

Acute Arterial Responses to Physiological and Psychological Stress

by

Thomas Sebastian Davies

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Abstract

Cardiovascular disease is the leading cause of death in the western world. As accumulating evidence emerges that risk of developing cardiovascular disease increases with higher levels of blood pressure, the early detection of those with hypertension becomes an increasing priority. Blood pressure is influenced by numerous factors, including the properties of the large arteries. This thesis sought to examine the effects of acute physiological and psychological stress on indices of arterial function. During likely elevation of sympathetic outflow following isometric exercise, indices of conduit and central artery function indicated stiffening in excess of 10%. During and following acute mental stress the large arteries exhibited a similar stiffening response, despite decreased resistance in the peripheral vasculature. These decreases in arterial compliance resulted in increased amplitude and premature return of arterial pressure waves and lead to a 15% augmentation in central systolic pressure during both forms of stress. These findings may have important clinical implications as increased central pressure elevates left ventricular workload. During graded dynamic exercise, reduced arterial compliance was shown to have progressive influence on the interaction between the heart and the vasculature. These studies provide valuable insight into the cardiovascular response to physiological and environmental stress.

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List of Abbreviations

Abbreviation	Meaning
AC	Arterial Compliance
AI	Augmentation Index
AE	Arterial Elastance
BP	Blood Pressure
CHD	Coronary Heart Disease
CO	Cardiac Output
CVD	Cardiovascular Disease
CNS	Central Nervous System
DBP	Diastolic Blood Pressure
EXSBP	Exaggerated systolic blood pressure response to exercise
PWV	Pulse wave velocity
HR	Heart rate
LV	Left Ventricular
MAP	Mean arterial pressure
NO	Nitric Oxide
PP	Pulse Pressure
SV	Stroke Volume
TPR	Total peripheral resistance
TR	Timing of the reflected wave

Chapter 1 – Literature Review

Introduction

Diseases of the heart and the circulatory system, such as heart attacks and strokes, are responsible for nearly half of all deaths in the United Kingdom (Office for National Statistics 2005). Of these cardiovascular diseases (CVD), coronary heart disease (CHD) is among the leading causes of death in both men and woman. This condition, in which the heart's blood vessels become blocked by cholesterol, calcium, plaques and blood clots, is also a leading public health concern in the UK in terms of the economic burden from the disease. By one estimate, the total annual cost of all CHD related burdens was over £7 billion, the highest for all diseases in the UK for which comparable analysis has been completed (Liu et al. 2002).

The likelihood that a person will develop CVD during their lifetime can be predicted by examining the various risk factors that the person possesses. The list of risk factors is the result of many years following thousands of people in numerous epidemiological studies such as the Framingham Heart Study which began in 1948. These risk factors can be divided into those over which the person has no control and those which can be controlled.

The risk factors that cannot be controlled include gender, age, and family history of early CVD. Men have a 3-4 times higher risk of developing CHD through middle age than woman and in general, the risk of CHD increases as people age. Heredity also plays an important role. If a person has one or more close relatives with CHD, their risk is significantly elevated. Diabetes mellitus, an inherited metabolic disorder that contributes to the development of fatty deposits in the blood vessels, also puts a person at risk of developing CHD. Risk factors that can be controlled include smoking, obesity, sedentary lifestyle, elevated serum cholesterol and triglycerides, untreated high blood pressure (hypertension) and to some extent diabetes

mellitus. Most of these risk factors contribute directly to the hardening of the arteries, also known as atherosclerosis.

Blood pressure is a significant risk factor for CV disease and epidemiological studies conducted over the past decades have shown independent and consistent relations between hypertension and CV risk (Lewington et al. 2002; Wang & Vasani 2005). The following literature review sets out to explain how hypertension and factors related to or influencing it may interact to cause increased CV risk.

