Thesis Submission to University of Wales College of Medicine

For the Degree of Philosophiae Doctor (PhD)

Arterial Function, Physiological Stress and the role of Nitric Oxide

Mr Ross I Campbell BSc (Hons)

Department of Cardiology

Wales Heart Research Institute

University of Wales College of Medicine

UMI Number: U585559

UMI
Dissertation Publishing

ProQuest

Declaration

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree

Signed....................Ross Campbell.............. (Candidate) Date...28/10/08.........

Statement 1

This thesis is being submitted in partial fulfilment of the requirements for the degree of PhD

Signed....................Ross Campbell.............. (Candidate) Date...28/10/08.........

Statement 2

This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references.

Signed....................Ross Campbell.............. (Candidate) Date...28/10/08.........

Statement 3

I hereby give my consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed....................Ross Campbell.............. (Candidate) Date...28/10/08.........

Statement 4

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans after expiry of a bar on access approved by the Graduate Development Committee

Signed.. (Candidate) Date......................

Dedicated to

Nicola, Ollie and Niamh for making everything worthwhile

Acknowledgments

Statement of Conjoint Work

Dr Barry McDonnell of the University of Wales College of Medicine carried out the Aortic Pulse Wave Velocity measurements

The acute mental stress study in Chapter 6 was a combined study with Mr Thomas Davies at the University of Birmingham

Table of Contents

List of Tables

List of Figures

Abstract

Cardiovascular disease is the leading cause of death in the western world. Hypertension is a major risk factor for cardiovascular diseases and blood pressure is in turn markedly influenced by large artery stiffness. Recently a number of studies have reported that apparently healthy normotensive individuals who exhibit an exaggerated systolic blood pressure response on exercise are at increased risk of developing subsequent sustained hypertension and cardiovascular disease. It is likely that an exaggerated systolic blood pressure response on exercise represents an abnormal response of the large artery during dynamic exercise. In this thesis the normal responses of large arteries to different types and intensities of exercise was investigated in healthy normotensive subjects. Whilst distensibility of limb conduit arteries was measured for up to 15 minutes following exercise aortic distensibility did not change. An exaggerated systolic blood pressure response on exercise was not observed in healthy subjects without other conventional cardiovascular risk factors, but was observed frequently in the presence of such risk factors. Subjects with an exaggerated systolic blood pressure response on exercise did not show increased limb conduit artery distensibility immediately following exercise (and by implication during exercise). Blockade of NO synthesis prevented the increase in limb conduit artery

distensibility seen in the first few minutes following exercise, but did not abrogate the more sustained increase in arterial distensibility following exercise. Systemic blockade of NO synthesis caused marked changes in systemic haemodynamics at risk, but these were markedly attenuated during exercise. The impact of mental stress on arterial function was also assessed. Whilst peripheral microvessels vasodilated, large arteries stiffened and this largely accounted for the observed increase in blood pressure.

Chapter One

Introduction

Heart Disease in the United Kingdom

Diseases of the heart and circulatory system (CVD) are the number one causes of death in the United Kingdom (UK), with over 240,000 deaths in 2001[1,2] (Table 1.1). Forty percent of all deaths in the UK are from CVD. CVD is made up of coronary heart disease (CHD) and stroke, with a 3:1 ratio of CHD to stroke. The cost of treatment of CVD is about £1.75 billion per annum. This is split between hospital care (53%), drug administration (34%), and 12% on other expenses. If the economic cost of days lost to illness, death and informal care of patients is included the total rises to just over seven billion pounds per year[1,2]. Less than one percent is spent on research into the primary prevention of CVD.

Blood Pressure and Cardiovascular Risk

Blood pressure is a significant risk factor for cardiovascular disease and the characteristics of the large arteries play a significant role in determining blood pressure[3]. Blood pressure is made up of two components, the systolic blood pressure (SBP) and the diastolic blood pressure (DBP). The SBP reflects the pressure exerted on the arterial wall as the ventricle ejects or is in contraction. The DBP is a measure of the pressure exerted on the arterial wall when the ventricle is filling or in relaxation. The guidelines from the American Heart Association (AHA), the World Health Organisation (WHO) and the British Heart Foundation (BHF) are that resting blood pressure

should be less than or equal to 120 over 80 (mmHg) and that

hypertension is defined as a blood pressure in excess of 140 over

90 (mmHg)[4, 5].

Table 1.1 Deaths by cause, sex and age, 2001, United Kingdom

Cause	Sex	All Ages	< 35	35-44	45-54	55-64	65-74	75 +
All causes	M	287,062	9,940	7,193	15,542	32,000	66,063	156,324
	F	315,960	5,179	4,453	10,310	20,402	47,027	228,589
	Total	603,022	15,119	11,646	25,852	52,402	113,090	384,913
All circulatory diseases	M	114,336	549	1,508	5,115	11,882	27,732	67,910
	F	125,931	291	675	1,783	4,849	16,398	101,935
	Total	240,267	840	2,183	6,898	16,731	43,770	169,845
Coronary Heart Disease	M	66,400	122	885	3,581	8,358	17,579	35,875
	F	54,491	26	195	758	2,514	8,642	42,356
	Total	120,891	148	1080	4,339	10,872	26,221	78,231
Stroke	M	25,208	118	249	675	1,588	4,724	17,854
	F	41,518	86	244	573	1,179	4,091	35,345
	Total	66,726	204	493	1,248	2,767	8,815	53,199
Diabetes	M	3,350	39	86	171	373	900	1,781
	F	3,628	29	44	69	219	650	2,617
	Total	6,978	68	130	240	592	1,550	4,398
Cancer	M	81,992	821	1,247	4,872	12,685	24,043	38,324
	F	76,531	805	1,852	5,354	10,487	18,533	39,500
	Total	158,523	1,626	3,099	10,226	23,172	42,576	77,824
Colo-rectal cancer	M	8,528	19	112	528	1,368	2,582	3,919
	F	7,627	28	96	331	812	1,625	4,735
	Total	16,155	47	208	859	2,180	4,207	8,654
Lung cancer	M	20,437	18	136	1,087	3,490	6,953	8,753
	F	13,027	11	119	822	1,991	4,183	5,946
	Total	33,509	29	255	1,909	5,481	11,136	14,699
Breast cancer	F	13,011	123	703	1,615	2,258	2,650	5,662
	Total	13,011	123	703	1,615	2,258	2,650	5,662
Respiratory Disease	M	34,848	249	271	722	2,246	6,702	24,658
	F	40,961	203	189	559	1,574	5,154	33,282
	Total	75,809	452	460	1,281	3,820	11,856	57,940
Injuries and poisoning	M	12,540	4,163	2,123	1,650	1,192	1,048	2,364
	F	7,580	1,075	648	603	514	667	4,073
	Total	20,120	5,238	2,771	2,253	1,706	1,715	6,437
All other causes	M	39,996	4,119	1,958	3,012	3,622	5,998	21,287
	F	61,329	2,776	1,045	1,942	2,759	5,625	47,182
	Total	101,325	6,895	3,003	4,954	6,381	11,623	68,469

Office for National Statistics (2002) Deaths registered in England 2001 by cause and area of residence, personal communication.
Office for National Statistics (2002) Deaths registered in Wales 2001 by cause and area of residence, personal communication.

The significance of raised blood pressure as a leading risk factor for CVD was highlighted in the recent WHO report[3]. Hypertension or raised blood pressure was identified as the second most influential risk factor for mortality and morbidity, exceeded only by tobacco. Within the UK about 50% of men and women aged between 65 and 74 are hypertensives or are receiving treatment for raised blood pressure. Approximately 80% of these patients are not receiving treatment (32% of this older population). Of note is that of those who are treated, 60% continue to be hypertensive despite therapy. Population based studies have consistently demonstrated that a continuous and positive relationship exists between blood pressure and mortality. However, studies examining the effects of pharmacological hypertensive treatments have shown a U-shaped relationship with an increased risk of events for subjects with low blood pressure[6]. The Framingham study into the effects of blood pressure on cardiovascular disease is the most cited piece of literature in this field[7], it has several updated publications and as a summary has concluded that of the three most related measures of blood pressure and mortality are SBP, DBP and Pulse Pressure (PP)[8, 9]. However, when analysed for age and blood pressure there is a difference in the predictive value of each measure (Table 1.2)[8]. The younger population of the Framingham study (<50 years old) show a significantly stronger predictive value for DBP than either SBP or PP (but all were prognostic), while all three pressures have similar

predictive values in the middle age group (50 –59 years old). In the older group (>60 years old) the strongest relationship was with PP, then SBP, with little or no value in adding DBP to the risk profile[8]. This led the authors to postulate that the under 50 group must have an abnormal systemic vascular resistance, whereas in older subjects the primary abnormality is one of large artery stiffness which fits with previous pathophysiological studies[10-13]. This also fits with the cardiovascular disease profile of the old with a high incidence of isolated systolic hypertensive with a wide pulse pressure. There have been similar findings in other large epidemiological trials[14, 15].

Table 1.2 Proportional-Hazard Regression Coefficients Relating Incidence of CHD to single BP Components of SBP, DBP, and PP by Age Groups[8, 10]

Single BP Components	ß†	SE†	Wald x2‡	HR (95% CI)†
<50 years				
SBP	0.13	0.04	10.8	1.14 (1.06-1.24)!
DBP	0.29	0.06	21.8	1.34 (1.18–1.51)¶
PP	0.02	0.07	0.1	1.02 (0.89–1.17)
50-60 years				
SBP	0.08	0.03	6.3	1.08 (1.02–1.15)§
DBP	0.10	0.06	2.9	1.11 (0.99–1.24)
PP	0.11	0.05	5.4	1.11 (1.02–1.22)§
>60 years				
SBP	0.16	0.03	30.0	1.17 (1.11–1.24)¶
DBP	0.11	0.06	3.2	1.12 (0.99–1.27)
PP	0.21	0.04	36.9	1.24 (1.16–1.33)¶

*SBP, DBP, and PP were entered in separate models, adjusted for age, sex, body mass index, cigarette smoking, diabetes mellitus, and ratio of total to HDL cholesterol. §=P<0.05, !=P<0.01, ¶=P<0.001.

Blood Pressure control

Blood pressure is controlled on both an acute and chronic basis. Acute changes occur with each beat of the heart, with inspiration and expiration and in response to changes in orthostatic position or other physiological stresses. Both chronic and acute changes share similar mechanisms to alter and control the pressure. The main acute and chronic mechanisms involved on the short and long-term control of blood pressure are highlighted below. The arterial Baroreceptor reflex is the single most important mechanism providing acute modulation of arterial pressure. As is usual in a reflex pathway there are sensory receptors, afferent and efferent pathways, integration with the central nervous system (CNS) and effector organs[16]. We include a simplified diagram to show the link between each of the stages of the pathway that we will discuss below (Fig 1.1).

Figure 1.1 Mechanisms of blood pressure control schematic

Decrease Mean Arterial Pressure

↓

- in baroreceptor discharge

↓

Medullary Cardiovascular Centres

+ sympathetic activity   - parasympathetic activity

+ arteriolar tone   + venous tone   + cardiac contractility

+ blood volume

+ peripheral venous pressure

transcapillary fluid reabsorption

+ ▼ -
central venous pressure

- capillary pressure

+ vasoconstriction   + stroke volume

+ heart rate

+ total peripheral resistance   + cardiac output

Increase mean arterial pressure

Note: - = decrease and + = increase

The afferent pathways are made up of sensory receptors commonly called the arterial baroreceptors. These receptors are found in the walls of the aorta, coronary and carotid arteries. The receptors themselves are mechanoreceptors that judge the degree of pressure from the degree of arterial stretch. Under normal conditions an increase in stretch causes an increased rate of firing of the baroreceptors. Baroreceptors are affected by both the mean arterial pressure but also by the arterial pulse pressure as they respond to both the change in pressure and the rate of change in pressure. The greatest changes in the rate of baroreceptor nerve activity occur between mean blood pressures of 90 and 110 mmHg, this demonstrates a marked ability to return the pressure to its normal values of around 95 mmHg. If arterial pressure remains elevated for a period of days the arterial baroreflex activity will return to normal levels (i.e. they adapt to a higher set point), thus arterial baroreceptors play no role in the chronic changes in arterial pressure. The medullary cardiovascular centre within the brain is where central integration occurs. The exact role and locations of each centre is not specifically known or wholly mapped out and is not of great significance to this review. We will therefore give an outline of their role in the responses to arterial pressure changes. The major external influence comes from arterial baroreceptors; this influence is continuous as baroreceptors are active at normal pressures. An increased input from the baroreceptors results in

inhibition of the spinal sympathetic outflow with concurrent stimulation of the parasympathetic inhibitory tract and preganglionic nerves. This leads to a reduction in sympathetic and an increase in parasympathetic nerve activity. If arterial pressure falls, the opposite response causes a decrease in parasympathetic activity and an increase in sympathetic activity.

The efferent pathways are the sympathetic and parasympathetic nerves that lead to the heart and blood vessels. These nerves contain preganglionic and postganglionic fibres, which are outside the CNS and form the terminal link to the heart and vessels[16]. Other reflexes and receptors influencing blood pressure including the dive reflex, pain reflex and the so called "low pressure" receptors and chemoreceptors in the heart and lung receptors. These reflexes cause large and rapid alterations in arterial blood pressure, which are short term and are therefore not relevant to this review[16]. We outline below the cardiovascular changes that occur following a drop in mean arterial blood pressure (Fig 1.1).

Arterial Blood pressure

As the left ventricle contracts there is a rapid rise in ventricular pressure until the left ventricular pressure exceeds the aortic pressure and the aortic valve opens, the pressure in the ventricles then rises at a steady rate as the blood is ejected into the aorta.

The blood in the aorta causes the walls of the artery to stretch and therefore the pressure rises. As the ventricular pressure falls and the flow of blood from the ventricle falls there is maintenance of arterial pressure due to the elastic recoil of the arteries. On closure of the aortic valve the pressure in the aorta falls slowly (Diastole) as the blood flows continually through the peripheral vessels and back to the veins.

As briefly described above there are two elements that maintain this arterial pressure, the elasticity of the arteries and the downstream resistance vessels. If either of these physiological mechanisms were not present the arterial pressure trace would mirror that of the ventricular pressure volume curve. These two elements have been described as the pulsatile and static components or arterial blood pressure. The static component is frequently measured as systemic vascular resistance, defined as the mean blood pressure (Diastolic BP plus 1/3 of the Pulse Pressure) divided by the cardiac output and at rest ranges from 16 – 24 mmHg/L. The pulsatile component or pulse pressure is affected by three factors, firstly the actual stroke volume, secondly the arterial compliance (or distensibility) and thirdly the character of ejection from the heart during systole. In general the greater the stroke volume, the greater the pulsatile component, while the opposite is true of compliance. We will discuss further the interaction between the ventricles and the large

arteries at the end of this chapter and the measurements we use to access arterial compliance is covered in chapter 2. Arterial elastance can be measured using the ratio of end systolic pressure to stroke volume and is a measure of the combined effects of pulsatile and static afterload/elastance, but is also influenced by heart rate.

Cardiovascular Responses to Exercise

At rest, cardiac output (CO) is approximately 5 (L/min) with a mean blood pressure of 90 (mmHg) resulting in a calculated systemic vascular resistance (SVR) of 18 (mmHg/L). On exercise, CO increases to meet the increased energy demand of the locomotor muscles. CO is increased by two mechanisms; the first is by an increase in heart rate (HR) that will generally increase up to a maximal heart rate of 220 minus age in years[17]. The initial increase in HR is due to parasympathetic withdrawal, up until a HR of 100 and then the continued increase is due to sympathetic activation[18, 19]. The second mechanism to increase CO is an increase in stroke volume (SV), which will generally increase during exercise up until a HR of 120-140[20]. The SV rises because of an increase in the end diastolic volume (via the frank-Starling mechanism) as a result of a translation of blood from the venous component to the heart as a result of venoconstriction, and also because of a small increase in ejection fraction[20].

A healthy untrained, 20 year old male will generally be able to generate a CO_{max} of 20 (L/min) that is made up of a HR of 200 (bts/min) and a SV of 100 (ml). If SVR had maintained its basal tone (18 mmHg/L), the net result would be a mean blood pressure of 360 (mmHg) at peak exercise (20(L)*18 (mmHg/L)). In fact SVR falls during exercise and at maximal exercise is about 6 (mmHg/L) resulting in a MBP of approximately 120 (mmHg)[18, 19, 21]. Figure 1.2 demonstrates the graded response in cardiovascular variables from rest to maximal exercise. As depicted, Systemic Vascular Resistance falls by almost three-fold from baseline to peak exercise. This, however, is the composite of two very different responses in exercising versus non-exercising vascular beds. The exercising beds demonstrate profound vasodilation, so much so that the limb blood flow can increase 2000% from baseline[18, 22]. This is believed to be through a series of local metabolic factors, which include adenosine, augmented by shear-related nitric oxide release. There is marked vasoconstriction in the non-exercising regions (forearm, kidneys and intestine). This is due to a complex interaction of opposing vasoconstrictor and vasodilator neural, and neurohumoral influences. The "central command reflex" and input from skeletal muscle ergoreceptors and metaboreceptors both promote sympathetic outflow from the brainstem (vasoconstriction). As exercise duration and/or intensity increases plasma arginine

vasopressin, angiotensin II and endothelin rise substantially and contribute to increased vasoconstriction of the non-exercising regions. These constrictor influences may be attenuated by neural input from arterial baroreceptors and from mechanoreceptors in the atria and left ventricle, in response to the rise in arterial stretch[18, 22-24].

Figure 1.2   Cardiovascular   Responses   to   Execise[18-22]

Figure showing BP (mmHg) on the y-axis (50 to 250) versus Percentage of VO2max on the x-axis (0% to 100%), with three lines: SBP, DBP, and MBP.

Exaggerated Systolic Blood Pressure Response to Exercise

In 1991 Faggard et al showed that in a hypertensive group of men (n=143) who had been assessed for resting and exercise blood pressure between 1972 and 1982, that exercise blood pressure was a significant predictor of mortality and CV events. However when resting blood pressure and age were added to the analysis the exercise blood pressure carried no additional effect[25]. In contrast, Filipovsky et al showed that an exaggerated systolic blood pressure response to exercise (ExSBP) was of prognostic significance in middle-aged men, independent of resting blood pressure. They studied 4,907 men over a 17-year period and independent of other risk factors the rise in blood pressure from rest to peak exercise was positively associated with cardiac and all cause mortality[26]. Using Cox regression analysis they demonstrated that systolic blood pressure after five minutes of cycling and the increase in systolic

pressure (Exercising – Resting) was significantly associated with cardiovascular mortality. Of note is that they found no relationship between resting systolic pressure or exercise heart rate and mortality. 2-years later, Mundal et al reported a study of 1,999 men followed up over a 16-year period, the exercise blood pressure was measured as the systolic pressure after 6 minutes of cycling at 100-Watts[27]. They demonstrated that when casual blood pressure and ExSBP were analysed simultaneously as continuous variables the independent predictive value of the casual BP became non-significant. The same group later published a further follow up to this study and demonstrated that in subjects with a moderate increase in resting blood pressure (140 mmHg) that those with a more marked increase in ExSBP (>200 mmHg) were at a significantly greater risk for myocardial infarction than those with a the same moderate rise in resting BP but an ExSBP < 200 (mmHg)[28]. The most recent papers from this same study group were in 1997 and 2001 and included the 21 year follow up results, this demonstrated that only supine and 6 minute exercise blood pressure were independently predictive of cardiovascular events[29,30].

The ExSBP in subjects with a high-normal BP can be used to predict future essential hypertension as Miyai et al showed in 239 men over a 5 year follow up[31]. Kurl and colleagues looked at the rate of blood pressure increase and the risk of stroke during upright cycle ergometry (20 watt increase per minute) and found that those men with an increase in ExSBP of greater than 19.7 mmHg per minute had a 2.3 fold increased risk of stroke and also a 2.3 risk of ischemic stroke when compared to the men whose SBP rise was less than 16.1 mmHg per minute[32]. Blood pressure following exercise in 6557 normotensive (<130/85) and borderline hypertensives Japanese (130-140 / 85-90 mmHg) was shown to confer a relative risk of developing hypertension of 1.55 per 10 mmHg increase in post exercise SBP and DBP[33]. These associations were independent of resting BP. Even after stratifying subjects according to blood pressure at rest, SBP or DBP 4 minutes post exercise was associated with an increased risk of long-term development of hypertension. The exercise performed, is called the "Two-step" exercise test, and involves walking up two steps, each 23 centimetres high and then going down the other side. The number of trips undertaken is dependent on age and weight. To determine the prognostic significance of an ExSBP (>60 mmHg SBP rise at 5 min, or >70 SBP mmHg at 10 min, or >10 mmHg in DBP a any time) and the risk of future hypertension in normotensive men, Matthews and colleagues reviewed 5386 treadmill tests carried out

over a twelve year period and showed that those who had an ExSBP had an Odds Ratio of 3.0 (CI 1.5-6.1) when sitting systolic and diastolic pressures, age, familial history and BMI were controlled for[34]. In subjects (n=239) with an already high-normal BP (135/86) Miyai et al demonstrated that those with the greatest increase in ExSBP had a greater risk of developing hypertension, independently of other resting measures[35]. In the first controlled study Sharabi et al re-analysed the data from a three-year period of male subjects whom had completed an exercise stress test with BP readings. They defined an ExSBP as an excess of 200 (mmHg) and an ExDBP being in excess of 100 (mmHg) at peak exercise. From this data they identified 73 subjects with an ExSBP and matched them with 117 controls. Over the follow up period 22% of subjects with an ExSBP developed hypertension compared to just 2.6% of the control group (P<0.05)[36].

All of the tests used to predict future events or hypertension have one common flaw and that is they use a standard workload that is independent of fitness levels. As we demonstrated earlier blood pressure and exercise intensity are linearly related and therefore the higher the increase in intensity the higher the ExSBP. Miyai et al addressed this by analysing 1033 normotensive males SBP and DBP during sub-maximal exercise at different heart rates[31]. Even after taking into account the exercise intensity, they still

demonstrated a 3.8 fold relative risk with an ExSBP above the 90th percentile.

We are only aware of one study to date that has failed to show a link between ExSBP and risk/prognosis, this was carried out in a highly trained (Vo^2_{max} 59.4, ml/kg/min) group and a sedentary control group (44.7 ml/kg/min). They used the peak blood pressure of each individual for analysis and showed no prognostic value. It is important then to understand that subjects with athletic training can exercise to higher levels of exertion at higher cardiac outputs and for a greater duration, all of which can lead to a significant rise in ExSBP, hence making this result questionable.

In summary ExSBP not only predicts which normotensive and borderline hypertensive subjects will become hypertensive it also can be used as a tool to predict cardiovascular death from myocardial infarction and stroke, independent of resting blood pressure

Table 1.3 Summary of Exercise Blood Pressure and Cardiovascular Disease

Author and Year	Sub (n)	Blood Pressure Studied	Outcome Measure
Faggard 1991	143	5th minute of exercise	Positive Relationship between ExSBP and CV Mortality
Filipovsky 1992	4907	Delta Rest to Peak Exercise SBP	Positive Relationship between ExSBP and CV Mortality
Mudal 1994	1999	6 minute SBP at 100 Watts (Cycling)	Positive Relationship between ExSBP and Myocardial Infarction
Kjelsden 1997	1999	6 minute SBP at 100 Watts (Cycling)	Positive Relationship between ExSBP and Myocardial Infarction, Stroke and Hypertension
Kjelsden 2001	1999	6 minute SBP at 100 Watts (Cycling)	Positive Relationship between ExSBP and Myocardial Infarction, Stroke and Hypertension
Miyai 2002	239	Submaximal Blood Pressures	Positive Relationship between ExSBP and Hypertension
Kurl 2001	1026	Change in SBP of 19.7 mmHg V 16.1 mmHg per stage	Positive Relationship between ExSBP and Stroke
Tsumura 2002	6557	1.55 increased risk for every 10 mmHg change in BP	Positive Relationship between ExSBP and Hypertension
Matthews 1998	5386	>60mmHg - 5 min >70mmHg - 10 min >10mmHg – DBP	Positive Relationship between ExSBP and Hypertension
Sharabi 2001	190	SBP>200 DBP>100	Positive Relationship between ExSBP and Hypertension
Miyai 2000	1033	Submaximal Blood Pressures	Positive Relationship between ExSBP and Hypertension
Sharabi 2001	76	Maximal SBP	No Relationships

Large Artery Properties and Blood Pressure

Large artery function plays a significant role in determining pulse, systolic and exercise blood pressure and as such will play a prominent role in our understanding of the ExSBP. In fact recent work has shown that arterial stiffness per se is an independent marker of risk[37-41]. The increase in large artery, particularly aortic, stiffness is of profound importance to the occurrence of increased PP with age and disease status. The consequences of this stiffness lead to both reduced arterial compliance and increased PWV. Reduced compliance will increase PP due to a reduced "buffering" capacity available from the arterial wall, while an increase in PWV will increase systolic pressure augmentation. These important consequences can be understood further with the analysis of the pulse pressure waveform. Before we continue with the assessment of large artery stiffness it is important to understand the basic anatomy and physiology of the arteries.

The overall function of the arterial system is to distribute blood from the heart to the peripheral arteries as efficiently as possible, with minimal mean pressure drop from input to termination, and with minimal pressure fluctuations at the heart and minimal pulsatile energy losses throughout. This is usually achieved in the young and healthy. However, aging and various disease states progressively impair arterial function. Elevated pulse pressure (PP) is increasingly

being recognised as a risk for cardiovascular particularly coronary disease[9, 13, 42]. PP can be discussed in terms of the Windkessel and distributive models of arterial circulation. In its simplest, two element form, the Windkessel model describes the circulation in terms of parallel resistance and capacitance components. The resistance element corresponds to measured peripheral vascular resistance, while the capacitance element corresponds to the compliance (C) of the arterial circulation. Compliance is simply a measure of the capacity of a volume-containing structure, such as the arterial system, to accommodate further increases in volume. While C is widely distributed throughout the arterial tree, total systemic arterial C is predominantly determined by the aorta and its major branches. Arterial C can be estimated from the decay in diastolic pressure as well as by the simpler approach of: C = Stroke Volume (SV)/PP. It is apparent from this approximation that elevations of PP can be secondary to a rise in SV or a fall in C. The rise in PP (or systolic pressure) with age in healthy subjects relates to a fall in C, whereas elevation of PP in the young appears to be related to increases in SV and bears no clinical risk[43]. The value of the Windkessel model is limited as a comprehensive explanation of arterial behaviour under differing circumstances. Analysis of the arterial system solely using the Windkessel model is restricted since it assumes that all pressure changes occur instantaneously, whereas the circulation serves to distribute cardiac output through a

series of branching networks. The elastic properties described by the model are not present at just one site but are distributed along the aorta and major arteries. Therefore, the Windkessel model is unable to explain important phenomena such as wave reflection and frequency dependence[44]. The pressure wave has a finite wave velocity in arteries, and in addition, pressure waveforms are different in amplitude and contour in central and peripheral arteries[45]. While the advantage of the two-element Windkessel model is its simplicity, it fails to characterise other important determinants. Considering the arterial system as comprising of a distributed C overcomes these limitations to a large extent[44].

Ejection of blood into the aorta generates a pressure wave that propagates to other arteries throughout the body. One of the most basic physiological properties of the arterial pulse is that its shape becomes modified as it travels along the arterial tree. The amplitude generally increases, and the pulse waveform features are altered[46]. Conventional measurement of cuff blood pressure fails to take into account these essential properties of the arterial pressure pulse. This means that the conventional pressure measurement, which gives only the maximum (systolic) and minimum (diastolic) values of the peripheral pressure pulse, is not an accurate measure of the pressure load on the heart. Pulse pressure (PP) is the consequence of intermittent ventricular ejection from the heart. PP

is influenced by several cardiac and vascular factors, but it is the role of large conduit arteries, mainly the aorta, to minimise pulsatility. In addition to the pattern of left ventricular ejection, the determinants of PP (and SBP) are the capacity of arteries and the timing and intensity of wave reflections[46]. The capacities of arteries are influenced by arterial stiffness, usually expressed in the quantitative terms of compliance and/or distensibility[44]. The timing and intensity of wave reflections result from the summation of a forward wave coming from the heart and propagating at a given speed toward the origin of resistance vessels and a backward wave returning toward the heart from particular sites of reflection along the arterial tree.

Large Artery stiffness is an important, independent predictor of cardiovascular risk in subjects with hypertension[37], diabetes[39], end-stage renal disease[38, 47] and older (>70 years) subjects[40, 41] and in the most recent of these studies was a better predictor of cardiovascular events than brachial blood pressure[48]. Large artery stiffness is in part determined by structural components (collagen and elastin), and by the transmural pressure. With age comes disruption of the elastin load bearing structures within the arterial wall, with the net result of transfer of stress to collagenous tissue and a stiffening of the artery. Collagen cross-linking also occurs via glycation, large arteries are not simply structural elements

however; they also have vascular smooth muscle cells in their wall, which are under endothelial control and as such can be influenced through various local or circulating mediators. These arteries are also highly innervated with sympathetic vessels, and adrenaline and noradrenaline have been shown to increase and decrease large artery tone[49]. Therefore arterial stiffness as such is regulated and modifiable by local and central stimuli. It has also been demonstrated in animals (rat) that removal of the endothelium causes significant changes in large artery pulse wave velocity, demonstrating the role of the endothelium in large artery stiffness[50].

Active Regulation of Arterial Stiffness

Traditionally, the stiffness of a vessel was viewed as only a function of the structural elements of the vessel and distending (mean arterial) pressure. However, the large arteries also have a generous coat of smooth muscle, which can alter the stresses between the elastic and collagenous fibres of the artery wall and thus alter the arteries stiffness[51]. The muscular arteries have a large sympathetic innervation and because smooth muscle tone is also influenced by circulating and local vasoactive substances mediators, arterial stiffness may be actively regulated, and modified, at least in the short term[51].

Arterial Stiffness and Sympathetic Nerve Activity

Gerova[52] reported that sympathetic nerve activity exerts a "tonic" stiffening influence on the arterial walls. In man, arterial wall distensibility can be reduced by an increased sympathetic drive, as shown by the effects on the radial and carotid arteries during cold pressor and mental arithmetic tests[53], and smoking[54], i.e. manoeuvres that cause sympathetic activation. Failla et al. have shown that in man, anaesthesia of the brachial plexus, lower spinal chord and lumbar sympathectomy caused an increase in the distensibility of the radial or femoral artery respectively[55]. These observations reflect the stiffening effect exerted by neural influences on the vessel wall. Significantly, arterial distensibility changes were limited to the vessel from which sympathetic activity was removed. It is important to note that these changes were not associated with significant reductions in BP or HR, therefore excluding the possibility that the results originated from a shift in the distensibility-pressure curve[56], or a reduction in the viscoelastic opposition of the arterial wall to inside pressure when HR is increased[57]. The authors concluded that in man, the sympathetic nervous system exerts a pronounced restraint in the distensibility of medium and large sized arteries. This restraint is observed not only when its activity is increased in a short-term manner as demonstrated by behavioural or emotional stimuli, but also when the vessel is exposed to long lasting sympathetic tone. It was also

speculated that since increases in distensibility were observed within a few minutes after the withdrawal of sympathetic influences, that functional rather than structural mechanisms are involved. It is thought that this functional mechanism may involve the tonic contraction of medial smooth muscle because *in vitro* human studies[53] have shown that arteries have a greater elastic modulus when smooth muscle is contracted. It is finally speculated that this smooth muscle contraction is accounted for by the direct influence of sympathetic medial wall innervation[55]. Further evidence for the influence of sympathetic nerve activity influencing arterial stiffening is provided by the recent hand transplantation studies by Giannattasio et al. which report that radial denervation is accompanied by an increased arterial distensibility[58].

The Endothelium and the Role of NO in Arterial Stiffening

The systemic infusions of drugs that promote or inhibit NO release have been used to investigate the role of NO in regulating large artery stiffness. Many studies have clearly demonstrated that NO donors, such as GTN, reduce augmentation index (AI), a marker of arterial stiffness (see later), independently of any effect on BP, in both healthy subjects, and in those with a range of CV risk factors including hypertension and hypercholesterolemia[59, 60]. NO donors have also been shown to reduce other measures of large artery stiffness such as PWV in hypertensive individuals, but not always

independently from changes in distending pressure[61, 62]. The contribution of basal NO to resting large artery stiffness has been assessed by infusions of NOS inhibitors such as L-NMMA and L-NAME. Wilkinson et al have shown that systemic infusion of L-NMMA increases AI in healthy normal volunteers[59, 60]. The findings of this study must be interpreted with caution however, since the changes in stiffness observed were accompanied by increases in MAP and reflect changes in heart rate.

More definitive evidence for the role of NO in regulating large artery stiffness may come from local intra-arterial infusions of L-NMMA and GTN. Such techniques overcome the methodological limitations of systemic infusions, because the drug doses used are much lower and, if infusion periods are relatively short, MAP and HR and normally unaffected[51]. Studies using techniques such as intravascular PWV measurement, using pressure or flow waveforms, or direct ultrasound measurements of distensibility or compliance, have shown that endothelium-derived NO regulates the stiffness of the ovine iliac artery[60]. Kinlay et al. also report similar findings in the human brachial artery when elasticity, compliance and PWV were observed[63]. Conversely GTN or Acetylcholine administrations reduce arterial stiffness in the muscular arteries[63, 64]. Together these studies suggest that basal, simulated, or exogenous NO acts to reduce arterial stiffness in humans *in vivo*, independently from

any changes in BP, highlighting the importance of the vascular endothelium in the functional regulation of arterial stiffness.

Endothelial Derived Hyperpolarizing Factor (EDHF)

By definition, an EDHF is a substance synthesised in, and released from, the endothelium which hyperpolarizes vascular smooth muscle cells thus reducing the open probability of voltage-dependent Ca^{2+} channels so that $[Ca^{2+}]_i$ is lowered, and relaxation can take place. Although it is generally assumed that EDHF opens Ca^{2+}-dependent K^+ (K_{Ca}) channels to hyperpolarize the smooth muscle cell membrane, the mechanism of action of EDHF is controversial as the exact type of K_{Ca} involved remains unclear and some evidence now suggests that the EDHF-activated K_{Ca} are localised on the endothelium. Moreover, the evidence suggesting the involvement of a Na^+-K^+-ATPase cannot be ignored and many of the EDHF's described in different arteries are able to activate this electrogenic pump[65].

Endothelial Nitric Oxide Production

The key factor controlling vascular blood flow at rest is nitric oxide. Furthermore NOS production increases in response to many stimuli; which include shear stress[66-69], acetylcholine[70], bradykinin[71] and prostaglandins[72]. Other stimuli inhibit the release of NOS, including angiotensin II (via inhibition of bradykinin), tumour necrosis factor

(TNF) which inhibits NOS and increases endothelial cell apoptosis[73], norepinephirene, endothelin[74] and vasopressin[75, 76]. It has been demonstrated that the administration of ACE inhibitors[77], the production of prostaglandins[72], bradykinin[78] and L-Arginine[79] all help to improve vascular conductance and/or exercise performance. It is quite logical to assume that part of this improvement is due to the agonistic or antagonistic effects these substances are known to have on the factors effecting endothelial function.

Nitric Oxide first appeared in the scientific literature in the mid 17th century in the writings of J van Helmont. Its chemical properties were not characterised until the mid to late 18th century when J Priestley gave it the name "nitrous air". Finally, in 1980 the physiological role of NO and the endothelium was revealed[80]. We concentrate on NO and its function within the vascular system from this point forward.

The vascular endothelium is a barrier between the blood and the vascular smooth muscle; the endothelium actively partakes in the control of vascular tone, blood flow and the regulation of blood fluidity. NO is synthesised in endothelial cells and plays a critical role in the maintenance of vascular tone, fluidity and the anti-adhesive properties of the endothelium. NO acts via a cyclic guanosine monophosphate (cGMP) mechanism to cause vasorelaxation, which in turn increases perfusion and therefore

oxygen flow. At rest, low levels of NO are continuously synthesised and released generating a constant counter against sympathetic vasoconstriction. During hypoxia, shear stress or activation of endothelial receptors NO synthesis increases.

Shear stress is the result of the frictional force applied to the endothelium by blood flow though a vessel. This can increase in one of two ways 1) a decrease in vessel diameter (vasoconstriction) without a decrease in flow, 2) or as an increase in blood flow without an increase in vessel diameter. Shear stress has been shown to potentiate agonist-stimulated NO release[66, 68] and chronic shear stress (exercise training) up regulates type III NOS expression[81, 82]. Unlike the other forms of NOS, type III NOS is membrane bound, this positioning of type III NOS appears to enable NO synthesis to be regulated by shear stress.

There are three main isoforms of NOS of these nNOS and eNOS are constitutively expressed and are found in skeletal muscle. The effects of NO on skeletal muscle perfusion are the most significant. Perfusion depends on the calibre of the resistance vessels and the perfusion pressure applied. These are in the main mediated by neural and metabolic effects with small but significant changes also coming from the endothelium and the muscle pump. Therefore the main determinant of resistance vessel calibre is the ongoing

vasodilatory effects (Hydrogen, Oxygen pressure, Carbon Dioxide Pressure, Adenosine, Magnesium and Potassium) balanced against the sympathetic activation from metabolite and mechanical receptors in the muscle.

Indices of Arterial Stiffness

O'Rourke et al highlighted a number of reservations in the measurement and interpretation of many of the indices used to calculate arterial stiffness[45]. These common misgivings include the use of an inappropriate arterial model (e.g. Windkessel), and problems when changes in proximal arterial diameter are related to pressure changes at a distant site. Arterial stiffness cannot be fully understood without examining the factors that control and regulate it.

