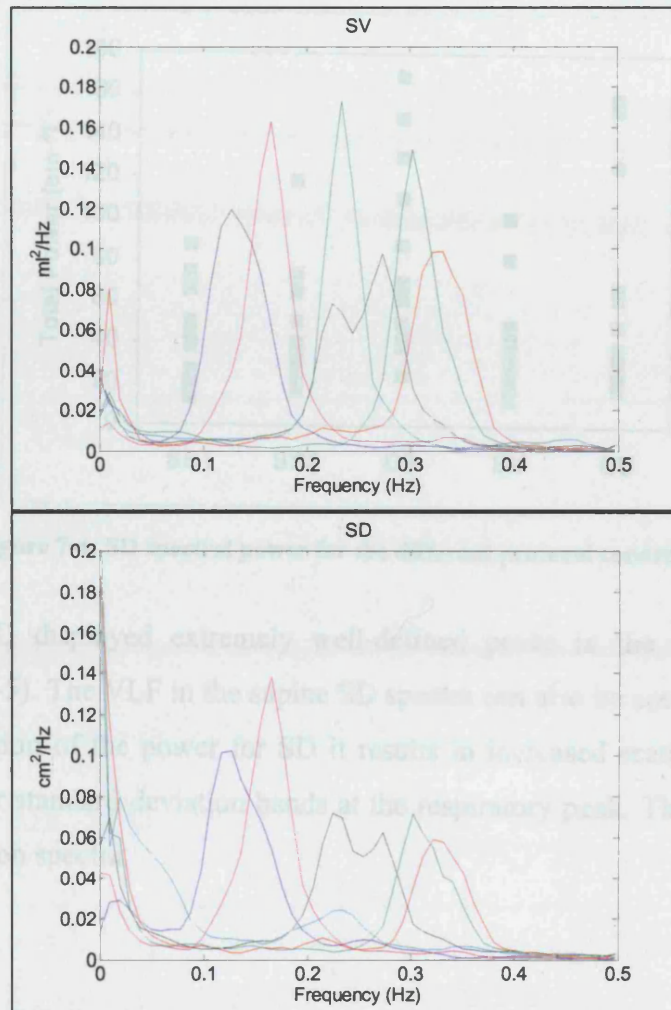


Figure 7-3: SV_{PC} (top) and SD (bottom) autospectra for SS1

Included in Figure 7-3 is one of the recordings with a very poor correlation between beat-to-beat SV_{PC} and SD values (pale blue). The SV_{PC} spectra for this subject shows a very strong peak at 0.23Hz, but the Doppler SD spectra contains a considerable amount of VLF power and only a small respiratory peak at this frequency. This correlation was not particularly improved by the high pass filtering (0.31 to 0.42), probably because the VLF in this case extends up to 0.1Hz. The respiration spectra matches that for SV_{PC} suggesting that lack of beat-to-beat agreement between SV_{PC} and SD is due to errors in the Doppler method.

Overall variability, as measured by total spectral power, was not different between the supine and upright conditions for either breathing protocol. The change from spontaneous to controlled breathing increased the total spectral power in both positions, although this was

only statistically significant in the supine position ($p < 0.05$). These results are shown in Figure 7-4 for Doppler SD, but were similar for both SD and SV_{PC} .

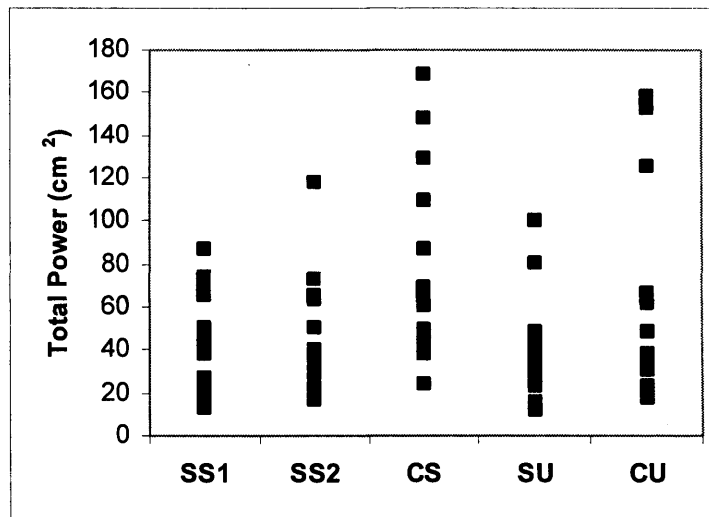


Figure 7-4: SD spectral power for the different protocol conditions

Both SV_{PC} and SD displayed extremely well-defined peaks in the controlled breathing protocols (Figure 7-5). The VLF in the supine SD spectra can also be seen in this figure. As it is a greater proportion of the power for SD it results in increased scatter in the spectra, as shown by the wider standard deviation bands at the respiratory peak. This was not generally seen in the respiration spectra.

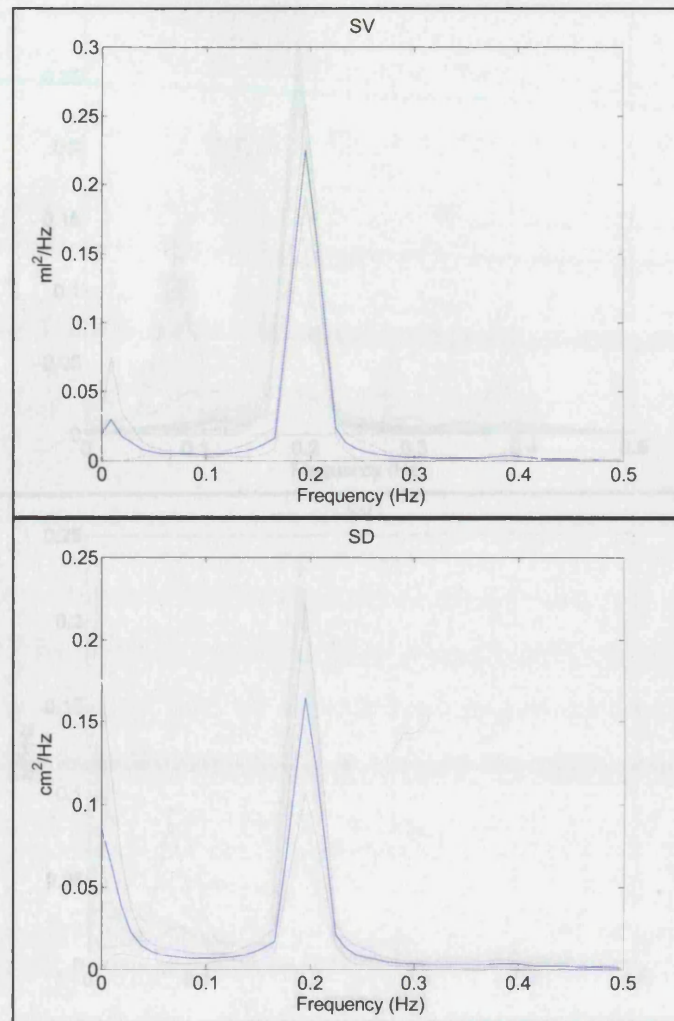


Figure 7-5: Group average autospectra for SV_{PC} and SD for CS (mean \pm stdev)

One subject was unable to maintain compliance with the controlled breathing protocol in the upright position and their main spectral peak occurred at around 0.08Hz (Figure 7-6, upper panel, green line). Another two subjects maintained a peak at the correct frequency but with a significant amount of power at lower frequencies (magenta lines). The spectra also show the presence of some harmonics at 0.4Hz.

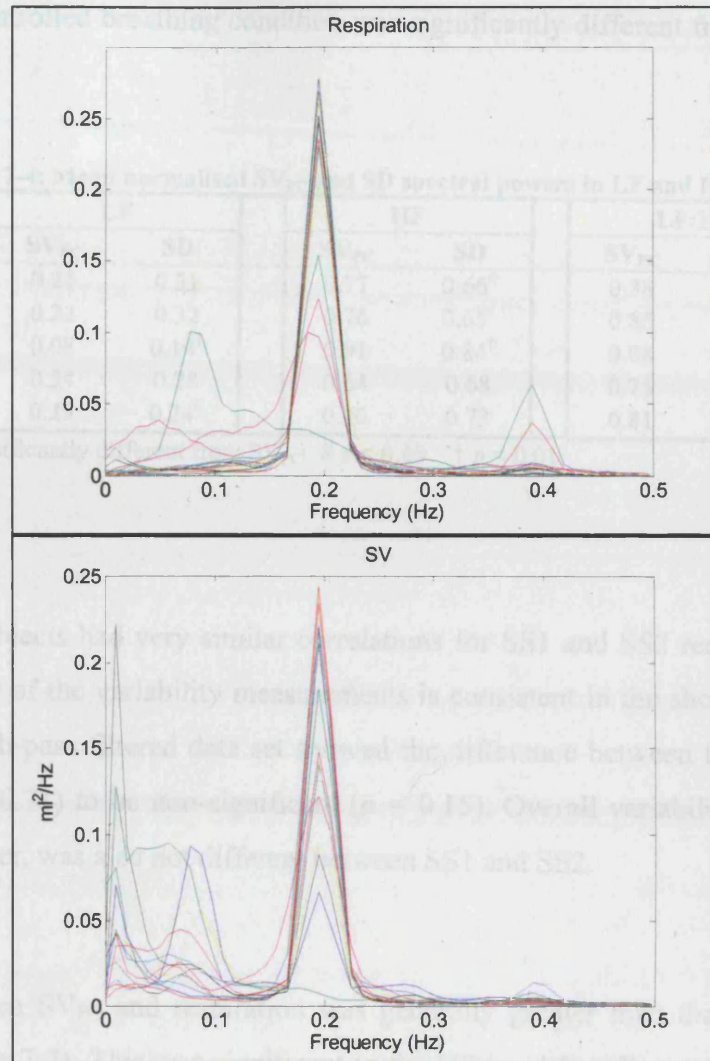


Figure 7-6: Respiration and SV_{PC} autospectra for CU

The SV_{PC} and SD autospectra both demonstrate the development of a peak at around 0.1 Hz in the upright posture for both spontaneous and controlled breathing which was not generally present in the respiration spectra (Figure 7-6). This LF peak was not as dominant as in the RR and SAP spectra.

Table 7-4 shows the mean normalised powers in each band for SV_{PC} and SD for the different protocols. In the supine position the LF power is smaller and the HF power greater for SV_{PC} than for SD. This is another manifestation of the VLF power which is present in the SD signal. It is especially significant in CS ($p < 0.01$), as those subjects whose spontaneous breathing rate was particularly low in SS1 and SS2 will no longer have considerable LF power in the SV_{PC} and SD spectra.

For the upright recordings the presence of the LF peak reduced the effect of the VLF discrepancy and ensured that the power distributions were more similar. Only the SD LF

power for the controlled breathing condition was significantly different from the SV_{PC} power ($p < 0.05$).

Table 7-4: Mean normalised SV_{PC} and SD spectral powers in LF and HF bands

	LF			HF			LF:HF	
	SV_{PC}	SD		SV_{PC}	SD		SV_{PC}	SD
SS1	0.21	0.31		0.77	0.66 [#]		0.38	0.59 [†]
SS2	0.22	0.32		0.76	0.65 [†]		0.85	0.78 [†]
CS	0.08	0.14 [†]		0.91	0.84 [†]		0.08	0.18 [†]
SU	0.34	0.28		0.64	0.68		0.75	0.54
CU	0.29	0.24 [#]		0.70	0.73		0.81	0.46

SD significantly different from SV_{PC} : [#] $p < 0.05$ [†] $p < 0.01$

Repeatability

Twelve of the subjects had very similar correlations for SS1 and SS2 recordings, indicating that the similarity of the variability measurements is consistent in the short term. A paired t-test using the high-pass filtered data set showed the difference between the first and second values (0.65 and 0.71) to be non-significant ($p = 0.15$). Overall variability, as measured by total spectral power, was also not different between SS1 and SS2.

Coherence

Coherence between SV_{PC} and respiration was generally greater than that between SD and respiration (Figure 7-7). This was significant in the HF band for all experimental conditions ($p < 0.05$), and for the LF band in all but CU. In some recordings the respiratory signal suffered from a low signal-to-noise ratio (SNR) probably due to incorrect fitting of the band or because chest movement was uneven. However, this will affect the coherence for SV_{PC} and SD equally. Coherence was also generally lower in the upright position, although this was only statistically significant for HF during controlled breathing. Again, this is most probably due to poorer registration of the breathing signal in this posture than an indication of reduced effectiveness of the mechanical modulation. Coherence between SV_{PC} or SD and respiration was not altered by the use of controlled respiration in either position.

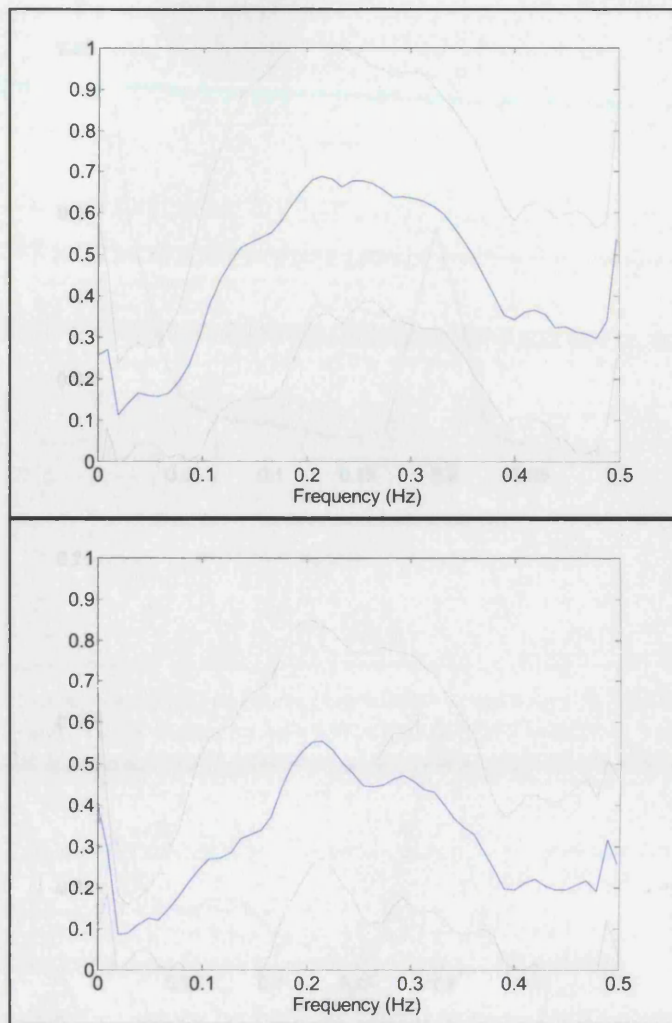


Figure 7-7: Group average coherence between respiration and SV_{PC} (top) or SD (bottom) for SS1 (mean±stdev)

Beat-to-beat Peripheral Resistance

Beat-to-beat cardiac output was calculated from the product of SV_{PC} and HR for each beat, and total peripheral resistance from the quotient of mean arterial pressure to cardiac output (Equation 5-1). The power spectra for PR for the controlled breathing conditions are shown in Figure 7-8. This parameter shows a significant peak at the respiratory frequency and also a significant amount of VLF power in the supine position. On the assumption of the upright posture a second smaller peak develops in the LF band.

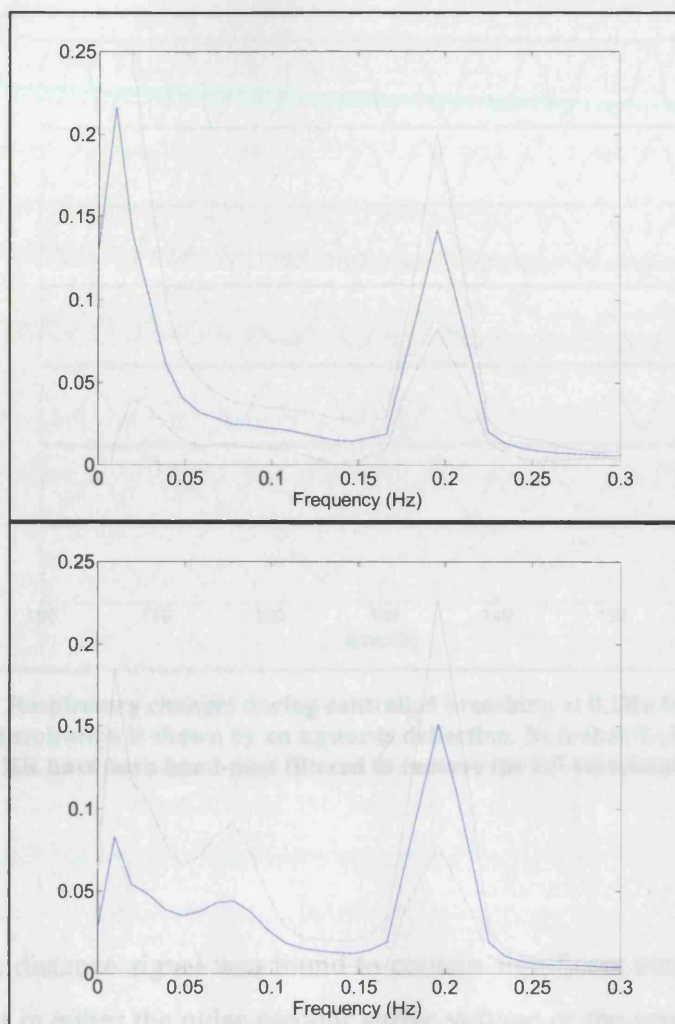


Figure 7-8: Group average autospectra for PR for CS (top) and CU (bottom). Note change of frequency scale to emphasise LF band (mean \pm stdev)

The respiratory changes in several cardiovascular parameters are shown in Figure 7-9 for CU conditions. The figure shows the previously-described changes in SAP, HR and SV. HR, MAP and SAP have been low-pass filtered to remove the LF variations that dominate in the upright position. Systolic blood pressure begins to fall at end-expiration and inspiration is associated with falling SV_{PC} . Heart rate and SV_{PC} are out-of-phase, and the changes in CO are in phase with SV_{PC} rather than HR. CO is also in phase with MAP but out of phase with PR. Therefore, as PR is reciprocally related to CO, the respiratory variations in PR are due to the dominance of CO over MAP.