Blood Pressure

The pressure created by ventricular contraction is the driving force for blood flow throughout the cardiovascular (CV) system. As blood leaves the left ventricle (LV), the aorta and arteries expand to accommodate it. When the ventricle relaxes the elastic arterial walls recoil, thereby sustaining the driving pressure during ventricular relaxation and producing constant blood flow through the downstream vessels. BP is highest in the arteries and falls continuously as blood flows throughout the circulatory system due to the resistance of the vessels to blood flow. The highest pressure occurs in the aorta and represents the pressure created by the LV. In clinical practice, peak pressure during ventricular contraction (systolic BP (SBP)) and pressure during ventricular relaxation (end-diastolic BP (DBP)) are used to define the CV risk factor. Guidelines from the World Health Organisation and British Heart Foundation are that pressures should not exceed 120/80mmHg. Hypertension is defined as chronically elevated BP with resting pressures in excess of 140/90mmHg (World Health Organisation 2003).

The ejection of blood from the LV into the aorta creates a pressure waveform (arterial pulse wave) that is transmitted throughout the arterial tree. This waveform is asymmetrical and can be considered as a summation of both a steady-state component, mean arterial pressure (MAP) and a pulsatile component, pulse pressure (PP) (Nichols and O'Rourke 2005). MAP represents the driving pressure required for the steady supply of blood to the tissues and is the product of cardiac output (CO) and total peripheral resistance (TPR). MAP is estimated as DBP plus one-third of the PP, where PP is defined as SBP minus DBP. The pulsatile component (PP) is the consequence of intermittent ventricular ejection and is influenced by several cardiac and vascular factors including the properties of the large arteries (Safar et al. 2003). This review will now explore the factors that determine both MAP and PP.

The reflex control of BP is coordinated by the central nervous system (CNS), with the main integrating centre in the medulla oblongata of the brain. The primary goal of this CV control centre is to minimise BP fluctuations and maintain adequate perfusion of the vital organs. Sensory input into the integrating centre comes from a variety of peripheral sensory receptors that detect and correct changes in arterial BP (baroreceptor reflex), blood volume or chemical composition (cardiopulmonary reflexes). Efferent outputs from the medullary CV control centre are carried via both sympathetic and parasympathetic nerves in order to elicit alterations in HR, cardiac contractility, and vascular resistance. Peripheral vascular resistance is under tonic sympathetic control, with increased sympathetic outflow causing vasoconstriction. The mammalian heart is innervated by both parasympathetic (vagus) and sympathetic nerves (Deighton et al. 1990) and heart function is regulated by antagonistic control. Increased sympathetic activity increases HR and enhances the force of myocardial contraction, whereas increased parasympathetic activity slows HR and can significantly decrease the inotropic state of the heart (Lewis et al. 2001). It has been proposed that part of the vagally mediated control of myocardial activity is due to the interaction of the vagus nerve with sympathetic nerve influences (Lewis et al. 2001). The nature of this interaction remains controversial and has not been fully elucidated.

Arterial baroreceptors are stretch-sensitive mechanoreceptors located in the walls of the aorta, coronary and carotid arteries where they monitor the pressure of blood flow. These receptors are tonically active and fire action potentials continuously at normal BP's. Thus arterial baroreceptors maintain a beat to beat control of both sympathetic and parasympathetic outflow and cause continual adjustments in HR, SV and TPR. When increased BP stretches the baroreceptors membrane, the firing of that receptor increases. This leads to an inhibition

of sympathetic outflow with concurrent stimulation of the parasympathetic autonomic neurons. In the circulation, decreased sympathetic outflow causes dilation of the arterioles, allowing blood flow out of the arteries, whilst increased parasympathetic outflow slows HR and decreases CO. The combination of reduced CO and decreased TPR lowers MAP. If MAP falls the opposite response is observed, characterised by a decrease in parasympathetic activity and an increase in sympathetic activity. This mechanism is summarised in figure 1.1 overleaf, which illustrates the CV changes following a drop in MAP.

The neural control of the CV system depends not only on the arterial baroreflex but also, and to an important extent on cardiopulmonary reflexes. Vagal pathways originating in cardiopulmonary receptors have been recognised for many years. Cardiopulmonary receptors are mainly located in low pressure regions of the CV system (such as the lungs, left ventricle and the great veins) and are stimulated by changes in the distension of the structures in which they are located (Mancia et al. 1973). The net effect of cardiopulmonary reflexes is thought to be a tonic depression of HR and TPR. In humans, baroreflex control of sympathetic nerve activity has often been studied using graded hypovolemia. Low levels of hypovolemia at which HR and BP are unaffected are thought to preferentially deactivate cardiopulmonary reflexes and evoke increased sympathetic activity and decreased parasympathetic activity (Zoller 1972; Davy et al. 1998), leading to elevated resistance in peripheral vasculature.