Arterial Stiffness Measurement

Many methodologies, both invasive and non-invasive, have been applied to the assessment of arterial elasticity in vivo. Non-invasive measures fall into three broad groups: 1) measuring PWV, 2) relating change in diameter or area of an artery to distending pressure, and 3) assessing arterial pressure waveforms. The most hallowed measure of arterial stiffness is pulse wave velocity (PWV). PWV is inversely related to distensibility and can be calculated using the Moens-Korteweg equation: PWV = $\sqrt{(Eh/2\rho R)}$, where E is

Young's modulus of the arterial wall, h is wall thickness, R is arterial radius at the end of diastole, and p is blood density. There are a number of ways to measure PWV, and these are generally easy to perform. For example, the arterial pulse wave is recorded at a proximal artery, such as the common carotid, as well as at a more distal artery such as the femoral. The superficial location of the carotid and femoral arteries means that their pulse waveforms are readily measured non-invasively, and between these two sites the pulse wave travels through most of the aorta. PWV is measured as the delay between corresponding points on the wave, which are not influenced by wave reflection between two recording sites in the line of pulse travel. The time delay between the arrival of a predefined part of the pulse wave (the wave front/foot or initial upstroke is the usual point of reference in the two waveforms[83]) at these two sites is obtained either by simultaneous measurement, or by recording the waveforms independently but comparing the time delay at both sites against a simultaneously measured QRS complex. The distance travelled by the pulse wave is measured over the body surface and PWV is then calculated as distance/time (m/s). Arterial pulse waves can be detected by using pressure sensitive transducers[83], Doppler ultrasound (the pressure pulse and the flow pulse propagate at the same velocity) or by applanation tonometry, where the pressure within a small micromanometer flattened against an artery equates to the pressure within an artery[84]. Practical problems in

measurement of PWV arise when convenient points of measurement (e.g., carotid and femoral artery) are not in the same line of travel, and in determining the actual arterial distance between recording sites form measurements on the surface of the body.

Pulse Wave Velocity

The "anatomical area" of the arterial tree determines the pulse wave velocity. It is usually recorded in metres per second (m/s). Higher velocity corresponds to higher arterial rigidity and therefore to lower arterial distensibility. The principal determinants of PWV are the elastic properties of the arterial wall, blood pressure and blood viscosity. PWV is generally greater in peripheral than central vessels due to differences in elastic properties and blood pressure amplification. Systolic Blood pressure in youth is lower in the aorta than in the periphery and as pressure increases so does PWV. Systolic pressure increases as we descend the arterial tree, but diastolic pressure remains constant, thus there is an increase in pulse pressure and PWV (Fig 1.3)[85, 86]. The internal cross sectional area of the aorta has been measured[87] at approximately 3 cm^2 whilst the smaller femoral arteries are as low as 0.17 cm^2. Also the ratio between arterial radius and wall thickness (Wall thickness /radius) increases with increasing distance from the aorta, especially in the elderly.[88]

Fig 1.3 Pulse Wave Velocities measured at Different Anatomical Sites[85, 86]
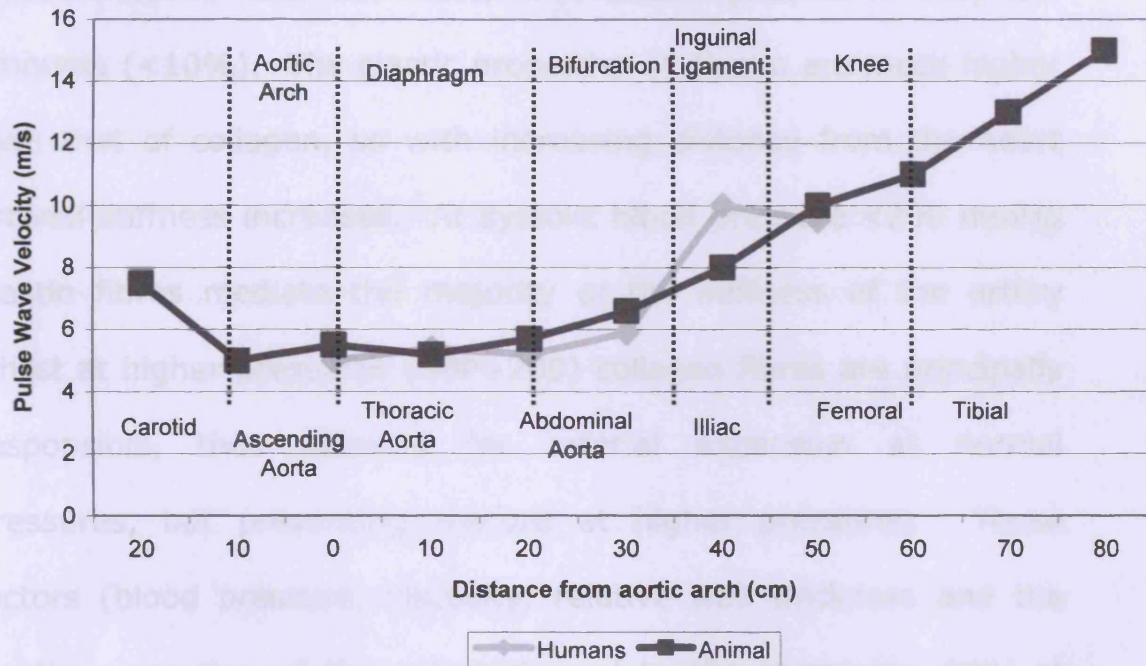
Blood viscosity and density is known to increase with higher rates of shear stress in smaller tubes when compared to larger ones[89]. However, this diminution with vessel size does not begin until the internal diameter is less than 1mm and persists until the vessels are smaller than 10 microns. The elastic properties of the arterial wall represent the ratio between stress/strain and this reflects the properties of the vessel walls. The major elastic properties of the arterial wall are determined by collagen and elastin[90]. It has been argued that smooth muscle also contributes to elastic properties but it is not a truly elastic material, nevertheless it does contribute to wall tension[90]. The proximal aorta has a large amount of elastin when compared to collagen, but further from the heart the

elastic/collagen ratio switches. In peripheral arteries collagen and smooth muscle cells dominate, with elastin present in very low amounts (<10%). The elastic properties of elastin are much higher than that of collagen, so with increasing distance from the heart arterial stiffness increases. At systolic blood pressure <200 mmHg elastin fibres mediate the majority of the stiffness of the artery whilst at higher pressures (SBP>200) collagen fibres are principally responsible, thus allowing for arterial expansion at normal pressures, but preventing rupture at higher pressures. These factors (blood pressure, viscosity, relative wall thickness and the elastic properties of the arteries) explain the increasing PWV at distal sites when compared to central PWV. PWV is primarily determined by the elastic properties of the artery as the pulse wave propagates against its walls. There are however many physiological and pathophysiological conditions that can increase and decrease velocity. These factors include age, blood pressure, heart rate and gender.

Age has a profound effect on both central and peripheral PWV, PWV increases gradually until the fourth decade but above this age the aortic PWV in particular appears to increase at a much faster rate than over the previous decades (Fig1.4). Aschoff demonstrated that when an aorta was released from its vertebral attachments and then sliced transversely through its midpoint the cut ends would

47

retract. In youth the cut ends retracted significantly further than in the elderly, suggesting a loss of recoil in the arterial wall with age[88, 91]. Also with increasing age the size of the aorta increases while extensibility decreases. Further studies have shown that collagenous tissue replaces elastic tissue in the aorta as age increases. We are born with all the elastin we will ever have in our arteries, it is broken down with a t½ of 30 years[92, 93].

Fig 1.4   **Age related changes in Aortic PWV[94]**
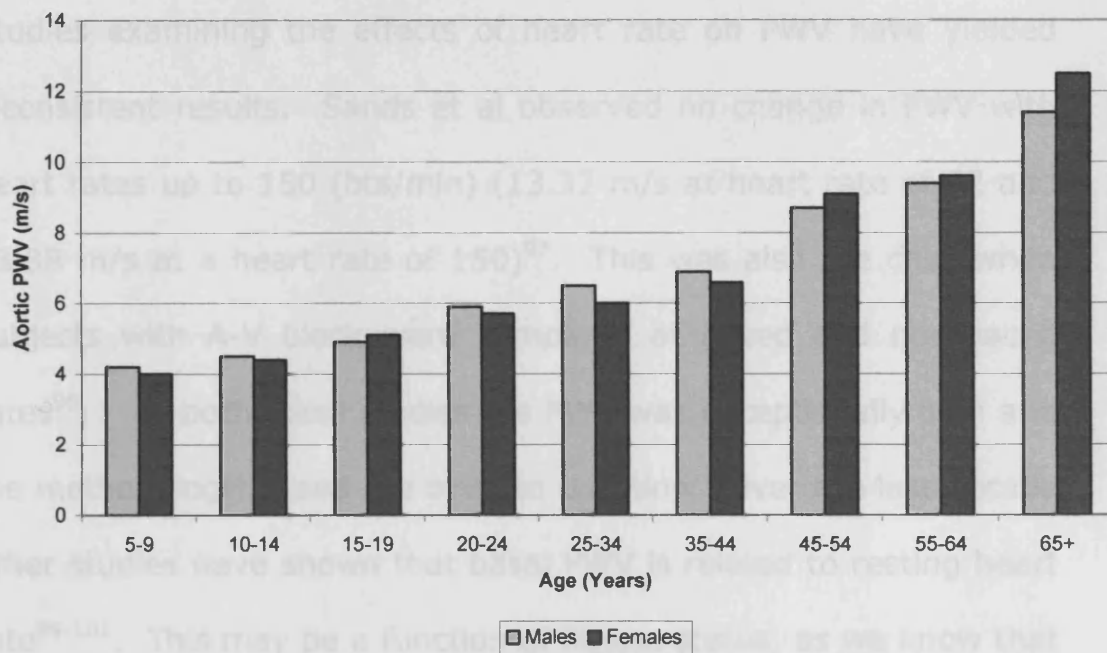
A study carried out in a Chinese population assessed brachial PWV, femoral PWV and aortic PWV across a large age range. Brachial PWV exceeded femoral PWV, which exceeded aortic PWV[95]. However, as the group aged the difference between the velocities become closer, because aortic PWV increases with age at a greater

rate than peripheral PWV[95]. This data agrees with the higher rate of atherosclerosis in the central arteries when compared to the periphery with increasing age. There are numerous studies independently demonstrating that the elasticity of an artery and PWV are both affected by changes in blood pressure[83, 96, 97]. Studies in large populations have shown that age and blood pressure are the two major factors influencing PWV[95]. Age has a greater effect on aortic than on brachial or femoral PWV.

Studies examining the effects of heart rate on PWV have yielded inconsistent results. Sands et al observed no change in PWV with heart rates up to 150 (bts/min) (13.32 m/s at heart rate of 92 and 13.38 m/s at a heart rate of 150)[97]. This was also the case when subjects with A-V block were compared at paced and non-paced rates[98]. In both these studies the PWV was exceptionally high and the methodologies used are open to question. Over the last decade other studies have shown that basal PWV is related to resting heart rate[99-101]. This may be a function of fitness status, as we know that both heart rate and PWV increase with worsening physical capacity[102, 103]. However in other recent studies pharmacological agents or pacing have been used to alter heart rate and these too have clearly shown that an increase in heart rate causes a concomitant increase in PWV. Liang et al showed that increasing heart rate (transoesophageal pacing) from 56 to 80 and then 100

(bts/min) resulted in significant increases in carotid to femoral (aortic) and femoral to dorsalis pedis PWV[104]. The increase in lower limb PWV was only significant when increasing heart rate from 56 to 80 (P<0.05), whilst the aortic PWV was significantly different at all three heart rates[104]. It should be noted that mean blood pressure (MBP) rose from 78 to 98 (P<0.05) and then to 102 (P=NS) (mmHg) during chronotropic stimulation, providing a confounding variable and hampering interpretation of the effect of heart rate alone. There have been numerous studies which have corroborated these results in both normotensive and hypertensive subjects[101, 105, 106]. In contrast, Wilkinson et al have claimed to show that aortic PWV does not change with pacing (80-120 bts/min)[107]. They used the timing of the reflection wave (T_R) to estimate PWV. Although T_R has been validated as a method for estimating PWV the error around this measurement (Approx 0.5 m/s) make it unreliable as an accurate determinant of PWV. Reflectance site as well as PWV influences TR.

One reason why increasing heart rate may increase PWV is the reduction in left ventricular ejection time (LVET) associated with increasing heart rate. Nurnbeger et al studied 102 young healthy men. From this cohort, 6 subjects who had been given α-methylnoradrenaline, were also given the β and alpha adrenoreceptor stimulator yohimbine and the β antagonist

propranolol. Basal PWV was related to age and LVET using multiple regression analysis (PWV was also related to heart rate using simple regression analysis (r=0.245, P=0.0125))[100]. Beta stimulation change in delta LVET was associated with delta PWV using simple regression analysis (r = -0.520, P= 0.0325), alpha stimulation produced significant correlations on simple regression between Age, DBP, HR, LVET and others (all P<0.05) whilst the multiple regression showed correlations between age, DBP and LVET only (all P<0.05)[100]. Most studies investigating gender differences in PWV have tended to show that women have a slightly lower PWV (5%) throughout early and mid-life until they reach the menopause where this difference is no longer evident. These gender differences are more marked in the peripheral arteries than in the Aorta, see Fig 1.5. As noted above PWV is linked with age, blood pressure and other cardiovascular risk factors, however there is still a question of cause and effect and whether this means that PWV is a valid marker for risk of future CHD. In 1996 Suzuki published the results from a large study (n >100,000) carried out in Japanese urban residents from 1983 to 1986[108]. During the follow up (2-years) there were 301 cardiovascular events. The results showed that blood pressure and Carotid-Femoral PWV were independent predictors of events in a multivariate model. Blacher et al showed that aortic PWV was significantly correlated with estimated risk for myocardial infarction, CHD, CVD and stroke (derived from the Framingham scoring

system)[37]. Aortic PWV correlated more closely with the Framingham risks than LV hypertrophy, plasma creatinine or total/HDL cholesterol. From their data they suggested an optimal cut off point of 13 m/s to predict ten-year CV events. This group have also published data showing that subjects with end-stage renal disease who have a raised aortic PWV (>12, m/s) have a 5.4 Odds ratio (CI 2.4-11.9) for all cause mortality and 5.9 for cardiovascular mortality (CI 2.3-15.5), when compared to subjects with an aortic PWV of <9.4 (m/s). They also showed that for every 1 m/s increase in PWV the increase in all cause mortality odds ratio was 1.39 (CI 1.19-1.62)[38]. These studies have shown the prognostic significance of aortic PWV in selected patient groups, and suggest the possibility of Aortic PWV being used as part of a multi-factorial stratification in apparently healthy subjects.

Fig 1.5   Aortic and Radial Pulse Wave Velocity for Age and Sex
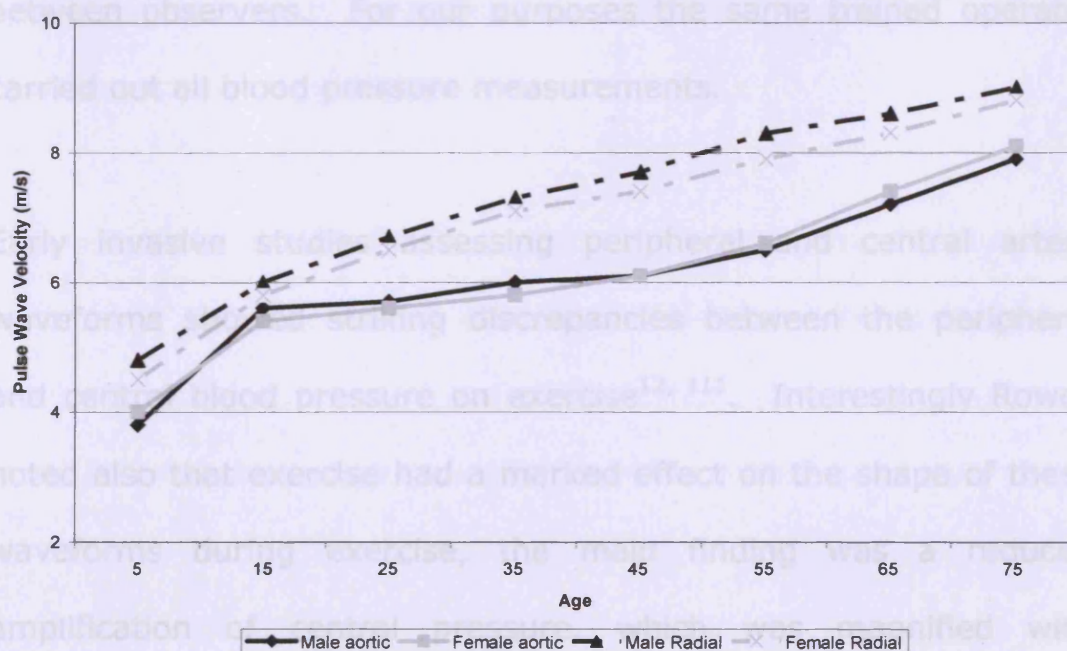
Peripheral and Central Arterial Blood Pressure

Historically the measurement of blood pressure has been from the brachial artery[109], however there are significant differences between aortic blood pressure and peripheral blood pressure with particular reference to Systolic Blood pressure[110, 111]. Diastolic and mean blood pressures are not usually significantly different in health[110, 111]. The reason for this is due to pressure wave reflection that results in amplification of peripheral systolic pressure. There are recent studies that demonstrate that this amplification increases on exercise and has been shown to be as high as 80 mmHg[12]. However given the complex nature of the equipment and the movement artefact involved in pulse wave analysis, brachial artery sphygmomanometery remains the gold standard for exercise blood pressure. It has been shown to yield reproducible results when carried out by a trained operator and has reasonable agreement between observers. For our purposes the same trained operator carried out all blood pressure measurements.

Early invasive studies assessing peripheral and central artery waveforms showed striking discrepancies between the peripheral and central blood pressure on exercise[12, 111]. Interestingly Rowell noted also that exercise had a marked effect on the shape of these waveforms during exercise, the main finding was a reduced amplification of central pressure, which was magnified with

increasing intensity[12]. Theoretically this was suggested to be due to a peripheral vasodilation and reduction in positive waveform reflections.

Pressure Wave Analysis

Modern clinicians on the whole pay little attention to the pulse wave itself, rate and rhythm apart, but it was originally studied in the late 19th century by Dr Mohamed, initially as a medical student in London. It was Dr Mohamed who developed the original Sphygmogram, this was a mechanical device which used levers to "draw" the peripheral pulse wave, in this case the radial artery waveform[112]. Mohamed showed that changes in peripheral waveforms were demonstrable following pharmaceutical intervention and that there were difference in health and disease. However the arrival of sphygmomanometery and the ease of application resulted in a reduction in the number of patients receiving sphygmogrametry.

The invention of high fidelity tonometers and hence "Applanation tonometry" has resurrected interest in this field. The radial artery has become the most used site of application and the application of a validated mathematical transfer function, which generates aortic waveforms and pressures from peripheral waveforms, has lead to widespread clinical use[113, 114]. The transfer function is still

questioned but it is becoming accepted that central systolic blood

pressures are accurate and any inaccuracy probably stems from the

error in the peripheral calibration pressure rather than the transfer

function itself. There is still no consensus on the accuracy of the

transfer function for central augmentation index and recent studies

have shown that peripheral augmentation is at least as accurate as

central estimates and could therefore be used instead.

Fig 1.6   Aortic Waveform and Augmentation Index

Augmentation Index (AI) is calculated as the difference between the
early systolic shoulder (P1) and the late or peak systolic shoulder
(P2), the augmentation index was then calculated as the difference in systolic shoulders (P2-P1) divided by the pulse pressure and expressed as a percentage.
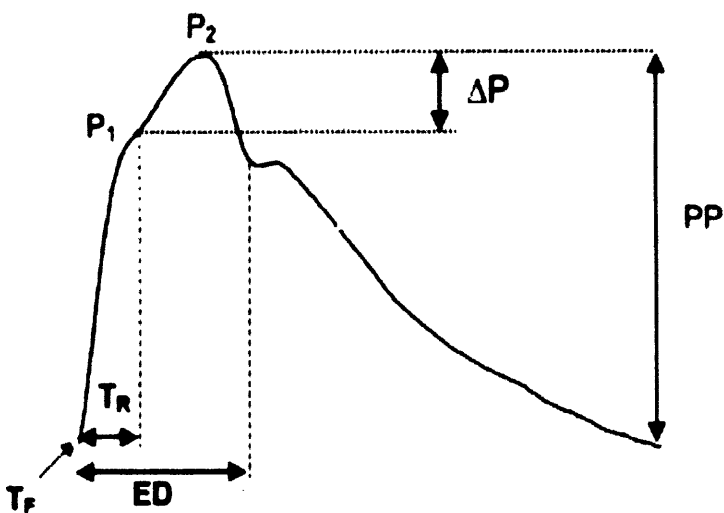
Arterial Wave Reflection

The left ventricle contracts and ejects blood into the circulation, this

creates a primary wave of forward travelling pressure. When

assessing the peripheral pulse wave there is a second pressure

wave and occasionally a third. The secondary pressure is largely

due to reflections of the initial wave and is smaller in magnitude than the primary wave but increases as the waveform reaches the periphery[115]. Wave reflections occur at branch points of the arterial tree and the reflected waves in the large vessels are the accumulation of the peripheral reflections. In health this wave reflection arrives during the diastolic phase of the cardiac cycle and augments diastolic coronary perfusion and does not negatively impact on ventricular ejection by increasing afterload[46].

In many disease states the arteries stiffen and pulse wave velocity increase. This increase in velocity and amplitude of the reflected waveforms can result in the returning waves arriving during the systolic component of the cardiac cycle[115, 116]. This results in an augmentation of the systolic pressure wave and can increase ventricular load and hence oxygen consumption. This increase in arterial pressure can be measured as the aortic augmentation index that is the augmentation pressure divided by the pulse pressure as depicted above (Fig 1.6).

Exercise and Pulse Wave Velocity

Exercise can of course be separated into acute exercise (Single bouts) and chronic exercise (Multiple bouts) and we would expect there to be different reactions to each. It can also be separated into aerobic (cycling, walking etc) and anaerobic (resistance

training, sprints etc) exercise, which are different in terms of intensity and duration. This review will concentrate mainly on acute and chronic aerobic exercise and its effect on markers of arterial function.

Chronic Exercise and Pulse Wave Velocity

Whilst aortic PWV was lower in athletes > 20 years old versus age matched sedentary controls, no differences were observed in younger subjects (Table 1.4)[117]. As the study demonstrates, older trained athletes have lower PWV than younger sedentary subjects. These data suggest that aerobic training reduces the pathophysiological effects of ageing on arterial stiffness.

When PWV and augmentation index (Aix, which is a measure of the effect of the reflected waves on systolic pressure) are analysed for the effect that they have on maximal exercise capacity there is an inverse relationship between both factors and exercise capacity. In the endurance-trained athletes the slope of this relationship was flatter, implying a delay in the age related decrease in fitness and arterial function[117]. In patients with dilated cardiomyopathy the aortic distensibility independently predicted exercise capacity ($r=-0.39$, $p=0.0007$) and in a multivariate model Aortic PWV and stroke volume accounted for 34% of the variance in Vo^2_{max}[118]. When the carotid, brachial and femoral arteries of elite cyclists were compared

to sedentary controls there was no difference between groups in carotid distensibility, but the brachial and femoral distensibilities were significantly greater in the cyclists[119]. This demonstrates a specific adaptation to the increased flow in the exercising limbs in this athletic population. Conversely Gauthier and colleagues showed that the resting BPWV was significantly higher in the arm in athletes than controls, suggesting decreased distensibility[120]. The authors felt that this finding may explain why competitive athletes sometimes develop cardiac hypertrophy (although they did not demonstrate athletic hypertrophy in this study). In a cross-sectional study involving 56 fun runners, 25 active subjects (>1500 kcal/week) and 83 control subjects (<1500 kcal/week) Kakiyama et al showed that the Aortic Pulse Wave Velocity Index (APWVI = Aortic PWV scaled for DBP) was negatively correlated with habitual physical exercise, as analysed from a seven day activity form[121]. They also showed that after accounting for age, the APWVI was significantly lower in the sub-group of runners compared to the active and sedentary groups and that the active group was significantly lower than controls. Tanaka et al confirmed these findings in pre and postmenopausal women. They showed that central markers of arterial function (Ai and Aortic PWV) were significantly lower in active women when compared to control but that peripheral markers (brachial an femoral PWV) were not different between the groups[122]. These observations demonstrate

that central arteries stiffen with increasing age much more than peripheral arteries and that this stiffening can be reduced by regular physical activity in women. In other studies in men central arterial compliance and β-index (a BP-corrected measure of aortic compliance) was shown to be 20-35% higher in endurance-trained subjects than in age-matched controls[123, 124].

A small number of studies have prospectively assessed the effects of exercise training in sedentary subjects. The Melbourne group carried out a randomised crossover study of four weeks training and four weeks de-training at 75% of maximal workload[125]. They showed that baseline systemic arterial compliance (sac) increased by an average of 0.26 (Arbitrary Compliance Units (ACU)) and that β-index decreased by an average of 1.03 following training. The increase in SAC was greater than could be explained by the reduction in blood pressure observed and the authors concluded that exercise training must cause an intrinsic alteration of arterial compliance. Subsequently Higashi et al showed that 12 weeks of walking for half an hour each day at an intensity equivalent to 52% of VO_{2max}, in hypertensive and normotensive subjects, elicited increases in forearm blood flow in response to acetylcholine (25.8 to 32.3 ml.min/100ml tissue, $P<0.05$) but no change in the response to the non endothelium dependent vasodilator isosorbide dismitrate, indicating an enhancement of endothelium dependent

vasodilatation, which was blocked with L-NMMA, a specific Nitric Oxide Synthase inhibitor[126]. This improvement in endothelial function with exercise training may at least in part explain the improvement in large artery stiffness. However data from the "Melbourne group" showed that an eight week exercise training programme (Cycle ergometry at 65% of MHR for 40 minutes three times a week) in hypertensive (ISH) subjects did not alter SAC (0.35 vs. 0.38 A.C.U, p=ns) [127] carotid femoral PWV (11.5 vs. 11.5 m/s, p=ns) or femoral-dorsalis PWV(9.02 vs. 9.23 m/s, p=ns) even after increasing VO_{2max} from 21.4 to 24.3 (ml/kg/min) (p<0.05)[128]. Studies in patients with chronic heart failure, who exhibit an impaired endothelial dependent vasodilation, have shown that L-Arginine supplementation[129], aerobic exercise training[81, 82, 130] and also combining training and supplementation correct endothelial dysfunction as assessed by radial artery diameter responses to Acetylcholine and Nitroglycerin[79].

Resistance Training

Although resistance training (Weight Training) is another form of chronic exercise it has been shown to have a negative impact on arterial function. Miyachi et al showed that in older men (40-60) who had resistance trained for an extended period of time (> 2 years) carotid arterial compliance was lower (0.11 vs. 0.15 p<0.05) when compared to age matched controls, but that no such

difference was seen between the young group (20-39) and controls (0.16 vs. 0.19, p=ns)[131]. They also showed that this decrease in compliance was associated with left ventricular hypertrophy. Bertovic et al assessed left ventricular mass (LVM), SAC, β-index and aortic and femoral PWV in strength trained versus control subjects[132]. They showed a significant increase in LVM and femoral PWV and a decrease in SAC and β-index in the resistance trained group when compared to controls (P<0.05 for all), conversely they showed no differences in Aortic PWV between groups (p=ns).

Acute exercise and Pulse Wave Velocity

Sebban et al evaluated PWV in elderly women and demonstrated that for a higher resting PWV, sub-maximal oxygen consumption and heart rate were significantly elevated at rest and during exercise when compared to those with a normal PWV[133]. This is probably due to the greater force needed to overcome the early returning waves from the periphery associated with the stiffening arteries and increased PWV. However the exercise was a 400m run which is neither wholly aerobic nor anaerobic and thus leaves the interpretation of this data open to methodological questioning[134]. Murgo and colleagues measured PWV invasively during supine exercise and demonstrated that aortic PWV increases during exercise, implying decreased compliance, despite a fall in total peripheral resistance from 1142 to 712 (dynes/cm^5). In this study

they demonstrated that the timing of the returning wave (T_R) from the periphery was shorter during exercise than at baseline[135]. Although an interesting finding, any increase in heart rate, and therefore a subsequent reduction in total waveform duration, will always lead to a reduced T_R when expressed in milliseconds, as was the case with this study.

Table 1.4 Influence of age and training on Aortic Pulse Wave Velocity[117]

Age (Years)	Untrained (m/s)	Trained (m/s)	P Value
10-19	6.17	6.17	P=NS
20-29	6.88	6.48	P<0.05
20-39	7.41	6.71	P<0.05

In the mid 1950's Simonsen and colleagues looked at aortic PWV pre and post-moderate treadmill exercise (3 m.p.h and 5% gradient), and observed no differences[136]. Recently our group demonstrated that after a maximal exercise test to volitional exhaustion, subjects who were free of cardiovascular disease showed a marked reduction in Femoral PWV[137]. This reduction lasted for up to an hour and implies an increase in femoral arterial distensibility following acute maximal exercise. Kingwell and colleagues demonstrated that half an hour after a single bout of cycle exercise (65% of VO2max) aortic (6.2 vs. 5.9 m/s, p<0.05) and femoral PWV (8.3 vs. 7.5 m/s, p<0.01) were both reduced and whole body arterial compliance was increased (0.9 vs. 1.4 ACU, p<0.05) compared to baseline[138]. Chen and colleagues have shown

that in elderly subjects with increased arterial stiffness and reduced exercise capacity, administration of Verapamil reduced baseline PWV and augmentation index (-5.9 and -31.7% respectively, $p<0.05$) and increased time to anaerobic threshold and maximal exercise capacity (48 and 13.4% respectively, $p<0.05$)[139]. In a recent study Suguwara et al have shown that acute single leg cycling at a very low intensity (20-30 watts for 5 minutes) reduces femoral PWV in the exercising (9.1 vs. 8.4, $p<0.05$) but not in the contra lateral non-exercising limb (9.0 vs.9.2, $p=ns$)[140]. Thus local exercise related factors (Nitric Oxide, adenosine, prostaglandins) might be responsible for the post exercise reduction in PWV, in the exercising limb.

Aerobic Exercise Physiology

Aerobic exercise is commonly described as continuous rhythmical exercise of a large muscle group or groups over a sustained period of time, where aerobic metabolism provides the majority of ATP needed for cross bridge activation. The gold standard for measuring aerobic capacity (Vo^2_{max}) is an incremental exercise test to volitional exhaustion, usually on a treadmill or cycle ergometer with breath-by-breath online analysis of oxygen consumption and carbon dioxide expiration. Vo^2_{max} is determined by two factors, the maximal cardiac output (CO_{max}) and the maximal arterio-venous oxygen difference ($A-Vo^2_{diff}$). Numerous factors can affect either

the COmax or the A-Vo2diff.   This has led to considerable disagreement between exercise physiologists as to which of the two factors (CO_{max} and A-Vo^2_{diff}) is responsible for limiting Vo^2_{max}.

$$Vo^2_{max} = CO_{max} \times A\text{-}Vo^2_{diff}$$

$$COmax = SV_{max} \times HR_{max}$$

$$SVmax = \text{End diastolic volume} - \text{End systolic volume}$$

$$A\text{-}Vo^2_{diff} = \text{Arterial oxygen load} - \text{Venous oxygen load}$$

We outline below the evidence gathered over the previous eighty plus years in relation to factors limiting maximal aerobic capacity.

As early as 1923 maximal cardiac output was indicated as the major rate-limiting step to $\dot{V}o_{2max}$[141, 142].   Since then maximal cardiac output (CO_{max}) has been shown to account for up to 85% of the variation in $\dot{V}o_{2max}$[143].   However, in recent years there has been disagreement between leading physiologists as to whether it is central or skeletal factors that limit $\dot{V}o_{2max}$[144-147].

Evidence for cardiac output as the rate-limiting step in aerobic exercise

CO_{max} in healthy adults is determined by maximal stroke volume (SV_{max}) and heart rate (HR_{max}).   Maximal heart rates have been demonstrated to be similar[18] and in some cases lower[148] in

endurance-trained athletes, when compared to sedentary controls. This indicates that the SV_{max} may be the limiting factor to CO_{max}.

There is clear evidence to indicate that CO is unable to increase further at maximal exercise. These studies have shown that the recruitment of additional muscle groups to the already maximal working lower body muscles results in no further increase in CO with only a slight increase in $\dot{V}O_{2max}$ due to a greater arterio-venous oxygen difference (A-$\dot{V}O_{2diff}$) between the resting arm's and the exercising arm's[149-151].

The administration of beta blockade, which decreases heart rate and marginally increases stroke volume, resulting in a lower CO by approximately 20%, has been demonstrated to lower $\dot{V}O_{2max}$. $\dot{V}O_{2max}$ decreases by only 5-15% in contrast to the CO drop of 20%, the difference is probably attributable to a slight increase in A-$\dot{V}O_{2diff}$, suggesting that A-$\dot{V}O_{2diff}$ is not usually at maximal levels and therefore not the limiting factor[152-154].

Data from Rowell demonstrates that the main difference between athletic and non-athletic healthy subjects is maximal stroke volume. As shown in table 1.5, both maximal heart rate and A-$\dot{V}O_{2diff}$ are similar between the athletic and non-athletic subjects. It is the 83% larger stroke volume which produces a 78% larger CO_{max}

(38.95 V's 21.84 L/min^{-1}), that accounts for the 78% difference in $\dot{V}$O$_{2max}$[18].

Table 1.5 Athletic and non-athletic haemodynamics at $\dot{V}$O$_{2max}$[18]

	$\dot{V}$O$_{2max}$ (L's/min)	Heart rate (Beats/min)	Stroke volume (ml's)	A-Vo$_{2diff}$ (ml's/100ml)
Athletic	6.25	190	205	16
Non-athletic	3.50*	195	112*	16

* = Significant difference between groups

In addition to having a lower maximal stroke volume the non-athletic subjects stroke volume reaches its maximal value at a heart rate between 120-140 (Beats/min) compared to athletic populations whose stroke volume can continue to increase to maximal heart rates[20]. Therefore, further increases in cardiac output can only be achieved through an increased chronotropic drive. This plateau in stroke volume is attributed to the decrease in end diastolic volume as a result of a decreased diastolic relaxation time[20]

The most conventional way to increase $\dot{V}$o$_{2max}$ is by aerobic exercise. In 1968 Ekblom and co-workers demonstrated that a 530 (ml/min^{-1}) increase in oxygen consumption following a 16-week training programme increased CO$_{max}$ by 8% while A-Vo$_{2diff}$ only increased by 3.6%[155]. The classic bed rest study of Saltin and colleagues has shown similar findings after 30 days of training

66

following extended bed rest[156]. Perhaps the strongest evidence for oxygen supply limitation (CO) not oxygen demand is demonstrated by comparing exercise of a small muscle mass (leg extension) to that of a large muscle mass (cycle ergometry). When exercising a small muscle mass (2.5-6kg) CO will not be a limiting factor therefore the oxygen consumption of the leg should remain the same if A-$\dot{V}O_{2diff}$ was a limiting factor. Contradicting this hypothesis is that oxygen consumption of that muscle is approximately 600 ml's (O_2/kg/min^{-1}) during exercise of a small muscle group[157]. In contrast, during exercise of the same muscle group during whole body exercise this value is 250 ml's (O_2/kg/min^{-1})[158]. The primary reason for this increase in oxygen consumption is the increase in blood flow to the working muscle (quadriceps), it was measured as 1.5 (L/kg/min^{-1}) by Knight and colleagues in 1992 during maximal cycling ergometry[158]. During leg extension exercise the blood flow to the quadriceps increases to 3.9 (L/kg/min^{-1}) as measured by Richardson and colleagues in 1995[157, 159]. This allows us to postulate that the 240% increase in leg $\dot{V}O_2$ during leg extension can be attributed to the 260% increase in cardiac output with a slight reduction in A-Vo2diff (16.6 cycling V's 15.3 leg extension). This reduction in A-Vo2diff was believed to be due to the decreased transit time through the capillary beds, though this has been reported as not being the case[160, 161]. It is important to note these experiments used the same technique to quantify muscle oxygen

consumption and blood flow. These experiments show us that when muscle blood flow and therefore oxygen delivery is increased that maximal oxygen consumption of the quadriceps muscle increases, demonstrating a central (Cardiac output) not peripheral (Skeletal muscle) limitation to whole body (Large muscle mass) exercise.

Evidence for skeletal metabolism as a determinant of Vo^2_{max}

Capillary density increases with exercise training and strongly correlates with $\dot{V}o_{2max}$, enabling the muscle to maintain A-Vo_{2diff} at a high cardiac output by reducing capillary transit times[162, 163]. A common example used to argue that skeletal muscle can limit maximal exercise is the increased number of mitochondria following exercise training. However despite the large increase in mitochondria density post exercise training and the strong correlation between Vo_2max and mitochondria density the proportional increase varies markedly. Saltin et al demonstrated that for a 220% increase in mitochondria there is only a $\leq$ 40% increase in Vo_{2max}[156]. More recently it has been demonstrated that subjects with a similar Vo_{2max} may have marked differences in their mitochondrial volumes and Coyle et al suggested that mitochondria were more important for endurance performance rather than for maximal exercise[164].

Does the Po_2 of the cell limit the rate of oxygen uptake?

Until recently is has been very difficult to examine the Po_2 of a muscle cell during dynamic exercise but with the use proton magnetic resonance spectroscopy this has become achievable. In 1995, Russell Richardson et al demonstrated for the first time that there are large differences between blood and intracellular tissue Po_2 and that this gradient is highly significant in determining oxygen uptake by the muscle[157, 159]. Since then he and others have demonstrated that it is only at very high metabolic rates that we come close to mitochondrial metabolic limits, even then by increasing oxygen delivery (Hyperoxic gas) metabolic rate can be increased[165].

In conclusion, during exercise in which a large muscle mass (>6 kg) has to be supplied with oxygen, cardiac output limits maximal exercise capacity. However, when a small muscle mass is isolated and flow can increase several fold the skeletal muscle characteristics appear to be the rate limiting step. It is important to note that walking, running, cycling, swimming and almost all commonly undertaken exercise will use a muscle mass in excess of 6kg and therefore will be limited by cardiac output. This is applicable in health and in the majority of cardiovascular states, however in the heart failure setting there are numerous peripheral factors, these are beyond the scope of this review.