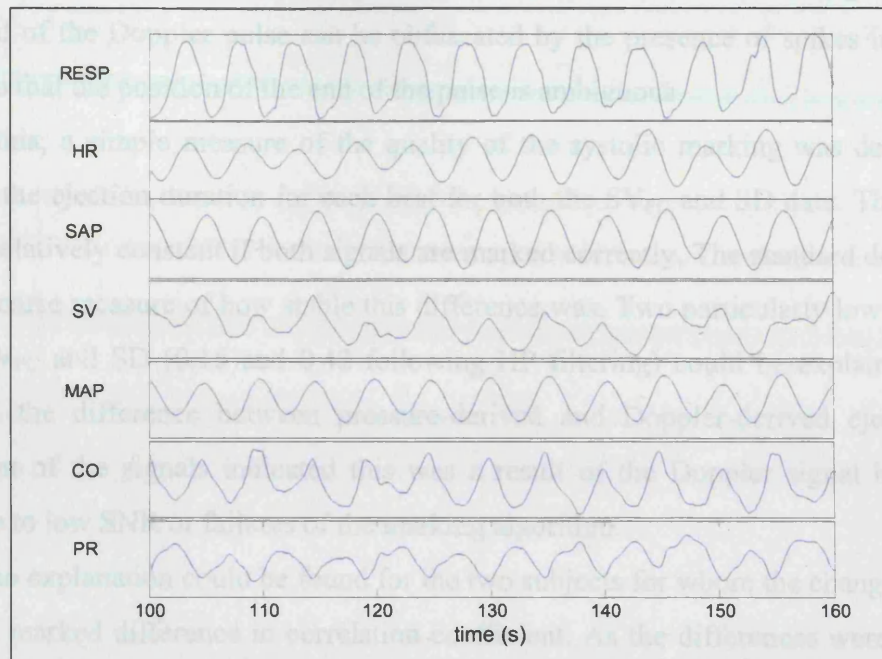


Figure 7-9: Respiratory changes during controlled breathing at 0.2Hz in the upright positions. Inspiration is shown by an upwards deflection. Note that SAP, MAP and HR have been band-pass filtered to remove the LF variations.

Discussion

The Doppler stroke distance signal was found to contain significant amounts of VLF power that was not present in either the pulse contour stroke volume or the respiration signals. This appeared to be the cause of low beat-to-beat correlations in several recordings. The application of a high-pass filter improved the correlations generally, especially in the supine position. Stroke volume is considered to be modulated by the direct mechanical effect of intrathoracic pressure, so the lower coherence between respiration and Doppler SD than between respiration and pulse contour SV suggests that discrepancies between the beat-to-beat results lie mostly with the Doppler method. This is also indicated by the power spectra, whereby the SV_{PC} autospectra always contained a strong peak at the respiratory frequency, but SD did not in all cases (Figure 7-3). It is most likely that the VLF variations were due to operator or subject movement, although efforts were made to minimise this.

Following high-pass filtering there remained some individual low correlation values, as indicated by the minimum correlations in Table 7-3. It was thought that the marking of the Doppler signal in recordings with poor SNRs may have been subject to errors that contributed to poor correlations. The gain and threshold values used for resampling the Doppler signal onto PC are set for the duration of the recording. Therefore if there is a variation in signal

quality during this time, the parameters will not be optimised for all beats. Figure 7-2 shows that the end of the Doppler pulse can be obfuscated by the presence of spikes in the IWMF envelope so that the position of the end of the pulse is ambiguous.

To assess this, a simple measure of the quality of the systolic marking was determined by comparing the ejection duration for each beat for both the SV_{PC} and SD data. The difference should be relatively constant if both signals are marked correctly. The standard deviation was used as a coarse measure of how stable this difference was. Two particularly low correlations between SV_{PC} and SD (0.15 and 0.42 following HP filtering) could be explained by large changes in the difference between pressure-derived and Doppler-derived ejection times. Examination of the signals indicated this was a result of the Doppler signal being poorly marked due to low SNR or failures of the marking algorithm.

However, no explanation could be found for the two subjects for whom the change in position produced a marked difference in correlation coefficient. As the differences were opposed in the two subjects there was probably no systematic error in the measurement procedure. Possibly postural differences in the orientation of the heart and aorta were more significant in these subjects than in others. Alternatively, the Finapres pressure or Doppler flow signals may have been more reliable for these subjects in one position than in the other.

Doppler measurement of aortic flow can be achieved with a high level of accuracy. Casadei *et al* (1992) claimed good agreement between Doppler IWMF and electromagnetic catheter measurements – the standard deviation of the difference was 3% and 4% for velocity and stroke distance respectively. Doppler-derived beat-to-beat variations in stroke volume have also been shown to follow electromagnetic measurements closely (Innes *et al*, 1986). In this latter study intrasubject correlation coefficients ranged from 0.77 to 0.97 and linear regression slopes from 0.46 to 1.37. The authors point out that these comparisons would have been affected by turbulent flow in their subject population (patients with cardiomyopathy and coronary heart disease).

The Doppler method used in this study appears to be less reliable than this however. The SNR may have been improved if the spectrogram had been available during the recording rather than the TAM envelope. This would have enabled the operator to quickly identify and correct any relative movement between the probe and the ascending aorta. This could have been achieved with the existing equipment but would have complicated the measurement process significantly. In addition, an adaptive method to alter the Doppler gain and threshold for individual beats or the ability to mark and therefore exclude poor quality Doppler or pulse

contour beats from the comparison would probably have improved the comparisons considerably.

Siebert *et al* (1999) measured SV by thoracic impedance but did not find any increase in LF spectral power in the standing position in patients before and after coronary artery bypass graft surgery (CABG). Liu *et al* (2004) found a small LF peak in SV variability in the supine position using an ultrasound method to determine LV volume. They determined that the normalised LF power was much smaller for SV than for heart rate. This is consistent with the finding here that the increase in LF power when upright is less for SV_{PC} than for SAP or RR interval. This is noteworthy, as the pulse contour stroke volume would be expected to be heavily dependent on systolic pressure values. The difference in spectra between SAP and SV_{PC} indicate that the information in the latter is not redundant.

The respiratory frequency variability of stroke volume shown here agrees with previously published results. I have also shown that left ventricular stroke volume as measured by pulse contour and Doppler methods demonstrates significant low frequency (0.04 – 0.15Hz) power in the upright position. Although the Doppler method used appeared to be affected by VLF noise and errors in marking systolic ejection times, the results for most subjects show strong similarity between beat-to-beat variations in Doppler stroke distance and pulse contour stroke volume. The dissimilar physical basis for the two methods indicates that this a real physiological phenomenon.

The extension of the pulse contour method to estimate beat-to-beat values of peripheral resistance was also shown. Although the PR autospectra showed strong respiratory variations, the sympathetic nervous system is not considered to be able to effect vascular changes at these frequencies. The respiratory peak may be the result of passive changes in vascular calibre (i.e. compliance) or an artifact of the derivation from several parameters that are known to vary at these rates.

CHAPTER 8 PERIPHERAL BARORECEPTOR

SENSITIVITY

Introduction

In Chapter 2 I introduced vascular calibre as the foremost determinant of arteriolar resistance and described why sympathetic effects on heart rate and resistance take much longer to achieve than the vagal effects on the heart rate. In Chapter 5 I explained why peripheral resistance applies to essentially steady flow and not to transient conditions and defined an appropriate bandwidth for resistive rather than capacitative vascular impedance. In this chapter I shall investigate how some of the methods for calculating cardiac chronotropic BRS, described in Chapter 4, can be adapted to apply to peripheral resistance data derived from pulse contour stroke volume (Chapters 6 and 7).

Peripheral Resistance as a Delayed Response

Due to the relatively slow sympathetic conduction and end-synaptic transmission, changes in peripheral resistance are known to take several seconds to effect. This delay is considered to be of the order of 5-10s (Ogoh *et al*, 2002) or 10-15s (Cooper *et al*, 2004). In Fourier cross-spectral analysis the total data record is often divided into subrecords to allow ensemble averaging (Chapter 5). The delay in the PR response to changes in blood pressure could represent a considerable portion of each subrecord and might result in reduced coherence between simultaneous data segments. Such an effect would be negligible for the cardiac chronotropic response, as the latencies for heart rate changes are much smaller.

Figure 8-1 shows this graphically. If there is a delay between the SAP stimulus and the PR response, the first few samples in the PR subrecord will be unrelated to the concurrent SAP subrecord, and the final few samples in the SAP segment will relate to the following PR subrecord. Therefore, if the PR response takes 8s to become evident, 16s of each simultaneous subrecord pair will effectively be unrelated noise. For a subrecord of 512 samples at 0.2s intervals this equates to approximately 15% of the data duration and could substantially degrade BRS estimates from spectral methods by reducing the signal-to-noise ratio (SNR). This effect might be mitigated by imposing a compensatory time lag between the pressure and resistance records. I hypothesised that cross-correlation analysis could be used to

determine the delay between SAP and PR, and that the value determined could be used as a time lag correction prior to cross-spectra determination to improve spectral estimates.

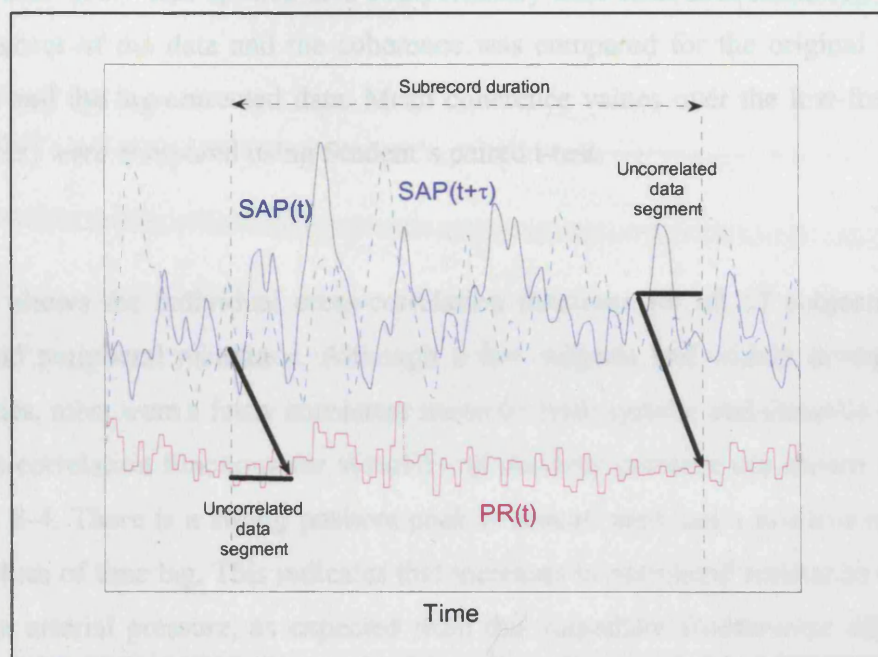


Figure 8-1: Uncorrelated portions of subrecords due to a time delay between SAP stimulus and PR response. The dashed blue line indicates the effect of imposing a time lag correction so that the stimulus and effect are roughly simultaneous.

Methods

Non-invasive finger blood pressure was recorded from fifty-seven healthy control subjects for ten minutes during supine rest²⁴. Uncalibrated pulse contour stroke volume (SV_{PC}) was determined from this by the method described in detail in Chapter 6. Peripheral resistance was calculated from the ratio of mean arterial pressure to cardiac output ($CO = SV_{PC} \times HR$) for each beat. The beat-to-beat data series were interpolated to 5Hz using a cubic spline for the pressure values and a zero-order hold method for peripheral resistance (Chapter 5).

Data Analysis

The interpolated data recordings were low-pass filtered to emphasise the low-frequency response of the sympathetic system. A second order Butterworth filter with a corner frequency of 0.25Hz was applied in the forward and reverse directions. The cross-correlation was determined between both systolic and diastolic blood pressures and peripheral resistance.

24. Demographic data and detailed protocol are the same as for the peripheral BRS analysis in the next experimental section.

A positive lag indicates that peripheral resistance leads pressure changes and a negative lag indicates that pressure leads resistance.

The mean time delay was applied as a compensatory time shift between blood pressure and PR for a subset of the data and the coherence was compared for the original synchronised time series and the lag-corrected data. Mean coherence values over the low-frequency band (0.04-0.15Hz) were compared using Student's paired t-test.

Results

Figure 8-2 shows the individual cross-correlation functions for all 57 subjects for systolic pressure and peripheral resistance. Although a few subjects had widely diverging function morphologies, most were a fairly consistent shape for both systolic and diastolic pressure. The mean cross-correlation functions for systolic and diastolic pressure are shown in Figure 8-3 and Figure 8-4. There is a strong positive peak at around zero and a positive relationship at positive values of time lag. This indicates that increases in peripheral resistance coincide with increases in arterial pressure, as expected from the immediate feedforward effect of PR on BP. The positive values at positive lags indicates that the effect of PR on pressure is relatively long-lasting.

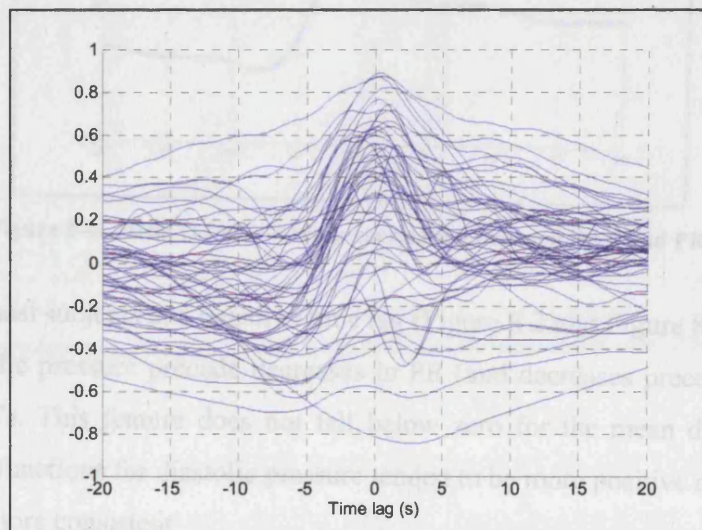


Figure 8-2: Individual cross-correlation functions between SAP and PR for 57 healthy control subjects

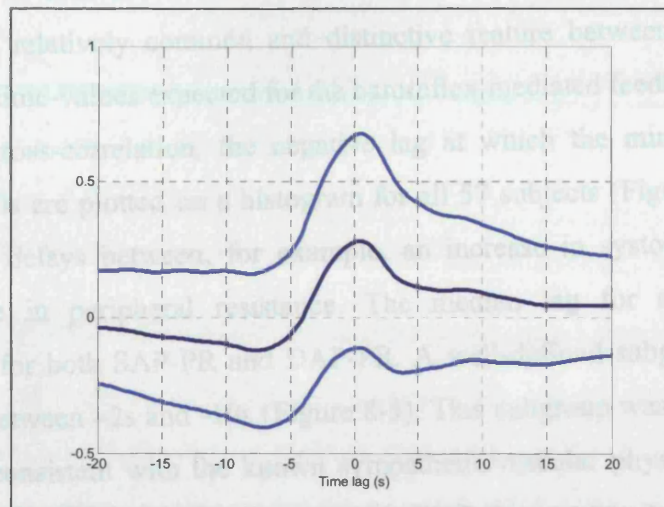


Figure 8-3: Mean (± 1 stdev) cross-correlation between SAP and PR

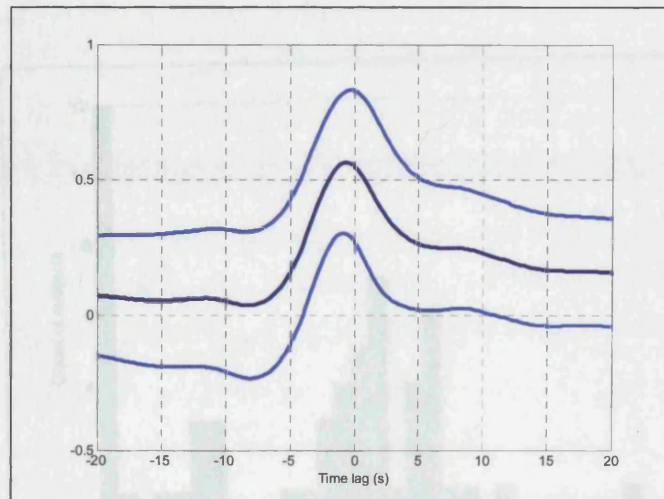


Figure 8-4: Mean (± 1 stdev) cross-correlation between DAP and PR

The minima for most subjects at a negative time lag (Figure 8-2 and Figure 8-3) indicates that increases in systolic pressure precede decreases in PR (and decreases precede increases) by approximately 6-7s. This feature does not fall below zero for the mean diastolic response (Figure 8-4). The functions for diastolic pressure tended to be more positive at all lags and the central peak was more consistent.