Figure 1.1:

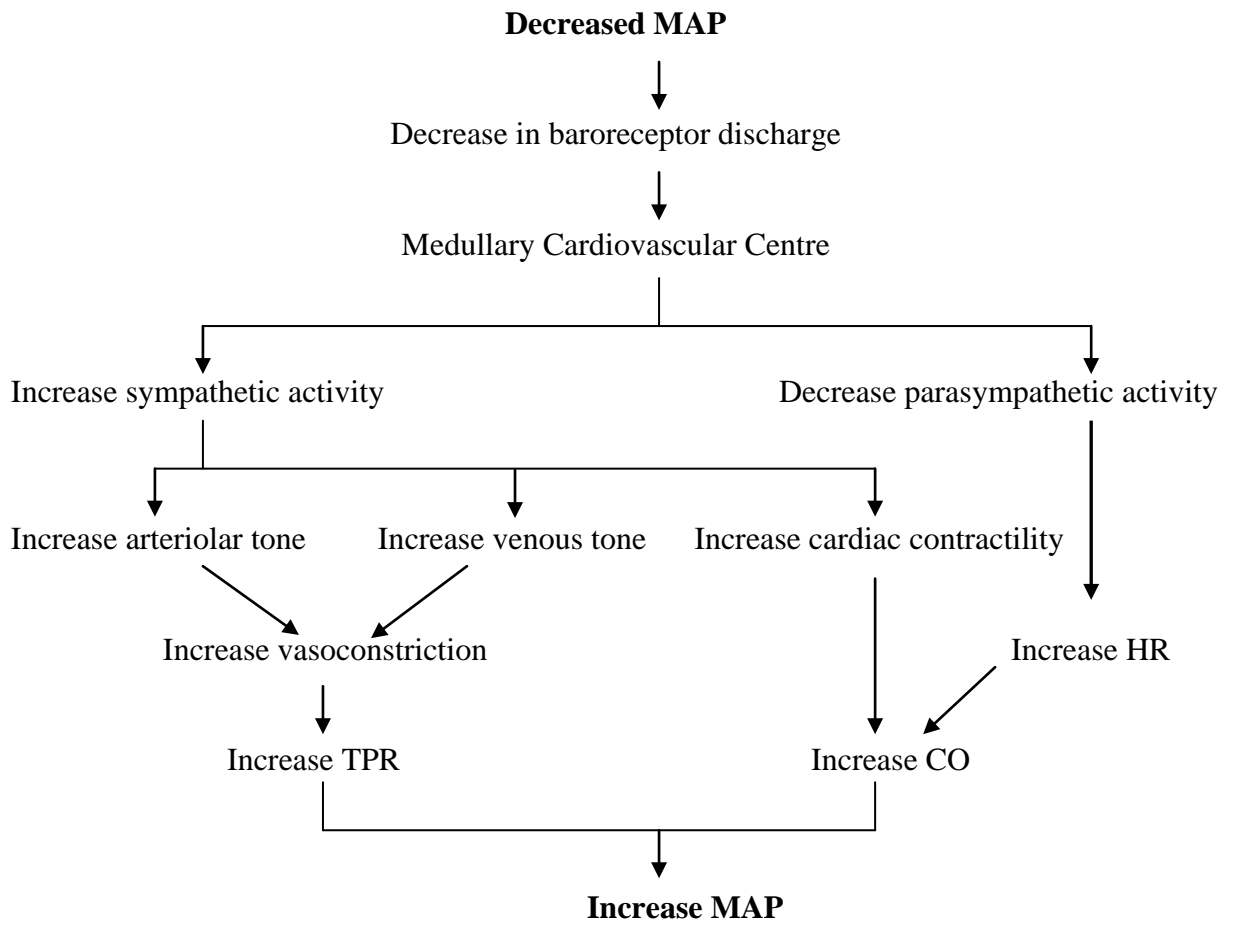


Figure 1.1. The baroreceptor reflex: responses to decreased MAP.