Ventricular Arterial Coupling

The interaction of the left ventricle and the vascular tree, termed ventricular-arterial coupling is a key determinant of physical performance. As we have clearly outlined there is a considerable role for the ventricle to play in outputting blood to allow for peak exercise performance, however the properties of the vasculature also play a significant role. The healthy ventricle and vasculature match properties so that near maximal cardiac output is achieved while maintaining adequate arterial pressure, this is ventricular arterial (V-A) coupling[166, 167]. The two measurable components are Ees (ventricle) and Ea (arterial) and the ratio of these two impacts on ventricular performance. Ea is a measure of both the pulsatile and static afterload, which is also influenced by heart rate. Ees is the ventricular contractile function. An Ea/Ees ratio of 0.7 to 1.0 in healthy humans allows for optimal stroke work, and importantly optimal metabolic efficiency[168, 169]. This ratio has been as high as 4.0 in patients with heart failure[170, 171], due to a rise in arterial resistance (Ea) and a fall in ventricular contractility (Ees). As we age our arteries stiffen as was initially reported by Avolio et al, using PWV, and has recently been reported using Ea[92, 95]. Mechanically this increases the load on the heart and this results in increases in diastolic and systolic ventricular stiffness, which may have accompanying hypertrophy. However not all people with stiff arteries have shortness of breath and this is explained by the fact

that the ventricular systolic stiffness also increases with the increase in arterial stiffness and thus the Ea/Ees ratio is preserved.

Although this preservation of coupling works within normal ranges and under normal physiological stimuli, it may affect cardiac reserve. The increase in Ea and Ees reduces left sided compliance resulting in a greater change in pressure for any given volume change. So as we exercise and increase venous return and diastolic filling we get a far greater increase in systolic developed pressure, this results in dramatic variations in arterial pressures and thus haemodynamic instability. During exercise the normal heart enhances output with maintenance of preload and an increase in systolic contraction. Ea typically rises due to heart rate and arterial pulsatility, even in the face of a fall in total peripheral resistance[172]. To match this the healthy heart usually increases its ventricular contractility to maintain the V-A coupling, however if resting Ea is already risen, as is the case with age and in heart failure, there is less reserve and any increase in Ea will only exacerbate the systolic pressure rises and decrease cardiac efficiency and increase metabolic demands[173].

Fig 1.7   **Pressure volume loops and Ea/Ees and the impact of age**

Example pressure-volume loops and Ea/Ees for a young versus elderly patient. In both instances the Ea and Ees values are similar to each other, with a relative ratio near 1.0. However, in the elderly subject both parameters are increased, consistent with both vascular stiffening and ventricular systolic stiffening. **Ea:** Arterial elastance / effective arterial stiffness. **Ees:** End systolic elastance / ventricular systolic stiffness
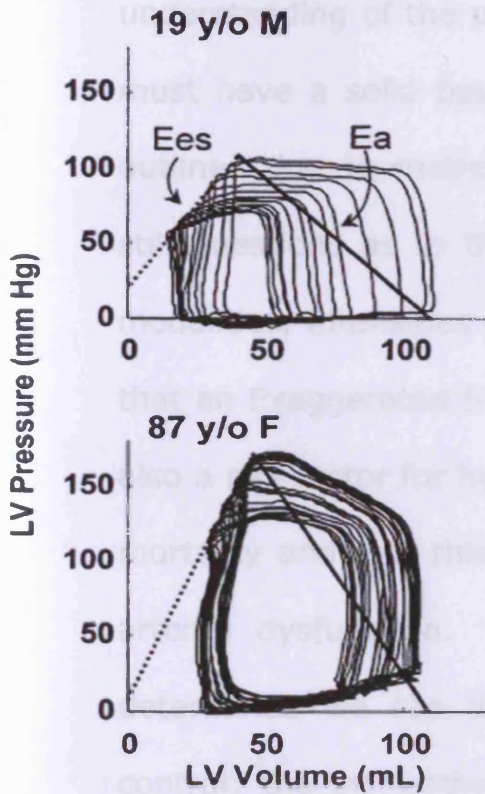
Overview

As we have outlined coronary heart disease is the single most influential disease in the Western world, and that blood pressure, be it pulse, diastolic, mean or systolic is linked with the risk of mortality and morbidity. Recent investigations have tended to assess systolic and pulse pressure, as they appear to have greater prognostic information that other measures of blood pressure. As these pressures are markedly influenced by arterial function it is unsurprising to note that arterial stiffness per se is also influential in the prognosis of chronic disease. Theoretically, at least, there is a

link between an early stiffening of the arteries and the development of isolated systolic hypertension through to the more serious consequences of diastolic heart failure. However important the understanding of the pathophysiology in these disease states is, we must have a solid base to examine these changes against and as outlined due to methodological and patient populations there are still questions as to the normal arterial responses to the different modalities, intensities and duration of exercise. It is also apparent that an Exaggerated Systolic Blood Pressure response to exercise is also a risk factor for hypertension, stroke, myocardial infarction and mortality and that this may be an early marker of disease or even arterial dysfunction. Once these normal responses have been determined we can look at the two key components of arterial control; the sympathetic nervous system and the endothelium in particular the role of Nitric Oxide.

Chapter 2

Physiological Measurements

Overview

It cannot be overstated the importance of understanding physiology, however physiology cannot be understood without the use and understanding of accurate and reproducible measurements. The aim of this chapter is to give an accurate and thorough understanding of the techniques that we used, the subsequent analysis that we carried out and the accuracy of our measurements.

Arterial Function

We used three techniques to assess arterial function in this thesis. In summary they were peripheral pulse wave velocity (Femoral and Brachial) using an oscillometric technique, aortic pulse wave velocity (Carotid-Femoral) and Pulse Wave Analysis both from the Sphygmocor system.

Pulse Wave Velocity

Pulse wave velocity (PWV) is defined as the speed of an arterial pulse wave as it travels from an initial arterial segment to a second segment along the same arterial tree. PWV is simply calculated as distance travelled (m) divided by time (s) and expressed as metres per second (m/s)

Peripheral Pulse Wave Velocity (Chapters 3-6)

We used one system to measure Brachial-Radial and Femoral-Tibial Pulse Wave Velocity (BPWV and FPWV respectively). We measured FPWV and BPWV simultaneously and non-invasively by oscillometry (time resolution +/- 2ms; QVL P84, SciMed, Bristol, UK). The right arm and leg were used for all measurements in these studies. Non-occlusive cuffs were placed over the upper arm (brachial), wrist (radial), mid thigh (femoral) and ankle (tibial); the cuffs were connected to computerised pressure transducers by non-compliant tubing. Pulse pressure waveforms caused by volume displacement were obtained from each of the four cuffs. A computer programme was developed to characterise the waveforms with respect to time at 30, 40 and 50% of peak pressure along the ascending limb, this then calculated the transit time between the cuffs. The transit times are the average of the 3 points (30, 40 +50) of ten continuous waveforms. PWV was then calculated by dividing the distance between the proximal points of each of the cuffs (mm) by the transit times (ms). This process was repeated every sixty seconds for periods of up to 15 minutes.

A colleague, Dr Nicholas Pegge, previously assessed this system. He investigated the changes in PWV following reactive hyperaemia (PWV minus PWV post reactive hyperaemia). BPWV and FPWV decreased in response to reactive hyperaemia ((7.3 to 6.4 and 8.7

to 7.6 m/s) (p<0.001) respectively) in keeping with endothelial control of large artery distensibility and the NO synthase inhibitor LNMMA blocked this response. PWV returned towards baseline over the subsequent 20 minutes. During L-NMMA infusion resting BPWV was increased by 0.8 (m/s) and the response to reactive hyperaemia decreased from 12.3% to 7.9% (P<0.005). Nitro-glycerine (GTN) administered 15 minutes after the last cuff measurement and following the L-NMMA infusion reduced BPWV by 18.1%. In addition it was shown that differences in PWV post reactive hyperaemia are correlated with other cardiovascular risk factors in a healthy population adding further evidence that changes in PWV following reactive hyperaemia are endothelium dependent.

Aortic Pulse Wave Velocity (Chapter 5)

Aortic pulse wave velocity (AoPWV) was determined by applanation tonometry and timed using the electrocardiogram. Applanation tonometry was performed using a Millar piezo-resistive pressure transducer (Millar SPT 301, Millar Instruments) coupled to a SphygmoCor device (PWV Medical, Australia), attached to a computer with specialised software (SCOR 2000, Version 7.0). AoPWV was calculated by sequential acquisition of the pressure waveforms from the carotid and femoral arteries. The timing of these waveforms was matched with that of the 'R' wave on a simultaneously recorded electrocardiogram. AoPWV was

determined by the calculation of the differences in carotid to femoral path length divided by the difference in R wave to waveform foot times. The difference in carotid to femoral path length can be measured in two ways. The first is the distance from the sternal notch to the femoral pulse (At the point of the applanation tonometer) measured in a direct line, this reduces between observer errors but reduces AoPWV by about 10%. The second method is the same sternal-femoral path length minus the distance from the sternal notch to the carotid pulse (At the point of the applanation tonometer). This allows for a more direct calculation of AoPWV but with a slightly higher level of between observer errors[84]. As we used the same operator for all studies, we used the femoral-carotid path length.

Pulse Wave Analysis (PWA) (Chapters 5 and 6)

Radial artery pressure waveforms were acquired and central pressure waveforms were generated using pulse wave analysis (SphygmoCor, AtCor Medical). The pulse wave analysis transfer function has been validated under differing haemodynamic states and recently during supine, low level exercise. The central waveform yields central aortic pressures (CBP), augmentation index (AI), heart rate corrected augmentation index (AI@HR75) and the timing of the reflected wave (TR). There have been studies[84, 174]

demonstrating the reproducibility and accuracy of these readings and a subset of the findings are reproduced in table 2.1

Central Blood Pressures (CBP) were derived using a mathematical transfer function that generates an aortic pressure waveform from the recorded radial waveform. This transfer function has been validated against invasive aortic pressure measurement at rest, during valsalva and from lying to sitting[175], it has also recently been validated during low level supine exercise[176].

Augmentation Index (AI) is calculated as the difference between the early systolic shoulder (P1) and the late or peak systolic shoulder (P2), the augmentation index was then calculated as the difference in systolic shoulders (P2-P1) divided by the pulse pressure and expressed as a percentage. As heart rate has been shown to affect augmentation index a correction factor was applied to the reference value augmentation index to a standard heart rate of 75 (AI@75).

Timing of the reflected wave (TR) is a measure in milliseconds of the arrival of the reflected wave, from the periphery, at the aorta. There is an inverse relationship between TR and Aortic Pulse Wave Velocity. However it is also influenced by heart rate and by height. TR is measured in milliseconds from the beginning of the systolic

upstroke, it is defined as a rapid increase in pressure during a plateau or decrease in pressure.

Table 2.1 Intra and Interobserver repeat and reproducibility of Sphygmocor[84, 174]

	Repeatability (%)	Reproducibility (%)	Interobserver (%)
Heart Rate	4.5 ± 1.7	9.1 ± 4.0	2.9 ± 1.7
Augmentation Index	4.5 ± 2.7	10.3 ± 5.5	5.1 ± 3.1
Mean Systolic Pressure	0.8 ± 0.4	6.2 ± 3.3	3.1 ± 1.9
Mean Diastolic Pressure	1.1 ± 0.5	6.6 ± 2.8	3.9 ± 3.2

*Coefficients of variation for replicate measures

Haemodynamics

The importance of accurate and reproducible haemodynamic measures during exercise was of great importance and this was problematic due to the human variance in blood pressure and heart rate but also the large amount of noise during high intensity exercise.

Blood Pressure

We assessed brachial blood pressure in the non-dominant arm of all our subjects. One of three cuffs (Child, Adult or Oversize Adult) was placed over the brachial artery and tested for size. This cuff was attached to a freestanding aneroid sphygmomanometer (Accoson CE 0120) with a pressure range from zero to three

hundred (mmHg). Systolic blood pressure was defined as the first "beat" heard during cuff deflation (Korotkoff I), diastolic blood pressure was defined as the last beat heard (Korotkoff V) rather than when noise became muffled (Korotkoff IV). At rest, the cuff was initially inflated to 160 (mmHg) and then during exercise it was inflated 30 (mmHg) more than the previous systolic reading. During all measurements, the sphygmomanometer was level with the subject's heart and the arm was kept in a relaxed but supported position.

Electrocardiogram (Chapters 3 and, 4)

We used the electrocardiogram to measure heart rate at baseline, during exercise and in recovery. During baseline and recovery, heart rate was recorded every second minute. During exercise, heart rate was recorded every minute throughout. The equipment we used (GE Marquette® Helliege Medical Systems, Model: CASE® 16 exercise testing system, Kettering, Northants) allowed for either requested or automatic recoding of the ECG and heart rate at any given time.

Heart Rate, Stroke Volume and Cardiac Output (Chapters 5 and 6)

Cardiac Output (CO) was measured using a newly developed, non-invasive, bio-impedance monitor (Task Force® Monitor). This

monitor allows determination of stroke volume (SV) and CO via impedance cardiography (ICG). In order to obtain the ICG signals $^{DZ}/_{DT}(t)$ and $Z_0(t)$ new electrodes were designed. The ICG signals $^{DZ}/_{DT}(t)$ and $Z_0(t)$ are used for the detection of stroke volume, whilst a newly developed signal processing tool is used to eliminate the electrical activity associated with breathing, to detect the maximum $^{DZ}/_{DT}$ signal (C-point), the aortic opening point (B-point) and the aortic closing point (X-point)[177]. This tool has been validated at rest against other non-invasive measures (BioZ) and invasive measures (Thermodilution, Baxter Explorer, Edwards Critical Care, Irvine, CA, USA)[178].

Haemodynamic and Arterial Calculations

From the haemodynamic and arterial data measured, we calculated other variables using standardised cardiovascular formulae.

Pulse Pressure was calculated as the systolic blood pressure minus the diastolic blood pressure.

Mean Blood Pressure was calculated as diastolic blood pressure plus one third of the pulse pressure.

Arterial Elastance, which is a measure of the total arterial load, including the pulsatile and static component, but is also influenced by heart rate, was calculated as the derived aortic end systolic pressure (From PWA) divided by the stroke volume[179].

Total peripheral Resistance, which is a measure of the static component of arterial load, is calculated as the mean blood pressure (mmHg) divided by the cardiac output (L/min).

Arterial Compliance, which is a measure of the pulsatile component of the total arterial load, was calculated as stroke volume divided by pulse pressure. This has been shown to accurately estimate the arterial compliance calculated by the more complex area method, which involves using the area under the curve of the pressure waveform[180]. There were no differences in the values obtained and the slope and intercept did not differ with each method in either healthy controls or two disease groups[181].

Expired Gas Analysis

Expired gases was sampled breath by breath and analysed as an average of thirty seconds during exercise using Pulmolab model Ex 670 mass spectrometry gas analysis (Morgan Medical Ltd., Kent, UK). The system comprised of a standard PC and appropriate software (Morgan medical Ex670 Interface Application, Version 1.4.1.0, release 2.11), attached to a Quadrupole Mass Spectrometer for gas analysis and a propeller type turbine cartridge for flow analysis (See table 2.2 for specifications). The theory and practice of gas analysis by mass spectrometry is summarised below:-

Table 2.2 Mass Spectrometer and Turbine Specifications

Quadrupole Mass Spectrometer		Propeller type Turbine Cartridge	
Sample Rate	50 data frames/ sec	Resistance	0.65 cmH2O/l/s @ 8.5 L/s
Transit time	200 ms	Output type	Volume signal
Response Time	30 ms (0-90% change)	Output Signal	0-4 volts – 0-7 L
Detection Limit	100 ppm	Sensitivity	2.2 ml per revolution
Stability	< 1%		
Linearity	> 1%		
Sample Rate	20-50 ml/min		

Gas Analysis

The equipment employs a continuous on-line quadrupole mass spectrometer to measure respiratory gas concentrations. Expirate is drawn into the tip of the sample capillary (inserted into the mouthpiece) at about 50ml/min, it travels the 2.5 m along the capillary tube and into the instrument in less than 250 ms. Inside the instrument, the sample passes by the high vacuum chamber and is drawn into a pump from which it is filtered and expelled to the cabinet air stream. A small portion of the sample is drawn into the high vacuum chamber where it is subjected to electron bombardment ionisation. The subsequent ions that are formed are projected into the influence of a complex electric field, across the quadrupole, which is dynamically altered to select ions of similar masses for short sequential periods.

During each period, the selected ions carry a current, which is collected and measured, and its value digitised and stored. The current in each period is proportional to the ionisation efficiency and partial pressure of the parent molecule, in turn proportional to the concentration of the associated gas. Ionisation efficiencies and total ion pressures can be kept constant, thus a measure of each component of the sample gas can be got in percentage terms via calibration factors.

Flow Measurements

The respiratory flow rate is measured with a turbine device connected to the end of the mouthpiece. The construction of the device is such that the airscrew rotates once for every 2.2ml passing through, over a wide range of flow rates. The electronics generate a volume signal, and by measuring the incremental volume over the gas sampling periods, small intra-breath volumes can be derived. The accumulation of these volumes within the phases of breathing, combined with analogue input measurements, provides primary data for breath-by-breath calculations.

Calculations

Inspirate and expirate volumes of each gas component in the sample are calculated for each breath, from the integration of running intra-breath partial volume values, thus oxygen uptake,

carbon dioxide production and respiratory exchange ratio are derived.

Calibration

For reproducible and reliable gas analysis we calibrated both flow and gas analysis prior to every study. Flow was calibrated using a standard syringe (3/L) filled and emptied at three different speeds, representing changes in breathing frequency (60, 30 + 15 Bths/min). On completion, the syringe was filled and emptied and the total volume of expiration and inspiration is displayed. We accepted calibration once readings were 1% of the syringe volume (2.97-3.03 L). The gas analysis was calibrated using a known concentration of gases. (O^2 14.99%, CO^2 8.12%, Ar 7.85% and N^2 69.04%). A two-stage check was then performed in which each gas was checked against room values (O^2 20.94%, CO^2 0.03% and N^2 78.9%) and again against the standard cylinder values.

Maximal Oxygen Consumption

Theoretically, maximal oxygen consumption describes an increase in workload without any resultant increase in oxygen consumption; this has been termed the oxygen plateau. Duncan et al demonstrated that failure to reach a plateau in $\dot{V}o_2$ does not mean that a true maximal effort has not been reached[182] and it is commonly recognised that only 50% of all subjects will reach a

plateau[183]. With this in mind, it is clear that we need secondary criteria to establish $\dot{V}O_{2max}$ when the plateau is not present. These criteria generally include a respiratory exchange ratio (RER) in excess of 1.10-1.15, a blood lactate in excess of 8 ($mmol/L^{-1}$) and a maximal heart rate equal to 220 ($Beats/min^{-1}$) – chronological age, a full review of these criteria are described by Howley et al[184].

Respiratory Exchange Ratio

The respiratory exchange ratio (RER) allows an accurate non-invasive measurement of substrate utilisation during aerobic exercise. RER is the ratio between expired carbon dioxide (Vco^2) and oxygen uptake (Vo^2) at the lungs. This is a different measurement from O^2 consumption (Qo^2) and CO^2 production (Qco^2) at the cellular level. RER is only an accurate reflection of Qco^2/Qo^2 (Respiratory Quotient) during steady state exercise. A normal resting RER (Assuming a Western diet) is approximately 0.8, this rises to approximately 0.95 during steady state exercise and then 1.0 at the anaerobic threshold. An RER in excess of 1.1 is a fair reflection that a maximal effort has been made in an exercise tolerance test.

Exercise Testing

As exercise testing was a key component of our studies we had to design rigorous protocols for each study that allowed the subject to

perform to the peak of their abilities but also to allow adequate time and conditions for collection of accurate data.

Maximal Strength Testing

The maximal strength tests were carried out for the biceps and quadriceps muscles using identical protocols. For biceps testing, we used a single dumbbell that could be increased or decreased in increments of two kilogram's. For quadriceps testing, we used an isotonic leg extension machine where we isolated each leg; the resistance could be altered with increments of 2.5 kilogram's. The protocol we applied for the testing consisted of three warm up sets and then our first maximal attempt of six repetitions. If this was successful, the subject chose the increment of weight increase until they could not complete six repetitions at the set weight. We completed all testing sessions in a randomised fashion.

Isometric Exercise Test

Subjects lay supine in a custom built isometric dynamometer designed to measure ankle plantar flexion force. The subjects were positioned with the right knee flexed by 50 deg, the ankle flexed at 90 deg, and the foot strapped to a footplate. Straps were aligned around the ankle in order to minimise lifting of the heel away from the footplate when performing plantar flexor exercise. Prior to each study the subjects performed maximal contractions with 2-

minute intervals until there was less than 5% difference between the two peak values. This maximal value was used to calculate the force required for each protocol.

Dundee Step Test

The Dundee step test is a simple procedure to measure increases in systolic blood pressure in response to sub-maximal exercise. The Dundee test, as may be expected, was first used in the University of Dundee in a lecture theatre. They used the first step of the lecture hall and had students step at approximately 90 steps per minute up and down this step and blood pressure was recorded before and at the end of the three-minute period. Since then, we have standardised the test on a 12" step, at a rate of 92 steps per minute (23 complete movements per minute). We measured blood pressure at the end of the third minute and every minute after that until two systolic readings within 5 mmHg of each other were recorded.

100-Watt Cycle Test

The 100-Watt cycle test was designed to duplicate the test used in the original Kjelsden paper in 1995. All tests were carried out on an upright stationary cycle ergometer ((874E; Monark Exercise AB, Varberg, Sweden). The test is a simple one that is carried out at 70-72 R.P.M. against a workload of 1.4kg (100 watts) for a total of

six minutes. We measured blood pressure after 90 seconds and every two-minutes till completion, heart rate was recorded every sixty seconds.

Maximal Exercise Test (Chapters 3-5)

All maximal exercise tests were carried out on an upright stationary cycle ergometer (874E; Monark Exercise AB, Varberg, Sweden) using a standardised protocol. After two minutes of rest, the subject began to cycle at 60 R.P.M. against a workload of 1kg (60 Watts), after three minutes the workload was increased to 1.3kg and the speed to 70 R.P.M. (90 Watts) for a further three minutes. There were then a further three stages at 120, 150 and 180 Watts for three minutes each. This was followed by one-minute stages of twenty-watt increments until the subject indicated fatigue. Expired gases, blood pressure and heart rate were measured throughout.

Arithmetic Stress Test (Chapter 6)

We used a widely used and validated mental arithmetic stress test. Briefly subjects continuously subtracted the number 7 from a random 4-digit number. Subjects answered verbally and were encouraged by an investigator to subtract as fast as possible. When subjects paused for more than 2 seconds or answered incorrectly they were given the correct answer and pressed to improve their performance.

Reproducibility and Reliability studies

The equipment we used has been shown to be accurate and reproducible within our group and others, but to confirm our confidence in the equipment that we were using we carried out a small reproducibility study.

Prior to the start of our studies, we carried out repeatability studies on arterial function and haemodynamics at baseline, during exercise and post maximal exercise. We chose maximal exercise, as it would be the modality with the greatest difficulty to get accurate and reproducible measurements. Ten subjects completed baseline readings (10 minutes), a maximal exercise test and then a further ten minutes of recovery readings. In the table below, we outline the reproducibility of the baseline values as an average, the exercise readings from three stages (60, 120 and 180 Watts) and the first recovery reading.

Table 2.3 Pulse Wave Velocity and Haemodynamics at rest, during exercise and recovery*

	Baseline A	60 Watts	120 Watts	180 Watts	Recovery
Brachial-Radial PWV	7.3%	N/A	N/A	N/A	9.4%
Femoral-Tibial PWV	6.8%	N/A	N/A	N/A	8.7%
Aortic PWV	5.9%	N/A	N/A	N/A	8.4%
Systolic blood pressure	2.8%	3.2%	4.6%	5.4%	3.6%
Diastolic blood pressure	3%	4%	5.2%	5.8%	3.9%
Heart Rate	2%	3.4%	4.3%	5.4%	3.6%
Stroke Volume	5%	7%	8%	8.9%	6.8%
Cardiac Output	4.8%	7.6%	9.2%	11.1%	8.4%

*Coefficient of variation for replicate measures

Chapter 3

Large Artery Responses to Exercise

The Influence of Modality, Intensity and Duration

Introduction

Pulse wave velocity is a non-invasive marker of arterial stiffness and is related to mortality and morbidity in health and disease[37, 38, 108], like other measures of arterial stiffness. It has been well established that increases in arterial stiffness cause increases in resting[7] and exercising blood pressure[185]. Individuals who carry out regular aerobic exercise have a reduced risk of mortality and morbidity from cardiovascular disease[3] and recent studies have shown that athletes have markedly higher arterial compliance and distensibility[120] when compared to age matched controls and that sedentary subjects who undertake short term (8-12 weeks) exercise training show increased distensibility independent of changes in resting blood pressure[125]. There have been few studies that have assessed acute arterial stiffness changes during and soon after exercise. These studies have in general shown a decrease in stiffness of the exercising limb that lasts for up to an hour. However these studies have generally looked at an individual artery and have used exercise of different modalities, intensities and durations in addition to the different methods of assessing arterial stiffness. Below we give a brief outline of these studies.

In the mid 1950's Simonsen and colleagues looked at aortic PWV pre and post-moderate treadmill exercise (3 m.p.h and 5% gradient), and reported no change in pulse transmission following

exercise[136]. More recently Naka et al demonstrated that after a maximal exercise test to volitional exhaustion, subjects who were free of cardiovascular disease showed a marked reduction in femoral PWV[137]. This reduction lasted for up to an hour and indicates a persistent increase in arterial distensibility in the exercising limb following acute maximal exercise. Also Kingwell and colleagues demonstrated that half an hour after a single bout of cycle exercise that aortic and femoral arterial compliance was increased[138]. In a recent study Suguwara et al have shown that acute single leg cycling at a very low intensity (20-30 watts for 5 minutes) reduces femoral PWV in the exercising but not in the contra lateral non-exercising limb[140].

The aim of this initial project was to evaluate whether exercise of different natures would reduce PWV similarly. During these studies we assessed where possible the responses in the exercising and non-exercising limb. We assessed the response into three forms of exercise. These sections would evaluate 1) aerobic, 2) resistance and 3) isometric exercise.

1:    We recruited 20 men to a study that accessed 6 different exercise intensities. All exercise studies were carried out on a cycle ergometer.

2:    Resistance exercise of a chronic nature has been shown to increase arterial stiffness and cardiovascular disease.   Recent studies have shown that there may be no difference between a resistance trained subjects arterial responses to exercise and a sedentary non-resistance trained control. To our knowledge no one has assessed the arterial response to acute resistance exercise in non-resistance trained subjects. Subjects completed four different resistance exercise bouts on different limbs to assess local and systemic responses.

3:    Isometric exercise provokes rapid responses in blood pressure far greater than the oxygen consumption increase of such work and without the changes in blood flow to the exercising leg. We carried out a study to assess whether isometric plantar flexion would reduce similar decreases in FTPWV and BRPWV as seen in aerobic exercise studies.

Methods

Recordings

Heart rate was recorded on a minute-by-minute basis at rest, during exercise and in recovery using a standard electrocardiogram. Femoro-Tibial and Bracho-Radial Pulse Wave Velocity were recorded for ten minutes at baseline and in recovery. Blood pressure was recorded every two minutes during rest and recovery and at various

time intervals during the exercise tests, ranging from every minute to every ten minutes. In all studies, subjects completed a familiarisation visit in which the exercise tests, arterial recordings and protocols were explained and subjects had any queries answered.

Study 1 – Aerobic Exercise (N=20)

The subject then returned for an initial study to assess their maximal exercise capacity. Following this study the subjects were randomly allocated a number by a member of staff not involved in the study. This decided the order that the subjects completed the exercise protocol in. The six protocols were A) 15 minutes at 25% of VO2max, B) 45 minutes at 25% of VO2max, C) 15 minutes at 50% of VO2max, D) 30 minutes at 50% of VO2max, E) 15 minutes at 75% of VO2max and F) maximal exercise test. Before each test, a fifteen-minute relaxation period was undertaken and then baseline measures were completed. The subject then completed the required exercise protocol; on cessation of exercise the subject had all baseline measures repeated over a ten-minute period. All studies were completed at least 72 hours apart and within a four week period.

Study 2 – Resistance Exercise (N=10)

All subjects completed maximal strength tests as outlined in chapter two. Briefly, single arm bicep curl and leg extension exercises were completed to assess maximal quadriceps and bicep strength capacity. The subjects then carried out four studies in a randomised order. The studies consisted of baseline, exercise and recovery measurements. The exercise was six minutes of muscular contractions on a single muscle bed at 40% of the maximal strength tests. Before each test, a fifteen-minute relaxation period was undertaken and then baseline measures were completed. The subject then completed the required exercise protocol; on cessation of exercise the subject had all baseline measures repeated over a ten-minute period. All studies were completed at least 72 hours apart and within a four week period.

Study 3 – Isometric Exercise (N=10)

On arrival the patient completed a maximal plantar flexion protocol as detailed in chapter 2. Following maximal plantar flexion a fifteen-minute relaxation period was undertaken and then baseline measures were completed. The subject then completed the required exercise protocol (Either 20, 40 or 60% of MVC for two minutes); on cessation of exercise the subject had all baseline measures repeated over a ten-minute period. All studies were completed at least 72 hours apart and within a four week period.

Statistical Analysis

For all studies the baseline data is presented as the mean of ten minutes, exercise data is presented as the mean of each study, unless otherwise noted, while recovery is presented as early, middle or late, which represents minutes 1-3, 4-6 and 7-10 respectively. The data is presented as the mean plus or minus the standard deviation of the mean. All comparisons were completed using repeated measures analysis of variance with post-hoc analysis completed with a significance value set at $P<0.05$.

Results

Table 3.1 Patient Characteristics

	Age (years)	Height (m)	Weight (kg)	BMI (kg/m2)	SBP (mmHg)	DBP (mmHg)
Study 1 (n=20)	26.4 (0.9)	1.76 (0.03)	73.6 (2.1)	22.9 (1.1)	119 (3)	77 (2)
Study 2 (n=10)	27.3 (1.1)	1.77 (0.04)	75.4 (1.8)	24.0 (0.7)	123 (3)	80 (2)
Study 3 (n=10)	26.8 (0.8)	1.74 (0.05)	74.2 (2.2)	24.5 (0.9)	119 (3)	81 (3)

There were no significant differences between baseline arterial and haemodynamic measures on any day of the studies (P=NS).

Study 1

Haemodynamics

All haemodynamic variables increased from baseline to exercise (P<0.05) with the exception of diastolic blood pressure which was slightly, but not significantly (P=NS), lower with the low intensity exercise (25-50% of VO2max) and significantly lower during maximal and high intensity exercise (75%VO2max) (P<0.05).

Table 3.2 Haemodynamic Responses to Aerobic Exercise

Intensity (%)	Duration (Mins)	SBP (mmHg)	DBP (mmHg)	PP (mmHg)	HR (Bts/min)
25	15	137 (8) *	65 (6)	72 (7) *	102 (9) *
25	45	135 (1) *	60 (3)	75 (5) *	103 (10) *
50	15	168 (5) *	67 (6)	101 (6) *	136 (9) *
50	30	172 (6) *	65 (7)	108 (6) *	142 (8) *
75	15	194 (7) *	65 (4) *	129 (7) *	174 (10) *
100	Varied	224 (8) *	62 (7) *	162 (8) *	191 (9) *

* = Different to baseline (P<0.05)

Pulse Wave Velocity

The femoral and brachial artery responses to exercise are presented in Figure 3.1 (a-d). There were no significant differences between baseline values for either femoral or brachial PWV (Table 3.3, P=NS) and exercise data is therefore presented as both the absolute changes (a) and (c) and the percentage change from baseline (b) and (d). There was no significant change in brachial PWV following sub-maximal exercise of any intensity, but maximal exercise resulted in a significant fall throughout the recovery period

100

(P<0.05). This was presumably due to the increase in upper-body work during maximal or supra-maximal exercise, converting the arms from being a non-exercising bed into an exercising bed. The three lowest levels of exercise (25-15, 25-45 and 50-15) resulted in no change in femoral PWV (P=NS), while the three higher intensities demonstrated significant decreases post exercise and through recovery (P<0.05).

Figure 3.1 (a+b) Absolute and Relative changes in Femoral Pulse Wave Velocity following sub and maximal cycle ergometry
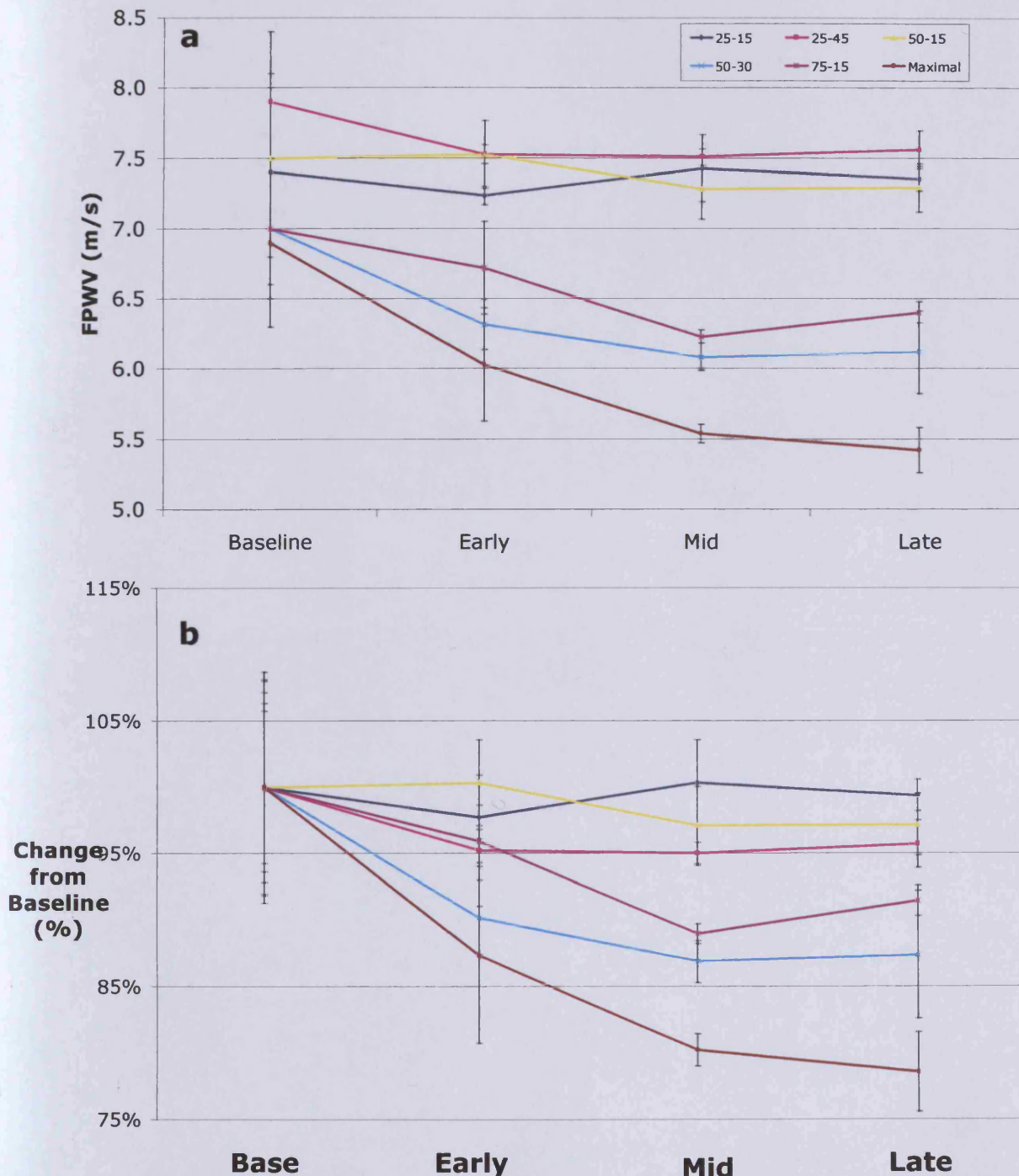
The three lowest levels of exercise (25-15, 25-45 and 50-15) resulted in no change in femoral PWV (P=NS), while the three higher intensities demonstrated significant decreases post exercise and through recovery (P<0.05).