The statistical significance of these features was tested in a similar manner to that for the coherence threshold (Chapter 5). The cross-correlation was determined for one thousand pairs of random data series. The values were normally distributed, therefore two standard deviations were used to determine the 95% confidence limits of -0.20 to $+0.20$ at 0 s and -0.19 to 0.19 at -7 s. The mean central peak at zero lag falls outside this range for both systolic and diastolic blood pressure. The mean and standard deviations of the trough at approximately -7 s

fall within the 95% confidence interval and therefore are not statistically significant. However, this is a relatively common and distinctive feature between subjects, which is consistent with the time-values expected for the baroreflex-mediated feedback of BP on PR.

For the SAP-PR cross-correlation, the negative lag at which the minimum value occurs between -15 s and 0 s are plotted on a histogram for all 57 subjects (Figure 8-5). This shows the distribution of delays between, for example, an increase in systolic pressure and the following decrease in peripheral resistance. The median lag for all 57 subjects was approximately -8 s for both SAP-PR and DAP-PR. A well-defined subgroup can be seen to have a minimum between -2 s and -10 s (Figure 8-5). This subgroup was selected as having a distinctive delay, consistent with the known sympathetic/vascular physiology. For SAP, 35 out of 57 subjects fell into this subgroup, and for DAP the number was 31 out of 57. The median lag was recalculated for these subgroups and was found to be 7 s.

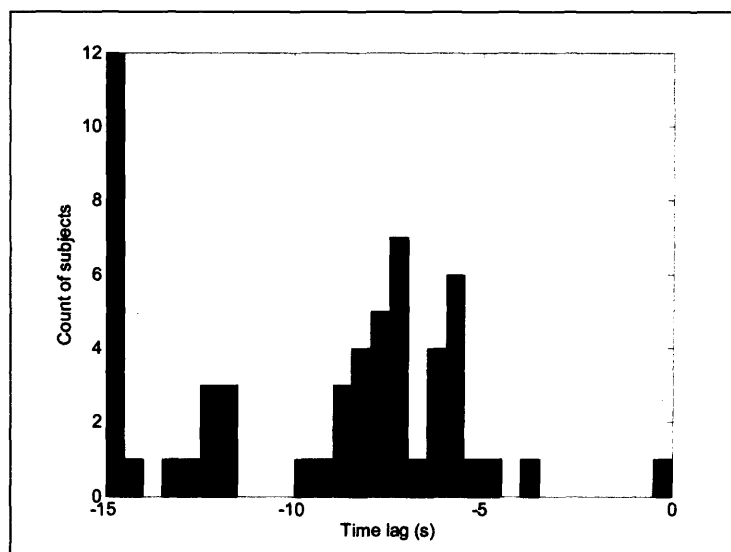


Figure 8-5: Histogram of time lags for the negative trough between systolic blood pressure and peripheral resistance. The subjects with values between -2 s and -10 s had the median lag applied as a correction.

This 7 s lag was applied as a correction to these subgroups of 35 and 31 subjects by removing the last 7 s from the blood pressure and the first 7 s from the peripheral resistance data. The cross-spectra were calculated between pressure and peripheral resistance and the mean and maximum coherence in the low frequency band (LF, 0.04 – 0.15 Hz) was determined for each subject. These coherence values were compared to the values determined before the 7 s correction.

The DAP-PR coherence was generally greater than SAP-PR coherence. Out of the original 57 subjects this was true for 48 for the mean LF coherence, and for 44 subjects for the maximum

coherence. Applying the lag correction to the subgroup data resulted in a decrease in coherence for almost all subjects. Mean coherence was decreased in 31 subjects and maximum coherence in 30 subjects out of 35 for systolic pressure. For diastolic pressure, the decreases were for 31 (mean) and 30 (maximum) out of 31 subjects. Mean SAP-PR coherence decreased from 0.44 to 0.37 and mean DAP-PR from 0.60 to 0.49. These differences were highly significant ($p < 0.001$).

Discussion

The morphology of the cross-correlation function between arterial pressure and peripheral resistance was consistent with the physiology and time delays involved in baroreflex-mediated sympathetic control of the vasculature. Most subjects displayed a reasonably well-defined minima at a negative lag, indicating that changes in systolic pressure preceded dissimilar changes in peripheral resistance by around 2-10s. The reason for the wide variety of response morphologies may be due to VLF variations in some subjects. Band-pass filtering for the LF range may have improved the consistency of the response shape. By using only the subgroup of subjects with well-defined delays, the application of the 7s time shift should have produced the most effective compensation for slow sympathetic control. However, the strength of the linear relationship between pressure and peripheral resistance, as measured by the ordinary coherence over the LF band, was found to be reduced by this pre-processing.

The imposition of a compensatory time shift into the cross-spectral analysis should not affect the strength of the linear relationship unless it significantly impairs the SNR. The hypothesis was that a compensatory time shift would remove uncorrelated data from the spectral analysis, thus improving the SNR and the coherence estimates. In fact the opposite result was found, indicating that useful information was lost by this 'compensation'. This does not necessarily indicate that the response of the vasculature is faster than 7s, but probably indicates that the effect on peripheral resistance is produced gradually and cannot be described as a pure time delay. It may also be due to the statistical non-significance of the minima. A positive outcome of the correction may have been achieved if it had been applied only to those subjects where the magnitude of the minimum exceeded the 95% confidence limits for zero correlation. However, these results indicate that this is not a necessary pre-processing procedure that should be applied to all subjects prior to calculating cross-spectral parameters between pressure and resistance.

A temporal correction for muscle sympathetic activity is usually applied to allow for the efferent conduction time when comparing MSA with other physiological variables – around

1.3s for the peroneal nerve (Sundlöf and Wallin, 1978). It could be considered that a compensatory time shift of this order would be more appropriate to correct for this pure delay in the peripheral resistance response. However, there are several reasons why this would be unnecessary:

- a delay of 1-2s represents a negligible proportion of the data subrecords used in the cross-spectral analysis – less than 4% of the duration used in this analysis
- the delay between pressure stimulus and vascular response will be variable depending on the distance of the vessel from the brain - the total vascular response may therefore be a cumulative effect
- a tapering window is applied to the data in each subrecord, therefore the first and last few samples contribute less information to the cross-spectra

A compensatory time shift is therefore determined to be unnecessary and detrimental to the cross-spectral analysis of peripheral resistance.

Peripheral BRS Estimates

When considering how to determine peripheral resistance BRS it is unclear which pressure parameter would provide the most suitable measure of the input stimulus. For cardiac chronotropic BRS it is systolic pressure that is most often taken to be the input. This is due to the greater coherence between systolic pressure and RR interval than for diastolic pressure, but may also be partly because the baroreceptors are known to respond more strongly to larger and more rapid changes. Peripheral resistance itself is calculated as the ratio between mean arterial pressure and cardiac output²⁵. However, the active control of vessel calibre is affected by the sympathetic nervous system, and muscle sympathetic activity has been shown to be most strongly linked to diastolic pressure. There appears to be no overwhelming *a priori* reason to choose one pressure variable over another.

Spontaneous methods for cardiac BRS determination were modified in order to investigate the feasibility of calculating peripheral baroreceptor sensitivity as a clinical indicator. Subjects with recurrent neurocardiogenic syncope (NCS) were used to assess the methods on the basis that such patients exhibit poor BP control. The pathophysiology of NCS is still contentious. Syncope is preceded by bradycardia and vasodilation (vasovagal reaction), although the bradycardia is not necessary for the development of hypotension or pre-syncope symptoms

25. Strictly speaking, it is the difference between MAP and central venous pressure or right atrial filling pressure that provides the driving pressure.

(Dietz *et al*, 1997; van Lieshout *et al*, 1997). Cardiac BRS in this patient group has been variously found to be reduced or comparable to control subjects depending on subject selection and experimental method (Morillo *et al*, 1997; Diehl *et al*, 1999; Mosqueda-Garcia *et al*, 2000; Flevvari *et al*, 2002). Resting MSA may be greater in predisposed subjects (Béchir *et al*, 2002), but there is consensus that the increases in MSA with orthostatic challenge are attenuated and that syncope is preceded by a dramatic withdrawal of efferent activity (Dietz *et al*, 1997; Morillo *et al*, 1997; Wieling *et al*, 1997; Béchir *et al*, 2002). Markers of vascular control also present mixed results (Thomson *et al*, 1995; Flevvari *et al*, 2002; Stewart and Weldon, 2003), although peripheral mechanisms are thought to be responsible for the orthostatic intolerance (Quan *et al*, 1997).

Methods

Subjects

Thirty-one adult patients were recruited from an outpatient syncope clinic. They were referred for recurrent syncope and diagnosed with vasodepressor neurocardiogenic syncope. Fifty-eight healthy adult controls were recruited from volunteers. Both groups were part of a separate investigation into cerebral autoregulation (Carey, 2001; Carey *et al*, 2001) and therefore underwent several tests of autonomic function that were not relevant to this study.

Protocol

Subjects were studied in a temperature-controlled room (approximately 21-24°C), at least two hours after breakfast and were asked to refrain from caffeine, alcohol and smoking for the previous 12hr. Subjects lay supine on a manually-controlled tilt table with a foot support and were allowed to acclimatise to the position for at least ten minutes, following which 10min of resting data were recorded. The subjects then underwent a 30min tilt test protocol. The subject was raised to a 70° tilt position within 3s and remained in this position for 30min or until presyncope. Five minutes of supine data were included before tilt-up and following tilt-back.

Instrumentation

Non-invasive finger blood pressure (Finapres) was measured in the non-dominant arm which was supported on an arm rest during the supine measurements. The lead II signal was recorded from a three-electrode ECG. Subjects were also instrumented to measure bilateral transcranial Doppler (QVL 842X, SciMed) and transcutaneous pCO₂ (Tina, Radiometer). A voltage spike to indicate the time-point of tilt and tilt-back was superimposed on the transcutaneous pCO₂ signal to allow synchronisation of the recordings. Additionally, subjects

wore a respiration mask during the tilt-test for the measurement of end-tidal CO₂. This was removed five minutes after tilt-up.

A shelf was attached to the tilt-table which provided security for the subjects during the tilt-up manoeuvre and allowed the arm with the Finapres to be rested at heart level in the upright posture. All signals were recorded simultaneously onto DAT. The rest and tilt recording were preceded with a calibration signal for the Finapres and the set-point mechanism for this device (Physiocal) was switched off during data recording.

Data Analysis

The signals were resampled onto a PC from DAT at 200Hz. Signal processing and spectral analysis were as described in Chapter 5 and pulse contour stroke volume was determined as described in Chapter 6.

Data for the tilt analysis were selected from the cessation of end-expiratory CO₂ measurement, five minutes after tilt-up. This was because the mask provided a significant resistance to air flow and is likely to have provoked a breathing pattern that was different to the rest of the recording. This also allowed adequate time for subjects to reach a steady cardiovascular state in the upright position (see Chapter 2)..Due to computational constraints, only 25min of data could be transferred onto PC at one time. The tilt analysis therefore comprised a maximum of 15min of data unless tilt-back occurred sooner. For those subjects who became pre-syncopal, the longest stationary section of tilt data following the removal of the respiratory mask was used.

Uncalibrated peripheral resistance was normalised to the mean for each record so that the pBRS estimates therefore have units of %/mmHg. The low frequency (LF) band was defined as 0.04-0.15Hz and the high frequency (HF) as 0.15-0.4Hz.

BRS Estimation

Spontaneous cardiac chronotropic (cBRS) and peripheral baroreceptor sensitivity (pBRS) were assessed in both the supine and tilted postures using alpha ratio, transfer function and sequence methods, as summarised in Table 8-1. Detailed information about the cardiac methods is given in Chapter 4. Peripheral estimates were determined for both systolic and diastolic input pressures. The coherence was determined for each record and spectral BRS estimates were only computed where this exceeded the non-zero threshold determined by the number of subrecords generated (Figure 5-4).

Table 8-1: Summary of cardiac and peripheral BRS estimates determined

Method	Cardiac	Peripheral §
Alpha	$\alpha = \frac{\sqrt{P_{RR}}}{\sqrt{P_{SAP}}} \text{ (LF, HF, mean)}$	$\alpha = \frac{\sqrt{P_{PR}}}{\sqrt{P_{BP}}} \text{ (LF)}$
Transfer function	$gain = \frac{ P_{SAP-RR} }{P_{SAP}} \text{ (LF, HF, mean)}$	$gain = \frac{ P_{BP-PR} }{P_{BP}} \text{ (LF)}$
Sequence	Parallel sequences of RR and SAP with a 1 beat delay	Opposing sequences of PR and BP with a 2-10s delay

P – autospectral or cross-spectral density

§ Where BP can be either SAP or DAP

Alpha Method

The cardiac alpha estimate was determined from the ratio of the square-roots of the RR interval and SAP spectral power over each of the LF and HF bands separately and as the mean of these. The peripheral estimate was taken as the ratio of the square-root of the PR power to the SAP or DAP power over the LF band only.

Transfer Function Method

The cardiac transfer function estimate was determined as the mean gain of the SAP-PR cross-spectrum divided by the autospectrum of the systolic pressure over each of the LF, HF and combined bands. The peripheral estimate was the mean gain between PR and SAP or DAP divided by the pressure autospectrum over the LF band only.

Sequence Method

The cardiac sequence method used spontaneous parallel sequences of RR interval and systolic pressure of at least three beats in length and with a 1 beat delay (i.e. the interval sequence starts a beat later than the systolic pressure sequence). Two consecutive identical RR intervals (i.e. a gradient of zero) were allowed for sequences longer than three beats. No minimum change in RR interval or SAP was defined. Estimates were obtained separately for up-pressure, down-pressure and all baroreflex slopes combined.

For the peripheral BRS estimate, sequences of opposing direction were determined whereby the PR sequence began within 2-10s of the start of the pressure sequence. All sequences were a minimum of three beats long but were not required to be of equal length, as the peripheral response was considered to be slowly reacting. Unequal length sequences were interpolated to have the same number of points before linear regression analysis was carried out.

For both cardiac and peripheral sequence BRS, linear regression was applied to each sequence pair and only those with a p -value of 0.05 or less were accepted. A minimum of

three statistically significant slopes were required and the median slope was used as the estimate for each subject²⁶. As the pressure and peripheral resistance sequences were opposite in direction, the gradients determined were negative but the sign was omitted to maintain consistency with the spectral estimates.

The peripheral sequence estimates were calculated using the raw beat-to-beat data and also following low-pass filtering. To achieve this, the 5Hz interpolated data were filtered in a forward and reverse direction using a second order Butterworth filter with a corner frequency of 0.25Hz. This produced a 50% amplitude decrease at 0.25Hz, so that the active sympathetic control of the resistance at frequencies below 0.2Hz would be emphasised and the 'artifactual' high frequency variations in peripheral resistance would be reduced. The low-pass filtered data were then re-interpolated to the original time points.

Statistical Analysis

Demographic data were tested for differences using Student's t-test. The subjects were classified into four 'independent groups' depending on the outcome of the tilt test (control non-fainters, control fainters, NCS non-fainters and NCS fainters) and two sets of 'pooled groups' (controls vs. patients and non-fainters vs. fainters). As BRS values are not normally distributed the differences in BRS between the four independent groups were tested using the Kruskal-Wallis variation of ANOVA for ranked data. The pooled groups were tested using the Wilcoxon rank sum test. The Wilcoxon signed rank test was used for paired BRS comparisons between tilt and supine data. BRS values are presented as the median and interquartile range (IQR), and using box and whisker plots. Statistical analysis was carried out using MATLAB and MS Excel.