Cardiovascular Responses to Exercise

The CV system must respond to physical stress in order to maintain the appropriate match between tissue metabolism and perfusion. Dynamic exercise can however present competing local demands for blood flow that challenges the balance between CO and total vascular conductance in the maintenance of BP. This section of the review will briefly discuss the mechanisms believed to be responsible for regulating skeletal muscle blood flow during exercise and will outline how central and local controls are integrated in the maintenance of arterial BP during exertion.

At rest, skeletal muscle receives approximately 20% of CO. Estimates of CO distribution demonstrate that this may increase to 85-90% during exercise at VO_2 max (Rowell 1996). Increases in muscle perfusion during exercise are achieved through 1) elevations in CO via increased HR and SV, 2) increased vascular conductance in active muscle, known as exercise hyperaemia, and 3) redirection of CO towards active muscle through decreased conductance in less active tissues and viscera.

Cardiac Output

At rest CO is approximately 5l/min in untrained individuals. This may increase 7 to 8 fold during vigorous exercise in order to meet the metabolic demand of active muscle tissue. Increased CO is achieved via two mechanisms; the first is by an increase in HR. The SA node of the heart is innervated by both parasympathetic and sympathetic nerve fibres. Initial increases in HR during exertion are due to parasympathetic withdrawal, with increases above 100bpm due to sympathetic activation (Rowell & O'Leary 1990). The second mechanism by which CO is increased during exercise is enhanced SV. In 1994, Gledhill et al. clearly

demonstrated that the SV of untrained individuals initially increases during progressive exercise but generally plateaus at HR's of 120-140bpm, whilst the SV of endurance athletes increases throughout incremental to maximal exercise. Considerable previous and subsequent evidence strongly support the ability of the healthy human heart to increase or maintain SV during short-term incremental upright exercise. This SV response has been reported by several laboratories using invasive and non-invasive techniques in a variety of populations (Krip et al. 1997; Proctor et al. 1998; Warburton et al. 2007). Nevertheless, this evidence is often debated and considerable variability between individuals in the SV response to exercise certainly exists and the response appears to be highly dependent on fitness levels (Warburton & Gledhill 2008). Body position (and therefore loading conditions of the heart) also clearly has an effect on the SV response to exercise and may explain some of the discrepancies in the literature. With increasing exercise intensity, diastolic filling time as well as systolic ejection duration is reduced, and it is thought that this decreases leads to a plateau in SV. Several well-described mechanisms that collectively increase or maintain SV during exercise in endurance trained subjects have been proposed. These include increased venous return, increased atrial and ventricular inotropy, and enhanced ventricular relaxation.

Exercise Hyperaemia

Dynamic exercise has a significant impact on the muscle vascular bed homeostasis via the phenomenon of exercise hyperaemia, which is a net result of the interaction between various neurogenic and local factors (Joyner & Dietz 1997; Saltin et al 1998). The increase in muscle blood flow during exercise is tightly matched to muscle metabolic demand (Hamann et al. 2005) and is known to begin immediately following the first contraction of exercise (Tschakovsky et al. 1996; Hamann et al. 2004). Although the phenomenon is incompletely

understood, we have come a long way in identifying the vascular control mechanisms that may contribute to exercise hyperaemia. These mechanisms may be classified as: substances released by nerves that remove or interfere with sympathetic vasoconstriction (Thomas & Victor 1998; Tschakovsky et al. 2002; Dinunno & Joyner 2004), potential mechanical interactions between blood vessels and contracting muscle (Tschakovsky et al. 1996; Van Teeffelen & Segal 2006), substances released by or near active muscle (Dyke et al. 1995) and/or substances carried in the blood (Stamler et al. 1997). In addition, it is now understood that the propagation of vasodilation from sites of metabolic demand to upstream vessels is a critical component of the integrated vascular response in exercising muscle (Segal et al. 1989; Segal 1994). The importance of this mechanism is highlighted if the arterioles and resistance arteries are considered as a series of resistors. In the presence of high feed artery resistance, dilation of the arterioles will only increase tissue blood to a certain extent, with the magnitude of the hyperaemic response limited by the constriction of the feed arteries. Therefore, with resistances arranged in series, maximal perfusion can only be obtained with concomitant dilation of upstream and downstream segments (Segal et al. 1994). The resistance arteries have been proposed as key sites in the network for integrating the vasodilator stimuli 'ascending' from active muscle (Segal et al. 1992). Evidence for conducted vasodilation in conduit arteries has also been presented. Dilation of the cat femoral artery subsequent to gastrocnemius muscle contraction was observed by Hilton in 1959 and this was originally interpreted as the transmission of an electrical signal along the smooth muscle cells of the vessel media (Hilton 1959). However, later studies have ascribed femoral artery dilation to changes in blood flow through the vessel lumen, a response subsequently shown to be mediated by the endothelial cells (Pohl et al. 1986).