Figure 3.1 (c+d)   Absolute and relative changes in Brachial Pulse Wave Velocity following sub and maximal cycle ergometry
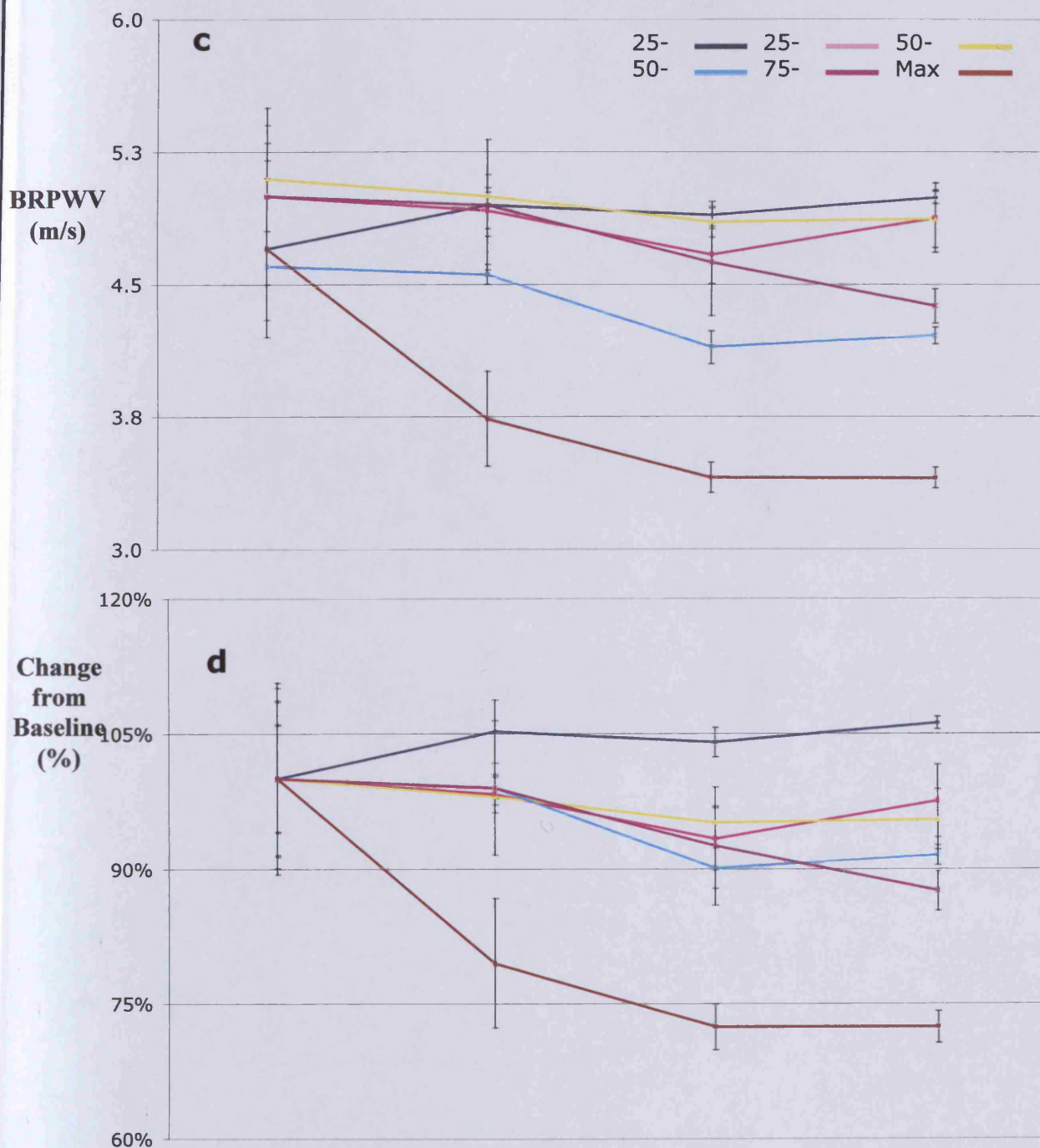
There is no significant change in brachial PWV following sub-maximal exercise of any intensity, but maximal exercise has resulted in a significant fall throughout recovery (P<0.05).

Table 3.3 Baseline brachial and femoral Pulse Wave Velocity

Study	Brachial PWV (m/s)	Femoral PWV (m/s)
25-15	4.7 (0.4)	7.4 (0.6)
25-45	5.0 (0.6)	7.9 (0.6)
50-15	5.1 (0.3)	7.5 (0.7)
50-30	4.6 (0.5)	7.0 (0.5)
75-15	5.0 (0.5)	7.0 (0.6)
Maximal	4.7 (0.2)	6.9 (0.4)

P=NS for all studies

Study 2

Haemodynamics

As shown in table 3.4 all the variables increased from baseline (P<0.05) apart from DBP, which showed a moderate non-significant decrease (P=NS). The systolic pressure and heart rate were significantly higher during leg exercise when compared to the values obtained during arm exercise (P<0.05). This is presumably due to the significantly larger muscle mass and therefore oxygen consumption of the lower limb.

Table 3.4 Haemodynamic Responses to Resistance Exercise

Exercising Limb	SBP (mmHg)	DBP (mmHg)	PP (mmHg)	HR (Bts/min)
		Baseline		
Right Arm	122 (7)	80 (5)	42 (3)	74 (6)
Left Arm	119 (8)	81 (6)	38 (4)	77 (7)
Right Leg	116 (7)	76 (8)	40 (3)	74 (8)
Left Leg	121 (9)	78 (7)	43 (3)	72 (6)
		Exercise		
Right Arm	166 (9) *	70 (6)	96 (5) *	122 (9) *
Left Arm	168 (10) *	68 (5)	100 (9) *	120 (5) *
Right Leg	182 (12) * !	74 (5)	108 (8) * !	136 (7) * !
Left Leg	180 (12) * !	72 (6)	108 (7) * !	140 (6) * !

* = Significantly different from baseline (P<0.05), ! = Significantly different from arm (P<0.05)

Pulse Wave Velocity

Following exercise the first reading of PWV was completed within two minutes. The graph below (Fig 3.2) demonstrates baseline and immediate post exercise values for all of the limbs during each study. All pulse wave velocities had returned to baseline within 3 minutes of cessation of exercise and therefore only immediate recovery is presented.

Fig 3.2   Pulse Wave Velocity following resistance exercise

First Letters (E.g. LA) is studied limb second letters (E.g. RA) equals exercised limb. RA = Right arm, LA = Left arm, RL = Right leg, LL = Left leg * = Different from baseline (P<0.05)

Study 3

Haemodynamics

Table 3.5 shows that there is a marked pressor and chronotropic response to isometric exercise, with the heart rate and blood pressure being significantly different from baseline and increasing with each exercise intensity.

Table 3.5 Haemodynamic responses to acute isometric plantar flexion

	BP Base	BP Ex	BP Rec	HR Base	HR Ex	HR Rec
20%	116/68	138/66 *	122/71	65	87 *	71
	(8/4)	(8/5)	(5/6)	(8)	(9)	(8)
40%	119/64	144/69 * !	130/72	67	94 * !	72
	(7/4)	(10/8)	(6/4)	(11)	(10)	(7)
60%	121/64	153/74 * !	138/71	63	99 * !	72
	(7/6)	(11/6)	(7/4)	(9)	(12)	(9)

*= Different to baseline   ! = Different to previous exercise intensity

All exercise systolic blood pressures and heart rates are significantly higher than baseline, and each value at each exercise intensity, is significantly greater than the previous intensity (P<0.05). Diastolic blood pressure only shows significant increases from baseline and between exercise at 60% (P<0.05).

Figure 3.3 Pulse Wave Velocity following isometric exercise of increasing intensity
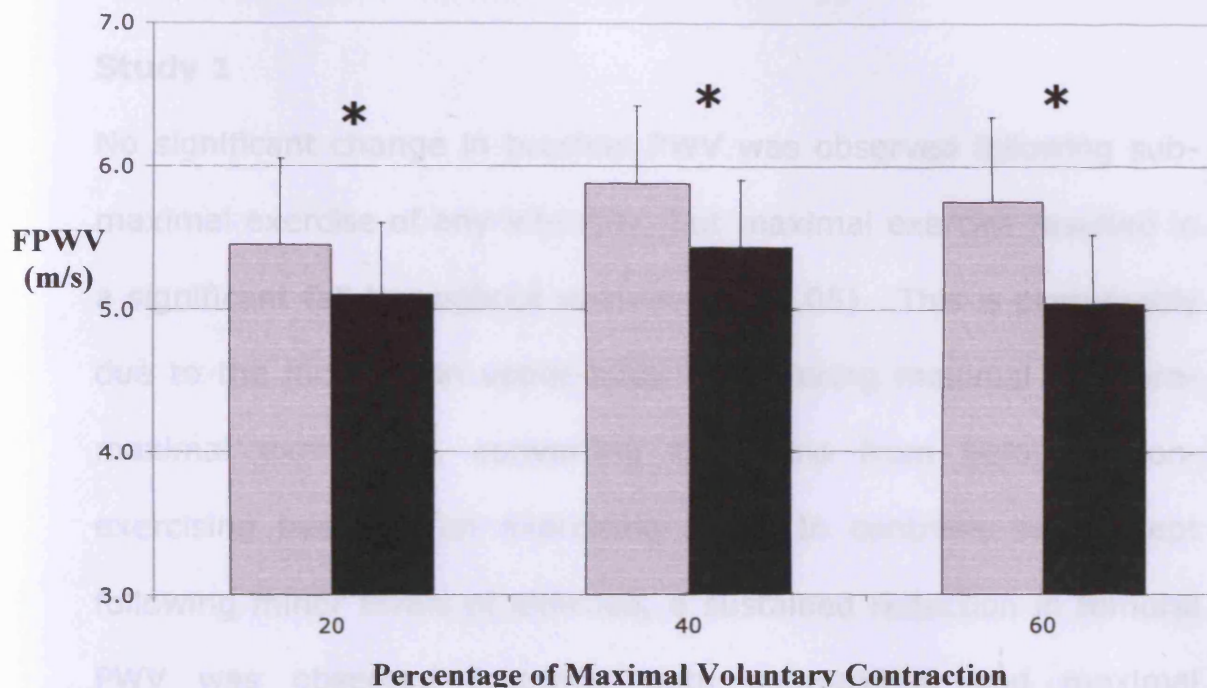
Grey columns = Baseline Black Columns = Recovery

Pulse Wave Velocity

As shown in figure 3.3 there was a significant reduction in Femoral Pulse Wave Velocity following isometric exercise (Average of 2 minutes readings) (P<0.05), there were no significant differences between baseline readings. The Bracho-Radial PWV did not change (P=NS).

Discussion

Study 1

No significant change in brachial PWV was observed following sub-maximal exercise of any intensity, but maximal exercise resulted in a significant fall throughout recovery ($P<0.05$). This is presumably due to the increase in upper-body work during maximal or supra-maximal exercise[186], converting the arms from being a non-exercising bed into an exercising bed. In contrast, and except following minor levels of exercise, a sustained reduction in femoral PWV was observed following both submaximal and maximal exercise.

The most likely explanation for this increase in large artery distensibility is a significant increase in release and/or formation of a local metabolite from the vascular endothelium (e.g. Nitric Oxide, Prostaglandins), or from the exercising muscle (Lactate, Potassium, adenosine), although a neural mechanism is also possible. The vascular endothelium is a barrier between the blood and the vascular smooth muscle and the endothelium actively modulates vascular smooth muscle tone via release of NO, prostaglandins and endothelial derived relaxation factors[65, 187, 188]. NO activates guanylate cyclase to increase cyclic guanosine monophosphate (cGMP) and this causes vasorelaxation. At rest, low levels of NO

are continuously synthesised and released generating a constant counter against sympathetic vasoconstriction.

Shear stress is the result of the frictional force applied to the endothelium by blood flow though a vessel. This can increase in one of two ways 1) a decrease in vessel diameter (vasoconstriction) without a decrease in flow, 2) or as an increase in blood flow without an increase in vessel diameter. Shear stress has been shown to potentiate agonist-stimulated NO release[66, 68], and chronic shear stress (exercise training) up regulates type III NOS expression[81, 82, 189, 190]. Unlike the other forms of NOS, type III NOS is membrane bound, this positioning of type III NOS appears to enable NO synthesis to be regulated by shear stress[191] (Transduction mechanisms sheer related NO release mediated by the kinase AKT)

Prostaglandins (PG's) belong to the family of prostanoids and are produced mainly from arachadonic acid by the enzyme cyclooxygenase. PGs are known to modulate platelet aggregation and inflammation and oedema. PG's also modulate tissue blood flow at rest and during increases in metabolic demand such as exercise and reactive hyperaemia[192]. Their role in regulating the large increases in muscle blood flow during exercise has not been clearly demonstrated, but this may be because there are multiple

redundant mechanisms[193]. Several interactions are known to exist between specific vasodilator substances, and therefore PGs can act in synergy with other substances and contribute to functional hyperaemia. Furthermore, there is evidence for differential, tissue-specific influences of PGs where their influence on blood flow during exercise may be profound. PG's have been shown to exhibit a role in the exercise hyperaemia in both human and animal models, with the magnitude of the response varying with the technique involved (Doppler ultrasound versus plethysmography) and the route of administration (local or systemic)[194].

Study 2

Sub-maximal resistance exercise was associated with an immediate post exercise reduction in the PWV of the exercising limb, implying an increase in the arterial distensibility. This may be a consequence of the increase in flow to the exercising limb. Exercising limb blood flow increases markedly in association with a large fall in local vascular resistance, due to local metabolic stimuli. Local skeletal muscle metabolic factors cannot of course be immediately responsible for the increase in large artery distensibility, because these vessels are upstream.

Although these factors have yet to be fully elucidated, local vasodilator mechanisms involving the muscle vascular endothelium

and limited retrograde travel of dilator signals have been postulated[195]. Once local intramuscular vasodilatation has occurred then flow in the femoral artery will increase and therefore cyclic wall stress on its endothelium will follow. This is a well-known vasodilator stimulus, which would facilitate the increased muscle blood flow. Therefore the observed increase, in distensibility immediately post exercise, could be attributed to endothelium dependent vasodilation. An alternative idea to explain post exercise increases in arterial distensibility is that an exercise driven increase in sympathetic vasoconstrictor tone is decreased at this time

Certainly systemic sympathoexcitation caused by muscle metaboreflex activation during the handgrip and calf exercise would be expected to oppose vasodilatation during exercise [52, 53, 58, 196]. However, rapid wash out of the metabolites on cessation of exercise removes this reflex response and sympathetic activity quickly returns to baseline, as is supported by the rapid recovery of BP in the control experiments. In addition the fact that there was no change in the non-exercising limbs suggest that a local factor must be involved.

Study 3

Consistent with previous observations of isometric exercise there was a marked pressor response and moderate rise in heart rate, which increases with increasing intensity[197]. This is the first study that we are aware of that demonstrates that isometric exercise, even in the face of a marked pressor and chronotropic response, augments arterial distensibility in the exercising limb.

Conclusions

Acute exercise of a significant intensity leads to increases in arterial distensibility, primarily in the exercising limb, even in the presence of a marked pressor and chronotropic response. As study 1 and 2 show the change is predominantly in the exercising limb and following aerobic exercise last for at least ten minutes. This may be an indication of a local response overriding any systemic sympathetic response, however this will require further investigation.

Chapter 4

The Exaggerated Systolic Blood Pressure Response to Exercise in Health and Disease

The role of the conduit arteries

Introduction

As discussed in the introduction and previous chapter, studies have shown that acute and chronic exercise can improve arterial distensibility. This may be an important factor in the control of blood pressure, particularly systolic pressure, on exercise. In otherwise healthy individuals an exaggerated Systolic Blood Pressure (ExSBP) response to exercise is a marker of increased risk for cardiovascular disease, including stroke and myocardial infarction[27-29, 34, 35]. However what is not clear from these studies was the risk factor status of these subjects and whether they had normal arterial function at rest. Below we summarise the data from a few of these studies and outline the reason for this study.

Normotensive subjects with an ExSBP have been shown to be at increased risk of developing future essential hypertension (ESH), Isolated Systolic Hypertension (ISH) and suffer a greater risk of future stroke and myocardial infarction[27-29, 34, 35]. However, it is not always clear whether this is because such patients have other cardiovascular risk factors or whether ExSBP can occur in the absence of other conventional cardiovascular risk factors. Recently Stewart et al demonstrated that subjects with an ExSBP demonstrate a high resting PWV and an impaired flow-mediated dilatation of the brachial artery[198]. This backs up the data from the Dundee group in which subjects with an ExSBP showed an

impairment of NO dependent forearm vasodilation compared with a group of controls with a normal blood pressure response[199].

The literature regarding exercise and PWV is very scarce with most studies looking at subjects whom have exercised chronically (athletes) versus their sedentary counterparts. These studies have shown that in older athletes (>20 years) there is a significant reduction in aortic and peripheral PWV in the athletic population[120]. The few studies that have looked at acute exercise and its effects on PWV have looked at exercise of a very low intensity, which showed no change, or exercise of a moderate intensity which resulted in a decrease in PWV thirty minutes into recovery[138]. More recently Naka et al have shown that healthy subjects who complete a maximal treadmill test to volitional exhaustion exhibit a marked reduction in femoral PWV, which persists for an hour post exercise[137]. We have also shown in chapter 3 that single limb exercise using continuous rhythmical exercise results in a reduction in the exercising limb PWV without any subsequent change to the other non-exercising arterial beds. Pathologically we know that subjects with an ExSBP suffer a greater rate of stroke, myocardial infarction and hypertension. Subjects with hypertension and heart failure have increased PWV compared to healthy controls.

In chapter three we have shown that subjects with a normal blood pressure response to exercise and no cardiovascular risk factors acutely improve arterial distensibility immediately after assessed maximal and sub-maximal exercise. However we have studied relatively small groups (10-20 subjects) and the subjects were mainly young, and were totally free of cardiovascular risk factors, so we cannot draw any conclusions as to whether this would be normal for the population.

In this study we recruited two separate groups into two separate studies. Study 1 aimed to define the prevalence of an ExSBP in healthy subjects without risk factors during two submaximal tests that have been used in previous studies of ExSBP, the Dundee step test and the 100-Watt Cycle test. The second part of study 1 was to determine the resting, exercise and recovery haemodynamics and arterial function in this group. We recruited 100 consecutive visitors to an open access risk factor clinic, who had been found to be free of conventional risk factors and consented to the two study visits.

Study 2 aimed to assess prevalence of ExSBP during the Dundee Step Test in patients with borderline cardiovascular risk factors and to assess the arterial responses to controlled sub-maximal exercise.

Methods

Subject Recruitment

Study 1: We recruited from an Open Access Risk Factor clinic at the Wales Heart Research Institute. All subjects were male, aged between 18 and 50, normotensive (<130/90), had normal cholesterol (<5.2, mmol/dl), were non-smokers, were free of diabetes and not taking cardiovascular medication. They also had to consent to the both arms of the study. There were 723 visitors to the clinic before our recruitment was complete.

Study 2: We recruited 20 males from the same Open Access Risk Factor clinic, however subjects included had borderline hypertension (130-140/80-90 mmHg) and/or mildly elevated cholesterol (5.2-6.0, mmol/dl), but were free of diabetes, non-smokers and on no cardiovascular medication.

Measurements

Heart rate was recorded on a minute-by-minute basis at rest, during exercise and in recovery using a standard electrocardiogram. Femoro-Tibial and Bracho-Radial Pulse Wave Velocity were recorded for ten minutes at baseline and in recovery. Blood pressure was recorded every two minutes during rest and recovery and at various time intervals during the exercise tests, ranging from every minute

to every ten minutes. In all studies, subjects completed a familiarisation visit in which the exercise tests, arterial recordings and protocols were explained and subjects had any queries answered.

Study 1 (a) – Haemodynamic responses to sub-maximal exercise (N=100) (No risk-factor group)

Following positioning of the sphygmomanometer and electrocardiogram subjects rested for fifteen minutes. A ten-minute baseline reading was completed, before the subject completed the Dundee Step Test. After a further 45 minutes of recovery baseline readings were re-measured and then the 100-Watt cycle test was completed.

Study 1 (b) – Haemodynamic and arterial responses to maximal exercise (N=100) (No risk-factor group)

Subjects had the sphygmomanometer, Scimed system and electrocardiogram positioned and rested for fifteen minutes. A ten-minute baseline reading was completed, before the subject completed a maximal exercise test on a cycle ergometer. On cessation of exercise the subject returned to the supine position for ten minutes of recovery readings (The first recovery reading was always complete within 2 minutes of exercise cessation).

Study 2 (a) – Haemodynamic responses to Dundee Step test (N=20)

Subjects had the sphygmomanometer and electrocardiogram positioned and then rested for fifteen minutes. A ten-minute baseline reading was undertaken, before the subject completed the Dundee Step Test. The results of this test used to divide the group into ExSBP (SBP >180 mmHg during test) or Normal Systolic Blood Pressure (NSBP) (SBP <180 mmHg during test).

Study 2 (b) – Arterial responses to sub-maximal exercise (N=20)

Subjects had the sphygmomanometer, Scimed system and electrocardiogram positioned and then rested for fifteen minutes. A ten-minute baseline reading was completed, before the subject completed 15 minutes of walking at 75% of age predicted maximal heart rate ((220-age)*0.75). On cessation of exercise the subject returned to the supine position for ten minutes of recovery readings (The first recovery reading was always complete within 2 minutes of exercise cessation).

Statistical Analysis

For all studies the baseline data is presented as the mean of ten minutes, exercise data is presented as the mean of each study, unless otherwise noted, while recovery is presented as early, middle

or late, which represents minutes 1-3, 4-6 and 7-10 respectively. The data is presented as the mean plus or minus the standard deviation of the mean. All comparisons were completed using repeated measures analysis of variance with post-hoc analysis completed with a significance value set at $P<0.05$.

Results

Study 1 (No risk-factor group)

Subject Data

The mean data is presented for the 100 male subjects who were free of all risk factors in Table 4.1

Table 4.1 Subject's Characteristics

	Age (Years)	Height (m)	Weight (kg)	BMI (Kg/m^2)	SBP (mmHg)	DBP (mmHg)
Mean	38	1.75	74.5	24.3	124	82
(SD)	(8)	(0.09)	(4.8)	(2.5)	(9)	(8)

Dundee Step Test and 100-Watt Cycle Test

For both tests we employed the definition of an ExSBP which had previously been shown to be associated with an increase risk of development of resting hypertension, myocardial infarction and stroke or had demonstrated an abnormal arterial function in this patient group i.e. > 180 mmHg during the Dundee Step Test, or > 200 mmHg after 6 minutes of cycling at 100 watts. In the Dundee step test the mean systolic pressure at end of test was 162 (11)

mmHg with a mean heart rate of 108 (11) BPM. During the 100-Watt Cycle Test the mean systolic blood pressure following six minutes was 166 (8) mmHg with a heart rate of 114 (13) BPM. None of our subjects had an SBP in excess of 180 or 200 mmHg at the end each respective test and neither test resulted in a significantly greater pressor or chronotropic response than the other (P=NS between tests). Table 4.2 tabulates the baseline and exercise responses for each study.

Table 4.2 Haemodynamics at rest and during sub-maximal exercise

	Heart Rate (Bts/min)	SBP (mmHg)	DBP (mmHg)	PP (mmHg)
Baseline 1	73 (7)	123 (7)	81 (8)	42 (7)
Dundee	108 (11)*	162 (11)*	78 (8)	84 (8)*
Baseline 2	70 (6)	119 (8)	80 (7)	39 (6)
100-Watt	114 (13)*	166 (8)*	76 (10)	90 (10)*

* = Different from baseline

Arterial and Haemodynamic Responses to Maximal Exercise

Heart rate and SBP rose from baseline and through each stage of testing ($P < 0.05$ for all), the diastolic blood pressure fell ($P < 0.05$ for group baseline vs. group max BDP). Baseline femoral and brachial pulse wave velocity was 7.1 (0.6) and 4.7 (0.8) m/s and fell following maximal exercise and was lower throughout recovery ($P < 0.05$ compared to baseline). Figures 4.1 and 4.2 depict these results.

Fig 4.1   **Haemodynamic responses to Maximal exercise**

Legend: — = SBP, — = DBP, — = HR

Y-axis: mmHg/ bts.min (250, 200, 150, 100, 50)

X-axis: Watts (N) — Seated, 60 (100), 90 (100), 120 (100), 150 (100), 180 (92), 200 (78), 220 (54), 240 (32), 260 (18), 280 (8), 300 (4)
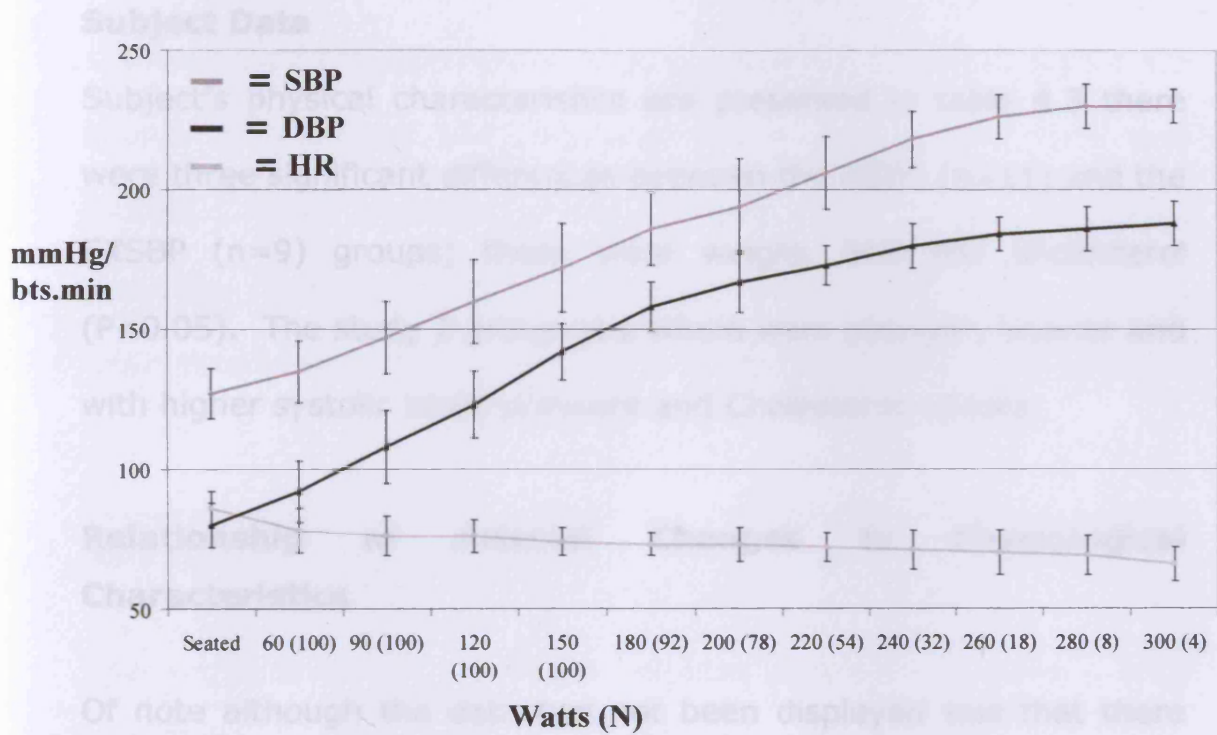
Fig 4.2   **Brachial and Femoral Pulse Wave Velocity Pre and Following Maximal Exercise**

Legend: — = FPWV, ■ = BPWV

Y-axis: PWV (m/s) (10, 8, 6, 4, 2)

X-axis: Baseline, Early, Mid, Late
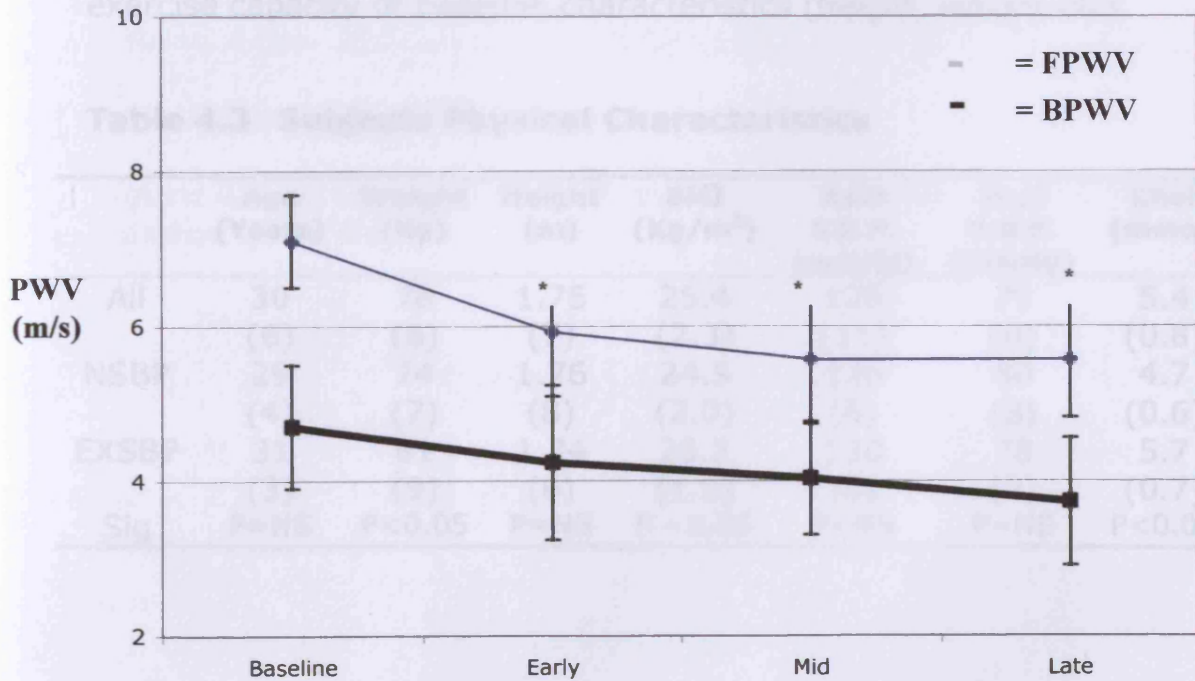
* = Significantly different to baseline

Study 2

Subject Data

Subject's physical characteristics are presented in table 4.3 there were three significant differences between the NSBP (n=11) and the EXSBP (n=9) groups; these were weight, BMI and cholesterol (P<0.05). The study 2 group as a whole were younger, heavier and with higher systolic blood pressure and Cholesterol, values.

Relationship of Arterial Changes to Physiological Characteristics

Of note although the data has not been displayed was that there was no relationship between change in PWV or augmentation as such when compared to any resting or exercise blood pressure, exercise capacity or baseline characteristics (height, weight etc).

Table 4.3 Subjects Physical Characteristics

	Age (Years)	Weight (Kg)	Height (m)	BMI (Kg/m^2)	Rest S.B.P. (mmHg)	Rest D.B.P. (mmHg)	Chol (mmol)
All	30	78	1.75	25.4	128	79	5.4
	(6)	(8)	(7)	(2.3)	(11)	(8)	(0.8)
NSBP	29	74	1.76	24.5	126	80	4.7
	(4)	(7)	(8)	(2.0)	(6)	(8)	(0.6)
EXSBP	31	81	1.74	26.2	130	78	5.7
	(3)	(9)	(6)	(1.9)	(9)	(7)	(0.7)
Sig	P=NS	P<0.05	P=NS	P <0.05	P=NS	P=NS	P<0.05

Dundee Step Test

As discussed the subjects were split into NSBP and ExSBP depending on the SBP response to the Dundee step test, figure 4.3 shows the different SBP response during stepping for NSBP and ExSBP. There were no significant differences in diastolic blood pressure (P=NS) but pulse pressure, mean blood pressure and heart rate were higher in ExSBP than in NSBP (P<0.05 for all).

Haemodynamic responses to controlled sub-maximal exercise

The subjects completed fifteen minutes of treadmill walking at a pace that resulted in the subjects walking with the speed and incline adjusted so that each individual walked at 75% of APMHR for 15 minutes. ExSBP had a significantly higher SBP during treadmill walking when compared with NSBP (197 (16) V 172 (19) mmHg, P<0.05), see figure 4.4.

Figure 4.3 NSBP and ExSBP systolic pressure response to Dundee Step Test
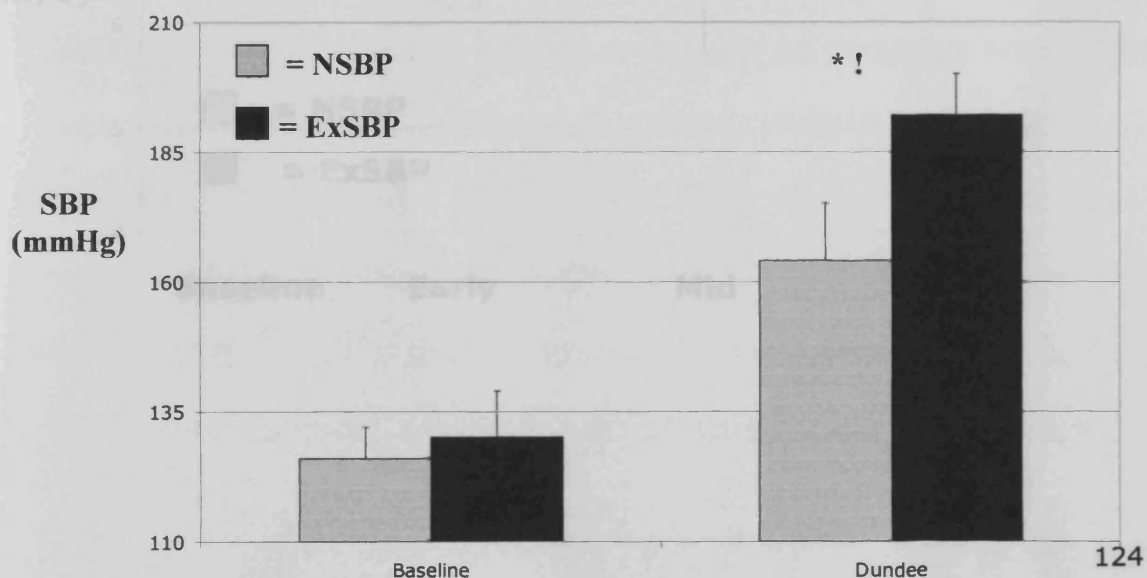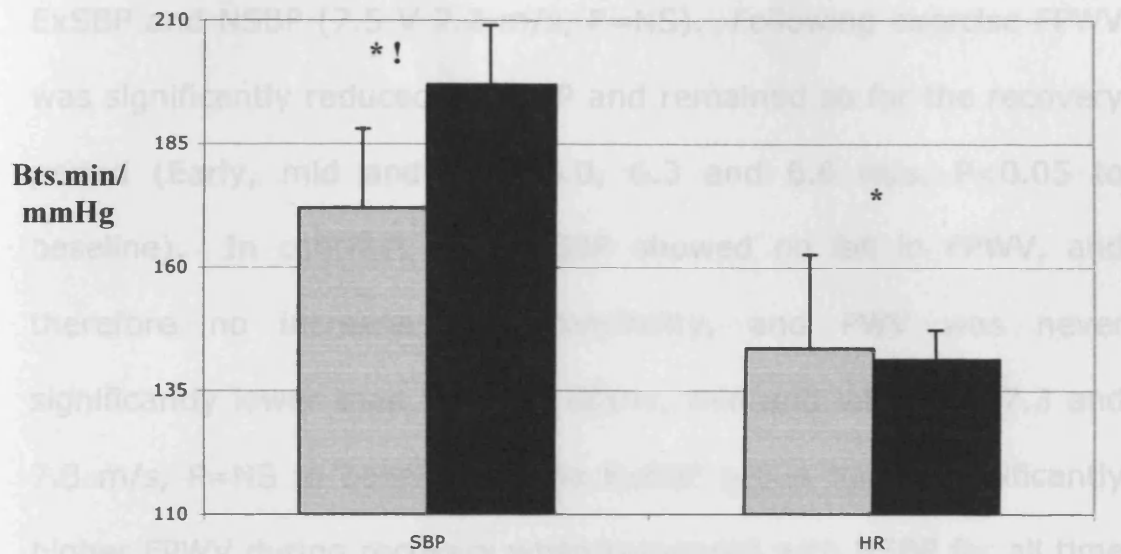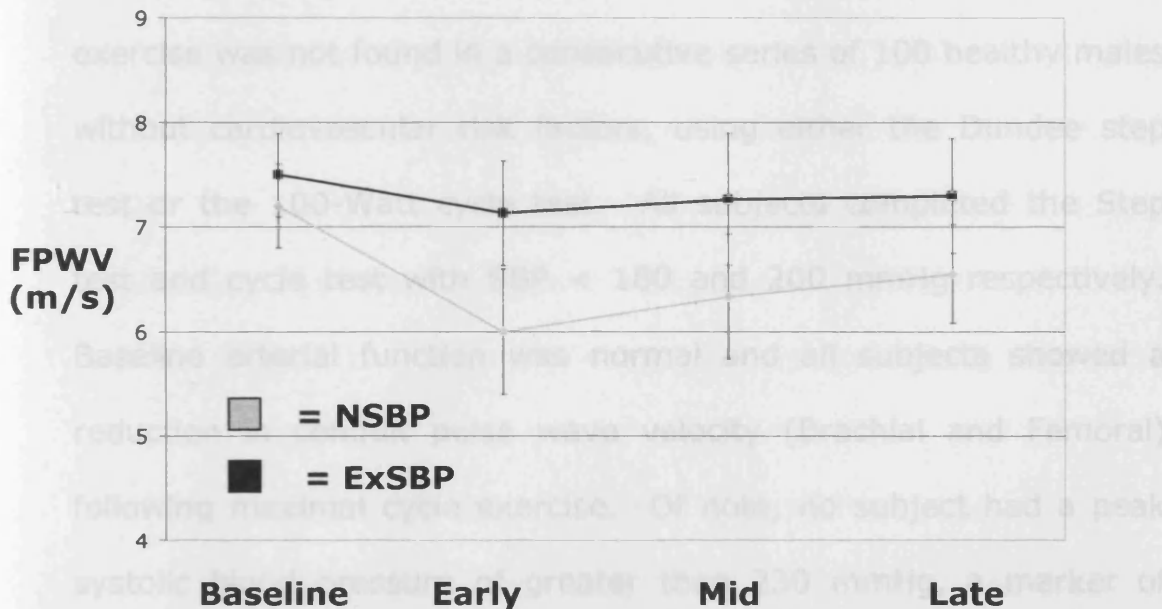

124

Figure 4.4 NSBP and ExSBP heart rate and systolic pressure response to sub-maximal treadmill exercise (75% of APMHR)

* = Significantly different to baseline, ! = Significantly different to NSBP

Fig 4.5 NSBP and ExSBP FPWV pre and post sub-maximal exercise

Arterial Responses to controlled sub-maximal exercise

Baseline Femoral Pulse Wave Velocity was not different between ExSBP and NSBP (7.5 V 7.2 m/s, P=NS). Following exercise FPWV was significantly reduced in NSBP and remained so for the recovery period (Early, mid and late (6.0, 6.3 and 6.6 m/s, P<0.05 to baseline). In contrast, the ExSBP showed no fall in FPWV, and therefore no increase in distensibility, and PWV was never significantly lower than baseline (Early, mid and late (7.1, 7.3 and 7.3 m/s, P=NS to baseline). The ExSBP group had a significantly higher FPWV during recovery when compared with NSBP for all time points Fig 4.4 (P<0.05).