The purpose of the investigation is to examine the properties of peripheral BRS rather than to determine the differences between the subject groups. As pBRS has not previously been estimated for different subject populations this can be considered to be a pilot study. Multiple comparisons between subject groups were carried out. The *p*-values for these are given on the tables and no threshold for statistical significance was used for these cases. Instead the *p*-values are used to indicate the degree of separation of the results. See Discussion section for further details.

26. BRS slopes are skewed towards zero and the median is relatively impervious to occasional outliers compared with the mean.

Results

Subjects

One non-fainting control subject was found to have significant periods of bigeminy during all recordings and was excluded from the analysis. Data from 57 control subjects and 31 NCS patients were available for analysis for the rest recordings. Of these, 45 healthy controls and 15 patients lasted the full duration of the tilt without presyncopal symptoms or a significant drop in blood pressure. On this basis subjects were also classified as 'fainting' and 'non-fainting'. Only three subjects (all in the control group) were considered to have too many ectopic beats in their tilt recordings for the data to be useful. Technical or protocol errors resulted in three other tilt data sets being discarded (2 NCS and 1 control subjects). In the fainting subjects, 13 (4 controls and 9 patients) did not have sufficiently long stationary data sections following the mask removal to allow analysis of the tilt data. Therefore, there were 50 control subjects and 20 NCS patients for whom both rest and tilt data could be compared. The demographic data for these subjects is summarised in Table 8-2. There were no significant differences between control and patient groups for age, systolic or diastolic pressures for either of the analysis groups.

Table 8-2: Demographic data for subject groups (mean and range)

	Supine		Tilt	
	Controls	Patients	Controls	Patients
Number	57	31	50	20
Gender (M:F)	29:28	11:20	25:25	6:14
Age (yrs)	46.7 (20-90)	50.8 (18-86)	45.7 (20-79)	50.3 (18-86)
SAP (mmHg)	130.2 (103-166)	134.2 (94-217)	130.0 (103-166)	133.7 (94-217)
DAP (mmHg)	79.3 (61-103)	77.9 (60-100)	79.1 (61-103)	79.0 (62-100)
No. of fainters	12 (21%)	16 (52%)	8 (16%)	7 (35%)

Rest Results - Cardiac BRS

Spectral analysis of the ten minute recordings produced 7 subrecords so that the coherence threshold was 0.11 (Figure 5-4). Out of all 88 subjects, only one subject (NCS, fainter) did not have mean coherence values for either LF or HF bands above this threshold. This subject had remarkably little heart rate variability, with a maximum variation over the ten minute recording of only 2bpm. Another subject (NCS, non-fainter) did not reach this threshold for the HF band only.

Table 8-3 and Table 8-4 show the group results for the cardiac BRS estimates in the supine position. There was no difference between any independent or pooled groups for any method of estimating cardiac chronotropic BRS ($p \gg 0.05$). The HF spectral estimates significantly overestimate the LF estimates ($p \ll 0.01$), and the alpha estimates are greater than those using the transfer function method. There was no difference between up and down-slope sequence estimates. Table 8-3 and Table 8-4 also show the number of results for each method, n , for each subject group. The numbers for the sequence estimates indicate how many subjects produced at least three statistically significant parallel sequences. All cBRS estimates decreased with increasing age and with increasing systolic blood pressure (linear regression, $p \ll 0.001$, Figure 8-6 and Figure 8-7).

**Table 8-3: Cardiac BRS estimates for independent subject groups, supine data
(median and IQR, ms/mmHg)**

Method		Control Group				NCS Patient Group				p
		NF	n (45)	F	n (12)	NF	n (15)	F	n (16)	
Alpha	LF	7.6 4.2 - 10.2	45	8.4 4.3 - 12.4	12	8.7 4.8 - 14.6	15	6.8 5.4 - 13.8	15	0.620
	HF	12.0 9.1 - 24.8	45	13.0 9.3 - 15.6	12	16.8 7.5 - 32.0	14	13.1 10.2 - 16.3	15	0.828
	Mean	10.4 6.3 - 16.4	45	9.9 6.9 - 14.1	12	13.2 5.7 - 21.4	15	9.3 8.2 - 15.0	15	0.952
TF	LF	5.1 2.7 - 9.2	45	7.6 2.7 - 12.5	12	6.5 4.0 - 11.7	15	5.4 3.3 - 9.0	15	0.702
	HF	9.4 6.4 - 18.3	45	8.5 7.5 - 12.4	12	12.3 4.8 - 25.2	14	9.9 5.0 - 11.5	15	0.612
	Mean	8.1 5.5 - 15.1	45	7.2 5.8 - 11.7	12	12.0 3.7 - 19.0	15	7.7 4.7 - 11.9	15	0.781
Sequence	Up	9.3 7.7 - 15.8	33	8.6 5.2 - 12.4	10	16.0 5.2 - 24.1	13	7.4 5.8 - 8.7	11	0.340
	Down	11.4 6.7 - 18.5	36	8.9 6.0 - 17.8	8	19.1 10.0 - 29.1	11	10.5 9.9 - 21.7	9	0.634
	All	10.1 7.0 - 17.4	40	9.1 5.2 - 15.0	10	19.9 5.2 - 25.2	15	8.8 8.0 - 13.9	14	0.612

NF - non-fainter, F - fainter

Table 8-4: Cardiac BRS estimates for pooled subject groups, supine data

Method		Controls vs. NCS Patients					Non-Fainters vs. Fainters				
		Control	n (57)	NCS	n (31)	p	NF	n (60)	F	n (28)	p
Alpha	LF	7.8 4.3 - 10.4	57	7.4 4.3 - 10.7	30	0.212	7.7 4.3 - 10.7	60	7.2 5.0 - 13.8	27	0.466
	HF	12.3 9.0 - 24.2	57	14.5 8.3 - 18.6	29	0.985	13.6 8.6 - 25.6	59	13.1 10.0 - 16.3	27	0.457
	Mean	10.2 6.5 - 16.4	57	10.3 6.6 - 17.1	30	0.848	10.5 6.3 - 17.4	60	9.6 7.6 - 15.0	27	0.801
TF	LF	5.1 2.7 - 9.3	57	5.7 3.3 - 11.7	30	0.553	5.3 2.7 - 9.7	60	5.6 2.9 - 12.0	27	0.837
	HF	9.0 6.4 - 18.0	57	10.2 4.4 - 14.4	29	0.756	9.7 6.2 - 19.7	59	8.9 6.1 - 12.0	27	0.268
	Mean	7.8 5.4 - 14.9	57	8.6 4.6 - 12.5	30	0.691	8.9 5.1 - 15.6	60	7.6 5.0 - 11.9	27	0.406
Sequence	Up	9.1 6.8 - 15.7	43	7.7 5.4 - 17.7	24	0.392	10.0 6.7 - 20.9	46	7.4 5.5 - 11.9	21	0.087
	Down	11.1 6.6 - 18.5	44	12.4 9.7 - 26.2	20	0.422	11.5 7.1 - 22.4	47	10.5 6.3 - 21.7	17	0.494
	All	10.1 5.8 - 16.6	50	9.6 6.7 - 21.5	29	0.883	10.3 6.3 - 21.7	55	8.8 5.4 - 15.2	24	0.239

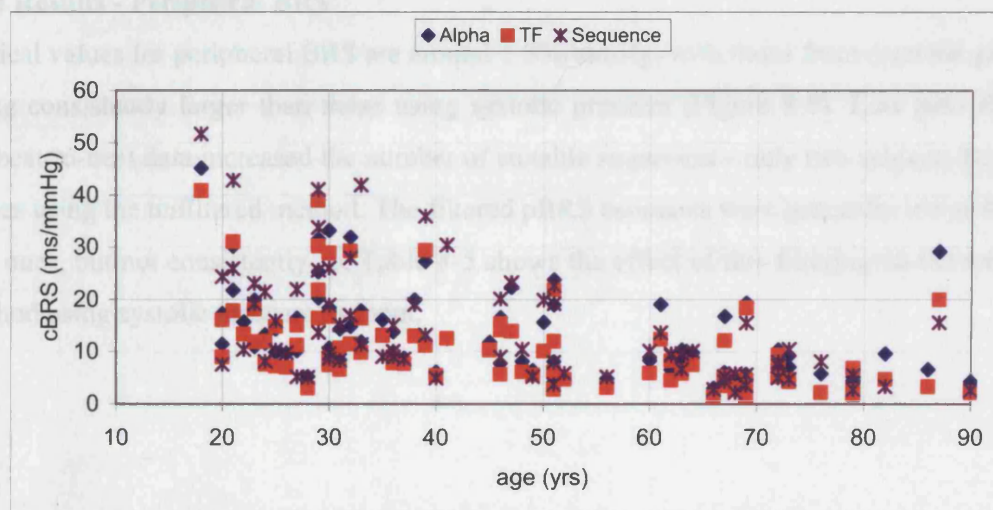


Figure 8-6: Variation of cBRS with age for all 88 subjects, supine data

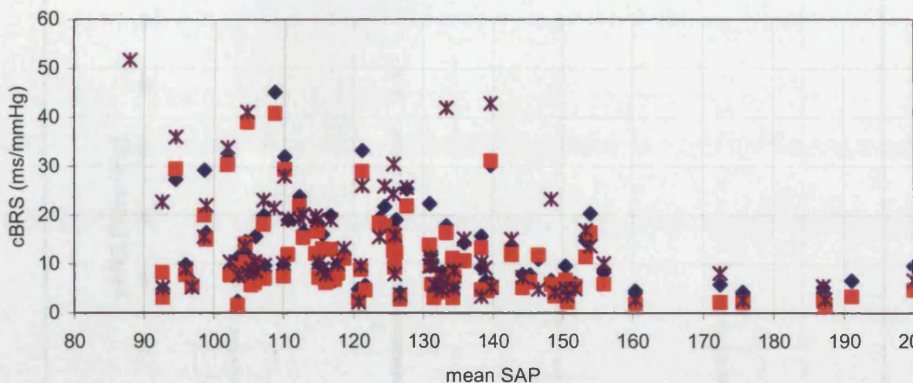


Figure 8-7: Variation of cBRS with mean resting SAP for all 88 subjects, supine data

Rest Results - Peripheral BRS

Typical values for peripheral BRS are around 1-3%/mmHg, with those from diastolic pressure being consistently larger than those using systolic pressure (Figure 8-8). Low pass filtering the beat-to-beat data increased the number of suitable sequences - only two subjects had more slopes using the unfiltered method. The filtered pBRS estimates were generally lower than the raw ones, but not consistently so. Table 8-5 shows the effect of this filtering on the sequence method using systolic pressure as input.

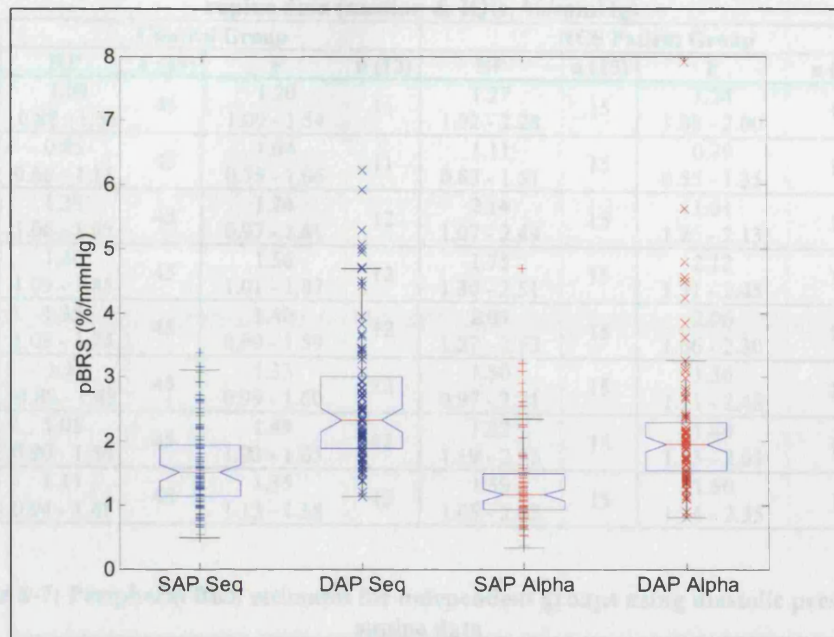


Figure 8-8: Difference between pBRS estimates using systolic and diastolic pressures as input. Boxes show IQR and median values (red horizontal line), whiskers define 1.5x IQR, notches indicate uncertainty of the median.

Table 8-5: Effect of low-pass filtering on the sequence estimate of pBRS, supine data, SAP as input pressure

	Mean no. of suitable slopes per subject		Combined-slope pBRS (%/mmHg)		No. of subjects with lower filtered estimate
	Filtered	Raw	Filtered	Raw	
Healthy controls	97	67	1.12	1.36	36 / 57
NCS patients	93	61	1.57	1.93	17 / 31
Non-fainters	99	67	1.13	1.42	38 / 60
Fainters	88	61	1.41	1.44	15 / 28

Table 8-6 and Table 8-7 give the peripheral BRS estimates for the independent groups and Table 8-8 and Table 8-9 for the pooled groups, using systolic and diastolic pressure as input respectively. There were many more sequences for peripheral BRS than there were for cardiac BRS, as a result of which there were no subjects for whom a sequence estimate of pBRS could not be determined. For pBRS there was a missing peripheral spectral estimate for only one subject (control, fainter), using SAP as input (Table 8-6 and Table 8-7). Compare this with Table 8-4, where cardiac sequence estimates were unavailable for 21 subjects for the up-pressure slopes, 24 for the down-pressure slopes and 9 for the combined slopes.

the sequence estimate for between 7 and 14 out of 57 control subjects, and between 4 and 11 out of 28 fainters.

Table 8-6: Peripheral BRS estimates for independent groups using systolic pressure, supine data (median & IQR, %/mmHg)

Method		Control Group				NCS Patient Group				P
		NF	n (45)	F	n (12)	NF	n (15)	F	n (16)	
Alpha LF		1.09 0.88 - 1.35	45	1.20 1.09 - 1.54	11	1.27 1.02 - 2.28	15	1.24 1.08 - 2.00	16	0.108
TF LF		0.85 0.66 - 1.13	45	1.04 0.75 - 1.06	11	1.11 0.83 - 1.51	15	0.79 0.55 - 1.35	16	0.323
Sequence	Up	1.38 1.06 - 1.65	45	1.24 0.97 - 1.61	12	2.14 1.07 - 2.49	15	1.61 1.26 - 2.13	16	0.117
	Down	1.40 1.09 - 1.85	45	1.56 1.01 - 1.87	12	1.75 1.36 - 2.51	15	2.12 1.71 - 2.45	16	0.014
	All	1.35 1.09 - 1.74	45	1.40 0.99 - 1.59	12	2.07 1.27 - 2.52	15	2.06 1.66 - 2.30	16	0.008
Filtered sequence	Up	1.11 0.89 - 1.48	45	1.33 0.99 - 1.60	12	1.50 0.97 - 2.21	15	1.36 1.11 - 2.48	16	0.434
	Down	1.08 0.90 - 1.56	45	1.49 1.23 - 1.63	12	1.52 1.19 - 2.35	15	1.44 1.13 - 2.09	16	0.127
	All	1.11 0.94 - 1.48	45	1.35 1.13 - 1.58	12	1.59 1.08 - 2.02	15	1.50 1.14 - 2.35	16	0.128

Table 8-7: Peripheral BRS estimates for independent groups using diastolic pressure, supine data

Method		Control Group				NCS Patient Group				P
		NF	n (45)	F	n (12)	NF	n (15)	F	n (16)	
Alpha LF		1.88 1.46 - 2.13	45	2.02 1.68 - 2.22	12	2.00 1.73 - 2.96	15	1.98 1.73 - 2.97	16	0.297
TF LF		1.51 1.18 - 1.78	45	1.64 1.42 - 2.02	12	1.61 1.28 - 1.97	15	1.40 1.04 - 2.48	16	0.544
Sequence	Up	2.10 1.69 - 2.56	45	2.26 1.77 - 3.19	12	2.45 1.79 - 2.79	15	3.34 2.23 - 4.17	16	0.023
	Down	2.45 1.81 - 2.90	45	2.29 1.99 - 3.40	12	2.94 2.02 - 3.17	15	3.19 2.08 - 4.91	16	0.170
	All	2.11 1.80 - 2.74	45	2.46 1.87 - 2.73	12	2.69 1.94 - 2.82	15	3.24 2.18 - 4.77	16	0.052
Filtered sequence	Up	1.78 1.41 - 2.12	45	1.90 1.33 - 2.31	12	1.82 1.46 - 2.60	15	2.20 1.72 - 3.09	16	0.460
	Down	1.92 1.37 - 2.19	45	1.79 1.53 - 2.39	12	2.11 1.53 - 2.90	15	2.25 1.80 - 3.28	16	0.238
	All	1.80 1.56 - 2.13	45	1.85 1.53 - 2.29	12	2.10 1.51 - 2.80	15	2.31 1.76 - 3.39	16	0.237

The most significant difference between the independent groups achieved were found for the unfiltered sequence method: down-pressure and combined slopes using SAP (Table 8-6 and Figure 8-9) and up-pressure slopes using DAP (Table 8-7). For the pooled groups, with the exception of the transfer function method, peripheral BRS values were consistently higher for NCS patients than for healthy controls. This difference was statistically significant for six out of eight methods using SAP (Table 8-8). The unfiltered sequence estimates using DAP were also significantly larger for NCS patients for up-pressure and combined slopes (down-pressure slope $p = 0.051$), whereas the filtered sequence was only significant for the down-pressure slopes (Table 8-9).