Since the discovery by Furchgott & Zawadski (1980) of endothelial-derived relaxing factor, later identified as vascular Nitric Oxide (NO), the role of NO in exercise hyperaemia has received much attention and is often debated. The evidence is conflicting, with some studies supporting a significant role (Dyke et al. 1995), whilst others have not (Wilson & Kapoor 1993; Endo et al. 1994). Although the nature of the vasodilatory mechanism interaction is now thought to be one of redundancy and (or) synergy (Clifford & Hellsten 2004), NO has been postulated to play a part in flow-mediated mechanisms (Maiorana et al. 2003), mechanical vessel distortion mechanisms (Clifford et al. 1996), and muscle-activation mechanisms (Van Teeffelen & Segal 2006).

Autonomic Responses to Exercise

Central CV control mechanisms are of paramount importance to the integrated haemodynamic response to exercise. These mechanisms contribute to the effective redirection of CO to active muscle and are also key in the effective BP control in response to a potentially hypotensive challenge (described in more detail later). Exercise presents a potent stimulus to the autonomic nervous system, decreasing parasympathetic nerve activity whilst enhancing sympathetic nerve activity. Four distinct neural mechanisms contribute to the regulation of this autonomic response. These are the arterial baroreflex and cardiopulmonary reflexes described previously, as well as central command and the exercise pressor reflex. Central command is a mechanism whereby higher areas of the brain responsible for recruiting motor units, activate CV control areas in the brainstem to modulate sympathetic and parasympathetic activity (Goodwin et al 1972). Recent research has also demonstrated that the perception of effort may contribute to this mechanism (Williamson et al. 2006). These feedforward inputs converge on the CV centres of the brainstem along with feedback arising

from small afferents located in active skeletal muscle (exercise pressor reflex) (Coote et al. 1971, McCloskey & Mitchell 1972). Traditionally, group III muscle afferents have been classified as predominantly mechanically sensitive (mechanoreceptors), whilst group IV muscle afferents are more chemically sensitive (metaboreceptors; Mense & Stahnke 1983). During exercise, these respective signalling pathways combine to modulate alterations in autonomic outflow that induce changes in cardiac activity (HR and contractility), and alter the diameter of resistance and capacitance vessels within both non-active and active skeletal muscle.

Functional Sympatholysis

The interaction between functional vasodilation and sympathetic vasoconstriction in the microvascular has long driven studies of blood flow control during exercise (Remensnyder et al. 1962; Hansen et al. 1996; Buckwalter & Clifford 2001; Tschakovsky et al. 2002; Dinunno & Joyner 2003). In 1962, Remensnyder et al. coined the term “functional sympatholysis” to describe the markedly reduced vasoconstrictor responses observed in exercising muscle in response to activation of sympathetic nerves or local intra-arterial infusion of noradrenalin. It is proposed that this phenomenon ensures the tight linkage between the metabolic demands of active muscle and blood flow.

Although not yet fully clear, the mechanism behind functional sympatholysis is thought to be a substance produced in the contracting muscle that inhibits α -1 and or α -2 activation by the noradrenalin released from nerve terminals. Some experimental evidence exists for NO to be the blocker of α -receptors, and the use of NO blockers such as N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine (L-NAME) has provided support for NO playing a role in

rat muscles (Thomas & Victor 1998). In this study of anaesthetised rats, inhibition of NO synthase using L-NAME partially reversed the attenuation of sympathetic vasoconstriction by hindlimb contraction. In humans, support for NO playing a role in eliciting functional sympatholysis comes from studies of Duchenne muscular dystrophy patients who are lacking n-NOS. In these patients, muscular contraction does not block lower body negative pressure-induced sympathetic activity (Sander et al. 2000).