Discussion

An Exaggerated Systolic Blood Pressure (ExSBP) response to exercise was not found in a consecutive series of 100 healthy males without cardiovascular risk factors, using either the Dundee step test or the 100-Watt cycle test. All subjects completed the Step test and cycle test with SBP < 180 and 200 mmHg respectively. Baseline arterial function was normal and all subjects showed a reduction in conduit pulse wave velocity (Brachial and Femoral) following maximal cycle exercise. Of note, no subject had a peak systolic blood pressure of greater than 230 mmHg, a marker of exaggerated blood pressure during maximal stress. This finding infers that subjects who are free of cardiovascular risk factors,

including smoking, diabetes, hypertension, and abnormal lipids are able to augment their arterial distensibility on exercise and this may maintain a normal blood pressure response.

When subjects with borderline hypertension and/or cholesterol were exercised according to the Dundee step test, 45% had an ExSBP as defined as >180 mmHg. The difference between the two groups is only partly explained by physical fitness as the difference in systolic blood pressure between NSBP and ExSBP remained, if slightly blunted, when exercise intensity was controlled. As the groups had similar resting blood pressure and arterial function it was interesting to note that whilst the group with borderline risk factors and an NSBP were able to augment their arterial distensibility as shown by a significant fall in FPWV, the group with ExSBP were unable to augment distensibility in the exercising limb. This inability to increase arterial distensibility may be the mechanism behind the ExSBP and as Lim et al showed an ExSBP group have resting endothelial dysfunction, raising the possibility that endothelial function may be an important factor. Finally the resting FPWV and BP were similar in both groups which results in difficulties in identifying which patients are at risk of ExSBP and therefore CHD and those that are not from simple resting measures of haemodynamics or arterial function such as peripheral pulse wave velocity.

Pathophysiology of ExSBP

Although sparse there is some data assessing physiological responses in groups with ExSBP and control. The date is split between vascular, cardiac and metabolic function and is discussed below.

Vascular function and ExSBP

Stewart and colleagues showed in men (n=38) with untreated high normal BP or mild hypertension that the variance in maximal exercise SBP and pulse pressure (PP) is due to resting SBP (34 + 23%, P<0.01) and that flow-mediated dilation (FMD) explained an additional 11 and 10% respectively (P<0.01)[198]. In men FMD was the only independent correlate of SBP augmentation (Maximal – Resting)(R^2 = 0.20, P<0.05). Resting aortic pulse wave velocity did not correlate with exercise blood pressure responses. Chang et al showed in patients with exercise induced hypertension (n-35) that they have impaired endothelial dependent vasodilation, as measured by FMD, compared to controls (n=35) (3.14 V's 6.5%, P<0.05) and that the systolic difference was significantly correlated with the extent of vasodilation (r=-0.36, P<0.05)[200]. They also measured the concentration of NO_2^-/NO_3^- and cyclic guanosine monophosphate (GMP) at baseline, peak exercise and in recovery (30 min post), showing that although NO_2^-/NO_3^- increased during exercise the rise was the same in both control and patients[185].

However the rise in cyclic GMP was significantly greater in controls than ExSBP (8.3 V's 10, pmol/ml, P<0.05), suggesting that the increase in ExSBP may be in part due to an inhibition of vasodilation via an inadequate increase in cyclic GMP (Fig 4.6). The Dundee group have also shown similar findings when they compared ExSBP with age matched controls and demonstrated a 150% increase in forearm blood flow (FBF) in the control group following acetylcholine infusion (100 mmol/min) whilst the ExSBP group demonstrated no significant increase (110%, P=NS)[201]. They also showed that complete NO inhibition with high dose L-NMMA (8mmol/kg) significantly inhibited FBF in the control group (-44%, P<0.01) whilst the ExSBP demonstrated a non-significant drop (-18%, p=ns) whilst the differences between the groups were significant (P<0.01)[199]. All of these studies have demonstrated an impairment in endothelial derived arterial dilatation, in 1999 Fossum et al showed a distinct link between ExSBP and the structure of the vascular wall[202]. In 27 draft recruits whom underwent exercise screening, echocardiography, venous occlusion plethysmography and biochemical analysis they demonstrated that 19% of the variation in blood pressure at the end of six minutes cycling (Mundal et al used this test to determine risk of myocardial infarction) was related to minimal forearm vascular resistance (MFVR). MFVR has been previously demonstrated to detect peripheral structural

vascular changes and is the mean arterial pressure divided by the maximal forearm blood flow[203, 204].

Figure 4.6 NO$_2^-$/NO$_3^-$ and cyclic GMP in NSBP and ExSBP at peak exercise[185]
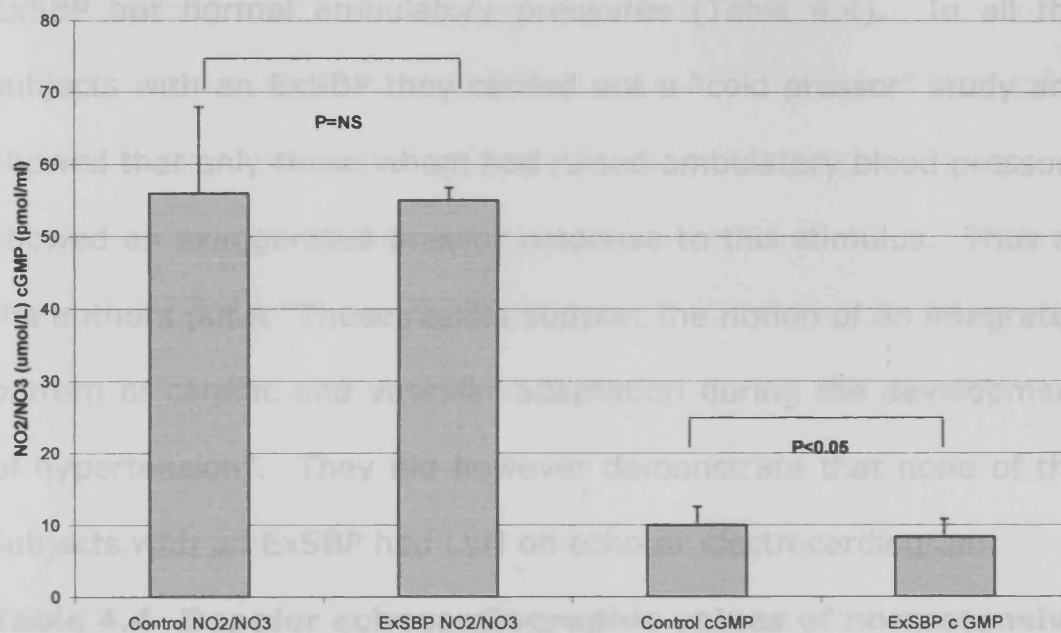
Cardiac function and ExSBP

Many studies have demonstrated that blood pressure responses to exercise are related to left ventricular mass, postulating that this may be a discreet marker to assess subjects with left ventricular hypertrophy[26]. However until recently no one has looked at cardiac function in subjects with an ExSBP. Using standard echo measurements Herkenhoff and colleagues showed that subjects with ExSBP (n=36) can be split into two groups[205]. Those with normal blood pressure at rest (128/81 mmHg, (n=19) and those

with raised blood pressure (144/91 mmHg, (n=17) P<0.05 between groups). Of those with an ExSBP and a raised ambulatory blood pressure they showed an increase in fractional shortening, a longer isovolumetric relaxation time and a reduction in the early to late flow velocity ratio when compared to controls and subjects with an ExSBP but normal ambulatory pressures (Table 4.4). In all the subjects with an ExSBP they carried out a "cold pressor" study and showed that only those whom had raised ambulatory blood pressure showed an exaggerated pressor response to this stimulus. Thus as the authors put it "These results support the notion of an integrated pattern of cardiac and vascular adaptation during the development of hypertension". They did however demonstrate that none of the subjects with an ExSBP had LVH on echo or electrocardiogram.

Table 4.4 Doppler echocardiographic values of normotensive controls (NT) and ExSBP with normal (ExSBP[1]) or elevated (ExSBP[2]) ambulatory blood pressure[205]

	NT (n=36)	ExSBP[1] (n=19)	ExSBP[2] (n=17)
LVDd (mm)	50.6 ± 2.8	49.5 ± 3	51 ± 2.6
LVMi (g/m^2)	81 ± 9.3	79 ± 12	83 ± 4
PWT (mm)	8.9 ± 0.6	9.0 ± 0.8	9.1 ± 0.7
IVST (mm)	8.9 ± 0.6	9.2 ± 0.8	9.2 ± 0.8
RWT	35 ± 2	37 ± 3	36 ± 4
FS (%)	40 ± 3	41 ± 4	43 ± 3*
E/A	1.4 ± 0.02	1.3 ± 0.03	1.0 ± 0.02*
IVRT (ms)	79 ± 7	81 ± 4	85 ± 4*

LVDd   =   Left ventricular diastolic diameter   LVMi   =   Left ventricular mass index
PWT   =   Posterior wall thickness   IVST   =   Interventricular septal thickness
RWT   =   Relative wall thickness   FS   =   Fractional shortening
E/A   =   Early to late peak flow ratio   IVRT   =   Isovolumetric relaxation time
* = P<0.05 (ANOVA and Tukey test)

Metabolic function and ExSBP

It has been shown that fasting blood glucose is independently associated with resting and exercise blood pressure in healthy normotensive, non-diabetic men. Bjornholt et al used a cross-sectional and prospective cohort of 2014 healthy middle aged men and evaluated resting and exercise blood pressure, intravenous glucose tolerance and fasting blood glucose[206]. After adjusting for age, BMI, blood lipids and glucose tolerance, fasting blood glucose was strongly associated with exercise (coefficient 6.57, P<0001) and resting blood pressure (coefficient 2.83, P<0.0005). It also predicted future development of treated hypertension or elevated blood pressure (Odds ratio 1.17, CI 1.05-1.31) seven years post baseline screening. As we demonstrated above the Framingham study found no relationship between Exaggerated Systolic Blood Pressure during exercise, however they did discover a link between exercise diastolic blood pressure and future hypertension[207]. Brett et al showed that diastolic pressure changes during cycle ergometry exercise (50,75 and 100 watts) are positively correlated with serum cholesterol (R>0.47, P<0.0001 for each workload) and insulin resistance (R>0.38, P<0.01, for each workload) in healthy active men[208]. They also showed that in subjects with type II diabetes the rise in DBP was greater than in control (13.6 mmHg vs. 2.7 mmHg, P<0.005).

Although this data demonstrates that subjects with an ExSBP appear to show an inhibition of endothelial derived relaxation factors, and certain cardiac abnormalities, it must be remembered that all of these mechanistic studies were carried out at rest (Excluding the cGMP and NO_2^- /NO_3^- data), which may have little relevance to what we see during exercise. We believe that our study demonstrates for the first time an abnormal arterial response immediately post exercise, and probably during, results in an ExSBP.

Chapter Five

Vascular Responses to Maximal Exercise

The Influence of Nitric Oxide

Introduction

Previous work from our group and that of others has shown that in healthy subjects there is a reduction in pulse wave velocity of the exercising limb/s following, acute and chronic, exercise[121, 125, 209-211]. This is true of most modalities of exercise where the intensity is sufficient (Chapt 3). The degree of change is directly related to the exercise intensity (Chapt 3). Our previous work has also shown that it is likely to be a locally mediated mechanism, as the change in arterial distensibility is not seen in control arterial beds (Chapt 3). We have also demonstrated that an Exaggerated Systolic Blood Pressure Response to Exercise (ExSBP) is not present in subjects without risk factors (Chapt 4), but is present in those with a number of borderline risk factors (Chapt 4). The group with ExSBP failed to augment their arterial distensibility following (and presumably during) exercise. It has previously been demonstrated by our group that patients with heart failure also fail to augment their arterial distensibility in response to exercise (Unpublished finding). Given the fact that patients with heart failure, and those with certain cardiovascular risk factors, have an impairment of Nitric Oxide synthesis and that a magnitude of treatments improve the endothelial function and exercise capacity[77, 79, 81, 82, 129, 212, 213]. Tzemos et al showed that subjects with an ExSBP have an impaired endothelial response to acetylcholine it is likely that Nitric Oxide may play a key role in arterial distensibility during and after

exercise and therefore the blood pressure response to exercise[199].

We therefore hypothesised that blockade of Nitric Oxide Synthesis with N^G-monomethyl-L-arginine would blunt the arterial response to exercise and that subjects with an NSBP would develop an ExSBP and that it may reduce maximal exercise capacity.

As discussed in the introduction, ventriculo-arterial interaction markedly influences cardiac performance. Increased arterial elastance leads to an increase in ventricular systolic stiffness and results in an exaggerated increase in left ventricular end systolic pressure on exercise, in turn leading to impaired left ventricular relaxation[167, 214]. This is believed to be a key factor of the pathophysiology of heart failure with preserved ejection fraction. When we discuss interaction of the arteries and ventricle, it is important to note that the primary artery that affects this interaction will be the aorta and as yet we have not shown the influence that exercise has on this vessel.

The aim of this study was two-fold. First we wished to assess the large central arteries, as measured as changes in Aortic PWV and augmentation index, response during maximal exercise. Secondly we aimed to establish what influence Nitric Oxide has on resting, exercise and post-exercise haemodynamics and arterial function. To do this we carried out a randomised, double blind, placebo controlled, crossover study.

Methods

Subject Recruitment

10 male subjects volunteered to participate in this study. None smoked, were hypertensive (blood pressure <130/80mmHg), or were taking medication (Table 5.2). All subjects gave informed written consent and were habituated with the experimental procedures, which were approved by the local ethics committee and conformed to the Declaration of Helsinki. Subjects were asked to refrain from consuming food and caffeine in the eight hours preceding the experiments and to have avoided strenuous exercise for 48 hours.

Measurements

Beat to beat stroke volume (SV) and HR were measured using a non-invasive, bio-impedance monitor and cardiac output (CO) was calculated as SV x HR using Task Force® Monitor as has been previously described. Blood pressure was measured using an aneroid sphygmomanometer as described in the methodology. Radial artery pressure waveforms were acquired using applanation tonometry (Millar Instruments) and central pressure waveforms were generated using pulse wave analysis (Sphygmocor, AltCor Medical). Aortic Pulse Wave Velocity was calculated from the carotid and femoral waveforms as previously described. BPWV and FPWV were measured simultaneously and non-invasively by oscillometry

(Scimed) as described previously. Due to the complex and sensitive nature of arterial measurements we were unable to make recordings during exercise and we have tabulated below the frequency of each recording (Table 5.1). Please note that the quality of the impedance signal was only acceptable for the first five stages of exercise and we therefore do not have data for stroke volume, cardiac output and total peripheral resistance after 180 Watts.

Table 5.1 Recordings taken during each stage

	Baseline	Post-Infusion	Exercise	Recovery
HR, SV, CO BP	Continuous 3 Times	Continuous 3 Times	Continuous Every stage	Continuous Every 2 minutes
PWA and AoPWV	Every 2 minutes	Every 2 minutes	N/A	Every 2 minutes
FTPWV	Every Minute	Every Minute	N/A	Every Minute

Calculations

Total peripheral resistance (TPR), an index of the static component of arterial load was calculated as mean arterial pressure (dBP + 1/3 PP) divided by CO. Arterial compliance, an index of the pulsatile component of arterial load was calculated as stroke volume divided by the pulse pressure. Effective arterial elastance (AE) an index of arterial load that incorporates both static and pulsatile components was calculated as central end-systolic pressure (obtained from the

aortic pressure waveform) divided by SV. All calculations are fully described in the methodology.

Protocol

We carried out a randomised, double blind, placebo-controlled, crossover trial to assess the impact of Nitric Oxide synthesis inhibition on resting, exercise and recovery vascular function and haemodynamics. All patients carried out a familiarisation visit and "practice" maximal exercise test and then returned for two studies. Each visit was identical apart from the nature of the infusion. Subjects had haemodynamic and arterial assessment equipment attached and a 14-gauge venflon was inserted into a forearm vein of the left arm. Each trial began with a 15-minute acclimation period and was followed by 10 minutes of baseline measurements; this was followed by a 5-minute infusion period and 10 minutes of post infusion measurements, a maximal exercise stress test and 15 minutes of recovery readings.

Infusions

A bolus infusion of either the nitric oxide synthase blocker N^G-monomethyl-L-arginine (L-NMMA) (3mg/kg) mixed with 20ml saline, or a saline only solution, was infused over a 5-minute period following completion of baseline readings. This was followed by a

further 50ml infusion of saline with 3 mg/kg/hour L-NMMA, or saline only, infused over the remaining study period.

Assessment of Nitric Oxide Blockade

To ensure that the dose we had chosen was sufficient to block Nitric Oxide synthesis we carried out a randomised trial with three separate doses of L-NMMA (6/12/18 mg/kg/min) and completed the protocol from this study. However we took no arterial or haemodynamic measurements but took bloods for analysis of cGMP at each stage. There were no significant differences between cGMP with any of the doses at any stage of the study (P=ns for all).

Statistical Analysis

All values are expressed as mean plus or minus standard deviation. Data is expressed as mean of baseline, post infusion, as stage by stage for the exercise protocol and as five-minute averages in recovery (Early, Mid Late). Statistical analysis was performed using repeated measures ANOVA and *post hoc* analysis using paired t-tests with Bonferroni correction. Significance levels were set at $P < 0.05$.

Results

Subjects

Subjects were all healthy non-smokers who did not participate in regular vigorous exercise and were free from medication, the characteristics are tabulated below (Table 5.2). There were no significant differences in baseline characteristics between studies (P=NS) apart from stroke volume (P<0.05) (Table 5.3, Figure 5.3 + 5.4).

Table 5.2 Subject Characteristics

	Age (years)	Height (m)	Weight (kg)	BMI (kg/m2)	SBP (mmHg)	DBP (mmHg)	Cholesterol (mmol/L)
Mean	30.6	1.77	76.6	24.4	120	74	4.6
SD	5	0.07	9.3	2.7	6	7	1.3

Maximal Exercise Capacity and Haemodynamics

Before establishing the effects of L-NMMA during sub-maximal exercise, it is important to note that any differences in heart rate or blood pressure and not due to a change in workload as there was no difference in time to peak exercise, maximal exercise capacity, anaerobic threshold or V_E/V_{CO_2} slope between saline and vehicle (P=NS). As expected in a double-blind study there was no significant difference in the effort made as defined by the RER at peak exercise (1.18 vs. 1.2, saline vs. LNMMA, P=NS) (Fig 5.1), Figure 5.2 also shows that there were no significant differences between the maximal pressor or chronotropic response between saline and L-NMMA.

Sub-Maximal Exercise Haemodynamics

Table 5.3 Resting, Post Infusion and Sub-Maximal Haemodynamics

	Baseline	PI	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
S SBP	120 (7)	118 (7)!	131 (9)	142 (9)	159 (11)	172 (8)	184 (10)
L SBP	120 (6)	126 (7)	138 (8)	149 (7)	164 (9)	177 (11)	189 (9)
S DBP	74 (5)	73 (6)	69 (6)	67 (7)	64 (7)	65 (9)	64 (8)
L DBP	73 (6)	78 (6)	73 (6)	72 (8)	73 (8)	72 (4)	69 (7)
S MBP	89 (5)	88 (7)	90 (7)	92 (8)	96 (8)	101 (10)	104 (9)
LMBP	89 (4)	94 (6)	95 (6)	98 (9)	103 (9)	107 (9)	109 (8)
S SV	86 (6) !	86 (6)	89 (4)	95 (4)	92 (4)	91 (4)	101 (4)
L SV	93 (6)	86 (6)	85 (3)	91 (4)	90 (4)	90 (4)	99 (3)
S HR	60 (5)	60 (7)!	91 (8)	104 (10)	121 (10)	139 (11)	155 (13)
L HR	60 (5)	54 (6)	88 (8)	102 (10)	118 (8)	135 (9)	150 (11)
S CO	5.2 (0.7)	5.1 (0.7)!	8.1 (1.2)	9.8 (1.7)	11.1 (1.5)	12.7 (1.8)	15.7 (2.1)
L CO	5.6 (0.6)	4.6 (1.2)	7.5 (1.3)	9.3 (1.4)	10.6 (1.8)	12.1 (1.6)	14.8 (1.9)

S = Saline, L = L-NMMA, SBP = Systolic Blood Pressure (mmHg), DBP = Diastolic Blood Pressure (mmHg), SV = Stroke Volume (ml), HR = Heart Rate (Bts/min), CO = Cardiac Output (L/min) ! = different to L-NMMA ($P < 0.05$)

Arterial Responses to Maximal Exercise
As depicted in Figure 5.3 and 5.4

Influence of Nitric Oxide on the Arterial Responses to Maximal Exercise
As depicted in Figure 5.5 and 5.6

Fig 5.1 Maximal Exercise Capacity, Anaerobic Threshold, V_E/Vco^2 slope and Respiratory Exchange Ratio with and without NO inhibition
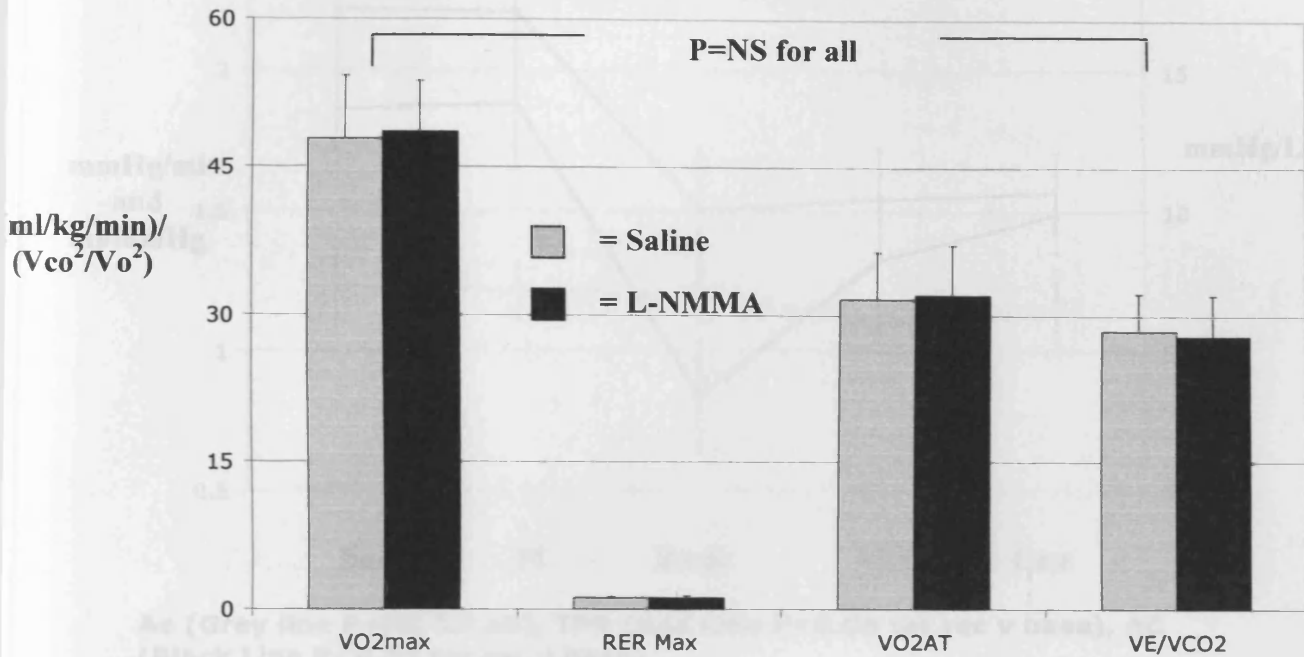

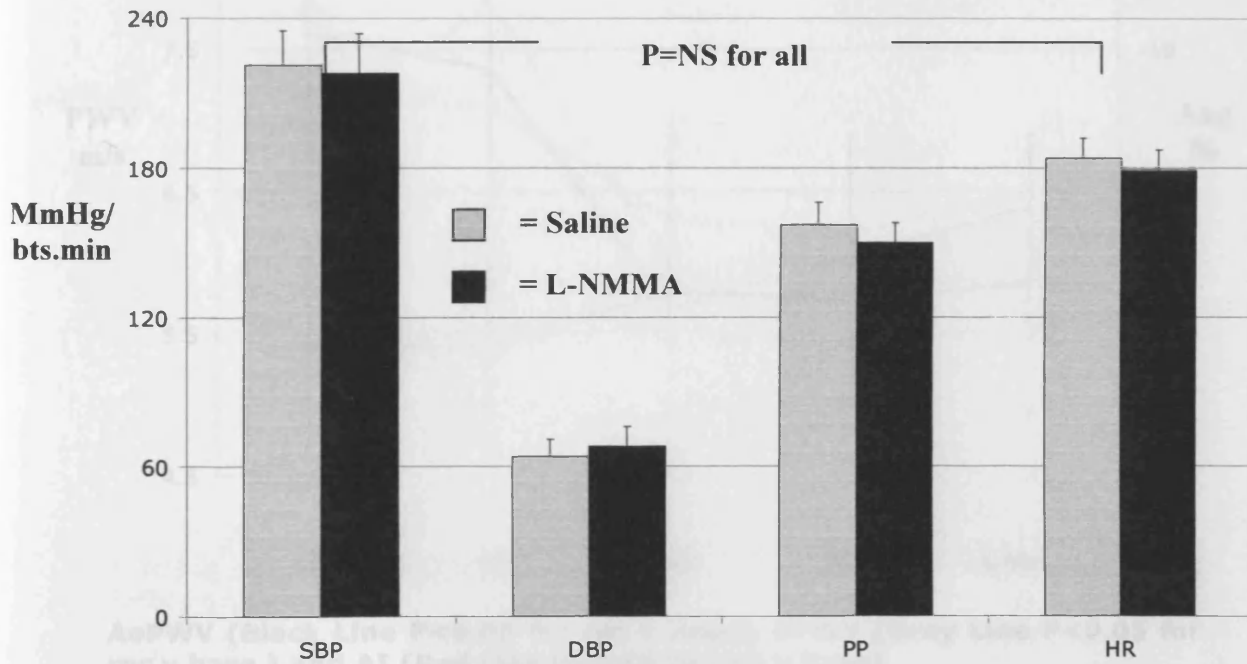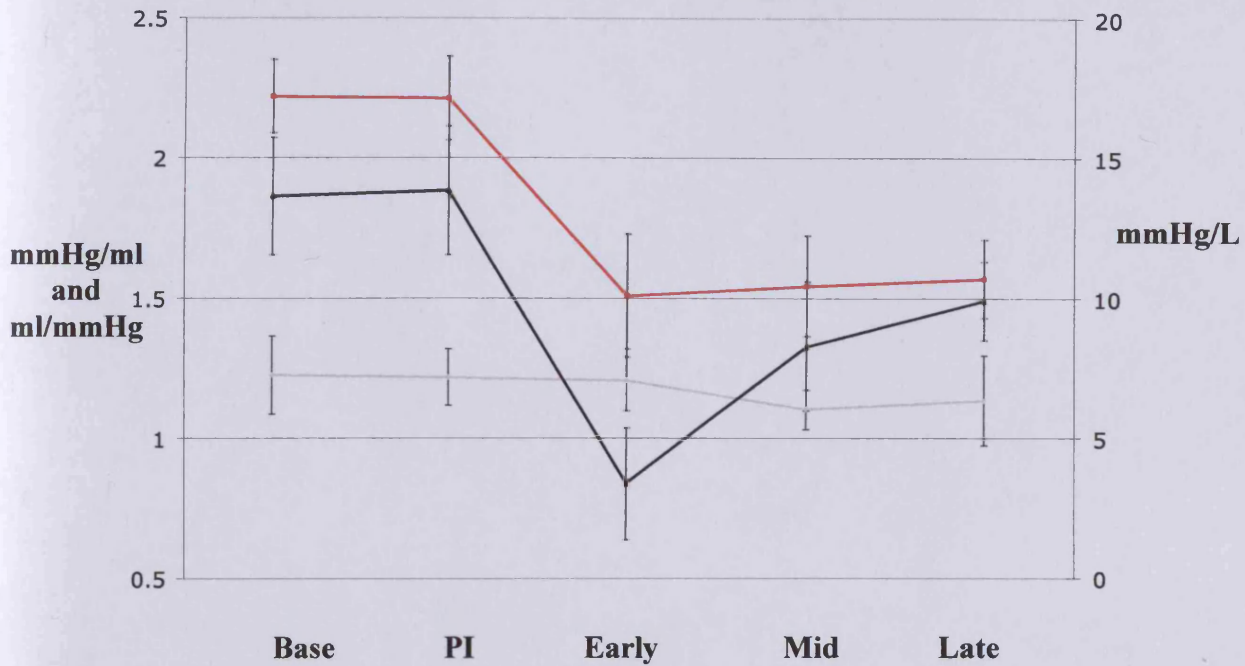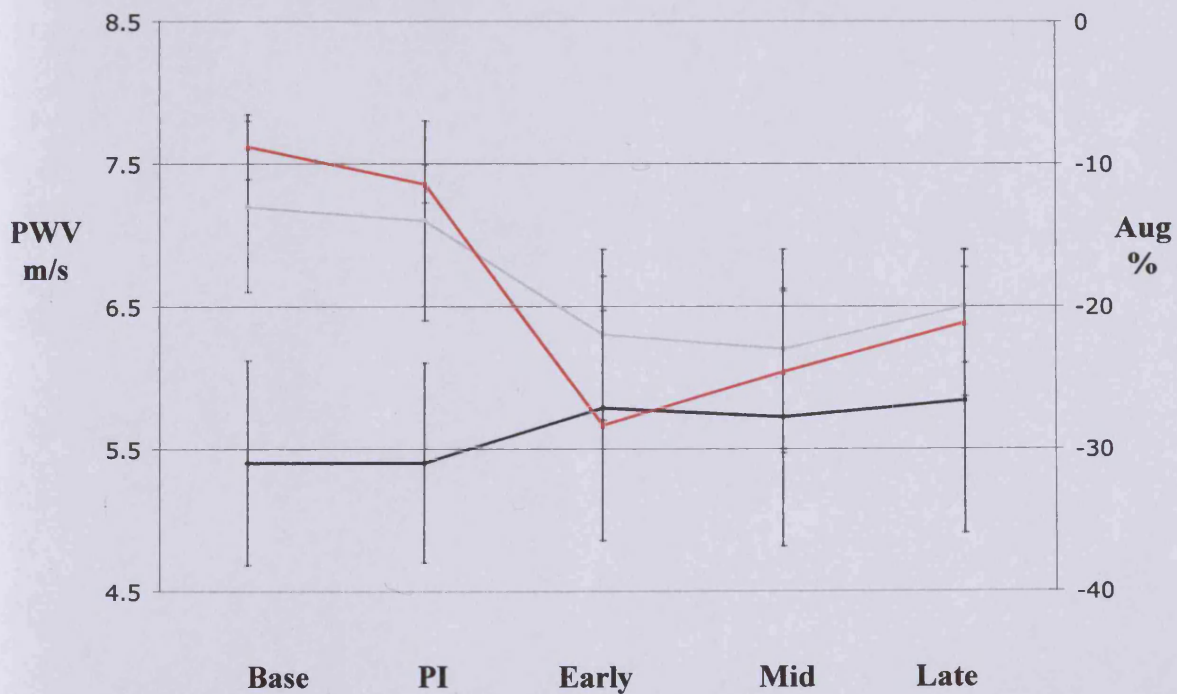## Fig 5.2 Maximal Haemodynamics with and without NO inhibition

Fig 5.3   Arterial Load at rest and post maximal exercise

Ae (Grey line P=NS for all), TPR (Red Line P<0.05 for rec v base), AC (Black Line P<0.05 for rec v base)

Fig 5.4   Arterial Stiffness at rest and post maximal exercise

AoPWV (Black Line P<0.05 for rec v base), FPWV (Grey Line P<0.05 for rec v base) and AI (Red Line P<0.05 for rec v base)

Fig 5.5   Arterial Load at rest and post maximal exercise influence of L-NMMA
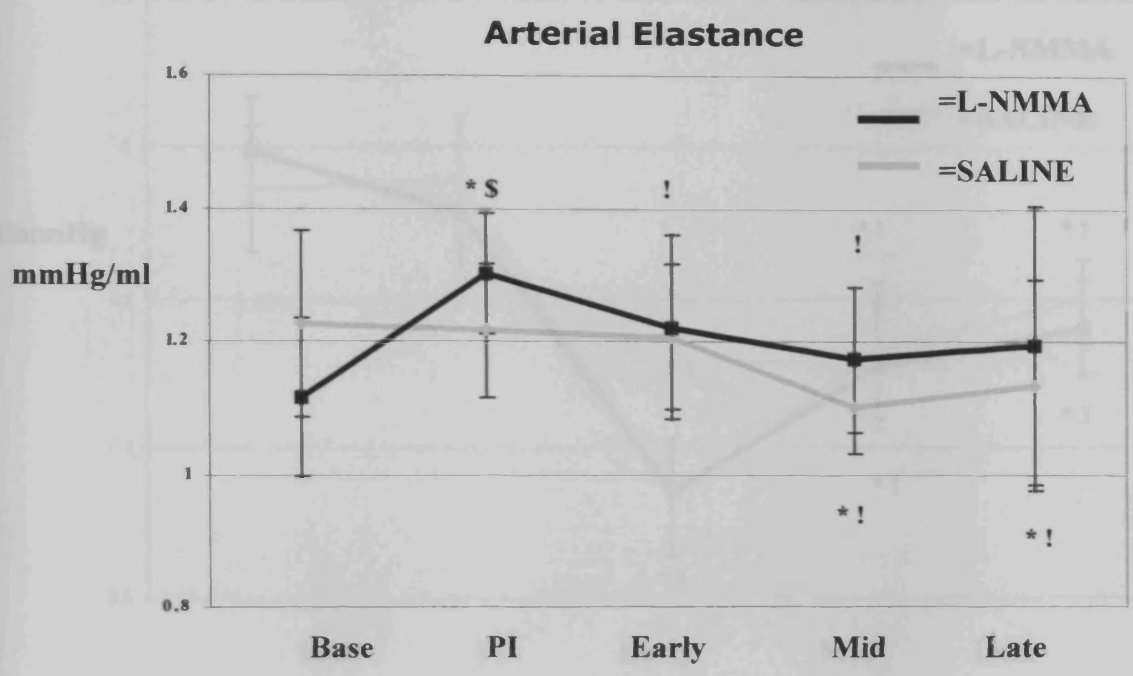
Arterial Elastance

Total Peripheral Resistance
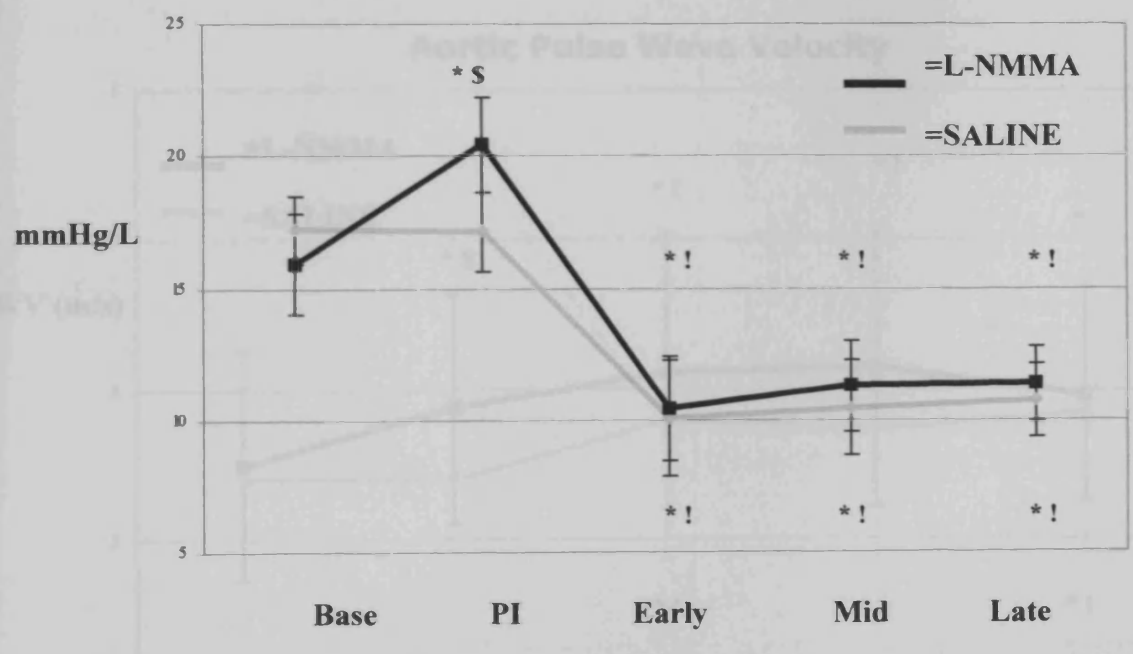
* = Sig dif from baseline, ! = Sig dif from PI and $ = Sig dif between saline and L-nMMa