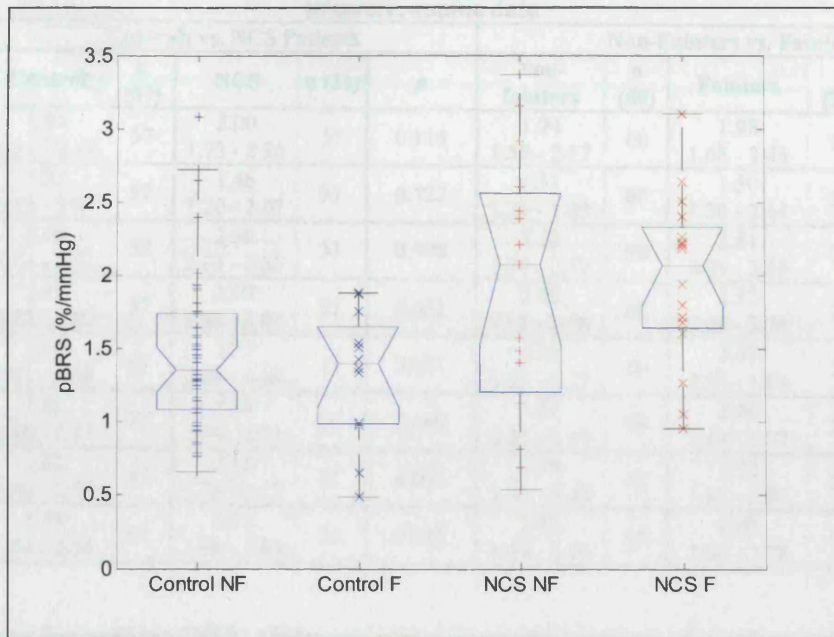


Figure 8-9: Combined-slope sequence pBRS showing significant differences between the independent groups, supine data, SAP as input pressure ($p = 0.008$, F - fainter, NF - non-fainter)

Table 8-8: Peripheral BRS estimates for the pooled data using SAP as the input pressure, supine data

Method		Controls vs. NCS Patients				Non-Fainters vs. Fainters					
		Control	n (57)	NCS	n (31)	p	NF	n (60)	F	n (28)	p
Alpha LF		1.13 0.89 - 1.35	56	1.27 1.06 - 2.28	31	0.027	1.13 0.91 - 1.46	60	1.20 1.08 - 1.75	27	0.116
TF LF		0.91 0.67 - 1.09	56	0.94 0.65 - 1.51	31	0.240	0.93 0.67 - 0.17	60	0.86 0.60 - 1.13	27	0.989
Sequence	Up	1.33 1.06 - 1.65	57	1.76 1.14 - 2.29	31	0.020	1.44 1.06 - 1.75	60	1.42 1.11 - 1.93	28	0.875
	Down	1.45 1.05 - 1.85	57	2.07 1.55 - 2.45	31	0.002	1.47 1.10 - 2.12	60	1.81 1.47 - 2.20	28	0.120
	All	1.36 1.06 - 1.74	57	2.07 1.45 - 2.42	31	0.001	1.42 1.12 - 1.90	60	1.70 1.32 - 2.19	28	0.221
Filtered sequence	Up	1.14 0.89 - 1.53	57	1.43 0.97 - 2.39	31	0.108	1.14 0.88 - 1.70	60	1.33 0.99 - 1.70	28	0.465
	Down	1.16 0.90 - 1.59	57	1.48 1.10 - 2.11	31	0.044	1.19 0.90 - 1.64	60	1.46 1.20 - 1.93	28	0.164
	All	1.12 0.98 - 1.58	57	1.57 1.13 - 2.25	31	0.025	1.13 0.95 - 1.72	60	1.41 1.14 - 1.85	28	0.177

Table 8-9: Peripheral BRS estimates for the pooled groups using DAP as the input pressure, supine data

Method		Controls vs. NCS Patients					Non-Fainters vs. Fainters				
		Control	n (57)	NCS	n (31)	<i>p</i>	Non-fainters	n (60)	Fainters	n (28)	<i>p</i>
Alpha LF		1.94 1.51 - 2.14	57	2.00 1.73 - 2.96	31	0.116	1.94 1.50 - 2.17	60	1.98 1.68 - 2.46	28	0.230
TF LF		1.51 1.25 - 1.81	57	1.46 1.20 - 2.07	31	0.727	1.51 1.21 - 1.83	60	1.50 1.26 - 2.14	28	0.539
Sequence	Up	2.11 1.71 - 2.58	57	2.59 2.03 - 3.85	31	0.008	2.12 1.70 - 2.59	60	2.81 2.05 - 3.58	28	0.027
	Down	2.41 1.83 - 3.07	57	2.99 2.06 - 4.01	31	0.051	2.46 1.83 - 3.09	60	2.92 2.02 - 3.74	28	0.110
	All	2.16 1.82 - 2.74	57	2.73 2.08 - 4.08	31	0.013	2.18 1.83 - 2.77	60	2.47 2.09 - 3.64	28	0.066
Filtered sequence	Up	1.80 1.40 - 2.12	57	2.10 1.49 - 2.71	31	0.160	1.81 1.45 - 2.19	60	2.00 1.49 - 2.77	28	0.327
	Down	1.82 1.46 - 2.20	57	2.11 1.74 - 3.17	31	0.047	1.98 1.36 - 2.35	60	2.02 1.62 - 2.87	28	0.276
	All	1.84 1.54 - 2.14	57	2.11 1.69 - 3.03	31	0.062	1.82 1.54 - 2.24	60	2.03 1.65 - 2.78	28	0.199

As was the case for cardiac BRS, alpha estimates exceeded transfer function estimates. Unlike cBRS the down-pressure slopes tended to be greater than the up-pressure slopes. This difference was significant for systolic input pressure for all 88 subjects (median of 1.57 vs. 1.47 %/mmHg, $p = 0.008$), but could be decomposed to show that there was no difference for non-fainters ($p = 0.293$), whereas for fainters the difference was highly significant (1.81 vs. 1.42 %/mmHg, $p = 0.0009$, Figure 8-10). For diastolic input pressure, the down-pressure slopes were also significantly higher for the entire sample (2.47 vs. 2.24 %/mmHg, $p = 0.005$). In contrast to the systolic results, this was due to non-fainters (2.46 vs. 2.12 %/mmHg, $p = 0.007$, Figure 8-10) rather than fainters ($p = 0.306$). The DAP down-slopes were also significantly greater for the healthy control subjects (2.41 vs. 2.10 %/mmHg, $p = 0.001$). There were no differences between up and down-pressure slopes for the low-pass filtered sequence method.

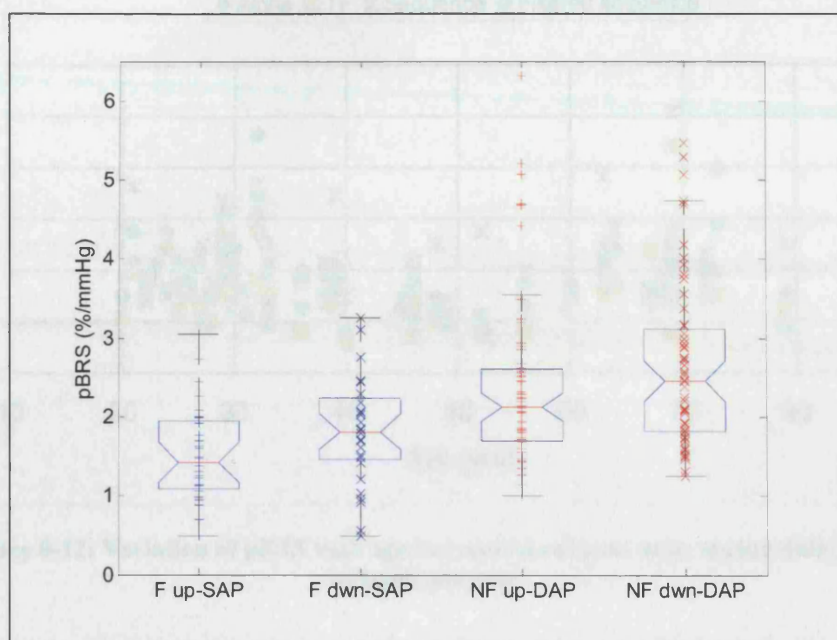


Figure 8-10: Differences between up and down-pressure slopes for sequence pBRS, supine data (F – fainters, NF – non-fainters)

There was no linear relationship between pBRS and either age or mean systolic pressure for the entire data range. However, there may be a tendency for pBRS to be higher in the oldest and youngest populations (Figure 8-11). This was consistent for all methods of estimating pBRS, for both systolic and diastolic input pressure, but the interpretation is complicated by the greater concentration of NCS patients at these age extremes. When only normal subjects are examined (Figure 8-12) there is still a suggestion of an increase at the extremes of age.

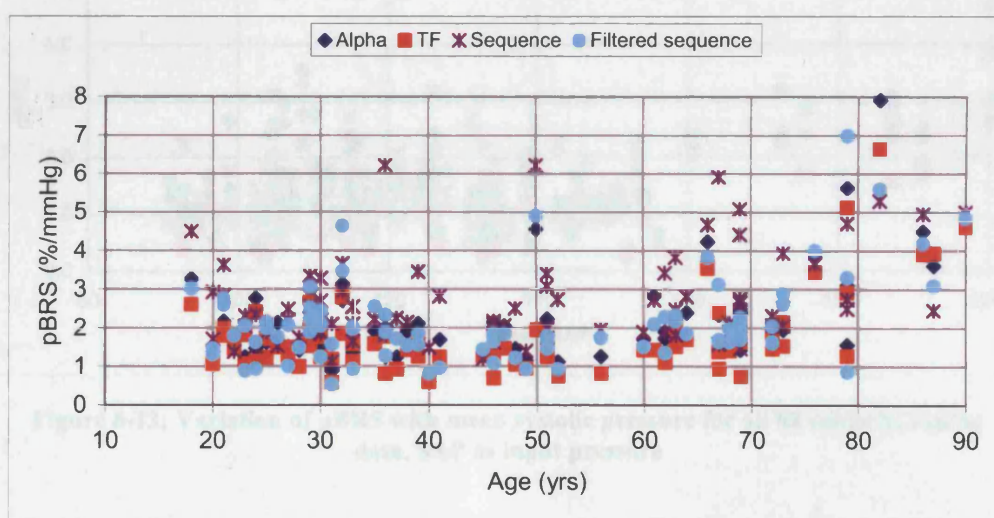


Figure 8-11: Variation of pBRS with age for all 88 subjects, supine data, DAP as input pressure

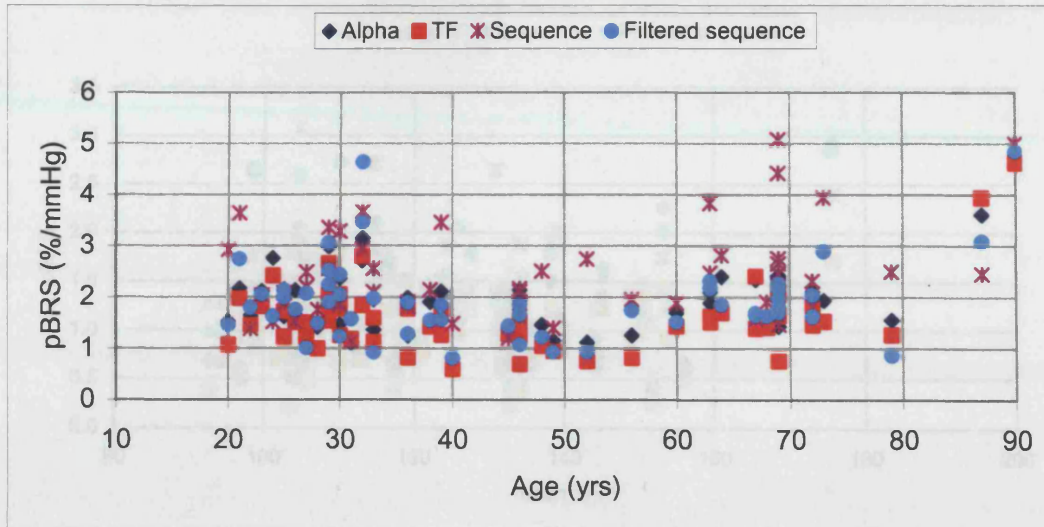


Figure 8-12: Variation of pBRS with age for control subjects only, supine data, DAP as input pressure

Peripheral BRS also appears to increase linearly with mean resting SAP above approximately 155mmHg, but is unrelated to pressure below this value (Figure 8-13). These patterns were visible for both systolic and diastolic input pressures and suggest that peripheral BRS would be increased in hypertensive subjects. If only control subjects are considered (Figure 8-14) there is only one subject in this pressure range.

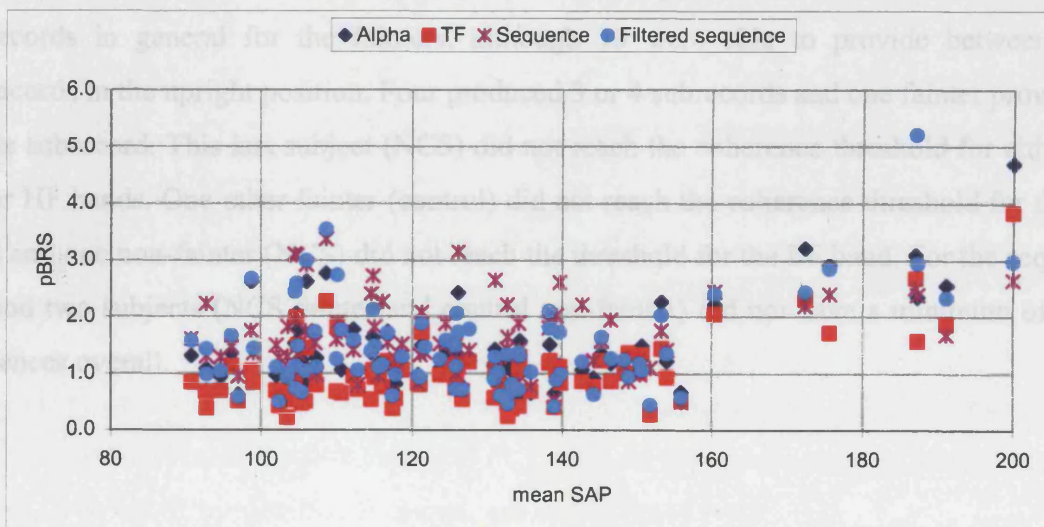


Figure 8-13: Variation of pBRS with mean systolic pressure for all 88 subjects, supine data, SAP as input pressure

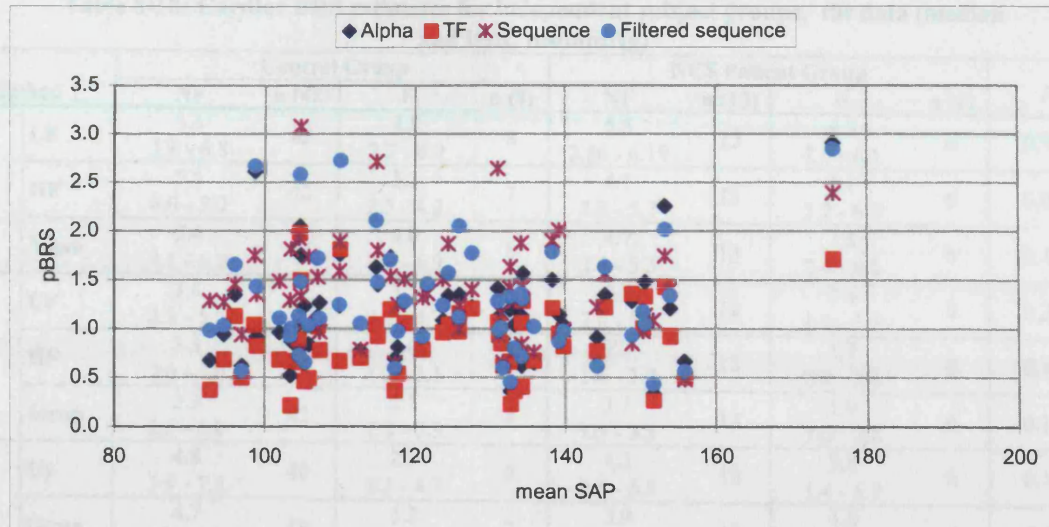


Figure 8-14: Variation of pBRS with mean resting systolic pressure for control subjects only, supine data, SAP as input pressure

Tilt Results - Cardiac BRS

Table 8-10 and Table 8-11 show the cardiac BRS estimates for 50 healthy controls and 20 NCS patients and the number of subjects for whom estimates were produced. There were 8 to 10 subrecords for all non-fainting subjects, except one. Due to technical problems with the Finapres, this single recording was truncated and only provided 5 subrecords. However, this subject still exceeded the coherence threshold for the spectral estimates. There were fewer subrecords in general for the fainters, although 10 were able to provide between 7-10 subrecords in the upright position. Four produced 3 or 4 subrecords and one fainter provided a single subrecord. This last subject (NCS) did not reach the coherence threshold for either the LF or HF bands. One other fainter (control) did not reach the coherence threshold for the HF band and one non-fainter (NCS) did not reach the threshold for the LF band. For the sequence method two subjects (NCS fainter and control non-fainter) did not have a minimum of three sequences overall.