However, in spite of the evidence supporting the existence of sympatholysis, this phenomenon may present a problem in the setting of whole body dynamic exercise. During dynamic exercise, peak muscle perfusion in active muscles may increase up to 100-fold from resting values (in the range 2-4l/kg/min) (Andersen & Saltin 1985). If sympatholysis was absolute (i.e. vasoconstriction was abolished in active muscle) the demands of a large dilated muscle mass would soon overcome an athletic heart capable of a CO of 40 l/min and arterial BP would be challenged. This effect is dramatically illustrated in the classical observation that patients with autonomic failure cannot maintain arterial pressure during exercise, even when performing light activity whilst supine (Marshall et al. 1961; Shepherd 1987). This poses an important question, is it possible to regulate blood flow to active muscles and protect arterial BP at the same time? An insight into this important issue was gained by the study of Klausen et al. (1982) which examined the circulatory responses to one-leg and two-leg cycling exercise following a one-leg training programme with each of both legs. The training programme resulted in larger increases in maximum oxygen uptake and cardiac output during one-leg than two-leg cycling (19% vs. 11% and 16% vs. 11% respectively). During submaximal one-leg exercise, HR decreased by 11%, whereas a fall of only 2% was seen during two-leg exercise. Leg blood flow and leg oxygen uptake were smaller during two-leg

exercise than one-leg exercise and this difference increased after the eight week training programme. These findings imply that oxygen supply to one large exercising muscle mass may be limited by vasoconstriction when another large muscle mass is simultaneously exercising.

Interestingly, recent studies have demonstrated that the interaction between sympathetic nerve activity and local vasodilatory stimuli appears to vary with vessel branch order (Haug et al. 2003). This may provide valuable insight into a regulatory system that enables BP to be adequately maintained whilst simultaneously providing active muscles with ample blood flow. This phenomenon may be explained by the heterogeneous distribution of α -adrenoreceptors throughout the human vasculature. It has been demonstrated that large upstream arteries contain a predominance of α 1-receptors whilst the arterioles contain mainly α -2 receptors. Whilst α -2 mediated vasoconstriction has been shown to be sensitive to metabolic inhibition, α -1 mediated vasoconstriction seems able to impair ascending vasodilation (Anderson & Faber 1991; Haug & Segal 2005; VanTeeffelen & Segal 2003; Wray et al., 2004). It is therefore proposed that α -1 receptors are ideally located in large upstream vessels for the control of arterial BP (Wray et al. 2004), whilst α -2 receptors are likely to play a key role in the fine control of tissue perfusion (Anderson & Faber 1991; McGillivray-Anderson & Faber 1990).

In summary, the present knowledge of the regulatory mechanisms for the CV responses to exercise may be that: 1) Peak muscle perfusion may increase up to 100-fold above resting values during intense exercise; 2) Vasodilation in active muscle is closely regulated to match oxygen demand. This is achieved through the combined effects of inhibiting sympathetic

vasoconstriction in active muscle (functional sympatholysis) and vasodilation induced by locally released vasoactive substances; 3) During vigorous exercise of a large muscle mass, the human CV system is simply not capable of supporting all muscles with sufficient blood flow if BP is to be effectively maintained; 4) Preserved vasoconstrictor responses in the arteries may be of prime importance in the active regulation of arterial BP during exercise.

Exaggerated BP Response to Exercise

As accumulating evidences emerges that risk of developing CVD disease increases progressively with higher levels of BP, the early detection of those at risk from developing hypertension becomes an increasing priority. It has been suggested that the development of hypertension is preceded by a prehypertensive state that may be manifested by abnormal CV reactivity to environmental and physiological stress (Menkes et al 1989; Fredrikson et al. 1991). Indeed, accumulating pathological evidence suggests that an exaggerated BP response to dynamic exercise is a powerful marker of CV disease that predicts the future onset of hypertension (Wilson et al. 1990; Manolio et al. 1994; Matthews et al. 1998; Miyai et al. 2002), stroke (Kurl et al. 2001), and CV mortality (Filipovsky et al. 1992; Mundal et al. 1996).