Arterial Compliance
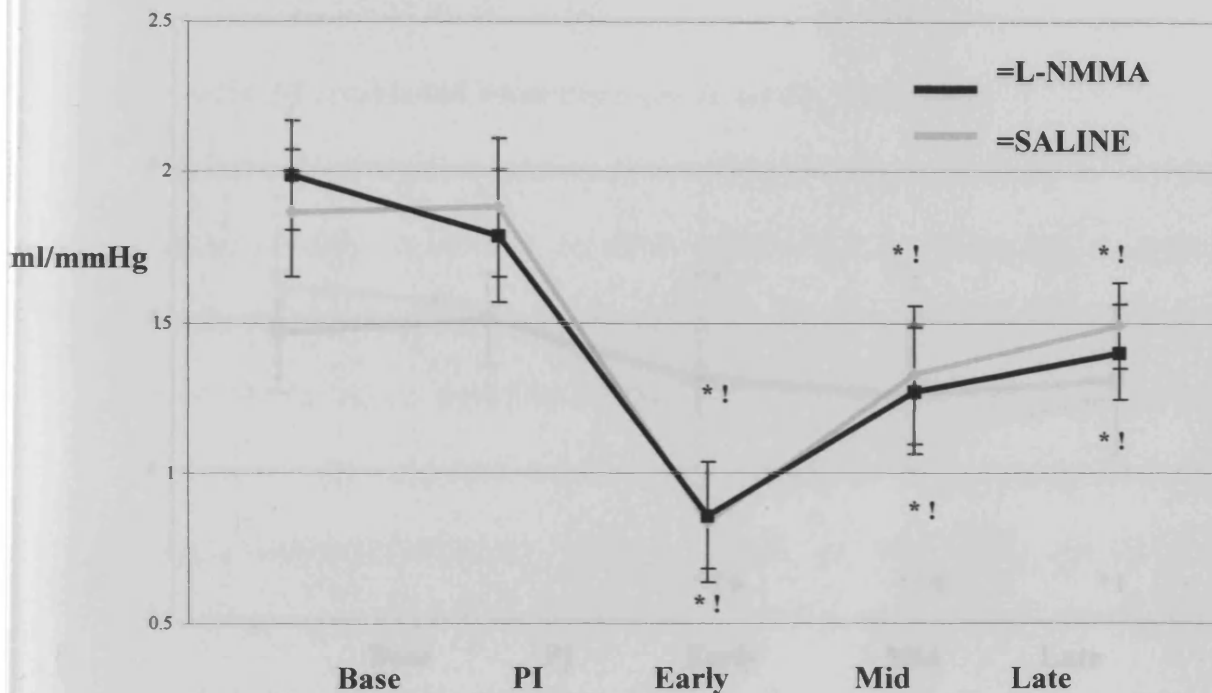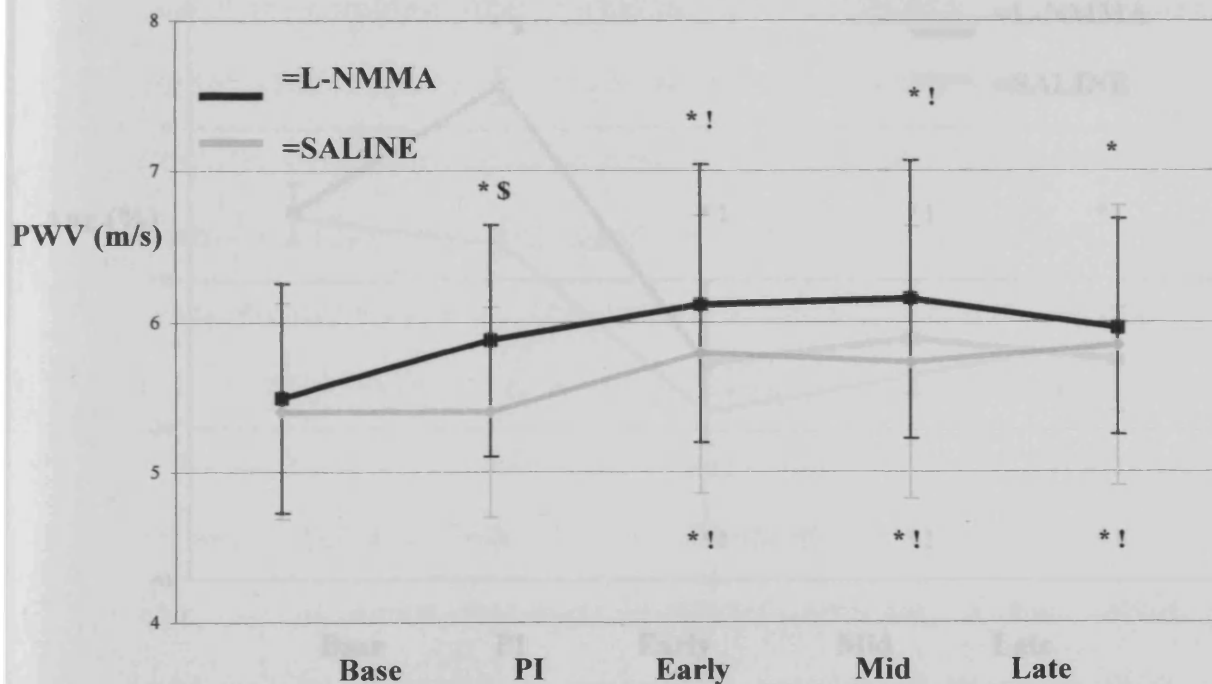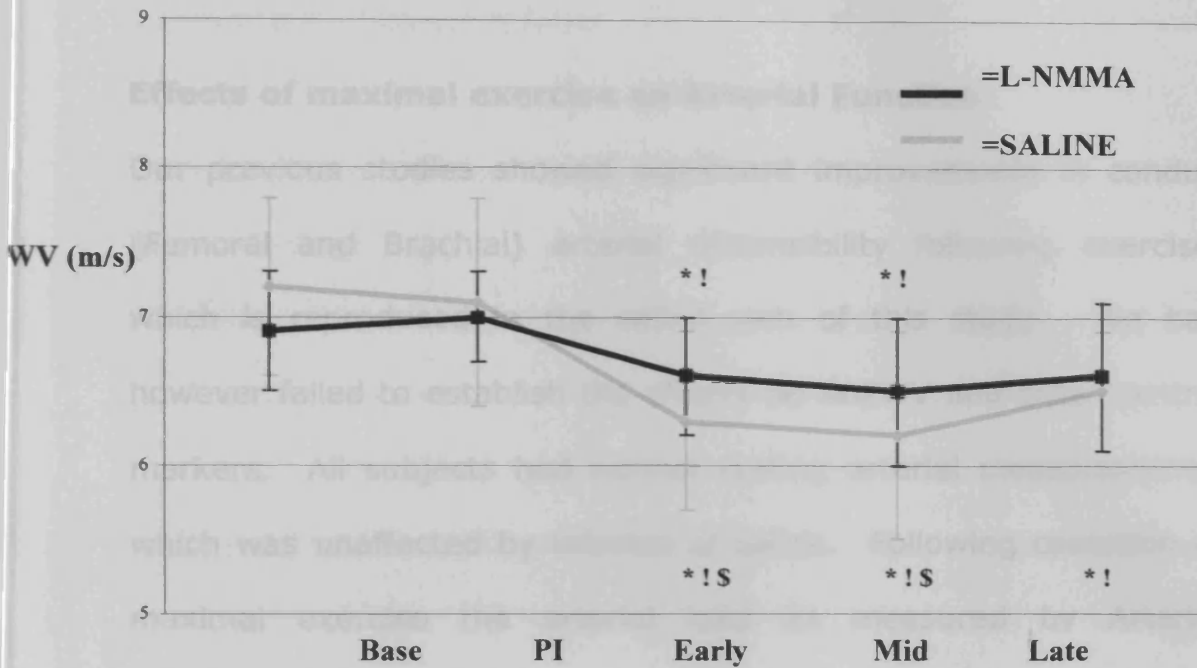
Fig 5.6   Arterial Stiffness at rest and post maximal exercise influence of L-NMMA
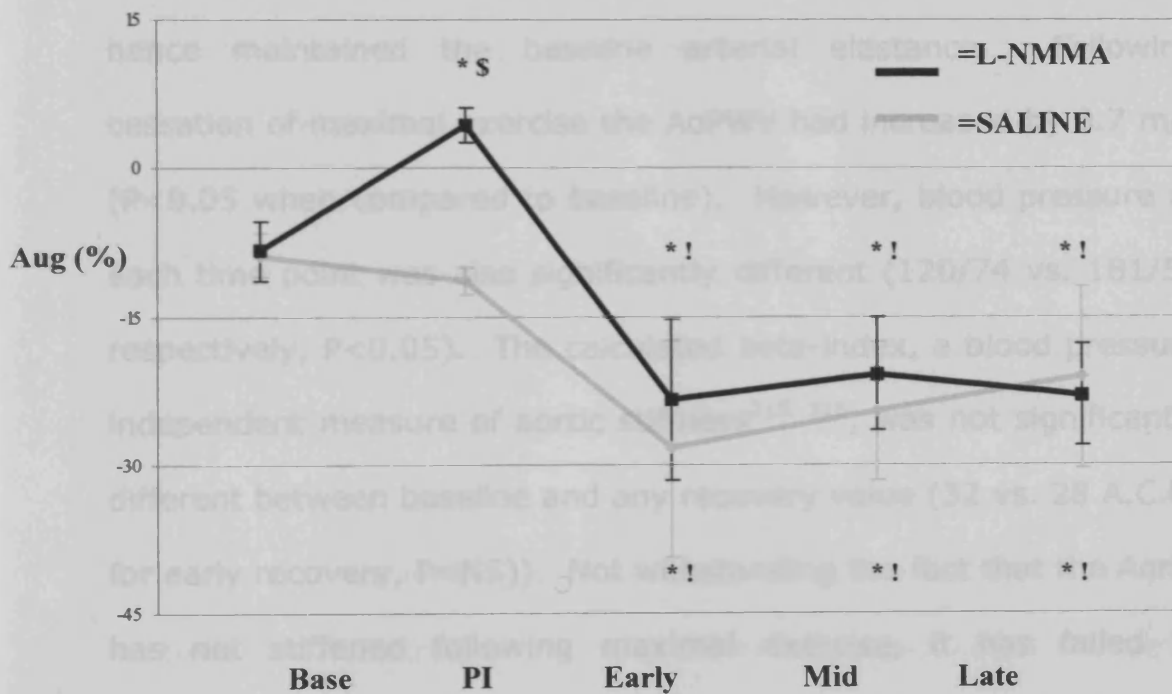

Aortic Pulse Wave Velocity

* = Sig dif from baseline, ! = Sig dif from PI and $ = Sig dif between saline and L-nMMa

Femoral Pulse Wave Velocity

Augmentation Index

* = Sig dif from baseline, ! = Sig dif from PI and $ = Sig dif between saline and L-nMMa

Discussion

Effects of maximal exercise on Arterial Function

Our previous studies showed significant improvements in conduit (Femoral and Brachial) arterial distensibility following exercise, which is reproduced in the saline arm of this study. We had however failed to establish the effects on AoPWV and other central markers. All subjects had normal resting arterial measurements, which was unaffected by infusion of saline. Following cessation of maximal exercise the arterial load as measured by Arterial Elastance had not changed from baseline. This is due to the significant reduction in total peripheral resistance or static load which counter balanced a significant fall in arterial compliance and hence maintained the baseline arterial elastance. Following cessation of maximal exercise the AoPWV had increased by 0.7 m/s (P<0.05 when compared to baseline). However, blood pressure at each time point was also significantly different (120/74 vs. 181/57 respectively, P<0.05). The calculated beta-index, a blood pressure independent measure of aortic stiffness[215, 216], was not significantly different between baseline and any recovery value (32 vs. 28 A.C.U. for early recovery, P=NS)). Not withstanding the fact that the Aorta has not stiffened following maximal exercise, it has failed to augment its distensibility unlike the conduit arteries, which is an unexpected finding. When we analyse the arterial data further both

the peripheral arterial distensibility has increased and the augmentation index has fallen.

Effects of Nitric Oxide Synthesis Inhibition on Maximal

Exercise Capacity

As we have shown inhibition of NO with L-NMMA causes endothelial dysfunction and caused an increase in arterial stiffness and load at rest. This might be expected to result in an arterial and ventricular mismatch, impairment in cardiac output with the coupled decrease in blood flow with NO inhibition and therefore maximal exercise capacity, but we have showed that no performance related measure of exercise capacity was affected (time to peak exercise, maximal exercise capacity, anaerobic threshold or V_E/V_{CO_2} slope, p=ns for all). This was because although the L-NMMA infusion resulted in a fall in heart rate, stroke volume and therefore cardiac output at rest this was not the case during all levels of sub-maximal, and presumably maximal, exercise. Other methodologies that have affected endothelial function at rest, such as Methionine loading have also failed to affect maximal exercise capacity[217].

This may be due to the multiple methods that allow for control of vascular smooth muscle. Many studies have blocked adenosine, acetylcholine and Nitric Oxide in isolation and reduced exercise blood flow but not to the extent expected. In a series of three

studies by the Minnesota group, coronary flow was measured in dogs at rest and during exercise, with inhibition of three possible stimuli for increasing flow. The first study showed that infusion of glibenclamide, an ATP sensitive K^+ channel blocker, decreased basal coronary flow but had no effect on the exercise hyperaemia[218]. In a separate study carried out using the same protocol they infused 8-phenyl theophylline, an adenosine receptor inhibitor, and showed that although basal flow was reduced (45 vs. 35 ml/min, $p<0.05$) and exercise flow was reduced, there was no reduction in delta flow (Exercise-Basal)[219]. In the same study they then infused both glibenclamide and 8-phenyl theophylline and demonstrated no further decrease in basal flow but a significant reduction in exercise induced hyperaemia (92 vs. 49 ml/min, $p<0.05$)[219]. In the final study they infused a combination of glibenclamide, 8-phenyl theophylline, and L-NMMA (NO inhibitor) and showed that baseline flow was reduced (49 vs. 20 ml/min, $p<0.05$) and that this multiple blockade resulted in a complete inhibition (20 vs. 21, 22, 26 +29 ml/min, baseline and 4 increasing exercise intensities all p=ns) of the hyperaemia in response to exercise[220].

Effects of maximal exercise L-nMMa infusion on Arterial Function

The femoral PWV post maximal exercise showed a significant reduction throughout the fifteen-minute recovery period during the

control and L-NMMA study. However, the first ten minutes of recovery were significantly different from each other. This demonstrates that the immediate increase in arterial distensibility post maximal exercise is at least partly mediated by NO synthesis. It also demonstrates that NO plays no role in the more prolonged reduction in PWV and therefore other factors must play a prominent role. The first of these findings suggests that NO almost certainly plays a significant role during exercise as well, due to the closeness to peak exercise, which would be a logical conclusion. The second finding, which is much more surprising, suggests a role for another metabolite, a change in sympathetic or parasympathetic tone or some unknown mechanism.

Haemodynamic Effects of L-nMMa infusion at Rest

Basal inhibition of Nitric Oxide with L-NMMA results in a significant increase in blood pressure, systemic vascular resistance and aortic pulse wave velocity. However, the heart rate and stroke volume drop significantly following L-NMMA infusion (60-54 and 93-86 HR and SV respectively), a mechanism that effectively blunts the blood pressure rise by approximately 28% (Fig 5.7). There appear to be two potential mechanisms, which could account for the reduction in heart rate and stroke volume. The first, and more obvious explanation is a baroreceptor-induced response to the initial increase in mean blood pressure. As we discussed in chapter one,

an increase in mean blood pressure causes an increase in the firing rate of the arterial baroreceptors. Two of the responses to this increased rate of firing are a reduction in sympathetic activity and a concurrent increase in parasympathetic activity, both of which would reduce the heart rate and stroke volume. Recent data suggest that the reduction in heart rate when blood pressure is increased by L-NMMA is less than blood pressure is similarly increased by Phenylephrine because of inhibition of brainstem Nitric Oxide[221].

The second potential mechanism is that L-NMMA itself will reduce NO availability not only in the arterial tree, but also in the ventricles. It has been demonstrated that NO is important for maintenance of both diastolic volume and ventricular ejection. Harrison et al assessed the effects of inhibition of NO with L-N(G)-methylarginine hydrochloride (LNMH) on cardiac function in dogs. These findings show that at doses of L-NMH and Phenylephrine which produce equivalent increases in afterload (Arterial elastance) LNMH caused a much lower increase in left ventricular end diastolic dimensions (Preload), consistent with previous studies reporting a lusitropic effect of NO[222, 223]. L-NMMA also caused a rightward shift of the end-systolic pressure relationship that is consistent with a negative inotropic response[224]. This data suggests a preload

dependent impairment of cardiac output following infusion of L-NMMA.

Haemodynamic Effects of L-nMMa infusion during Sub-Maximal Exercise

As exercise began both the systolic and diastolic pressures were higher with L-NMMA when compared to baseline. However, analysis using the post infusion values as baseline demonstrated a non-significant rise in SBP (P=0.13) and DBP (P=0.20). It therefore appears that during exercise the impact of inhibition of NO synthesis on haemodynamic variables progressively diminishes.

Fig 5.7   Estimated Changes in Mean Blood Pressure when Cardiac Output is unaffected by NO inhibition

$$Q * R = MBP$$

Baseline L-NMMA

5.6 (L/min) * 16.5 (mmHg/L) = 89 (mmHg)

Post Infusion L-NMMA

4.6 (L/min) * 21.4 (mmHg/L) = 94 (mmHg)

Post Infusion L-NMMA if we substitute baseline cardiac output into equation

5.6 (L/min) * 21.4 (mmHg/L) = 120 (mmHg)

Conclusion

Exercise of a maximal nature improves arterial distensibility in the exercising limb, but has no beneficial effects on the aorta as assessed by pulse wave velocity. Inhibition of Nitric Oxide synthesis with L-nMMa resulted in an increase in arterial stiffness and blood pressure at rest but there were no additional effects during exercise. The immediate post exercise arterial distensibility is influenced by Nitric Oxide but the sustained reduction >10 minutes is due to other factors. No measure of aerobic performance was influenced by L-nMMa infusion.

Chapter Six

Vascular Responses to Acute Sympathetic Activation

The Role of Nitric Oxide

Introduction

Central aortic blood pressure (BP) reflects left ventricular afterload, and is a key determinant of myocardial oxygen consumption and coronary perfusion pressure. Aortic BP may be analysed according to both its static and pulsatile components. The static component, estimated by mean arterial pressure, is accurately described as a function of cardiac output and total peripheral resistance (TPR)[225]. The pulsatile component, estimated by pulse pressure, is affected by the patterns of left ventricular ejection, large artery stiffness and arterial pulse wave reflection[226]. Therefore, it is clear that for a given cardiac performance, the entire vascular tree influences aortic BP.

Chronic mental stress is increasingly recognised as a novel risk factor for coronary artery disease[227], left ventricular dysfunction[228], and sudden cardiac death[229]. Furthermore, there is a clustering of cardiovascular events following stresses such as earthquakes or bereavement[229-231]. The mechanistic link between stress and increased cardiovascular risk however, remains controversial. The haemodynamic response to acute mental stress (AMS), which is partly mediated by sympathetic nervous system activation, includes increased heart rate, cardiac output and BP. AMS also induces endothelial dysfunction[232] that persists for approximately 2 hours after the stressor. Endothelial dysfunction following mental stress

may be mediated via increased oxidative stress, or through the release of potent vasoconstrictors, such as endothelin[233, 234] and angiotensin II[235]. Cortisol may also be involved in provoking these changes[236, 237]. It is suggested that prolonged impairment of endothelial-dependent relaxation resulting from AMS, may represent an important link between repeated or chronic stress and the acceleration of the atherogenic process[232].

The responses of various vascular beds to AMS have been studied independently in a number of studies and have suggested differential responses of the vasculature to AMS. Through this approach AMS has been shown to elicit vasodilation in the resistance beds of the forearm, but not the calf[238-241]. To our knowledge, the only previous study looking at large artery function and AMS has shown an increase in aortic stiffness and pulse wave reflections[242]. Given the important role that vascular dysfunction plays in the development of risk factors for cardiovascular disease, it is perhaps surprising that concurrent measurement of central and peripheral vascular responses to AMS has not yet been reported. Furthermore, the vascular and haemodynamic patterns underlying the BP response to mental stress are not yet completely understood, and in particular the role of endothelial dysfunction in modulating these changes is unknown.

Despite years of investigation, the precise mechanisms through which AMS may lead to vasodilation in some vascular beds are not yet clear. Previous studies have suggested that sympathetic withdrawal[240], beta-adrenergic vasodilation[240-243] and in particular flow-induced nitric oxide (NO) release[244, 245] may be of prime importance. Vascular NO, known to be a key regulator of basal vascular tone, significantly contributes to circulatory modulation during various physiological stimuli, including forearm exercise hyperaemia[246]. Although local NO release has been shown to contribute to the forearm dilator response to mental stress (approximately two thirds of the response was blunted with NO synthase blockade)[247], its role in the responses of other vasculature to AMS is yet to be determined.

This study was split into two sections with all 14 subjects completing study 1 and 10 of the initial 14 subjects completing study 2. Study 1 assessed the haemodynamic and arterial responses to acute sympathetic activation (Mental Stress) while study 2 assessed the impact of Nitric Oxide on these responses.

Methods

Subject Recruitment

A total of 14 subjects (9 male) aged 27 ± 1.3 yr (mean ± standard error of the mean, SEM) volunteered to participate in this study. None smoked, were hypertensive (blood pressure <140/80mmHg), or were taking medication. All subjects gave informed written consent and were habituated with the experimental procedures, which were approved by the local ethics committee and conformed to the Declaration of Helsinki (2002). Subjects were asked to refrain from consuming food and caffeine in the eight hours preceding the experiments.

Measurements

Stroke volume (SV), beat-to-beat BP and HR were measured using the Task Force® Monitor. Continuous beat-to-beat BP was assessed using the Flying V cuffs of the Task Force® Monitor at the middle finger of the left hand. Central Aortic pressure waveforms were derived for, Augmentation Index (AI), end systolic pressure (ESP), and the timing of the reflected wave (TR), from radial applanation tonometry using the Sphygmocor System. FTPWV was measured simultaneously and non-invasively using the SciMed.

Calculations

Total peripheral resistance (TPR), an index of the static component of arterial load was calculated as mean arterial pressure (DBP + 1/3 PP) divided by CO. Arterial compliance an index of the pulsatile component of arterial load is calculated as the stroke volume divided by the pulse pressure. Effective arterial elastance (AE), an index of arterial load, which incorporates both static and pulsatile components, was calculated as central end-systolic pressure (obtained from the central pressure waveform), divided by SV.

Study 1 – Haemodynamic and Arterial responses to Acute Mental Stress (N=14)

Subjects entered the laboratory and were prepared for all measurements while in the supine position. During each phase of the study we measured pulse wave analysis, cardiac output, heart rate, blood pressure, and femoral pulse wave velocity (FPWV). Each trial began with a 15-minute acclimation period followed by 10 minutes of baseline measurements, 6 minutes of acute mental stress (Arithmetic Stress Test as detailed in the methodology) and 10 minutes of recovery readings.

Study 2 – The Influence of Nitric Oxide on these Responses (N=10)

This was a randomised, double blind, placebo controlled, crossover study that was similar to study 1. Prior to each trial in study 2, a 14-gauge Venflon was inserted into a forearm vein of the left arm. A bolus infusion of either the nitric oxide synthase blocker N^G-monomethyl-L-arginine (L-NMMA) (3mg/kg) mixed with 20ml saline, or a saline only solution, was infused over a 5-minute period. This was followed by a further 50ml infusion of saline with 3 mg/kg/hour L-LNMMA, or saline only, infused over the remaining study period. Each trial began with a 10-minute acclimation period and 10 minutes of baseline measurements, this was followed by a 5 minutes infusion period and 10 minutes of post infusion measurements, acute mental stress (6 minutes) and 10 minutes of recovery readings.

Statistical Analysis

All values are expressed as mean ± SEM. For study 1, data was analysed as the mean baseline and stress measurement periods. For study 2, data is expressed as mean of baseline post infusion, stress and recovery for both saline and L-NMMA infusion protocols. Statistical analysis was performed using repeated measures ANOVA and *post hoc* analysis using paired t-tests with Bonferroni correction. Significance levels were set at $P<0.05$.

Results

Subject Data

Table 6.1 Subject Data

	Male	Age (Years)	SBP (mmHg)	DBP (mmHg)	Wt (kg)	Ht (m)
Study 1 (n=14)	9	27 (1.3)	121 (3)	75 (3)	72 (2.6)	1.74 (.03)
Study 2 (N=10)	7	26 (1.6)	120 (2)	77 (2)	74 (2.1)	1.76 (.02)

Study 1

Haemodynamic Responses to Acute Mental Stress

Table 6.2 shows averaged haemodynamic data for baseline and stress periods. AMS increased HR, SBP, DBP, MAP and CO ($p<0.05$ for all). AMS also caused significant increases in central SBP and central DBP ($p<0.05$ for both).

Table 6.2 Haemodynamic responses to AMS

	Baseline	AMS	Rec		Base	AMS	Rec
HR (bpm)	67±4	82±4*	65±4	CO (l/min)	6.3±0.5	7.7±0.5*	6.2±0.4
SBP (mmHg)	121±3	133±4*	125±4	cSBP (mmHg)	106±4	116±3 *	109±3
DBP (mmHg)	75±3	86±3 *	77±2	cDBP (mmHg)	76±5	87±7 *	78±5
SV (ml)	95±6	95±8	97±4	ESP (mmHg)	96±3	105±4*	98±4

HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SV, stroke volume; CO, cardiac output; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; ESP, end-systolic pressure; * indicates significantly different from respective baseline value.

As shown in figures 6.1 and 6.2 it is also clear that AMS increased AI@HR75, AE, and FTPWV and caused a significant decrease in TPR, and TR (p<0.05 for all).

Study 2

Haemodynamic Response to Mental Stress with and without L-NMMA

L-NMMA infusion significantly increased central and peripheral BP's, whilst decreasing both HR and SV and therefore CO (all p<0.05). The magnitude of the responses to AMS was not different during infusion of saline versus infusion of L-NMMA. That is, the absolute change from post-infusion to AMS was statistically indistinguishable with either vehicle (Table 6.3)

Figure 6.1 Effects of Sympathetic Activation on arterial load

Arterial Elastance
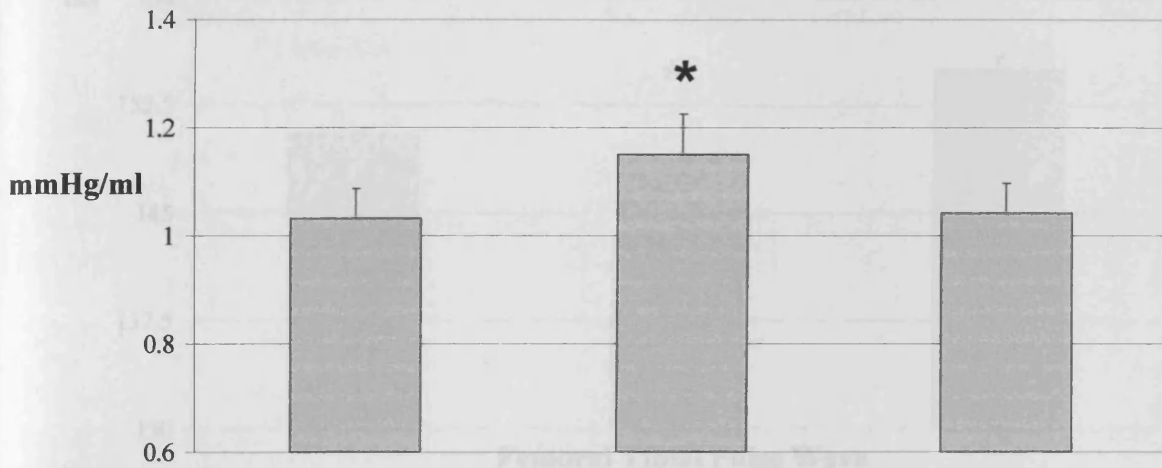
Total Peripheral Resistance
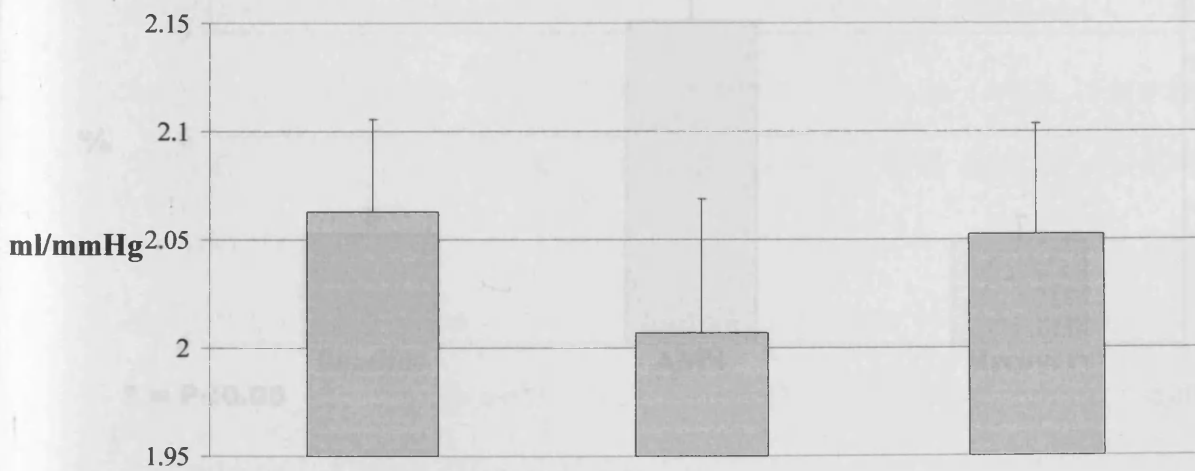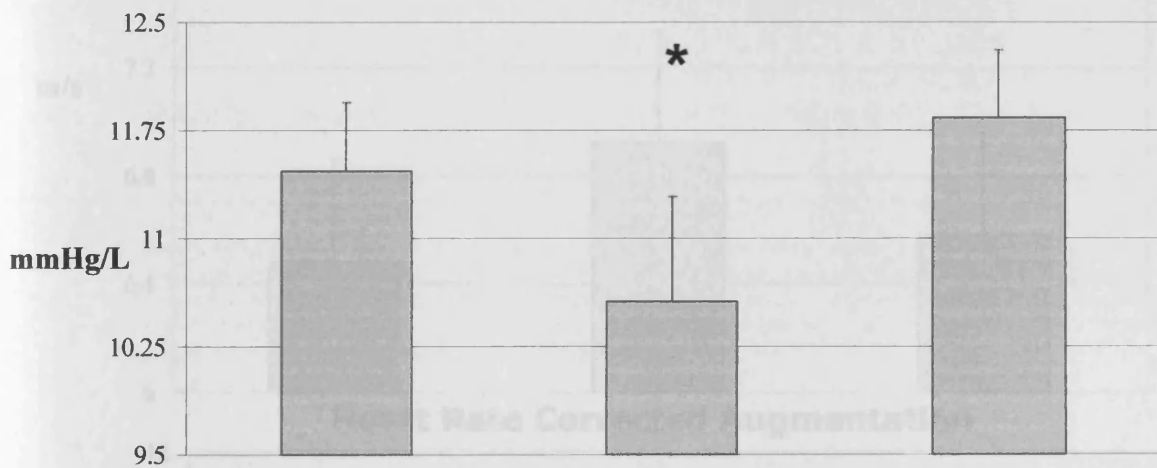
Baseline   AMS   Recovery

* = P<0.05

Figure 6.2 Effects of Sympathetic Activation on arterial stiffness

Timing of the Reflected Wave

Femoral Tibial Pulse Wave

Heart Rate Corrected Augmentation

* = P<0.05

Table 6.3 Haemodynamic responses to AMS following saline or L-NMMA infusion

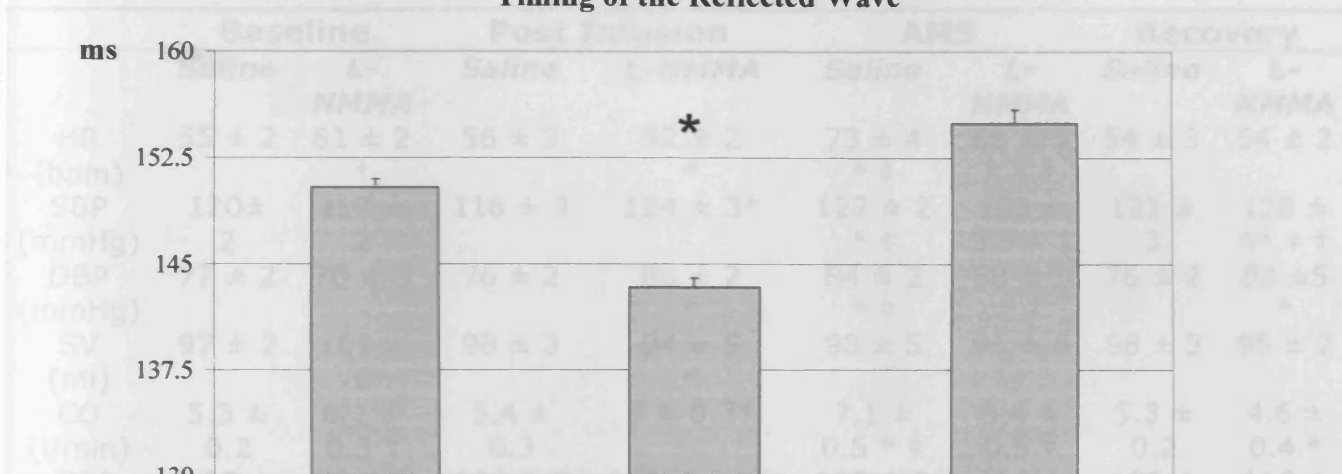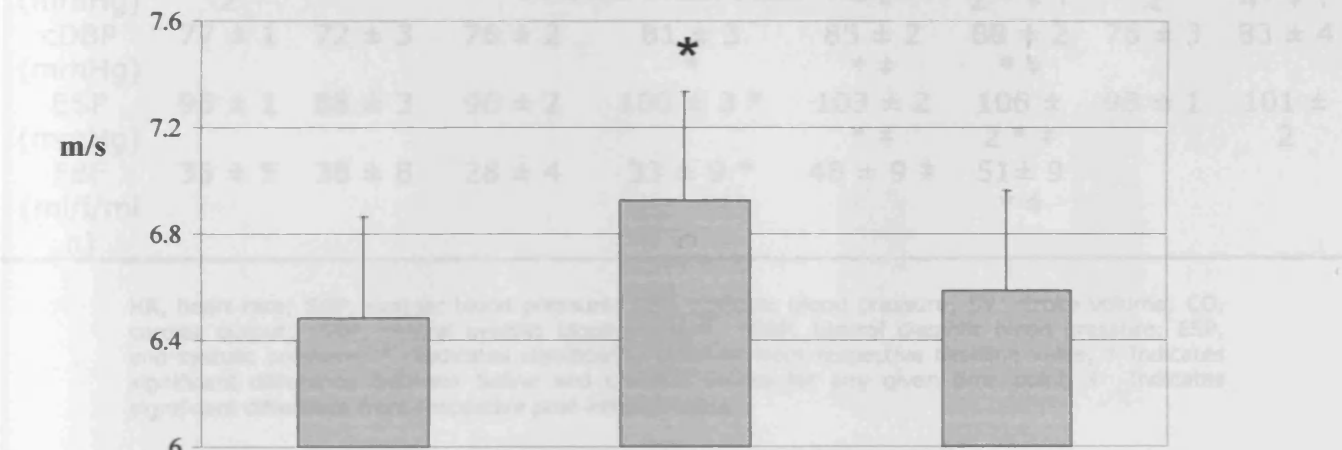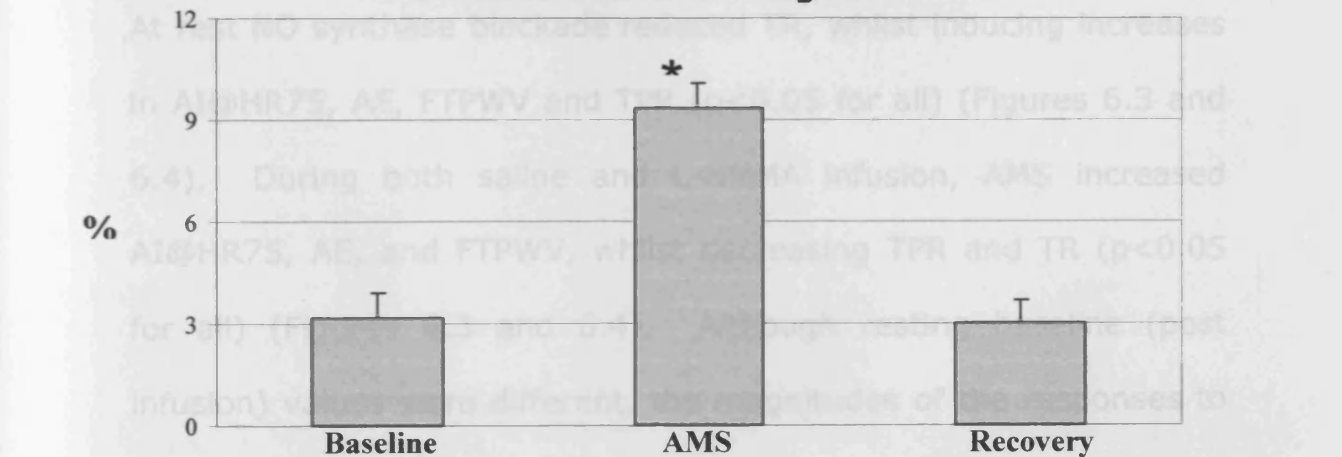|	Baseline		Post Infusion		AMS		Recovery	
	Saline	L-NMMA	Saline	L-NMMA	Saline	L-NMMA	Saline	L-NMMA
HR (bpm)	55 ± 2	61 ± 2 †	56 ± 3	52 ± 2 *	73 ± 4 * ‡	66 ± 3 * ‡ †	54 ± 3	54 ± 2
SBP (mmHg)	120± 2	117 ± 2	118 ± 3	124 ± 3*	127 ± 2 * ‡	133 ± 3 * ‡ †	121 ± 3	128 ± 4* ‡ †
DBP (mmHg)	77 ± 2	70 ± 3	76 ± 2	80 ± 2 *	84 ± 2 * ‡	88 ± 3 * ‡	76 ± 2	83 ±5 *
SV (ml)	97 ± 2	101 ± 6	98 ± 3	94 ± 5 *	98 ± 5	96 ± 6	98 ± 3	95 ± 2
CO (l/min)	5.3 ± 0.2	6.1 ± 0.3 †	5.4 ± 0.3	5 ± 0.3*	7.1 ± 0.5 * ‡	6.4 ± 0.5 ‡	5.3 ± 0.2	4.6 ± 0.4 *
cSBP (mmHg)	105 ± 2	99 ± 2	104 ± 3	110 ± 3* †	112 ± 2 * ‡	118 ± 2 * ‡ †	106 ± 2	116 ± 4* ‡ †
cDBP (mmHg)	77 ± 1	72 ± 3	76 ± 2	81 ± 3 *	85 ± 2 * ‡	88 ± 2 * ‡	76 ± 3	83 ± 4
ESP (mmHg)	95 ± 1	88 ± 3	96 ± 2	100 ± 3 *	103 ± 2 * ‡	106 ± 2 * ‡	98 ± 1	101 ± 2
FBF (ml/l/min)	33 ± 5	38 ± 8	28 ± 4	33 ± 9 *	48 ± 9 ‡	51± 9 * ‡		

HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; CO, cardiac output; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; ESP, end-systolic pressure; * Indicates significantly different from respective baseline value, † Indicates significant difference between Saline and L-NMMA values for any given time point, ‡ Indicates significant difference from respective post infusion value

At rest NO synthase blockade reduced TR, whilst inducing increases in AI@HR75, AE, FTPWV and TPR (p<0.05 for all) (Figures 6.3 and 6.4). During both saline and L-NMMA infusion, AMS increased AI@HR75, AE, and FTPWV, whilst decreasing TPR and TR (p<0.05 for all) (Figures 6.3 and 6.4). Although resting baseline (post infusion) values were different, the magnitudes of the responses to AMS were not significantly different with infusion of saline versus infusion of L-NMMA (Figure 6.5).