An expected, cardiac BRS estimates were reduced in the upright position for all subjects. The difference between supine and tilt tended to be greater for NCS patients than healthy controls, this was most significant for the LF spectral estimates (Table 8-12).

Table 8-12: NCS patients have a greater difference in cardiac BRS (supine to tilt) than healthy controls for LF spectral estimates (p < 0.05, Wilcoxon, median & IQR)

	Control	NCS
LF Alpha	1.6 0.2 - 3.2	0.5 0.2 - 3.5
LF TF	1.2 0.4 - 3.5	0.6 0.1 - 3.3

Table 8-10: Cardiac BRS estimates for independent subject groups, tilt data (median and IQR, ms/mmHg)

Method		Control Group				NCS Patient Group				p
		NF	n (42)	F	n (8)	NF	n (13)	F	n (7)	
Alpha	LF	5.3 3.8 - 6.8	42	4.8 2.7 - 6.0	8	5.3 2.86 - 6.19	12	4.7 2.5 - 6.1	6	0.585
	HF	5.5 4.0 - 8.0	42	3.3 2.5 - 4.2	7	4.1 2.0 - 5.2	13	3.1 2.2 - 6.7	6	0.044
	Mean	5.4 4.1 - 6.8	42	4.6 3.8 - 6.9	8	4.7 2.4 - 5.3	13	3.8 2.4 - 6.6	6	0.169
TF	LF	3.8 2.5 - 5.5	42	3.5 2.2 - 4.7	8	3.8 2.0 - 5.0	12	2.4 1.5 - 3.1	6	0.221
	HF	3.3 2.0 - 5.0	42	2.0 1.3 - 3.1	7	2.7 1.6 - 3.2	13	1.7 0.8 - 2.6	6	0.128
	Mean	3.3 2.5 - 5.0	42	2.7 2.2 - 3.5	8	3.1 1.5 - 3.5	13	1.9 1.0 - 2.8	6	0.152
Sequence	Up	4.8 3.9 - 7.5	40	2.9 2.5 - 4.7	8	4.3 2.5 - 5.0	12	2.8 1.4 - 5.7	6	0.106
	Down	4.7 3.67 - 6.94	39	5.2 3.8 - 6.7	7	3.9 2.8 - 5.1	13	4.0 3.5 - 5.4	4	0.491
	All	4.7 3.6 - 6.6	41	3.8 2.7 - 5.7	8	4.0 2.3 - 4.6	13	3.5 2.2 - 5.9	6	0.188

Table 8-11: Cardiac BRS estimates for pooled subject groups, tilt data

Method		Controls vs. NCS Patients					Non-Fainters vs. Fainters				
		Control	n (50)	NCS	n (20)	p	NF	n (55)	F	n (15)	p
Alpha	LF	5.1 3.6 - 6.8	50	5.3 2.4 - 6.2	18	0.257	5.3 3.5 - 6.8	54	4.8 2.6 - 6.1	14	0.336
	HF	5.2 3.3 - 7.8	49	3.9 2.0 - 5.2	19	0.048	5.2 3.1 - 7.9	55	3.3 2.3 - 4.8	13	0.073
	Mean	5.3 3.9 - 6.7	50	4.1 2.2 - 5.5	19	0.067	5.3 3.8 - 6.7	55	4.1 2.4 - 6.2	14	0.192
TF	LF	3.7 2.5 - 5.5	50	2.9 1.8 - 4.6	18	0.142	3.9 2.4 - 5.3	54	2.9 1.6 - 4.1	14	0.110
	HF	2.9 1.9 - 4.6	49	2.4 1.1 - 3.0	19	0.093	2.9 2.0 - 4.7	55	2.0 1.0 - 2.7	13	0.055
	Mean	3.2 2.3 - 4.7	50	2.9 1.3 - 3.5	19	0.067	3.3 2.1 - 4.6	55	2.5 1.4 - 3.8	14	0.096
Sequence	Up	4.6 3.0 - 6.8	48	4.0 1.7 - 5.9	18	0.115	4.5 3.6 - 7.4	52	2.9 1.8 - 5.8	14	0.038
	Down	4.8 3.6 - 7.3	46	3.8 3.5 - 5.1	17	0.131	4.5 3.5 - 6.3	52	4.4 3.5 - 6.7	11	0.863
	All	4.6 3.4 - 6.1	49	4.0 2.2 - 4.9	19	0.061	4.6 3.3 - 5.9	54	3.8 2.2 - 5.9	14	0.246

As expected, cardiac BRS estimates were reduced in the upright position for all subjects. The difference between supine and tilt tended to be greater for NCS patients than healthy controls, this was most significant for the LF spectral estimates (Table 8-12).

Table 8-12: NCS patients have a greater decrease in cBRS (supine to tilt) than healthy controls, for LF spectral estimates ($p < 0.05$, ms/mmHg, median & IQR)

	Control	NCS
LF Alpha	1.6 0.3 - 4.2	3.5 2.4 - 6.5
LF TF	1.2 0.4 - 4.5	3.8 1.9 - 6.3

There was a tendency towards lower cBRS values for NCS patients and fainters in the upright posture. The difference between the independent groups was most significant for the HF alpha method, which could be seen to decompose into lower cBRS values for NCS patients and fainters (Figure 8-15 and Figure 8-16). The sequence method using up-slope sequences reached $p < 0.05$ for the difference between non-fainters and fainters.

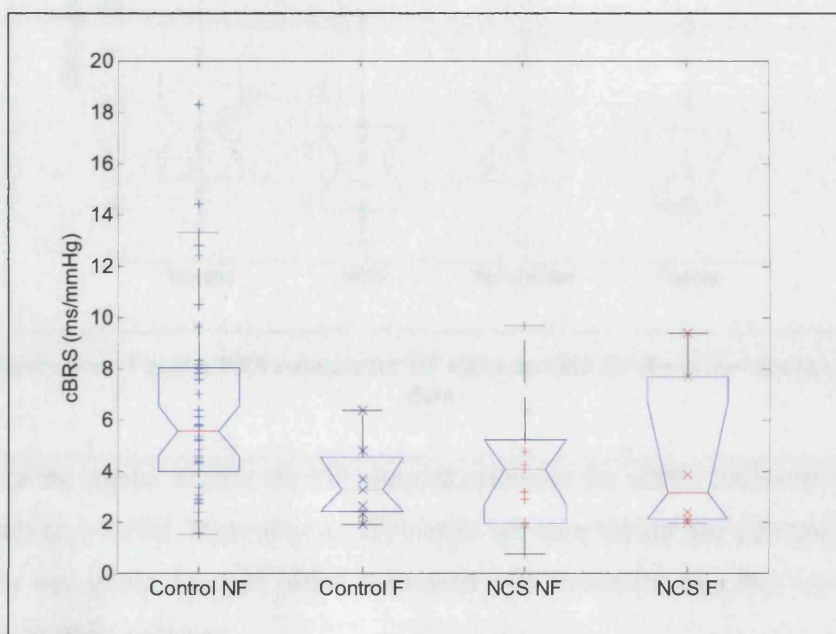


Figure 8-15: Cardiac BRS estimate for HF alpha method for the independent groups, tilt data

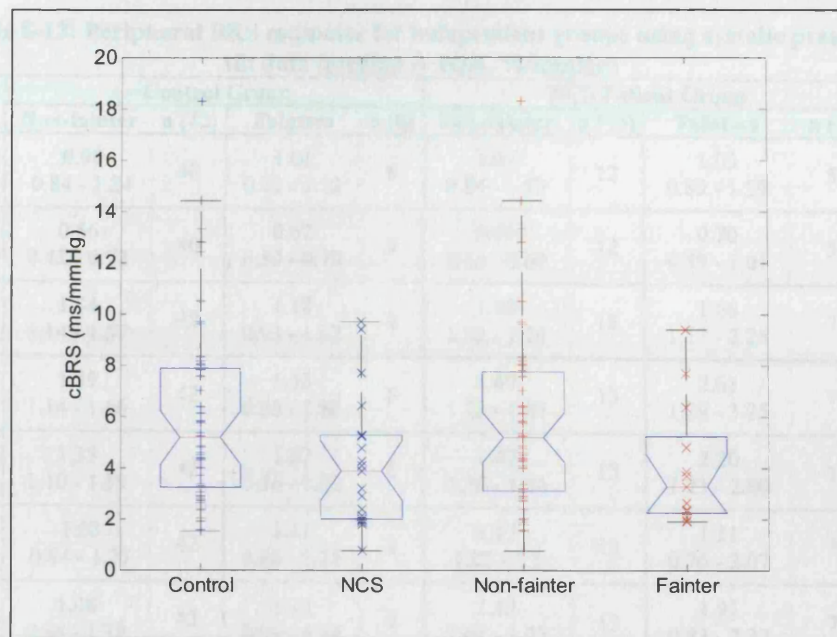


Figure 8-16: Cardiac BRS estimate for HF alpha method for the pooled groups, tilt data

In contrast to the supine results, the HF spectral estimates for cBRS tended to be lower than the LF values ($p > 0.05$). There was no difference between the up and down-slope sequence estimates for any group. Upright cBRS decreased with increasing age, but was unrelated to resting mean systolic pressure.

Tilt Results - Peripheral BRS

Table 8-13 to Table 8-16 show the results of the peripheral BRS estimates for the tilt recordings. Tilt pBRS was not consistently altered by the upright position. Approximately half of the subjects had higher tilt pBRS than supine for the alpha and sequence methods. For the TF method, two-thirds to three-quarters of the subjects had lower pBRS in the upright position.

As was the case for the supine data, orthostatic pBRS tends to be higher for NCS patients and for fainters. For the independent groups only the unfiltered DAP-sequence method using down or combined-slopes reached $p < 0.05$ (Table 8-14). In the pooled groups this method showed that NCS patients had higher pBRS values for all DAP sequence methods (Table 8-16). The down and combined-slope methods were also higher for fainters with respect to non-fainters. For SAP-input methods the difference between NCS patients and healthy controls using the unfiltered sequence method was also quite significant (Table 8-15).

Table 8-13: Peripheral BRS estimates for independent groups using systolic pressure, tilt data (median & IQR, %/mmHg)

Method		Control Group				NCS Patient Group				p
		Non-fainter	n (42)	Fainters	n (8)	Non-fainter	n (13)	Fainters	n (7)	
Alpha LF		0.98 0.84 - 1.24	40	1.01 0.78 - 1.12	8	1.07 0.84 - 1.59	12	1.05 0.89 - 1.59	5	0.868
TF LF		0.56 0.42 - 0.72	40	0.62 0.50 - 0.79	8	0.50 0.44 - 0.69	12	0.70 0.59 - 1.01	5	0.378
Sequence	Up	1.34 1.14 - 1.57	42	1.18 0.93 - 1.52	8	1.38 1.32 - 1.74	13	1.66 1.17 - 2.28	7	0.348
	Down	1.39 1.14 - 1.66	42	1.33 0.85 - 1.80	8	1.49 1.29 - 1.85	13	2.61 1.29 - 3.25	7	0.171
	All	1.35 1.10 - 1.59	42	1.27 0.86 - 1.62	8	1.47 1.30 - 1.66	13	2.20 1.23 - 2.60	7	0.190
Filtered sequence	Up	1.03 0.84 - 1.27	42	1.11 0.86 - 1.21	8	1.13 1.02 - 1.8	13	1.31 0.76 - 2.07	7	0.689
	Down	1.08 0.88 - 1.32	42	1.12 0.99 - 1.14	8	1.13 1.01 - 1.23	13	1.91 0.83 - 2.37	7	0.698
	All	1.04 0.90 - 1.24	42	1.12 0.93 - 1.18	8	1.11 0.93 - 1.33	13	1.57 0.79 - 2.21	7	0.759

Table 8-14: Peripheral BRS estimates for independent groups using diastolic pressure, tilt data

Method		Control Group				NCS Patient Group				p
		NF	n (42)	F	n (8)	NF	n (13)	F	n (7)	
Alpha LF		1.71 1.51 - 2.07	42	1.64 1.40 - 1.74	8	1.85 1.41 - 2.64	13	2.55 1.95 - 3.47	5	0.408
TF LF		1.20 0.98 - 1.50	42	1.22 1.11 - 1.40	8	1.13 0.97 - 1.71	13	1.57 1.35 - 2.54	5	0.707
Sequence	Up	2.20 1.80 - 2.51	42	2.03 1.82 - 3.27	8	2.04 2.12 - 3.31	13	3.48 2.34 - 3.94	7	0.079
	Down	2.03 1.71 - 2.55	42	2.43 2.06 - 2.94	8	3.03 2.47 - 3.75	13	4.42 4.04 - 5.90	7	0.005
	All	2.04 1.76 - 2.55	42	2.59 2.06 - 3.30	8	2.97 2.30 - 3.66	13	3.91 2.99 - 4.87	7	0.007
Filtered sequence	Up	1.61 1.37 - 1.89	42	1.58 1.57 - 2.10	8	1.59 1.17 - 2.10	13	2.47 1.55 - 3.58	7	0.224
	Down	1.58 1.28 - 1.89	42	1.36 1.32 - 2.25	8	1.59 1.23 - 2.49	13	3.62 1.87 - 5.58	7	0.357
	All	1.59 1.39 - 1.88	42	1.49 1.43 - 2.21	8	1.82 1.25 - 2.40	13	3.20 1.60 - 5.10	7	0.352

Table 8-15: Peripheral BRS estimates for the pooled data using SAP as the input pressure, tilt data

Method		Controls vs. NCS Patients					Non-Fainters vs. Fainters				
		Control	n (50)	NCS	n (20)	p	NF	n (55)	F	n (15)	p
Alpha LF		0.98 0.81 - 1.24	48	1.06 0.84 - 1.23	17	0.451	1.00 0.84 - 1.23	52	1.03 0.78 - 1.33	13	0.700
TF LF		0.57 0.44 - 0.73	48	0.52 0.47 - 0.86	17	0.935	0.53 0.42 - 0.72	52	0.64 0.51 - 0.92	13	0.090
Sequence	Up	1.34 1.06 - 1.57	50	1.45 1.31 - 1.82	20	0.117	1.34 1.16 - 1.61	55	1.49 0.92 - 1.69	15	0.886
	Down	1.39 1.02 - 1.69	50	1.56 1.29 - 2.38	20	0.037	1.42 1.24 - 1.68	55	1.62 1.10 - 2.32	15	0.567
	All	1.35 1.03 - 1.61	50	1.52 1.29 - 2.21	20	0.043	1.41 1.22 - 1.61	55	1.52 1.02 - 2.04	15	0.753
Filtered sequence	Up	1.05 0.84 - 1.27	50	1.17 0.84 - 1.54	20	0.229	1.05 0.87 - 1.29	55	1.11 0.81 - 1.37	15	0.731
	Down	1.09 0.88 - 1.29	50	1.14 0.96 - 1.96	20	0.266	1.09 0.88 - 1.32	55	1.14 0.91 - 1.68	15	0.537
	All	1.05 0.90 - 1.23	50	1.13 0.91 - 1.63	20	0.314	1.06 0.91 - 1.25	55	1.14 0.85 - 1.48	15	0.616

Table 8-16: Peripheral BRS estimates for the pooled groups using DAP as the input pressure, tilt data

Method		Controls vs. NCS Patients					Non-Fainters vs. Fainters				
		Control	n (50)	NCS	n (20)	p	NF	n (55)	F	n (15)	p
Alpha LF		1.68 1.49 - 2.03	50	1.99 1.43 - 2.86	18	0.219	1.73 1.48 - 2.11	55	1.69 1.40 - 2.55	13	0.827
TF LF		1.20 1.00 - 1.50	50	1.33 1.00 - 1.72	18	0.612	1.19 0.98 - 1.55	55	1.32 1.13 - 1.64	13	0.326
Sequence	Up	2.20 1.80 - 2.69	50	2.99 2.11 - 3.68	20	0.013	2.25 1.87 - 2.78	55	2.77 1.88 - 3.83	15	0.224
	Down	2.08 1.75 - 2.57	50	3.67 2.51 - 4.64	20	0.001	2.25 1.75 - 3.12	55	3.28 2.23 - 4.37	15	0.044
	All	2.11 1.77 - 2.76	50	3.12 2.47 - 3.99	20	0.002	2.24 1.80 - 2.95	55	3.13 2.31 - 4.06	15	0.034
Filtered sequence	Up	1.63 1.39 - 1.90	50	2.15 1.16 - 2.53	20	0.151	1.62 1.29 - 1.93	55	2.08 1.56 - 2.51	15	0.076
	Down	1.56 1.28 - 1.91	50	2.35 1.20 - 3.68	20	0.144	1.59 1.26 - 1.97	55	2.24 1.28 - 3.74	15	0.323
	All	1.57 1.40 - 1.90	50	2.27 1.12 - 3.24	20	0.174	1.61 1.34 - 1.95	55	2.20 1.43 - 3.38	15	0.179

There was no difference between up and down-pressure sequence estimates for upright pBRS. Upright pBRS was unrelated to age or mean resting systolic blood pressure, although this may be due to the smaller number of subjects at the extremes of these parameters in the tilt group.