Figure 6.3 Effects of Sympathetic Activation on arterial load with Saline and L-nMMa
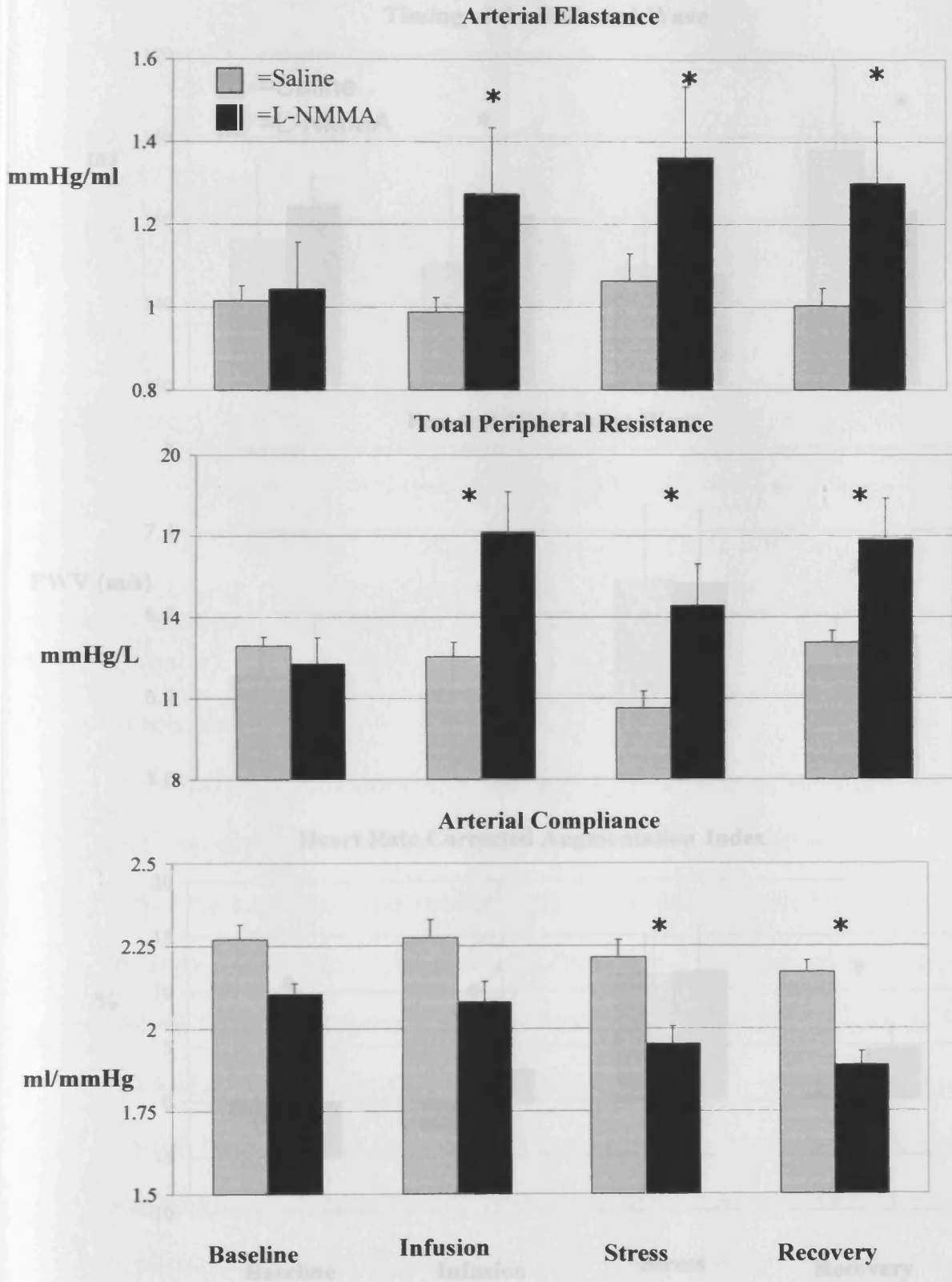
* = P<0.05 Saline V L-nMMa

Figure 6.4 Effects of Sympathetic Activation on arterial stiffness with Saline and L-nMMa

Timing of the Reflected Wave
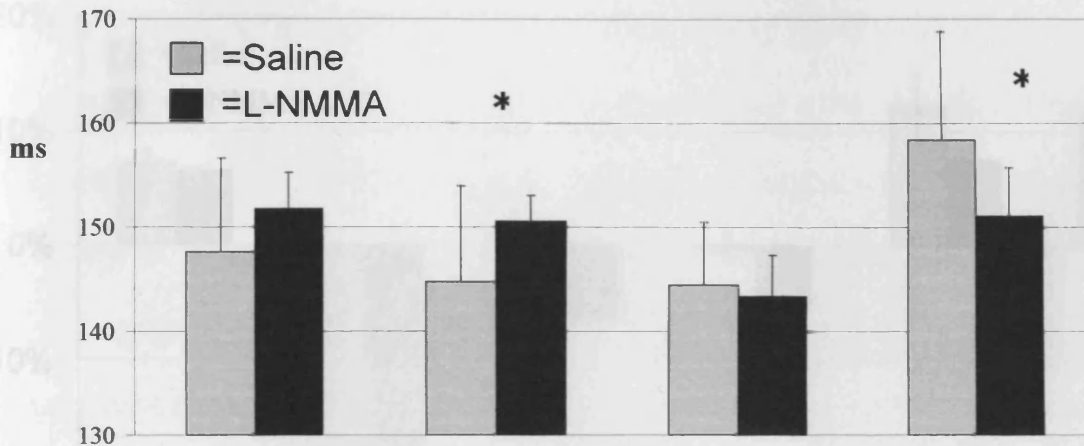
Femoral Tibial Pulse Wave
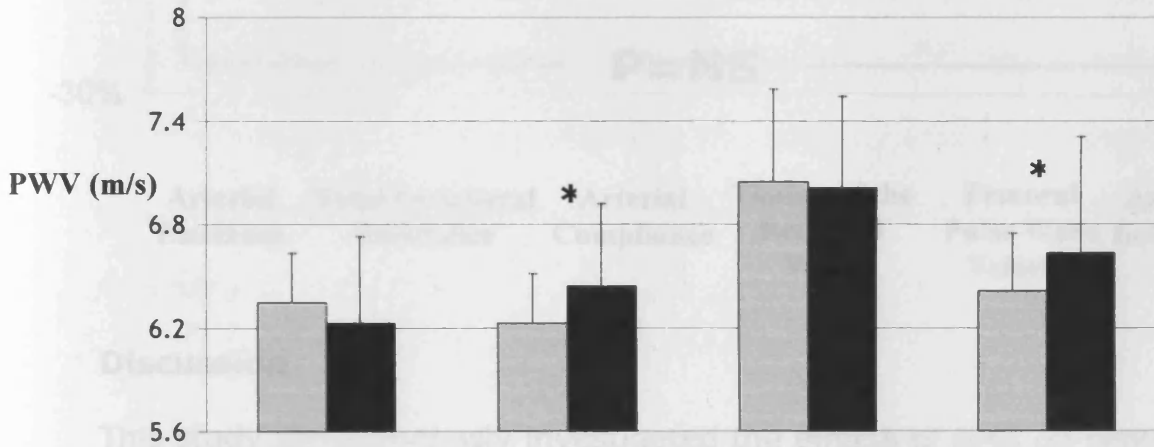
Heart Rate Corrected Augmentation Index
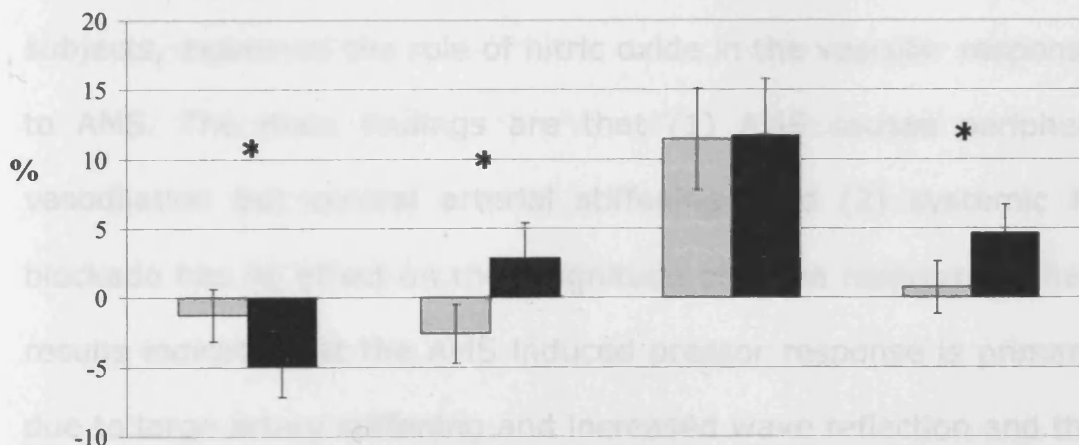
* = P<0.05 Saline V L-nMMa

Figure 6.5 Magnitude of Change with Saline and L-nMMa

Figure showing bar chart with =Saline and =L-NMMA, y-axis from -30% to 20%, categories: Arterial Elastance, Total Peripheral Resistance, Arterial Compliance, Timing of the Reflected Wave, Femoral Pulse Wave Velocity, Augmentation Index (@HR75). P=NS
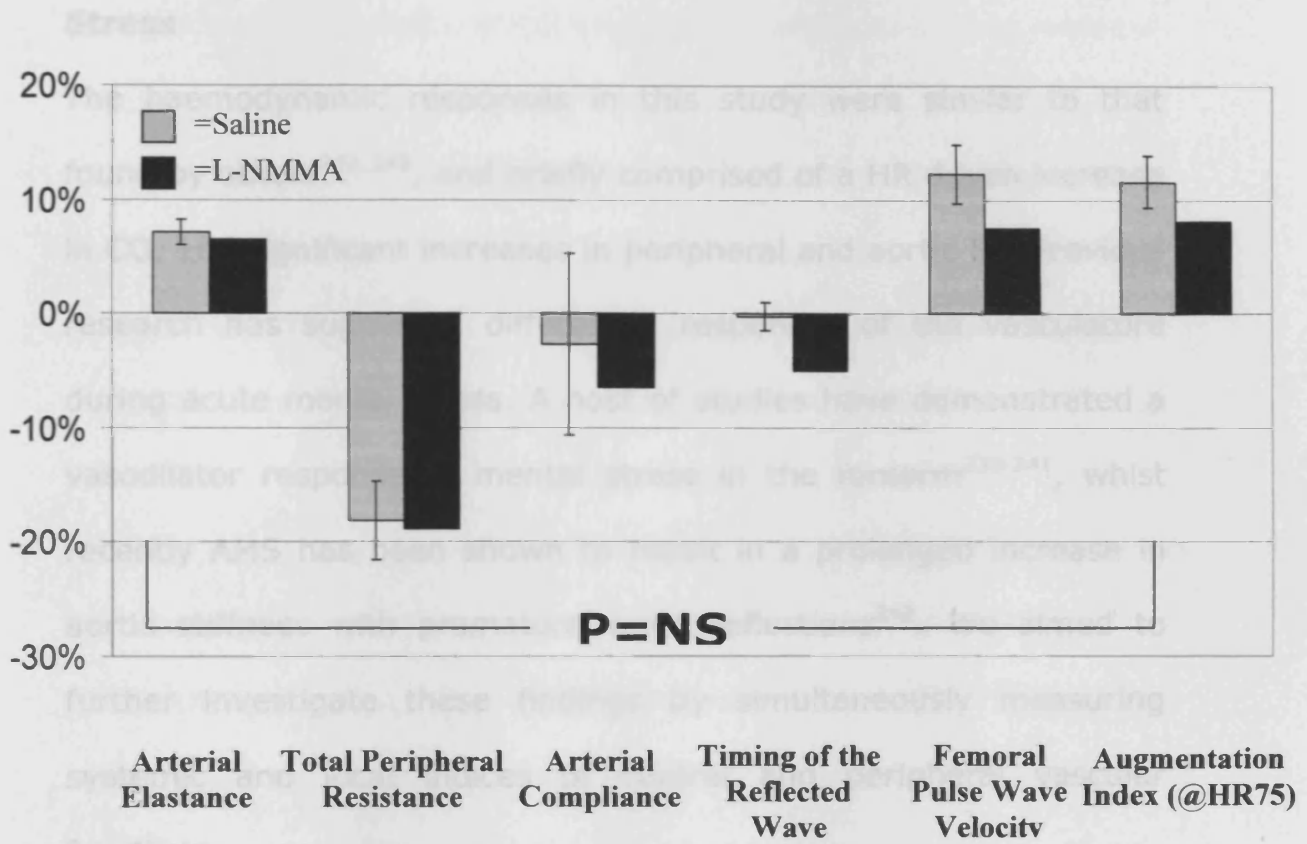
Discussion

This study simultaneously investigated the effects of AMS on central and peripheral vascular behaviour, and in a further subset of subjects, examined the role of nitric oxide in the vascular responses to AMS. The main findings are that (1) AMS causes peripheral vasodilation but central arterial stiffening; and (2) systemic NO blockade has no effect on the magnitude of these responses. These results indicate that the AMS induced pressor response is primarily due to large artery stiffening and increased wave reflection and that reduction in NO bioactivity does not appear to contribute significantly to these changes.

Haemodynamic and Vascular Responses to Acute Mental Stress

The haemodynamic responses in this study were similar to that found by others[242, 248], and briefly comprised of a HR driven increase in CO, and significant increases in peripheral and aortic BP. Previous research has suggested differential responses of the vasculature during acute mental stress. A host of studies have demonstrated a vasodilator response to mental stress in the forearm[238-241], whist recently AMS has been shown to result in a prolonged increase in aortic stiffness with premature wave reflections[242]. We aimed to further investigate these findings by simultaneously measuring systemic and local indices of central and peripheral vascular function.

In accordance with a wealth of literature, we have shown increased FBF with AMS. Mental stress also decreased TPR, further confirming the expected vasodilatation of the resistance vasculature. At the same time, AMS increased FTPWV and AI@HR75, and reduced the timing of the reflected wave (TR) indicating increased conduit and central arterial stiffness. These results clearly demonstrate differential responses of central and peripheral vasculature during AMS. Arterial elastance (AE), a measure of systemic arterial load that incorporates both peripheral resistance and arterial compliance, also increased with AMS. This was seen despite decreased

peripheral resistance suggesting that in the face of peripheral vasodilation, increased conduit and central artery stiffening increase the arterial load. Our results suggest that both the static and pulsatile components of aortic BP are altered with AMS, and we can conclude that arterial stiffening and the resulting premature return of the reflected waves from the periphery override decreased peripheral resistance to cause the observed increased aortic BP with AMS.

The Role of Nitric Oxide

NO is known to be a key regulator of vascular tone[244, 245], and has been shown in two studies, to significantly contribute to the forearm vasodilator response to AMS, via cholinergic stimulation of the vascular endothelium[247, 249].

In our study population of healthy subjects, despite the vasoconstrictor response to L-NMMA at rest, the haemodynamic response pattern to AMS was essentially unchanged during NO synthesis inhibition. This data is in accordance with Lindqvist et al. 2004[250]. Additionally the systemic (TPR) and local (FBF) vasodilatory response to AMS was not attenuated by L-NMMA infusion, in contrast to the studies of Dietz et al. and Cardillo et al[247, 249]. The different influences of local[247, 249] and systemic[250] NO synthesis inhibition on forearm vascular responses to AMS highlight

the importance of reflexogenic regulation of skeletal muscle blood flow in the control of arterial BP during stress. It must also be noted that FBF was only measured in 5 subjects. It appears that the forearm vasodilatory response may reflect the interaction between a host of redundant mechanisms. Although the precise contributions of each mechanism are not yet known and are often controversial, previous studies have suggested that sympathetic withdrawal[240] and circulating adrenaline[248, 251] may also be of prime importance. The fact that AMS increases large artery stiffness even when NO synthesis is blocked indicates that endothelial dysfunction is not responsible, or at most contributes only modestly.

To our knowledge, this is the first study to comprehensively investigate the role of NO in the vascular responses (other than forearm) to AMS. Our results suggest that impaired NO synthesis plays little or no part in the overall vascular response to AMS. Although NO significantly increased TPR, and both central and peripheral arterial stiffness at rest, and therefore is a key regulator of basal vascular tone, the responses of these indices to AMS were unchanged with NO synthase blockade. We therefore conclude that NO plays little role in the vascular responses to AMS and suggest that other mechanisms (see below) underlie the adverse effects of AMS on vascular function.

Potential Mechanisms

The increases in heart rate and arterial pressure induced by AMS are mediated in part by sympathetic neural activation[233] and substantial catecholamine (i.e. adrenaline and noradrenaline) release[252]. Circulating catecholamines are widely considered to cause vasoconstriction[253]. Indeed, noradrenaline infusion has been shown to result in increases in peripheral and central pulse pressure and an increase in augmentation index and aortic stiffness[254]. However circulating adrenaline in concentrations that can be produced by mental stress has been shown to cause β-adrenergic stimulation and a regional vasodilatory effect in the forearm[248, 251, 253].

The heterogeneous distribution of α-adrenoreceptors throughout the human vasculature may provide insight into the mechanisms underlying the differential vascular response to AMS. It has been demonstrated that large upstream arteries contain a predominance of α1-receptors for the control of arterial BP[255], whilst the arterioles contain α2-receptors for the fine control of tissue perfusion[256, 257]. Recent studies have also demonstrated that the interaction between sympathetic nerve activity and local vasodilatory stimuli appears to vary with vessel branch order[258]. Alpha 2-mediated vasoconstriction in the arterioles appears more sensitive to metabolic inhibition than

α1-mediated vasoconstriction further upstream where sympathetic nerve activity seems able to impair ascending vasodilation[255, 256, 259, 260]. Results from the present study may be in accordance with this concept, where local dilation in the forearm is able to override α2-mediated constriction, but α1-vasoconstriction upstream in the conduit and large arteries is maintained during AMS.

Clinical Implications

These findings may have important clinical implications. Chronic stress is increasingly recognised as a risk factor for coronary artery disease, left ventricular dysfunction, and sudden cardiac death[227-229], and there is also a clustering of cardiovascular events after acute stressful episodes[231, 261].

AMS can act as a trigger for acute cardiac events in susceptible individuals, with a vulnerable period of a few hours[231, 261]. Indeed it has been reported that relative risk of acute MI was more than doubled in the 2 hours following an acute episode of anger or severe work stress[230, 262]. Although the pathophysiological basis for these effects is not completely known, the onset of acute coronary syndrome is thought to involve the disruption of vulnerable plaques by rupture or erosion[263]. It is suggested that heightened platelet activation and increased haemodynamic shear stress may play a key role in this process[264], and that some individuals may be

particularly susceptible to the acute onset of acute coronary syndrome with AMS due to an impairment (heightened response) in these mechanisms[265].

From a practical perspective this study highlights the importance of adequate rest before assessment of large artery function and as such in situations such as open access risk factor clinics and clinical trials it is essential to allow a minimum of a fifteen-minute baseline period to allow for the effects of stress on the large arteries.

Conclusions

Acute mental stress results in a vasodilation of the peripheral vasculature. However, the observed stiffening of the conduit and central arteries may provide a mechanistic link between mental stress and increased risk factors for cardiovascular disease. Although others have shown that NO contributes to the forearm vasodilatory response to AMS, our results suggest its role in the responses of other vasculature appears to be of less importance.

Chapter Seven

Conclusions

Exercise Modality and Arterial Function

Although this study only looked at conduit artery function following exercise, it demonstrated that all healthy subjects who undertook exercise of a strenuous nature showed an increase in arterial distensibility in the early post exercise period. The magnitude of improvement was influenced by modality (Aerobic, resistance or isometric), duration (2-45 minutes) and intensity (25-100% of Vo^2_{max}). It also demonstrated that the response to resistance and isometric exercise resulted in changes in only the arterial bed of the exercising limb, suggesting a local control mechanism. In contrast the maximal cycle test resulted in brachial and femoral changes in PWV, this however may have been due to the increasing use of the upper body musculature as exercise intensity increases. This was the first study to look at multiple beds in a large group of subjects during differing exercise modalities, intensities and durations.

The prevalence of an Exaggerated Systolic Blood Pressure Response to Exercise in Health and the Impact of Cardiovascular Risk Factors

Previous research had shown a significantly independent prognostic value of ExSBP, but had failed to ascertain whether ExSBP occurs in subjects without risk factors. From 723 visitors to an open access

risk factor clinic we recruited 100 subjects who had no cardiovascular risk factors and demonstrated that no subject had an ExSBP and that all subjects without an ExSBP augmented their limb conduit artery distensibility and substantial proportion of post maximal exercise. The second part of this study demonstrated that subjects (45%) with borderline resting blood pressure and/or mild hypercholesterolemia developed an ExSBP, which was still present when exercise intensity (I.E. fitness) was accounted for. The subjects with ExSBP failed to augment there femoral distensibility immediately post and presumably during sub-maximal exercise, while the subjects with similar risk factors but a normal response had normal augmentation on the femoral distensibility. Interestingly there were no significant differences in baseline haemodynamics or arterial function between the NSBP and ExSBP groups.

The impact of maximal exercise on central (Aortic) arterial function

Unlike the fall in peripheral arterial stiffness, central arterial compliance fell in healthy subjects during exercise. However when the rise in blood pressure was accounted for there was no significant difference from baseline. Infusion of L-nMMa did not alter maximal exercise capacity or any other performance related marker but did increase basal blood pressure and arterial stiffness.

178

L-nMMA had no additional effects during exercise on blood pressure or heart rate but was influential in the post exercise arterial response.

The impact of acute mental stress on haemodynamics and arterial function

Acute sympathetic activation, mental stress, results in central and conduit arterial stiffening and may provide a link between stress and cardiovascular disease. Although Nitric Oxide influences resting arterial function and haemodynamics it appears to have any additional effect during stress.

Chapter Nine

Appendices

Table 9.1 Definition of terminology

Terms	Short	Definition
Arterial Compliance	C	The change in compliance of a vessel regardless of changes in blood pressure
Arterial Distensibility	Nil	The change in compliance of a vessel accounting for changes in blood pressure
Arterial Elastance	Ae	A measure of total arterial load influenced by arterial compliance and total peripheral resistance
Total Peripheral Resistance	TPR	The "stiffness" of the peripheral vasculature
Augmentation Index	Ai	A measure of the influence of wave reflection on aortic blood pressure
Timing of the Reflected Wave	Tr	The timing of the arrival of the reflected peripheral pressure wave onto the aortic waveform
Aortic Pulse Wave Velocity	AoPWV	The speed in m/s of the waveform between the Carotid and femoral artery
Bracho-Radial Pulse Wave Velocity	BRPWV	The speed in m/s of the waveform between the upper and lower leg
Femoral-Tibial Pulse Wave Velocity	FTPWV	The speed in m/s of the waveform between the upper and lower arm
Exaggerated Systolic Blood pressure	ExSBP	Subjects whom on exercise developed a significantly higher systolic blood pressure
Normal Systolic Blood pressure Responders	NSBP	Subjects whom on exercise developed a normal systolic blood pressure.

Chapter Nine

References

Reference List

(1) Office for National Statistics. Deaths registered in England 2001 by cause and area of residence. 2002.

(2) Office for National Statistics. Deaths registered in Wales 2001 by cause and area of residence. 2002.

(3) The World Health Report 2002. Reducing risks, Promoting healthy life. Geneva; 2008.

(4) Miller ER, III, Jehn ML. New high blood pressure guidelines create new at-risk classification: changes in blood pressure classification by JNC 7. *J Cardiovasc Nurs* 2004 November;19(6):367-71.

(5) Vasan RS, Kannel WB. Recent US and European blood pressure guidelines: implications for developing countries. *Natl Med J India* 2003 September;16(5):241-4.

(6) Kannel WB, D'Agostino RB, Silbershatz H. Blood pressure and cardiovascular morbidity and mortality rates in the elderly. *Am Heart J* 1997 October;134(4):758-63.

(7) Kannel WB, McGee D, Gordon T. A general cardiovascular risk profile: the Framingham Study. *Am J Cardiol* 1976 July;38(1):46-51.

(8) Franklin SS, Larson MG, Khan SA et al. Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. *Circulation* 2001 March 6;103(9):1245-9.

(9) Haider AW, Larson MG, Franklin SS, Levy D. Systolic blood pressure, diastolic blood pressure, and pulse pressure as predictors of risk for congestive heart failure in the Framingham Heart Study. *Ann Intern Med* 2003 January 7;138(1):10-6.

(10) Franklin SS, Gustin W, Wong ND et al. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation* 1997 July 1;96(1):308-15.

(11) Franklin SS, Khan SA, Wong ND, Larson MG, Levy D. Is pulse pressure useful in predicting risk for coronary heart Disease? The Framingham heart study. *Circulation* 1999 July 27;100(4):354-60.

(12) Rowell LB, Brengelmann GL, Blackmon JR, Bruce RA, Murray JA. Disparities between aortic and peripheral pulse pressures induced by upright exercise and vasomotor changes in man. *Circulation* 1968 June;37(6):954-64.

(13) Vlachopoulos C, O'Rourke M. Diastolic pressure, systolic pressure, or pulse pressure? *Curr Hypertens Rep* 2000 June;2(3):271-9.

(14) Lichtenstein MJ, Shipley MJ, Rose G. Systolic and diastolic blood pressures as predictors of coronary heart disease mortality in the Whitehall study. *Br Med J (Clin Res Ed)* 1985 July 27;291(6490):243-5.

(15) Tverdal A. Systolic and diastolic blood pressures as predictors of coronary heart disease in middle aged Norwegian men. *Br Med J (Clin Res Ed)* 1987 March 14;294(6573):671-3.

(16) Guyton AC, Hall JE. *Textbook of Medical Physiology*. 9th ed. Saunders; 2000.

(17) KARVONEN MJ, KENTALA E, MUSTALA O. The effects of training on heart rate; a longitudinal study. *Ann Med Exp Biol Fenn* 1957;35(3):307-15.

(18) Rowell LB. Regulation during Physical Stress. *Human Circulation*.New York: Oxford University Press; 1986. p. 213-86.

(19) Rowell LB. Integration of cardiovascular control systems in dynamic exercise. *Handbook of Physiology*.New York: Oxford University Press; 1996. p. 730-838.

(20) Gledhill N, Cox D, Jamnik R. Endurance athletes' stroke volume does not plateau: major advantage is diastolic function. *Med Sci Sports Exerc* 1994 September;26(9):1116-21.

(21) Rowell LB, Sheriff DD, Wyss CR, Scher AM. The nature of the exercise stimulus. *Acta Physiol Scand Suppl* 1986;556:7-14.

(22) Rowell LB, Saltin B, Kiens B, Christensen NJ. Is peak quadriceps blood flow in humans even higher during exercise with hypoxemia? *Am J Physiol* 1986 November;251(5 Pt 2):H1038-H1044.

(23) Armstrong RB, Vandenakker CB, Laughlin MH. Muscle blood flow patterns during exercise in partially curarized rats. *J Appl Physiol* 1985 March;58(3):698-701.

(24) Armstrong RB, Delp MD, Goljan EF, Laughlin MH. Distribution of blood flow in muscles of miniature swine during exercise. *J Appl Physiol* 1987 March;62(3):1285-98.

(25) Fagard R, Staessen J, Thijs L, Amery A. Prognostic significance of exercise versus resting blood pressure in hypertensive men. *Hypertension* 1991 April;17(4):574-8.

(26) Filipovsky J, Ducimetiere P, Safar ME. Prognostic significance of exercise blood pressure and heart rate in middle-aged men. *Hypertension* 1992 September;20(3):333-9.

(27) Mundal R, Kjeldsen SE, Sandvik L, Erikssen G, Thaulow E, Erikssen J. Exercise blood pressure predicts cardiovascular mortality in middle-aged men. *Hypertension* 1994 July;24(1):56-62.

(28) Mundal R, Kjeldsen SE, Sandvik L, Erikssen G, Thaulow E, Erikssen J. Exercise blood pressure predicts mortality from myocardial infarction. *Hypertension* 1996 March;27(3 Pt 1):324-9.

(29) Kjeldsen SE, Mundal R, Sandvik L, Erikssen G, Thaulow E, Erikssen J. Exercise blood pressure predicts cardiovascular death and myocardial infarction. *Blood Press Monit* 1997 June;2(3):147-53.

(30) Kjeldsen SE, Mundal R, Sandvik L, Erikssen G, Thaulow E, Erikssen J. Supine and exercise systolic blood pressure predict cardiovascular death in middle-aged men. *J Hypertens* 2001 August;19(8):1343-8.

(31) Miyai N, Arita M, Miyashita K, Morioka I, Shiraishi T, Nishio I. Blood pressure response to heart rate during exercise test and risk of future hypertension. *Hypertension* 2002 March 1;39(3):761-6.

(32) Kurl S, Laukkanen JA, Rauramaa R, Lakka TA, Sivenius J, Salonen JT. Systolic blood pressure response to exercise stress test and risk of stroke. *Stroke* 2001 September;32(9):2036-41.

(33) Tsumura K, Hayashi T, Hamada C, Endo G, Fujii S, Okada K. Blood pressure response after two-step exercise as a powerful predictor of hypertension: the Osaka Health Survey. *J Hypertens* 2002 August;20(8):1507-12.

(34) Matthews CE, Pate RR, Jackson KL et al. Exaggerated blood pressure response to dynamic exercise and risk of future hypertension. *J Clin Epidemiol* 1998 January;51(1):29-35.

(35) Miyai N, Arita M, Morioka I, Miyashita K, Nishio I, Takeda S. Exercise BP response in subjects with high-normal BP: exaggerated blood pressure response to exercise and risk of future hypertension in subjects with high-normal blood pressure. *J Am Coll Cardiol* 2000 November 1;36(5):1626-31.

(36) Sharabi Y, Ben-Cnaan R, Hanin A, Martonovitch G, Grossman E. The significance of hypertensive response to exercise as a predictor of hypertension and cardiovascular disease. *J Hum Hypertens* 2001 May;15(5):353-6.

(37) Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1999 May;33(5):1111-7.

(38) Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. *Circulation* 1999 May 11;99(18):2434-9.

(39) Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? *Circulation* 2002 October 15;106(16):2085-90.

(40) Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. *Arterioscler Thromb Vasc Biol* 2001 December;21(12):2046-50.

(41) Meaume S, Rudnichi A, Lynch A et al. Aortic pulse wave velocity as a marker of cardiovascular disease in subjects over 70 years old. *J Hypertens* 2001 May;19(5):871-7.

(42) Wilkinson IB, Prasad K, Hall IR et al. Increased central pulse pressure and augmentation index in subjects with hypercholesterolemia. *J Am Coll Cardiol* 2002 March 20;39(6):1005-11.

(43) Wilkinson IB, Franklin SS, Hall IR, Tyrrell S, Cockcroft JR. Pressure amplification explains why pulse pressure is unrelated to risk in young subjects. *Hypertension* 2001 December 1;38(6):1461-6.

(44) Dart AM, Kingwell BA. Pulse pressure--a review of mechanisms and clinical relevance. *J Am Coll Cardiol* 2001 March 15;37(4):975-84.

(45) O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values. *Am J Hypertens* 2002 May;15(5):426-44.

(46) Nichols WW, O'Rourke MF. **McDonald's Blood Flow in Arteries,** *Theoretical, Experimental and Clinical Principles*. 5th ed. Hodder Arnold Publications; 2005.

(47) Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation* 2001 February 20;103(7):987-92.

(48) Safar H, Mourad JJ, Safar M, Blacher J. Aortic pulse wave velocity, an independent marker of cardiovascular risk. *Arch Mal Coeur Vaiss* 2002 December;95(12):1215-8.

(49) Opie LH. *Heart Physiology From Cell to Circulation*. 4 ed. Philadelphia: Lippincott, Williams and Wilkins; 2004.

(50) Levy BI, Benessiano J, Poitevin P, Safar ME. Endothelium-dependent mechanical properties of the carotid artery in Wistar-Kyoto and spontaneously hypertensive rats. *J Hypertens Suppl* 1989 December;7(6):S118-S119.

(51) Wilkinson IB, McEniery CM. Arterial stiffness, endothelial function and novel pharmacological approaches. *Clin Exp Pharmacol Physiol* 2004 November;31(11):795-9.

(52) Gerova M. Gero J: Range of the sympathetic control of the dog femoral artery. *Circ Res* 1969 March;24(3):349-59.

(53) Boutouyrie P, Lacolley P, Girerd X, Beck L, Safar M, Laurent S. Sympathetic activation decreases medium-sized arterial compliance in humans. *Am J Physiol* 1994 October;267(4 Pt 2):H1368-H1376.

(54) Failla M, Grappiolo A, Carugo S, Calchera I, Giannattasio C, Mancia G. Effects of cigarette smoking on carotid and radial artery distensibility. *J Hypertens* 1997 December;15(12 Pt 2):1659-64.

(55) Mangoni AA, Mircoli L, Giannattasio C, Mancia G, Ferrari AU. Effect of sympathectomy on mechanical properties of common carotid and femoral arteries. *Hypertension* 1997 November;30(5):1085-8.

(56) Tardy Y, Meister JJ, Perret F, Brunner HR, Arditi M. Non-invasive estimate of the mechanical properties of peripheral arteries from ultrasonic and photoplethysmographic measurements. *Clin Phys Physiol Meas* 1991 February;12(1):39-54.

(57) Mangoni AA, Mircoli L, Giannattasio C, Ferrari AU, Mancia G. Heart rate-dependence of arterial distensibility in vivo. *J Hypertens* 1996 July;14(7):897-901.

(58) Giannattasio C, Failla M, Lucchina S et al. Arterial stiffening influence of sympathetic nerve activity: evidence from hand transplantation in humans. *Hypertension* 2005 April;45(4):608-11.

(59) Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolio AP, Cockcroft JR. Nitric oxide regulates local arterial distensibility in vivo. *Circulation* 2002 January 15;105(2):213-7.

(60) Wilkinson IB, MacCallum H, Cockcroft JR, Webb DJ. Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity in vivo. *Br J Clin Pharmacol* 2002 February;53(2):189-92.

(61) Safar ME, Laurent SL, Bouthier JD, London GM, Mimran AR. Effect of converting enzyme inhibitors on hypertensive large arteries in humans. *J Hypertens Suppl* 1986 December;4(5):S285-S289.

(62) Safar ME, Laurent S, Bouthier JA, London GM. Comparative effects of captopril and isosorbide dinitrate on the arterial wall of hypertensive human brachial arteries. *J Cardiovasc Pharmacol* 1986 November;8(6):1257-61.

(63) Kinlay S, Creager MA, Fukumoto M et al. Endothelium-derived nitric oxide regulates arterial elasticity in human arteries in vivo. *Hypertension* 2001 November;38(5):1049-53.

(64) Ramsey MW, Stewart WR, Jones CJ. Real-time measurement of pulse wave velocity from arterial pressure waveforms. *Med Biol Eng Comput* 1995 September;33(5):636-42.

(65) de WC, Wolfle SE. EDHF and gap junctions: important regulators of vascular tone within the microcirculation. *Curr Pharm Biotechnol* 2007 February;8(1):11-25.

(66) Joannides R, Haefeli WE, Linder L et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 1995 March 1;91(5):1314-9.

(67) Joannides R, Haefeli WE, Linder L, Luscher TF, Richard V, Thuillez C. [Role of nitric oxide in the regulation of the mechanical properties of peripheral muscular arteries in man]. *Arch Mal Coeur Vaiss* 1995 August;88(8):1149-52.

(68) Joannides R, Richard V, Haefeli WE, Linder L, Luscher TF, Thuillez C. Role of basal and stimulated release of nitric oxide in the regulation of radial artery caliber in humans. *Hypertension* 1995 August;26(2):327-31.

(69) Katz SD. The role of endothelium-derived vasoactive substances in the pathophysiology of exercise intolerance in patients with congestive heart failure. *Prog Cardiovasc Dis* 1995 July;38(1):23-50.

(70) Moncada S, Rees DD, Schulz R, Palmer RM. Development and mechanism of a specific supersensitivity to nitrovasodilators after

inhibition of vascular nitric oxide synthesis in vivo. *Proc Natl Acad Sci U S A* 1991 March 15;88(6):2166-70.

(71) Moncada S, Rees DD, Schulz R, Palmer RM. Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. *Proc Natl Acad Sci U S A* 1991 March 15;88(6):2166-70.

(72) Lang CC, Chomsky DB, Butler J, Kapoor S, Wilson JR. Prostaglandin production contributes to exercise-induced vasodilation in heart failure. *J Appl Physiol* 1997 December;83(6):1933-40.

(73) Gillham JC, Myers JE, Baker PN, Taggart MJ. TNF-alpha Alters Nitric Oxide- and Endothelium-Derived Hyperpolarizing Factor-Mediated Vasodilatation in Human Omental Arteries. *Hypertens Pregnancy* 2008;27(1):29-38.

(74) Banting JD, Friberg P, Adams MA. Acute hypertension after nitric oxide synthase inhibition is mediated primarily by increased endothelin vasoconstriction. *J Hypertens* 1996 August;14(8):975-81.

(75) Kostrzewska A, Modzelewska B, Kleszczewski T, Batra S. Effect of nitric oxide on responses of the human uterine arteries to vasopressin. *Vascul Pharmacol* 2008 January;48(1):9-13.

(76) Yamova L, Atochin D, Glazova M, Chernigovskaya E, Huang P. Role of neuronal nitric oxide in the regulation of vasopressin expression and release in response to inhibition of catecholamine synthesis and dehydration. *Neurosci Lett* 2007 October 22;426(3):160-5.

(77) Hornig B, Arakawa N, Drexler H. Effect of ACE inhibition on endothelial dysfunction in patients with chronic heart failure. *Eur Heart J* 1998 July;19 Suppl G:G48-G53.

(78) Bernier SG, Haldar S, Michel T. Bradykinin-regulated interactions of the mitogen-activated protein kinase pathway with the endothelial nitric-oxide synthase. *J Biol Chem* 2000 September 29;275(39):30707-15.

(79) Hambrecht R, Hilbrich L, Erbs S et al. Correction of endothelial dysfunction in chronic heart failure: additional effects of exercise training and oral L-arginine supplementation. *J Am Coll Cardiol* 2000 March 1;35(3):706-13.

(80) Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980 November 27;288(5789):373-6.

(81) Hambrecht R, Fiehn E, Weigl C et al. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation* 1998 December 15;98(24):2709-15.

(82) Hornig B, Maier V, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. *Circulation* 1996 January 15;93(2):210-4.

(83) Asmar R, Benetos A, Topouchian J et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation

and clinical application studies. *Hypertension* 1995
September;26(3):485-90.

(84) Wilkinson IB, Fuchs SA, Jansen IM et al. Reproducibility of pulse wave
      velocity and augmentation index measured by pulse wave analysis. *J
      Hypertens* 1998 December;16(12 Pt 2):2079-84.

(85) Latham RD, Rubal BJ, Westerhof N, Sipkema P, Walsh RA. Nonhuman
      primate model for regional wave travel and reflections along aortas. *Am
      J Physiol* 1987 August;253(2 Pt 2):H299-H306.

(86) McDonald DA. Regional pulse-wave velocity in the arterial tree. *J Appl
      Physiol* 1968 January;24(1):73-8.

(87) Patel DJ, DEFREITAS FM, Fry DL. Hydraulic input impedance to aorta and
      pulmonary artery in dogs. *J Appl Physiol* 1963 January;18:134-40.

(88) Learoyd BM, Taylor MG. Alterations with age in the viscoelastic
      properties of human arterial walls. *Circ Res* 1966 March;18(3):278-92.

(89) Gustafsson L, Appelgren L, Myrvold HE. Effects of increased plasma
      viscosity and red blood cell aggregation on blood viscosity in vivo. *Am J
      Physiol* 1981 October;241(4):H513-H518.

(90) Stehbens WE. *Hemodynamics and the Blood Vessel Wall*. Springfield;
      1979.

(91) Aschoff L, Hoeber B. *Lectures on pathology*. 1992.

(92) Benetos A, Asmar R, Gautier S, Salvi P, Safar M. Heterogeneity of the
      arterial tree in essential hypertension: a noninvasive study of the
      terminal aorta and the common carotid artery. *J Hum Hypertens* 1994
      July;8(7):501-7.

(93) Zon L. Effects of age on artery. 1924. Report No.: Dissertation.

(94) Hallock P. Arterial elasticity in man in relation to age as evaluated by the
      pulse wave velocity method. *Arch Int Med* 1934;54:770-98.

(95) Avolio AP, Chen SG, Wang RP, Zhang CL, Li MF, O'Rourke MF. Effects of
      aging on changing arterial compliance and left ventricular load in a
      northern Chinese urban community. *Circulation* 1983 July;68(1):50-8.

(96) Asmar R, Benetos A, London G et al. Aortic distensibility in
      normotensive, untreated and treated hypertensive patients. *Blood Press*
      1995 January;4(1):48-54.

(97) Sands J. Pulse Wave Velocity in pathological conditions. *Am J Physiol*
      1924;71:511-9.

(98) Eliakim M, Sapoznikov D, Weinman J. Pulse wave velocity in healthy
      subjects and in patients with various disease states. *Am Heart J* 1971
      October;82(4):448-57.

(99) Albaladejo P, Asmar R, Safar M, Benetos A. Association between 24-hour
      ambulatory heart rate and arterial stiffness. *J Hum Hypertens* 2000
      February;14(2):137-41.

(100) Nurnberger J, Opazo SA, Dammer S et al. Left ventricular ejection time: a potential determinant of pulse wave velocity in young, healthy males. *J Hypertens* 2003 November;21(11):2125-32.

(101) Sa CR, Pannier B, Benetos A et al. Association between high heart rate and high arterial rigidity in normotensive and hypertensive subjects. *J Hypertens* 1997 December;15(12 Pt 1):1423-30.

(102) Palatini P, Casiglia E, Julius S, Pessina AC. High heart rate: a risk factor for cardiovascular death in elderly men. *Arch Intern Med* 1999 March 22;159(6):585-92.

(103) Stefenelli T, Bergler-Klein J, Globits S, Pacher R, Glogar D. Heart rate behaviour at different stages of congestive heart failure. *Eur Heart J* 1992 July;13(7):902-7.

(104) Liang YL, Gatzka CD, Du XJ, Cameron JD, Kingwell BA, Dart AM. Effects of heart rate on arterial compliance in men. *Clin Exp Pharmacol Physiol* 1999 April;26(4):342-6.

(105) Lantelme P, Mestre C, Lievre M, Gressard A, Milon H. Heart rate: an important confounder of pulse wave velocity assessment. *Hypertension* 2002 June;39(6):1083-7.

(106) McGrath BP, Liang YL, Kotsopoulos D, Cameron JD. Impact of physical and physiological factors on arterial function. *Clin Exp Pharmacol Physiol* 2001 December;28(12):1104-7.

(107) Wilkinson IB, Mohammad NH, Tyrrell S et al. Heart rate dependency of pulse pressure amplification and arterial stiffness. *Am J Hypertens* 2002 January;15(1 Pt 1):24-30.

(108) Suzuki K. [Use of epidemiological data to predict the occurrence of arteriosclerotic diseases in urban residents]. *Nippon Ronen Igakkai Zasshi* 1996 May;33(5):360-70.

(109) Roguin A. Scipione Riva-Rocci and the men behind the mercury sphygmomanometer. *Int J Clin Pract* 2006 January;60(1):73-9.

(110) FULLER J, TAYLOR BE, CLAGETT OT, WOOD EH. Blood pressure in the aorta during resection, and repair of coarctation of the aorta. *J Lab Clin Med* 1952 January;39(1):10-25.

(111) KROEKER EJ, WOOD EH. Comparison of simultaneously recorded central and peripheral arterial pressure pulses during rest, exercise and tilted position in man. *Circ Res* 1955 November;3(6):623-32.

(112) O'Rourke MF. Frederick Akbar Mahomed. *Hypertension* 1992 February;19(2):212-7.

(113) Kelly R, Hayward C, Avolio A, O'Rourke M. Noninvasive determination of age-related changes in the human arterial pulse. *Circulation* 1989 December;80(6):1652-9.

(114) Kelly RP, Millasseau SC, Ritter JM, Chowienczyk PJ. Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. *Hypertension* 2001 June;37(6):1429-33.

(115) O'Rourke MF, Kelly RP. Wave reflection in the systemic circulation and its implications in ventricular function. *J Hypertens* 1993 April;11(4):327-37.

(116) O'Rourke MF. Pulsatile arterial haemodynamics in hypertension. *Aust N Z J Med* 1976;6 suppl 2:40-8.

(117) Vaitkevicius PV, Fleg JL, Engel JH et al. Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation* 1993 October;88(4 Pt 1):1456-62.

(118) Bonapace S, Rossi A, Cicoira M et al. Aortic distensibility independently affects exercise tolerance in patients with dilated cardiomyopathy. *Circulation* 2003 April 1;107(12):1603-8.

(119) Kool MJ, Struijker-Boudier HA, Wijnen JA, Hoeks AP, Van Bortel LM. Effects of diurnal variability and exercise training on properties of large arteries. *J Hypertens Suppl* 1992 August;10(6):S49-S52.

(120) Gauthier PJ, Chibatte F. [Value of the measurement of pulse wave velocity in high level sportsmen. Practical applications]. *Arch Mal Coeur Vaiss* 1989 August;82 Spec No 2:29-33.

(121) Kakiyama T, Matsuda M, Koseki S. Effect of physical activity on the distensibility of the aortic wall in healthy males. *Angiology* 1998 September;49(9):749-57.

(122) Tanaka H, DeSouza CA, Seals DR. Absence of age-related increase in central arterial stiffness in physically active women. *Arterioscler Thromb Vasc Biol* 1998 January;18(1):127-32.

(123) DeSouza CA, Shapiro LF, Clevenger CM et al. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation* 2000 September 19;102(12):1351-7.

(124) Tanaka H, Dinenno FA, Monahan KD, Clevenger CM, DeSouza CA, Seals DR. Aging, habitual exercise, and dynamic arterial compliance. *Circulation* 2000 September 12;102(11):1270-5.

(125) Cameron JD, Dart AM. Exercise training increases total systemic arterial compliance in humans. *Am J Physiol* 1994 February;266(2 Pt 2):H693-H701.

(126) Higashi Y, Sasaki S, Kurisu S et al. Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. *Circulation* 1999 September 14;100(11):1194-202.

(127) Ferrier KE, Waddell TK, Gatzka CD, Cameron JD, Dart AM, Kingwell BA. Aerobic exercise training does not modify large-artery compliance in isolated systolic hypertension. *Hypertension* 2001 August;38(2):222-6.

(128) Ferrier KE, Waddell TK, Gatzka CD, Cameron JD, Dart AM, Kingwell BA. Aerobic exercise training does not modify large-artery compliance in isolated systolic hypertension. *Hypertension* 2001 August;38(2):222-6.

(129) Rector TS, Bank AJ, Mullen KA et al. Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. *Circulation* 1996 June 15;93(12):2135-41.

(130) Katz SD, Yuen J, Bijou R, LeJemtel TH. Training improves endothelium-dependent vasodilation in resistance vessels of patients with heart failure. *J Appl Physiol* 1997 May;82(5):1488-92.

(131) Miyachi M, Donato AJ, Yamamoto K et al. Greater age-related reductions in central arterial compliance in resistance-trained men. *Hypertension* 2003 January;41(1):130-5.

(132) Bertovic DA, Waddell TK, Gatzka CD, Cameron JD, Dart AM, Kingwell BA. Muscular strength training is associated with low arterial compliance and high pulse pressure. *Hypertension* 1999 June;33(6):1385-91.

(133) Sebban C, Berthaux P, Lenoir H et al. Arterial compliance, systolic pressure and heart rate in elderly women at rest and on exercise. *Gerontology* 1981;27(5):271-80.

(134) Spencer MR, Gastin PB. Energy system contribution during 200- to 1500-m running in highly trained athletes. *Med Sci Sports Exerc* 2001 January;33(1):157-62.

(135) Murgo JP, Westerhof N, Giolma JP, Altobelli SA. Aortic input impedance in normal man: relationship to pressure wave forms. *Circulation* 1980 July;62(1):105-16.

(136) Simonsen E, Koff S, Keys A, Minckler J. Contour of the toe pulse transmission velocity: group and repeat variability, effects of age, exercise, and disease. *Am Heart J* 1955;27:271-80.

(137) Naka KK, Tweddel AC, Parthimos D, Henderson A, Goodfellow J, Frenneaux MP. Arterial distensibility: acute changes following dynamic exercise in normal subjects. *Am J Physiol Heart Circ Physiol* 2003 March;284(3):H970-H978.

(138) Kingwell BA, Berry KL, Cameron JD, Jennings GL, Dart AM. Arterial compliance increases after moderate-intensity cycling. *Am J Physiol* 1997 November;273(5 Pt 2):H2186-H2191.

(139) Chen CH, Nakayama M, Talbot M et al. Verapamil acutely reduces ventricular-vascular stiffening and improves aerobic exercise performance in elderly individuals. *J Am Coll Cardiol* 1999 May;33(6):1602-9.

(140) Sugawara J, Otsuki T, Tanabe T et al. The effects of low-intensity single-leg exercise on regional arterial stiffness. *Jpn J Physiol* 2003 June;53(3):239-41.

(141) Hill AV, Lupton H. Muscular exercise, lactic acid, and the supply and utilisation of oxygen. *Q J Med* 1923;16:135-71.

(142) Hill AVLCNHaLH. Muscular exercise, lactic acid and the supply and utilisation of oxygen:parts VII-VIII. *Proc Roy Soc* 1924;B97:155-76.

(143) Fishman AP, Farhi LE, Tenney SM. Gas exchange in exercise. *Handbook of Physiology*. 1987. p. 297-339.

(144) Bassett DR, Jr., Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 2000 January;32(1):70-84.

(145) Bergh U, Ekblom B, Astrand PO. Maximal oxygen uptake "classical" versus "contemporary" viewpoints. *Med Sci Sports Exerc* 2000 January;32(1):85-8.

(146) Noakes TD. 1996 J.B. Wolffe Memorial Lecture. Challenging beliefs: ex Africa semper aliquid novi. *Med Sci Sports Exerc* 1997 May;29(5):571-90.

(147) Noakes TD. Maximal oxygen uptake: "classical" versus "contemporary" viewpoints: a rebuttal. *Med Sci Sports Exerc* 1998 September;30(9):1381-98.

(148) Bailey DM, Davies B. Decreased chronotropic drive as an adaptation to chronic exercise; possible mechanisms. *Int J Sports Med* 1999 May;20(4):219-21.

(149) Hermansen L, Ekblom B, Saltin B. Cardiac output during submaximal and maximal treadmill and bicycle exercise. *J Appl Physiol* 1970 July;29(1):82-6.

(150) Hermansen L. Oxygen transport during exercise in human subjects. *Acta Physiol Scand Suppl* 1973;399:1-104.

(151) Stenberg J, Astrand PO, Ekblom B, Royce J, Saltin B. Hemodynamic response to work with different muscle groups, sitting and supine. *J Appl Physiol* 1967 January;22(1):61-70.

(152) Tesch PA, Kaiser P. Effect of beta-adrenergic blockade on maximal oxygen uptake in trained males. *Acta Physiol Scand* 1981 July;112(3):351-2.

(153) Tesch PA, Kaiser P. Effects of beta-adrenergic blockade on O2 uptake during submaximal and maximal exercise. *J Appl Physiol* 1983 April;54(4):901-5.

(154) Tesch PA. Exercise performance and beta-blockade. *Sports Med* 1985 November;2(6):389-412.

(155) Ekblom B, Astrand PO, Saltin B, Stenberg J, Wallstrom B. Effect of training on circulatory response to exercise. *J Appl Physiol* 1968 April;24(4):518-28.

(156) Saltin B, Blomqvist G, Mitchell JH, Johnson RL, Jr., Wildenthal K, Chapman CB. Response to exercise after bed rest and after training. *Circulation* 1968 November;38(5 Suppl):VII1-78.

(157) Richardson RS, Knight DR, Poole DC et al. Determinants of maximal exercise VO2 during single leg knee-extensor exercise in humans. *Am J Physiol* 1995 April;268(4 Pt 2):H1453-H1461.

(158) Knight DR, Poole DC, Schaffartzik W et al. Relationship between body and leg VO2 during maximal cycle ergometry. *J Appl Physiol* 1992 September;73(3):1114-21.

(159) Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, Wagner PD. Myoglobin O2 desaturation during exercise. Evidence of limited O2 transport. *J Clin Invest* 1995 October;96(4):1916-26.

(160) Ayappa I, Brown LV, Wang PM, Lai-Fook SJ. Arterial, capillary, and venous transit times and dispersion measured in isolated rabbit lungs. *J Appl Physiol* 1995 July;79(1):261-9.

(161) Ayappa I, Brown LV, Wang PM et al. Effect of blood flow on capillary transit time and oxygenation in excised rabbit lung. *Respir Physiol* 1996 September;105(3):203-16.

(162) Saltin B. Hemodynamic adaptations to exercise. *Am J Cardiol* 1985 April 26;55(10):42D-7D.

(163) Saltin B, Kiens B, Savard G, Pedersen PK. Role of hemoglobin and capillarization for oxygen delivery and extraction in muscular exercise. *Acta Physiol Scand Suppl* 1986;556:21-32.

(164) Coyle EF. Integration of the physiological factors determining endurance performance ability. *Exerc Sport Sci Rev* 1995;23:25-63.

(165) Richardson RS, Harms CA, Grassi B, Hepple RT. Skeletal muscle: master or slave of the cardiovascular system? *Med Sci Sports Exerc* 2000 January;32(1):89-93.

(166) Little WC, Cheng CP. Effect of exercise on left ventricular-arterial coupling assessed in the pressure-volume plane. *Am J Physiol* 1993 May;264(5 Pt 2):H1629-H1633.

(167) Little WC, Cheng CP. Left ventricular-arterial coupling in conscious dogs. *Am J Physiol* 1991 July;261(1 Pt 2):H70-H76.

(168) Asanoi H, Kameyama T, Ishizaka S, Miyagi K, Sasayama S. Ventriculoarterial coupling during exercise in normal human subjects. *Int J Cardiol* 1992 August;36(2):177-86.

(169) Starling MR. Left ventricular-arterial coupling relations in the normal human heart. *Am Heart J* 1993 June;125(6):1659-66.

(170) Asanoi H, Sasayama S, Kameyama T. Ventriculoarterial coupling in normal and failing heart in humans. *Circ Res* 1989 August;65(2):483-93.

(171) Feldman MD, Pak PH, Wu CC et al. Acute cardiovascular effects of OPC-18790 in patients with congestive heart failure. Time- and dose-dependence analysis based on pressure-volume relations. *Circulation* 1996 February 1;93(3):474-83.

(172) Sunagawa K, Maughan WL, Burkhoff D, Sagawa K. Left ventricular interaction with arterial load studied in isolated canine ventricle. *Am J Physiol* 1983 November;245(5 Pt 1):H773-H780.

(173) Kelly RP, Tunin R, Kass DA. Effect of reduced aortic compliance on cardiac efficiency and contractile function of in situ canine left ventricle. *Circ Res* 1992 September;71(3):490-502.

(174) Wilkinson IB, Hall IR, MacCallum H et al. Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. *Arterioscler Thromb Vasc Biol* 2002 January;22(1):147-52.

(175) Chen CH, Nevo E, Fetics B et al. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation* 1997 April 1;95(7):1827-36.

(176) Sharman JE, Lim R, Qasem AM et al. Validation of a generalized transfer function to noninvasively derive central blood pressure during exercise. *Hypertension* 2006 June;47(6):1203-8.

(177) Gratze G, Fortin J, Holler A et al. A software package for non-invasive, real-time beat-to-beat monitoring of stroke volume, blood pressure, total peripheral resistance and for assessment of autonomic function. *Comput Biol Med* 1998 March;28(2):121-42.

(178) Fortin JHGBAHWGRHAPRWPaSF. Validation and Verification of the Task Force monitor. FDA; 2001. Report No.: 510(k).

(179) Kelly RP, Ting CT, Yang TM et al. Effective arterial elastance as index of arterial vascular load in humans. *Circulation* 1992 August;86(2):513-21.

(180) Liu Z, Brin KP, Yin FC. Estimation of total arterial compliance: an improved method and evaluation of current methods. *Am J Physiol* 1986 September;251(3 Pt 2):H588-H600.

(181) Chemla D, Hebert JL, Coirault C et al. Total arterial compliance estimated by stroke volume-to-aortic pulse pressure ratio in humans. *Am J Physiol* 1998 February;274(2 Pt 2):H500-H505.

(182) Duncan GE, Howley ET, Johnson BN. Applicability of VO2max criteria: discontinuous versus continuous protocols. *Med Sci Sports Exerc* 1997 February;29(2):273-8.

(183) Rowland TW. Does peak VO2 reflect VO2max in children?: evidence from supramaximal testing. *Med Sci Sports Exerc* 1993 June;25(6):689-93.

(184) Howley ET, Bassett DR, Jr., Welch HG. Criteria for maximal oxygen uptake: review and commentary. *Med Sci Sports Exerc* 1995 September;27(9):1292-301.

(185) Chang HJ, Chung JH, Choi BJ et al. Endothelial dysfunction and alteration of nitric oxide/ cyclic GMP pathway in patients with exercise-induced hypertension. *Yonsei Med J* 2003 December 30;44(6):1014-20.

(186) Baker JS, Gal J, Davies B, Bailey DM, Morgan RM. Power output of legs during high intensity cycle ergometry: influence of hand grip. *J Sci Med Sport* 2000 December;3(4):360-8.

(187) Pohl U, Herlan K, Huang A, Bassenge E. EDRF-mediated shear-induced dilation opposes myogenic vasoconstriction in small rabbit arteries. *Am J Physiol* 1991 December;261(6 Pt 2):H2016-H2023.

(188) Radegran G, Saltin B. Nitric oxide in the regulation of vasomotor tone in human skeletal muscle. *Am J Physiol* 1999 June;276(6 Pt 2):H1951-H1960.

(189) Higashi Y, Yoshizumi M. Exercise and endothelial function: role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacol Ther* 2004 April;102(1):87-96.

(190) Kingwell BA, Jennings GL, Dart AM. Exercise and endothelial function. *Circulation* 2000 November 28;102(22):E179.

(191) Silacci P, Formentin K, Bouzourene K, Daniel F, Brunner HR, Hayoz D. Unidirectional and oscillatory shear stress differentially modulate NOS III gene expression. *Nitric Oxide* 2000 February;4(1):47-56.

(192) Wilson JR, Kapoor SC. Contribution of prostaglandins to exercise-induced vasodilation in humans. *Am J Physiol* 1993 July;265(1 Pt 2):H171-H175.

(193) Joyner MJ, Proctor DN. Muscle blood flow during exercise: the limits of reductionism. *Med Sci Sports Exerc* 1999 July;31(7):1036-40.

(194) Schrage WG, Joyner MJ, Dinenno FA. Local inhibition of nitric oxide and prostaglandins independently reduces forearm exercise hyperaemia in humans. *J Physiol* 2004 June 1;557(Pt 2):599-611.

(195) Green D, Cheetham C, Reed C, Dembo L, O'Driscoll G. Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *J Appl Physiol* 2002 July;93(1):361-8.

(196) Failla M, Grappiolo A, Emanuelli G et al. Sympathetic tone restrains arterial distensibility of healthy and atherosclerotic subjects. *J Hypertens* 1999 August;17(8):1117-23.

(197) Fisher JP, White MJ. Muscle afferent contributions to the cardiovascular response to isometric exercise. *Exp Physiol* 2004 November;89(6):639-46.

(198) Stewart KJ, Sung J, Silber HA et al. Exaggerated exercise blood pressure is related to impaired endothelial vasodilator function. *Am J Hypertens* 2004 April;17(4):314-20.

(199) Tzemos N, Lim PO, Farquharson CAJ, Struthers AD. Dundee step test predicts vasular endothelial dysfunction in subjects with mild to moderate essential hypertension. Circulation 102[(Supp II)], 606. 2000. Ref Type: Abstract

(200) Chang HJ, Chung J, Choi SY et al. Endothelial dysfunction in patients with exaggerated blood pressure response during treadmill test. *Clin Cardiol* 2004 July;27(7):421-5.

(201) Tzemos N, Lim PO, Farquharson C.A.J, Struthers AD. Dundee step test predicts vasular endothelial dysfunction in ubjects with mild to moderate

essential hypertension. Circulation 102 [Suppl II], 606. 2000.
Ref Type: Abstract

(202) Fossum E, Hoieggen A, Moan A, Rostrup M, Kjeldsen SE. Insulin sensitivity is related to physical fitness and exercise blood pressure to structural vascular properties in young men. *Hypertension* 1999 March;33(3):781-6.

(203) Pedrinelli R, Spessot M, Salvetti A. Reactive hyperemia during short-term blood flow and pressure changes in the hypertensive forearm. *J Hypertens* 1990 May;8(5):467-71.

(204) Rosei EA, Rizzoni D, Castellano M et al. Media: lumen ratio in human small resistance arteries is related to forearm minimal vascular resistance. *J Hypertens* 1995 March;13(3):341-7.

(205) Herkenhoff FL, Vasquez EC, Mill JG, Lima EG. Ambulatory blood pressure and Doppler echocardiographic indexes of borderline hypertensive men presenting an exaggerated blood pressure response during dynamic exercise. *Braz J Med Biol Res* 2001 October;34(10):1285-93.

(206) Bjornholt JV, Erikssen G, Kjeldsen SE, Bodegard J, Thaulow E, Erikssen J. Fasting blood glucose is independently associated with resting and exercise blood pressures and development of elevated blood pressure. *J Hypertens* 2003 July;21(7):1383-9.

(207) Singh JP, Larson MG, Manolio TA et al. Blood pressure response during treadmill testing as a risk factor for new-onset hypertension. The Framingham heart study. *Circulation* 1999 April 13;99(14):1831-6.

(208) Brett SE, Ritter JM, Chowienczyk PJ. Diastolic blood pressure changes during exercise positively correlate with serum cholesterol and insulin resistance. *Circulation* 2000 February 15;101(6):611-5.

(209) Delp MD. Effects of exercise training on endothelium-dependent peripheral vascular responsiveness. *Med Sci Sports Exerc* 1995 August;27(8):1152-7.

(210) Kakiyama T, Sugawara J, Murakami H, Maeda S, Kuno S, Matsuda M. Effects of short-term endurance training on aortic distensibility in young males. *Med Sci Sports Exerc* 2005 February;37(2):267-71.

(211) Delp MD, McAllister RM, Laughlin MH. Exercise training alters aortic vascular reactivity in hypothyroid rats. *Am J Physiol* 1995 April;268(4 Pt 2):H1428-H1435.

(212) Katz SD, Krum H. Acetylcholine-mediated vasodilation in the forearm circulation of patients with heart failure: indirect evidence for the role of endothelium-derived hyperpolarizing factor. *Am J Cardiol* 2001 May 1;87(9):1089-92.

(213) Katz SD, Schwarz M, Yuen J, LeJemtel TH. Impaired acetylcholine-mediated vasodilation in patients with congestive heart failure. Role of endothelium-derived vasodilating and vasoconstricting factors. *Circulation* 1993 July;88(1):55-61.

(214) Little WC, Cheng CP. Effect of exercise on left ventricular-arterial coupling assessed in the pressure-volume plane. *Am J Physiol* 1993 May;264(5 Pt 2):H1629-H1633.

(215) Lehmann ED, Gosling RG, Parker JR, deSilva T, Taylor MG. A blood pressure independent index of aortic distensibility. *Br J Radiol* 1993 February;66(782):126-31.

(216) Lehmann ED, Hopkins KD, Jones RL, Rudd AG, Gosling RG. Aortic distensibility in patients with cerebrovascular disease. *Clin Sci (Lond)* 1995 September;89(3):247-53.

(217) O'Dochartaigh C, Ong H, Lovell S et al. Changes in pulmonary vascular function after acute methionine loading in normal men. *Clin Sci (Lond)* 2004 April;106(4):413-9.

(218) Duncker DJ, van Zon NS, Altman JD, Pavek TJ, Bache RJ. Role of K+ATP channels in coronary vasodilation during exercise. *Circulation* 1993 September;88(3):1245-53.

(219) Duncker DJ, van Zon NS, Pavek TJ, Herrlinger SK, Bache RJ. Endogenous adenosine mediates coronary vasodilation during exercise after K(ATP)+ channel blockade. *J Clin Invest* 1995 January;95(1):285-95.

(220) Ishibashi Y, Duncker DJ, Zhang J, Bache RJ. ATP-sensitive K+ channels, adenosine, and nitric oxide-mediated mechanisms account for coronary vasodilation during exercise. *Circ Res* 1998 February 23;82(3):346-59.

(221) Chowdhary S, Harrington D, Bonser RS, Coote JH, Townend JN. Chronotropic effects of nitric oxide in the denervated human heart. *J Physiol* 2002 June 1;541(Pt 2):645-51.

(222) Paulus WJ, Vantrimpont PJ, Shah AM. Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. Assessment by bicoronary sodium nitroprusside infusion. *Circulation* 1994 May;89(5):2070-8.

(223) Paulus WJ, Kastner S, Pujadas P, Shah AM, Drexler H, Vanderheyden M. Left ventricular contractile effects of inducible nitric oxide synthase in the human allograft. *Circulation* 1997 November 18;96(10):3436-42.

(224) Harrison RW, Thakkar RN, Senzaki H et al. Relative contribution of preload and afterload to the reduction in cardiac output caused by nitric oxide synthase inhibition with L-N(G)-methylarginine hydrochloride 546C88. *Crit Care Med* 2000 May;28(5):1263-8.

(225) St John SM. Aortic stiffness: a predictor of acute coronary events? *Eur Heart J* 2000 March;21(5):342-4.

(226) McVeigh G, Finklestein S, Cohn J. Pulse Contour and Impedance Paramaters derived from Arterial Waveform Analysis. *Functional Abnormalities of The Aorta*. Futura; 1996. p. 183-93.

(227) Rozanski A, Blumenthal JA, Kaplan J. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation* 1999 April 27;99(16):2192-217.

(228) Wittstein IS, Thiemann DR, Lima JA et al. Neurohumoral features of myocardial stunning due to sudden emotional stress. *N Engl J Med* 2005 February 10;352(6):539-48.

(229) Leor J, Kloner RA. The Northridge earthquake as a trigger for acute myocardial infarction. *Am J Cardiol* 1996 June 1;77(14):1230-2.

(230) Mittleman MA, Maclure M, Sherwood JB et al. Triggering of acute myocardial infarction onset by episodes of anger. Determinants of Myocardial Infarction Onset Study Investigators. *Circulation* 1995 October 1;92(7):1720-5.

(231) Strike PC, Perkins-Porras L, Whitehead DL, McEwan J, Steptoe A. Triggering of acute coronary syndromes by physical exertion and anger: clinical and sociodemographic characteristics. *Heart* 2006 August;92(8):1035-40.

(232) Ghiadoni L, Donald AE, Cropley M et al. Mental stress induces transient endothelial dysfunction in humans. *Circulation* 2000 November 14;102(20):2473-8.

(233) Noll G, Wenzel RR, Schneider M et al. Increased activation of sympathetic nervous system and endothelin by mental stress in normotensive offspring of hypertensive parents. *Circulation* 1996 March 1;93(5):866-9.

(234) Noll G, Wenzel RR, Luscher TF. Endothelin and endothelin antagonists: potential role in cardiovascular and renal disease. *Mol Cell Biochem* 1996 April 12;157(1-2):259-67.

(235) Kosunen KJ. Plasma renin activity, angiotensin II, and aldosterone after mental arithmetic. *Scand J Clin Lab Invest* 1977 September;37(5):425-9.

(236) Broadley AJ, Korszun A, Abdelaal E et al. Inhibition of cortisol production with metyrapone prevents mental stress-induced endothelial dysfunction and baroreflex impairment. *J Am Coll Cardiol* 2005 July 19;46(2):344-50.

(237) Broadley AJ, Korszun A, Abdelaal E et al. Metyrapone improves endothelial dysfunction in patients with treated depression. *J Am Coll Cardiol* 2006 July 4;48(1):170-5.

(238) BARCROFT H, BROD J, HEJL BZ, HIRSJARVI EA, KITCHIN AH. The mechanism of the vasodilatation in the forearm muscle during stress (mental arithmetic). *Clin Sci* 1960 November;19:577-86.

(239) BLAIR DA, GLOVER WE, GREENFIELD AD, RODDIE IC. Excitation of cholinergic vasodilator nerves to human skeletal muscles during emotional stress. *J Physiol* 1959 October;148:633-47.

(240) Halliwill JR, Lawler LA, Eickhoff TJ, Dietz NM, Nauss LA, Joyner MJ. Forearm sympathetic withdrawal and vasodilatation during mental stress in humans. *J Physiol* 1997 October 1;504 (Pt 1):211-20.

(241) Rusch NJ, Shepherd JT, Webb RC, Vanhoutte PM. Different behavior of the resistance vessels of the human calf and forearm during contralateral

isometric exercise, mental stress, and abnormal respiratory movements. *Circ Res* 1981 June;48(6 Pt 2):I118-I130.

(242) Vlachopoulos C, Kosmopoulou F, Alexopoulos N, Ioakeimidis N, Siasos G, Stefanadis C. Acute mental stress has a prolonged unfavorable effect on arterial stiffness and wave reflections. *Psychosom Med* 2006 March;68(2):231-7.

(243) Lindqvist M, Melcher A, Hjemdahl P. Attenuation of forearm vasodilator responses to mental stress by regional beta-blockade, but not by atropine. *Acta Physiol Scand* 1997 October;161(2):135-40.

(244) Vallance P, Collier J, Moncada S. Nitric oxide synthesised from L-arginine mediates endothelium dependent dilatation in human veins in vivo. *Cardiovasc Res* 1989 December;23(12):1053-7.

(245) Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 1989 October 28;2(8670):997-1000.

(246) Dyke CK, Proctor DN, Dietz NM, Joyner MJ. Role of nitric oxide in exercise hyperaemia during prolonged rhythmic handgripping in humans. *J Physiol* 1995 October 1;488 (Pt 1):259-65.

(247) Dietz NM, Rivera JM, Eggener SE, Fix RT, Warner DO, Joyner MJ. Nitric oxide contributes to the rise in forearm blood flow during mental stress in humans. *J Physiol* 1994 October 15;480 (Pt 2):361-8.

(248) Lindqvist M, Davidsson S, Hjemdahl P, Melcher A. Sustained forearm vasodilation in humans during mental stress is not neurogenically mediated. *Acta Physiol Scand* 1996 September;158(1):7-14.

(249) Cardillo C, Kilcoyne CM, Quyyumi AA, Cannon RO, III, Panza JA. Role of nitric oxide in the vasodilator response to mental stress in normal subjects. *Am J Cardiol* 1997 October 15;80(8):1070-4.

(250) Lindqvist M, Melcher A, Hjemdahl P. Hemodynamic and sympathoadrenal responses to mental stress during nitric oxide synthesis inhibition. *Am J Physiol Heart Circ Physiol* 2004 November;287(5):H2309-H2315.

(251) Lindqvist M, Kahan T, Melcher A, Bie P, Hjemdahl P. Forearm vasodilator mechanisms during mental stress: possible roles for epinephrine and ANP. *Am J Physiol* 1996 March;270(3 Pt 1):E393-E399.

(252) Tidgren B, Hjemdahl P. Renal responses to mental stress and epinephrine in humans. *Am J Physiol* 1989 October;257(4 Pt 2):F682-F689.

(253) Kjeldsen SE, Petrin J, Weder AB, Julius S. Contrasting effects of epinephrine on forearm hemodynamics and arterial plasma norepinephrine. *Am J Hypertens* 1993 May;6(5 Pt 1):369-75.

(254) Wilkinson IB, MacCallum H, Hupperetz PC, van Thoor CJ, Cockcroft JR, Webb DJ. Changes in the derived central pressure waveform and pulse pressure in response to angiotensin II and noradrenaline in man. *J Physiol* 2001 February 1;530(Pt 3):541-50.

(255) Wray DW, Fadel PJ, Smith ML, Raven P, Sander M. Inhibition of alpha-adrenergic vasoconstriction in exercising human thigh muscles. *J Physiol* 2004 March 1;555(Pt 2):545-63.

(256) Anderson KM, Faber JE. Differential sensitivity of arteriolar alpha 1- and alpha 2-adrenoceptor constriction to metabolic inhibition during rat skeletal muscle contraction. *Circ Res* 1991 July;69(1):174-84.

(257) McGillivray-Anderson KM, Faber JE. Effect of acidosis on contraction of microvascular smooth muscle by alpha 1- and alpha 2-adrenoceptors. Implications for neural and metabolic regulation. *Circ Res* 1990 June;66(6):1643-57.

(258) Haug SJ, Welsh DG, Segal SS. Sympathetic nerves inhibit conducted vasodilatation along feed arteries during passive stretch of hamster skeletal muscle. *J Physiol* 2003 October 1;552(Pt 1):273-82.

(259) Haug SJ, Segal SS. Sympathetic neural inhibition of conducted vasodilatation along hamster feed arteries: complementary effects of alpha1- and alpha2-adrenoreceptor activation. *J Physiol* 2005 March 1;563(Pt 2):541-55.

(260) VanTeeffelen JW, Segal SS. Interaction between sympathetic nerve activation and muscle fibre contraction in resistance vessels of hamster retractor muscle. *J Physiol* 2003 July 15;550(Pt 2):563-74.

(261) Strike PC, Steptoe A. Behavioral and emotional triggers of acute coronary syndromes: a systematic review and critique. *Psychosom Med* 2005 March;67(2):179-86.

(262) Moller J, Theorell T, de Faire U, Ahlbom A, Hallqvist J. Work related stressful life events and the risk of myocardial infarction. Case-control and case-crossover analyses within the Stockholm heart epidemiology programme (SHEEP). *J Epidemiol Community Health* 2005 January;59(1):23-30.

(263) Monaco C, Mathur A, Martin JF. What causes acute coronary syndromes? Applying Koch's postulates. *Atherosclerosis* 2005 March;179(1):1-15.

(264) von Kanel R, Mills PJ, Fainman C, Dimsdale JE. Effects of psychological stress and psychiatric disorders on blood coagulation and fibrinolysis: a biobehavioral pathway to coronary artery disease? *Psychosom Med* 2001 July;63(4):531-44.

(265) Strike PC, Magid K, Whitehead DL, Brydon L, Bhattacharyya MR, Steptoe A. Pathophysiological processes underlying emotional triggering of acute cardiac events. *Proc Natl Acad Sci U S A* 2006 March 14;103(11):4322-7.