Peripheral versus Cardiac BRS

Complimentary measures of baroreflex function can be combined in multiple ways to provide further methods of discriminating between different subject groups. Peripheral BRS was plotted against cardiac BRS for similar methods of estimation (e.g. LF alpha pBRS against LF, HF and mean alpha cBRS, up-sequences against up-sequence) and for both pressure inputs. Most subjects had moderate values of cBRS and pBRS and showed no discernable relationship. There was no linear relationship between the two parameters, although several plots suggested that there may be a reciprocal relationship. Figure 8-17 shows the plot for (LF) TF pBRS against LF TF cBRS. There is a tendency for non-fainters (crosses) to occupy the highest cBRS values and for NCS patients (red) to have the highest pBRS values. These conditions (high cBRS or high pBRS) seem to be mutually exclusive.

There appeared to be no systematic pattern to the appearance of the plots regarding posture, pressure input or BRS estimation method. The sequence method in the supine position with SAP as input pressure is much less clear (Figure 8-18).

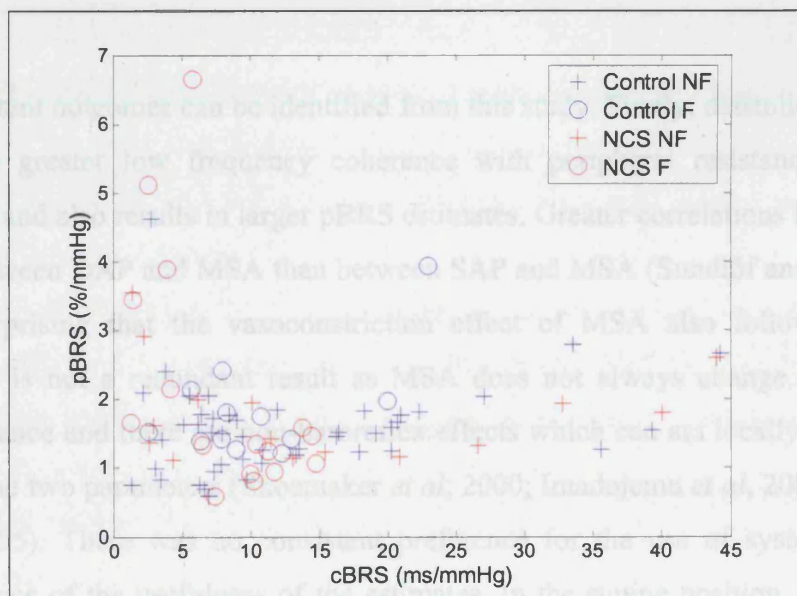


Figure 8-17: Peripheral vs. cardiac BRS, HF TF method, supine data, DAP as input pressure.

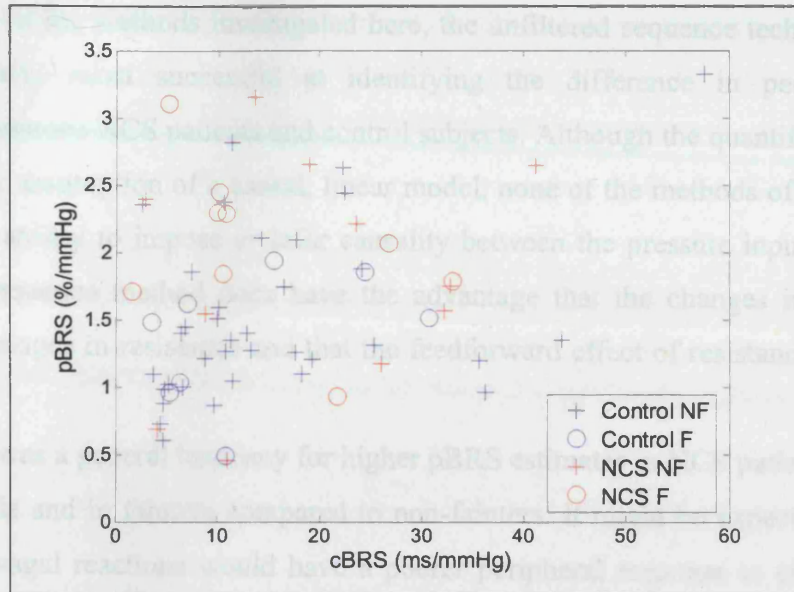


Figure 8-18: Peripheral vs. cardiac BRS, down-pressure sequence method, supine data, SAP as input pressure.

Discussion

Outcomes

Several consistent outcomes can be identified from this study. Firstly, diastolic blood pressure tends to have greater low frequency coherence with peripheral resistance than systolic pressure does, and also results in larger pBRS estimates. Greater correlations have been found previously between DAP and MSA than between SAP and MSA (Sundlöf and Wallin, 1978), so it is unsurprising that the vasoconstriction effect of MSA also follows this pattern. However, this is not a redundant result as MSA does not always change in parallel with vascular resistance and there are non-baroreflex effects which can act locally or systemically to dissociate the two parameters (Shoemaker *et al*, 2000; Imadojemu *et al*, 2001; Ichinose and Nishiyasu, 2005). There was no consistent preference for the use of systolic or diastolic pressure in terms of the usefulness of the estimates. In the supine position, pBRS estimates using systolic pressure appear to be slightly more discriminatory than diastolic (see particularly Table 8-8), but this is not the case in the tilt position. The pressure parameter that provides the most potent baroreceptor stimulus may vary depending on the condition of the subject. For example, in the upright position the low-pressure cardio-pulmonary baroreceptors are selectively unloaded and this may modify the response to the arterial receptors. Lucini *et al* (1999) determined that the decrease in the alpha parameter in the standing position was almost entirely due to cardiopulmonary effects.

Secondly, out of the methods investigated here, the unfiltered sequence technique for pBRS estimation seems most successful at identifying the difference in peripheral control mechanisms between NCS patients and control subjects. Although the quantification of pBRS is based on the assumption of a causal, linear model, none of the methods of estimation used here have the ability to impose or infer causality between the pressure input and resistance output. The sequence method does have the advantage that the changes in pressure must precede the changes in resistance and that the feedforward effect of resistance on pressure is eliminated.

Thirdly, there was a general tendency for higher pBRS estimates in NCS patients compared to healthy controls and in fainters compared to non-fainters. It might be expected that subjects prone to vasovagal reactions would have a poorer peripheral response to changes in blood pressure, and especially to decreases in blood pressure. These results suggest an alternative explanation, that syncopal subjects have an increased peripheral feedback gain, possibly leading to instability in the control loop. The difference seems to be less pronounced between fainters and non-fainters than between NCS-patients and control subjects. The fainting controls and non-fainting patients represent false positive and false negative outcomes respectively. The confounding effects of these subgroups could be eliminated by investigating only non-fainting controls and fainting patients. The pathological responses to tilt may be different between control and patient-group fainters. Also, the control group may have included subjects with undiagnosed NCS.

Fourthly, it was anticipated that the upright estimates of peripheral BRS would differ from the supine values. Tilt had a consistent reduction in cardiac BRS, as has been previously demonstrated (Kim and Euler, 1997), but no similar effect on pBRS. Orthostasis should result in activation of the sympathetic system and there is also some contention that the baroreflex is preferentially activated in the upright position (Chapter 3). If these statements are true, it would appear that this 'activation' is unlikely to be evident in the absolute value of the peripheral BRS.

Fifthly, the combination of cardiac and peripheral BRS is likely to provide better information and discriminatory capability than either parameter individually. Cardiac BRS is usually measured in the supine position, but in this study this was unsuccessful in distinguishing patients from controls or fainters from non-fainters. Plots of pBRS against cBRS indicate a tendency for some subjects to develop either high cBRS or high pBRS values, although most subjects have moderate values of both. There is also a suggestion that the highest cBRS values are most prevalent in control or non-fainting subjects. This is to be expected, as

subjects with high cardiac baroreflex gains will be able to effect arterial pressure regulation using changes in heart rate before a peripheral response has time to develop.

Whittam *et al* (2000) studied the effect of varying the parameters in de Boer's beat-to-beat cardiovascular model (de Boer *et al*, 1987) by comparing SAP and RR interval spectral powers from model simulations with those from real subjects. Their Figure 4 can be interpreted as showing that LF power in SAP and RR interval increases with both peripheral and cardiac BRS magnitudes. However, my results indicate that subjects are unlikely to have high values of both parameters. Their results could also indicate that for high values of cBRS there is a threshold for pBRS above which the LF variations become extremely augmented. For lower cBRS, pBRS can take a wide range of values without the model becoming unstable.

Methods

Diastolic pressure may evoke a larger percentage change in peripheral resistance because it is more closely related to mean pressure than systolic pressure is. The MAP-PR relationship was not evaluated here, but mean pressure may prove to be more appropriate as an input. Also, it has previously been shown that diastolic pressure contains little high frequency variation compared to systolic pressure (de Boer *et al*, 1985b), therefore using DAP may have a similar effect to low-pass filtering the input to the baroreflex. The systolic pressure signal contains a significant amount of HF power to which the sympathetic system cannot respond adequately and thus may constitute input noise. However, low-pass filtering the beat-to-beat data did not have a consistent effect on the sequence pBRS estimates and was less successful than the unfiltered method at differentiating any of the groups.

This is an unexpected result. The high frequency variability in peripheral resistance is considered here to be either an artifact of the beat-to-beat PR estimation procedure, or a manifestation of the compliance of the elastic vessels involved. Peripheral resistance is the steady-flow (dc) component of the complex-valued, frequency-dependent impedance of the vascular system and the active control of vascular resistance by the sympathetic nervous system is known to only be capable of responding at frequencies below around 0.2Hz (Chapter 5). Therefore, removing high-frequency variability from both the input and output was expected to improve the signal-to-noise ratio of the analysed data and provide a more reliable estimate of peripheral baroreceptor sensitivity.

It should be noted that the fainters who were affected by syncope soonest, and who therefore may have the most disturbed control system, could not be studied in the upright position as the stationary data segment was not sufficiently long for spectral analysis. One subject, for whom a single subrecord was used, did not reach the minimum coherence threshold and thus

had no spectral estimate of either cardiac or peripheral BRS in the upright posture. Neither did they have sufficient slopes to provide a sequence estimate of cBRS. However, there were many peripheral slopes available for a sequence estimation of pBRS.

Statistical Analysis

The results from this study have been analysed using many repetitions of statistical tests. Kruskal-Wallis and Wilcoxon rank tests were applied to the independent and pooled subject groups respectively: nine times for each cardiac BRS grouping and eight times for each peripheral BRS. Using a p -value of 0.05, random variation should result in a significant result one time in twenty independent tests, where no true difference exists (Type 1 error, false-positive). So the more tests there are carried out, the more likely a significant result is to be found. Statistical correction methods, such as Bonferroni corrections, exist to reduce the false positive rate in such cases (Perneger, 1998). However, different methods of calculating the same parameter are not independent tests and the Bonferroni method would be too conservative. Also (and more importantly), the purpose of this study was not to test the hypothesis that patients with recurrent neurocardiogenic syncope differed from healthy controls in their cardiac or peripheral BRS values (or fainters from non-fainters). I have assumed that the groups differ in their autonomic control of blood pressure and used them to test whether pBRS methods could detect or describe that difference. The hypothesis being tested is that peripheral BRS exists as a useful clinical parameter that can be measured using spontaneous variations in non-invasive cardiovascular parameters. To find no difference between the groups would indicate either that the methods are not suitable to detect the difference in physiology, or that the assumption about the groups was erroneous.

The p -value is useful here as an indicator of the separation of the groups. Median and inter-quartile ranges were used to illustrate the central tendency and spread of the skewed distribution of BRS values. However, these numbers can only provide a limited indication of group characteristics. The p -values take into account both the differences between the groups and the spread of values within the groups, providing a measure of how successfully pBRS discriminates between the groups.

Conclusion

These results indicate that methods used for estimating cardiac BRS non-invasively from spontaneous variability can be adapted to apply to peripheral control of blood pressure. Although previous authors have begun to quantify the sympathetic or peripheral response to blood pressure changes (Scher and Young, 1962; Wallin *et al*, 1975; Mukkamala *et al*, 2003;

O'Leary *et al*, 2004; Ichinose and Nishiyasu, 2005), there have been no previous attempts to evaluate different methods for discriminating between healthy and pathological states. The implications of these results and suggestions for improvements and further work are discussed in Chapter 9.

CHAPTER 9 DISCUSSION

Thesis Summary

The aim of this thesis was to investigate non-invasive methods for quantifying peripheral baroreceptor sensitivity, i.e. to determine the magnitude of the response of total peripheral resistance to changes in arterial blood pressure. A system identification approach to cardiovascular control relates measurable parameters to inputs and outputs of 'black box' filters which represent parts of the physiology which we are attempting to analyse. Characterising the entire autonomic blood pressure control loop is still an aspirational goal rather than a practicality, but would provide much better understanding of pathophysiological processes and therefore their control or cure. Xiao *et al* (2005) have recently produced a thorough review of system identification methods applied to this area of research.

Chapter 1 reviewed the physics of fluid resistance and the problems with investigating TPR in humans. The baroreflex was modelled as having three feedback loops, although only the cardiac chronotropic limb has been extensively studied. Chapter 2 described the known anatomy and physiology of the baroreflex autonomic control loop and the response to the upright posture. Chapter 3 examined the current state of knowledge regarding spectral variability analysis of cardiovascular parameters. Low and respiratory frequency variations are known to exist in blood pressure, HR/RR interval, MSA and stroke volume but the causal relationships between them are still not unequivocally identified.

Several methods of evaluating cardiac BRS were described and compared in Chapter 4. The advantages and disadvantages of each were described and the clinical uses to which they have been put provide a background for the methods used on the PR data.

Several topics for consideration prior to collection and analysis of the physiological data were brought together in Chapter 5. Non-invasive measurement of phasic blood pressure is a relatively recent development. Finapres-type devices (Finapres, Portapres, Finometer) have been used and evaluated under many experimental conditions and for subjects with a variety of pathologies. It is well-known that the absolute values of systolic and diastolic pressure determined with these devices are not identical to those measured intra-arterially, but absolute accuracy is not as important here as the reliable tracking of beat-to-beat changes in pressure and pulsatile systolic area.

The theoretical basis for the peripheral resistance analysis was also described in Chapter 5. Peripheral resistance is a concept that applies strictly only to steady-flow conditions. Using

typical values for human vascular quantities in models of vascular impedance a suitable frequency limit of $<0.15\text{-}0.2\text{Hz}$ for active sympathetic vascular control was established that was consistent with known results from animal data.

Techniques for the data pre-processing and analysis were also described in detail in Chapter 5 including a random surrogate data method to determine a 95% confidence threshold for non-zero coherence. Another random data method was used to determine experimental values for the coherence bias error, which was found to be significantly lower than the analytical values for the spectral analysis methods used here.

Other issues of cardiovascular data analysis dealt with in Chapter 5 centre on the signal sampling of beat-to-beat data. Phase (or time lag) results are affected by different methods of synchronising the cardiovascular variables and the details of these processing methods should be clearly defined where phase/lag information is presented. The treatment of ventricular ectopic beats was shown to have a very significant effect on cardiac BRS values. Supraventricular beats were shown to have little effect on cardiovascular parameters, whereas the turbulence following a ventricular premature beat provides significant information on baroreflex mechanisms. Excluding signal segments or subjects containing ectopics therefore removes useful data from the study, but including even small numbers of ventricular ectopics produces considerable augmentation of the spectral cBRS estimates compared to ectopic-free data. Linear interpolation of the non-normal intervals and re-synchronisation of all subsequent beats was shown to be an appropriate method of correcting these events for cBRS calculations.

Before using the pressure data for estimation of stroke volume it was necessary to determine that non-invasive finger pressure would provide suitably reliable data for the 'corrected impedance' pulse contour algorithm (Chapter 6). The algorithm had been developed using aortic pressure as the model input (Wesseling *et al*, 1983), therefore stroke volume values derived from Finapres pressures were compared with those from aortic pressure. The existing clinical method of measuring aortic pressure in the Cardiac Catheter Suite involved a fluid-filled catheter manometer. The aortic signals that were determined to be unaffected by the resonance of this system were used as the reference data.

Values for uncalibrated pulse contour stroke volume from a custom-built program were compared to those from a commercial implementation of the algorithm. Beat-to-beat pressure values showed excellent correspondence, although custom SV_{PC} tended to overestimate the commercial values at higher values. Two out of ten subjects had poor correlations between aortic and Finapres SV_{PC} , one of whom suffered from symptom indicating peripheral vascular

disease secondary to diabetes. All other subjects showed strong linear relationships between aortic and Finapres pulse contour stroke volume ($R = 0.63$ to 0.94), although there was a tendency for Finapres SV to underestimate aortic SV at higher values.

To further examine the reliability of the Finapres-derived pulse contour stroke volume it was compared to a Doppler measure of cardiac outflow in Chapter 7. Beat-to-beat uncalibrated stroke volume and stroke distance were compared under conditions of spontaneous and regular respiration in the supine and upright positions. Following high-pass filtering to remove VLF variations in the Doppler stroke distance, the correlations between SV and SD were good although there were isolated recordings with poor results. Both parameters mirrored the respiration power spectra, but the higher coherence between respiration and SV_{PC} and greater similarity of the spectra indicates the discrepancies between SV_{PC} and SD were primarily due to errors in the Doppler method (relative movement of the probe and poor marking of systole in recordings).

Pulse contour stroke volume was therefore used as the basis for the beat-to-beat calculation of total peripheral resistance for estimates of peripheral BRS in Chapter 8. Three methods for determining cardiac BRS from spontaneous variations were adapted in an attempt to measure the magnitude of the PR response to changes in arterial pressure. These methods were applied to subjects who were assumed to differ in their autonomic control of blood pressure. Peripheral BRS was shown to have discriminatory capability where cBRS indicated no difference, although these results were unable to indicate whether diastolic blood pressure was a more useful input than systolic. Some of the results are discussed below in reference to other published work.

Sympathetic and Peripheral Baroreceptor Sensitivity

Whereas cardiac BRS is dominated by the vagal branch of the autonomic system, the peripheral part of the feedback loop is sympathetically controlled, giving rise to terminology such as 'cardiovagal' and 'sympathetic' BRS (Rudas *et al*, 1999; O'Leary *et al*, 2003). The response of MSA to variations in blood pressure has been characterised as exhibiting a similar, but opposite, sigmoid shape as that for RR interval (Rea and Eckberg, 1987; Sato *et al*, 1999), although determining a parameter with which to describe this response is complicated by the variety of ways in which MSA has been quantified (Eckberg *et al*, 1988; Jacobsen *et al*, 1993). In most cases the MSA response is determined using diastolic pressure as the cardiac-synchronous MSA bursts are thought to originate during diastole. Also, steady-state changes in diastolic pressure are most strongly related to MSA burst frequency and total

activity (Sanders and Ferguson, 1989; Ebert and Cowley, 1992; Rudas *et al*, 1999; O'Leary *et al*, 2003). As only the frequency of MSA can be calibrated, sympathetic BRS is quantified in normalised but arbitrary units of activity or percentage changes.

Several results from studies into sympathetic BRS show similarities with my results in peripheral BRS.

The response to falling pressure has been found to be generally greater than that to increasing pressure. Rea and Eckberg (1987) found that the MSA response to decreases in systolic carotid distending pressure were greater than those for increases. It was determined that subjects' operating point pressure was close to the threshold for this relation so that increases in baroreflex input could not elicit large decreases in MSA during supine rest. However, as interindividual base levels of MSA are notoriously variable (Burke *et al*, 1977; Sundlöf and Wallin, 1977), this may not be universally true. Rudas *et al* (1999) found non-significantly larger sympathetic baroreflex slopes for decreasing diastolic pressures. This is consistent with the larger sequence pBRS estimates found in this thesis for decreasing pressure slopes. Such a result is also in keeping with results from animal studies, whereby the time constant for vasodilation is longer than that for vasoconstriction (Rosenbaum and Race, 1968). This is to be expected when the control of vessel calibre is only 'active' during vasoconstriction.

This suggests that the sequence method could be the most appropriate technique for investigating pBRS. The spectral methods used here are incapable of differentiating between increasing and decreasing pressure effects and this could be a contributory factor to the lack of discriminatory power that these methods showed for these subject groups. O'Leary *et al* (2004) concluded that the MAP-PR transfer function was unable to characterise the vascular baroreflex response and suggested that low coherence between these parameters could be explained by non-linear effects or disparate regional responses.

The difference between up and down pressure slopes was most pronounced in non-fainters and controls using diastolic pressure and in fainters using systolic pressure as input. It seems that the influence of the different pressure variables is not simple and requires significant further investigation before a coherent picture of the most appropriate baroreceptor input is defined.

Rudas *et al* (1999) found no correlation between vagal and sympathetic baroreflex gains. The plots of pBRS versus cBRS shown here, also indicate a lack of linear relationship. There was either no discernable relationship (Figure 8-18) or there was a tendency for subjects to lie along one of the axes (Figure 8-17). The relationship between these two parameters was not analysed further here but is likely to be an area that will provide a great deal of scope for

further investigation. Combinations of cardiac and peripheral BRS measures may have greater value than either one alone. Some refinement and additional testing of the peripheral methods will be necessary before reliable information concerning the balance and co-operation of these two control branches can be used to answer questions of physiology and pathophysiology. In the same paper, the authors also noted that sympathetic gain was not related to age.

Sympathetic BRS was found to increase from supine to 60° head-up tilt, mostly due to increases in burst frequency (O'Leary *et al*, 2003), whereas the MAP-TPR LF transfer function gain underwent a non-significant increase from supine to 45° tilt (O'Leary *et al*, 2004). In this thesis no consistent change in pBRS with tilt was detected. Burke *et al* (1977) determined that MSA burst incidence increases with tilt in inverse relation to the resting activity. Therefore changes in sympathetic or peripheral BRS with tilt may be related to base levels. Changes in mean TPR and differences between individuals could not be examined here as the measurements were uncalibrated. However, as there was a five minute supine period immediately preceding the tilt section, the percentage increase in mean TPR with tilt could have been determined.

The results from MSA experiments cannot simply be extrapolated to postulate the effects on systemic resistance. Firstly, sympathetic efferent activity is regionally differentiated (Esler, 1993; Grassi and Esler, 1999). Although MSA in the arm and leg have been found to increase in parallel upon tilt (Imadojemu *et al*, 2001), other vascular beds may be less affected by baroreceptor influences (Malpas *et al*, 2001). Secondly, the TPR response additionally includes the end-organ effect. Respiratory and cardiac frequencies account for most of the spectral power in MSA recordings, but the neuromuscular transmission and smooth muscle acts as low pass filter and are limited to LF variations. Using parametric multivariate analysis Nakata *et al* (1998) determined that LF MSA influenced systolic pressure, but that HF variations were passive and had no effect on pressure. Thirdly, vascular resistance is subject to influences from inputs other than MSA and can become dissociated from it (Delius *et al*, 1973).

There is probably a place for both 'sympathetic' and 'peripheral' BRS assessment techniques. Measurement of MSA provides information regarding the sensing and central processing of receptor afferents, but does not measure the end-organ effectiveness of the response. Additionally, it is invasive and can be uncomfortable, if not painful. The use of both methods could help determine the focal point of a pathological response. Particularly, the spectral variability of MSA is thought to provide complimentary information to the steady-state values

(Pagani and Malliani, 2000) so that spectral methods of BRS may be more appropriate for this parameter than for peripheral resistance.

The assessment of the pressure control loop could be extended full-circle into measuring the effect of changes in blood pressure on changes in blood pressure. The closed-loop can be broken physically in animal studies (Scher and Young, 1963; Sato *et al*, 1999) and pharmacologically or using neck pressure/suction in humans. Ogoh *et al* (2002) used the latter approach to determine the effect on mean arterial pressure of step changes in carotid distending pressure. They determined that the peak effect in MAP occurred after approximately 6s. Wallin and Nerhed (1982) determined that following spontaneous bursts in MSA, diastolic pressure began to increase after 1-2s and reached a peak value after 4.6-7s. The standard delay expected for pressure input to peroneal MSA response is 1.3s, so the total time for the pressure to pressure effect is again approximately 6-8s. An analysis of the delays between blood pressure and resistance slopes used in sequence pBRS could provide additional evidence that the slopes represent real baroreflex responses.

Study Limitations

Some of the limitations of this work have been described in earlier chapters.

The comparison between Finapres-derived and aortic-derived pulse contour values was marred by the poor dynamic response of the fluid-filled catheter system that was used in the clinical setting. The primary effect of the resonance was probably in small changes in the turning points on the pressure signal that were used as markers for the beginning and end of systole. At the time of the measurements catheter-tip manometers for high-fidelity aortic measurements were found to be too expensive.

The Doppler method against which Finapres SV_{PC} was compared could have been improved by displaying the spectrogram during the recording. Fixation of the transducer probe could also have considerably reduced any movement artifact, but due to the probe shape and anatomical location this was considered to be technically difficult and time-consuming. Every effort was made to reduce patient and operator discomfort and maintain a stationary position. Shorter recordings may have reduced movement artifact but would have resulted in spectral measures with greater variance.

The measurement of respiration during this protocol also suffered from poor quality control. The DAT recorder had a simple visual display of signal level in each channel, but this was insufficient to determine the quality of the signal from the thoracic impedance band. Again, being able to see the signal during the measurement would have allowed remedial action to

have been taken before the recording. Additionally, some subjects appeared to exhibit poor control of breathing, especially in the upright position. The visual stimulus was provided by two LEDs in a small box placed within easy sight of the subject. However, brief lapses in concentration may have allowed subjects to re-synchronise 180° out-of-phase with their original timing. Labelling the LEDs with 'IN' and 'OUT' would have been a simple but potentially highly effective means to minimise this.

The non-invasive determination of stroke volume could have been improved by using the Modelflow method (Wesseling *et al*, 1993) rather than the corrected impedance pulse contour (Wesseling *et al*, 1983). This is a model that generates a flow waveform from pressure signals, which is integrated over systole to determine stroke volume. Commercial software for this method was available during this study (Beatscope). However, the output from the commercial software was limited. For example, in Chapter 6, one recording had erroneously marked beats from this software and no manual correction was possible. Also, the outputs from the software would have had to be re-synchronised with the other signals following processing. By implementing the algorithm myself I had full access to and control of all stages of the procedure and was able to correct any mis-marking of the beats. The Modelflow application has, however, been more widely accepted and tested than the pulse contour method and is considered to have much better tracking of blood pressure and more accurate uncalibrated values (Wesseling *et al*, 1993; Bos *et al*, 1996; Ogoh *et al*, 2003; Sugawara *et al*, 2003).

Calibration of stroke volume (and its derivatives) may also have improved the results. This was unnecessary for the comparison protocols in Chapter 6 and Chapter 7 as the beat-to-beat variability would have been unaffected by the absolute values. However, the results in Chapter 8 may have been affected by the use of absolute TPR for calculating pBRS. Even where the differences between the subject groups achieved quite low *p*-values, the absolute difference in %/mmHg were too small to be of any clinical use. Greater differences may also be found in other patient groups, with the use of absolute measurements, or if conductance was used instead of resistance (Lautt, 1989).

Alternatively, the results may have been more positive if data from more fainting subjects were included in the tilt results. Using orthostatic stress in a patient population who are defined by their propensity for syncope has the advantage of acquiring data during the most pathological events, but the disadvantage of data loss when subjects are unable to tolerate the condition for a sufficient duration. The end-tidal mask excluded the use of the first five minutes of each tilt recording. If this had not been present, then data analysis could have

begun 3min following tilt-up to allow for the steady-state to become established. Additional tilt records from several fainters may then have been available for analysis. Alternative methods of activating the sympathetic system could also have been used that would be less likely to result in lost data (mental stress, isometric exercise, cold tests, lower body negative pressure, etc.).

Future Work

There are still very few published reports that have addressed the idea of peripheral baroreceptor sensitivity, and to my knowledge only one has previously attempted to determine quantitative values for human subjects (O'Leary *et al*, 2004). There are now relatively mature and methodologically independent techniques for determining beat-to-beat TPR (Doppler, pulse contour, electrical impedance) and these estimates should be compared to determine the reliability of the variability which is the basis for spontaneous baroreflex estimation. These methods should ideally be calibrated so that absolute values for TPR can be determined.

The original reference method for cardiac BRS using pharmacologically-induced pressure ramps is unsuited to peripheral BRS estimation, as the agents act via peripheral resistance (O'Leary *et al*, 2004). A potential surrogate for this approach could be slow, continuous tilt. This would need to be slow enough so that transient effects were not induced, but fast enough so that pressure was not restored to baseline values during the manoeuvre.

The results from this study would appear to indicate that the sequence method provides a pBRS estimate with the greatest potential for clinical success. This may be because it is the only method that imposes order and direction on the changes in pressure and peripheral resistance and as a result offers a potentially open-loop analysis of the system. The standard sequence method for cardiac BRS was adapted for peripheral estimates by permitting a variable delay and sequences of unequal length. Low pass filtering the input and output data seemed to have a detrimental effect on the discriminatory power of the method, although a lack of difference in one patient group should not necessarily be taken as a failure of the method. Other methods for emphasising the LF changes and reducing HF noise could include: filtering only the input or output, allowing for 'small' changes in slope in the middle of longer slopes, or increasing the minimum sequence length. A close examination of the beat-to-beat sequences and the respective slopes determined could help to inform such improvement of the algorithm. Experimental protocols that include alpha-adrenergic and β_2 blockade could provide firm evidence for or against the sympathetic origin of the TPR sequences.

Additionally, certain other pathologies may show greater influence on baroreceptor control of peripheral resistance due to their mode of effect. For example, patients with autonomic peripheral neuropathy, orthostatic hypotension, atherosclerosis and hypertension. Indeed, the results here indicate that there may be a strong relationship between high resting systolic pressure and pBRS. Extremes of age (adolescents and the elderly) are also often associated with increased incidence of syncope and there is some suggestion in these results that pBRS may be increased in these subjects.

The evidence indicates that pathological states may be characterised by a tendency for larger pBRS values, whereas for cBRS it is the lowest values that are of most prognostic value. This may simply be an expression of the interaction between the two branches of the baroreflex control loop and their relative speed of response. If a subject has a strong cardiac response to changes in blood pressure, the rapidity of the action may correct the deviation and prevent the peripheral branch from becoming fully activated. The resulting pBRS estimates may be either small or highly variable and unreliable. It is possible that a true estimate of the gain of the peripheral response can only be obtained when the cardiac vagal effect has been prevented, for example by the administration of atropine, cardiac pacing or in subjects with transplanted hearts. There will also be many ways in which the cardiac and peripheral BRS estimates can be combined for a fuller picture of the autonomic control of blood pressure. One possibility is to calculate a ratio of cardiac to peripheral to indicate proportional control, or to look at the relative changes between vagally-dominated conditions (supine rest) and sympathetically activated conditions.

Previous research into MSA responses has indicated the possibility of a gender effect in the sympathovagal balance of blood pressure control and in the response to tilt (Evans *et al*, 2001; Shoemaker *et al*, 2001). This was not investigated in this study although there were approximately equal numbers of males and females in the patient and control groups studied in Chapter 8 (with the exception of the NCS patient group during tilt: 6 females out of 20).

A particular advantage of the pulse contour or Modelflow approach is that, as they only require recordings of arterial pressure, there is a wealth of data sets, previously-recorded for cardiac BRS estimation, that can be reanalysed for peripheral BRS estimation.

Statement of Contribution

The data acquisition applications used during this thesis were written by members of the University of Leicester Medical Physics Department. Mr Lingke Fan wrote the FTNEW and STROKE01 software for analogue-to-digital conversion from DAT of the recordings

including Doppler signals (Chapter 7 and Chapter 8). Prof Ronney Panerai wrote the PHYSIDAS application used to download the data from the Cardiac Catheter Suite (Chapter 6). Dr David Simpson provided MATLAB functions that I adapted to read the binary data files from these programs and also the function for marking the R-waves on the ECG. All other MATLAB functions and script files were written by myself.

Subject data collection for Chapter 6 and Chapter 7 was conducted by myself. Mr Harry Hall assisted with the measurement of the dynamic response of the cardiac catheter system. The NCS patients in Chapter 8 were recruited by research registrars in the University of Leicester Cardiovascular Sciences Department (primarily Dr Brian Carey and Dr Penny Eames) for a separate study into cerebral autoregulation (Carey, 2001). For this protocol I provided some technical input and also assisted with recruitment and data collection for some of the control subjects.

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