In further detail, Filipovsky et al. (1992) demonstrated that an exaggerated SBP response to cycling exercise (ExSBP) was of prognostic significance in middle-aged men, independent of resting BP. This investigation studied 4,907 men over a 17-year period, and showed, using Cox regression, that independently of other CV risk factors, the BP rise from rest to peak exercise was positively associated with cardiac and all cause mortality. Mundal et al. (1996) later demonstrated that exercise BP was more strongly related to both morbidity and mortality from myocardial infarction than resting BP. Furthermore in those subjects with moderately elevated resting SBP (140mmHg), subjects who demonstrated ExSBP (>200mmHg) were at significantly greater risk for myocardial infarction than those with elevated resting BP but normal BP response to exercise (ExSBP < 200mmHg).

In 2002, Miyai et al. examined exercise test data from a population based sample of 1033 normotensive men. Cox proportional survival analysis revealed a significantly increased risk (3.8 fold) for developing hypertension associated with ExBP above the 90th percentile. Furthermore, as elevated SBP is a common risk factor for stroke, Kurl and colleagues (2001) examined the associations between the rate of SBP response to graded upright cycling and the risk of stroke in a population based sample of men with no history of CHD (n = 3433). They showed that those men with an ExSBP increase of greater than 19.7mmHg per minute had a 2.3 fold increased risk of stroke when compared to those men whose SBP increase was less than 16.1mmHg per minute.

Potential Mechanisms

Although several proposals have been made, the precise mechanism of an exaggerated BP response to exercise is poorly understood. This haemodynamic response may be explained by a hyperactivity of the sympathetic nervous system (Polonia et al. 1992; Miyai et al. 2005), an increased vascular response to adrenergic stimulation or by a thickening of the arteriolar wall that alters its ability to respond to vasodilator or vasoconstrictor stimuli (Miyai et al. 2002). Recent reports have observed the presence of impaired vasodilator function among individuals with significant exercise BP elevation (Stewart et al. 2004; Tzemos et al. 2009). These abnormalities of autonomic control and vasoreactivity have been found to contribute to the pathogenesis of hypertension at an early stage (Julius 1996; Smith et al. 2004). We may therefore speculate that an exaggerated BP response to exercise is one of the manifestations of the pathophysiological changes during the prehypertensive state.

Arterial Properties and Blood Pressure

Increased arterial stiffness is emerging as the most important determinant of increased SBP and PP in our aging community and therefore as the root cause of a host of cardiovascular complications and events. In fact, accumulating evidence suggests that arterial stiffness per se is an independent marker of CV risk (Blacher et al. 1999; Meaume et al. 2001; Cruickshank et al. 2002).

The overall function of the arterial system is to distribute blood from the heart to the peripheral vasculature as efficiently as possible. A healthy network of arteries will minimise the drop in MAP from input to termination, reduce pressure fluctuations at the heart and minimise pulsatile energy losses throughout the cardiac cycle. The elastic wall of the aorta transforms the pulsatile on-off blood flow of the left ventricle into less pulsatile flow in distal vessels that results in smooth non-pulsatile flow in capillaries (Safar 2004). This system protects the microcirculation from pressure-induced damage and ensures that the heart receives adequate blood flow in the coronary arteries during diastole to meet its metabolic requirements. The ability of the aorta and the proximal arteries to buffer the pulsatile flow depends largely on the compliance characteristics of these vessels. Stiffening of the aorta results in an elevation in central SBP and a lowering of DBP (i.e. an increase in PP) via the effects of wave reflection (discussed below). This, in turn increases LV afterload and alters coronary artery perfusion (Nicols & O'Rourke 1998) and therefore substantially increasing the risk for CV events. Although there is no precise definition for afterload, this concept represents the factors that contribute to total myocardial wall stress (or tension) during systolic ejection. Afterload is often simply considered to be the ventricular pressure at end-systole.

Ejection of blood into the aorta generates a pressure wave that propagates throughout the arterial tree. This forward travelling wave is reflected at all points of functional and structural discontinuity, generating a reflected wave that travels backward towards the heart. The sites of major wave reflection are determined by the impedance mismatch between the large central arteries, such as the aorta, and the smaller conduit (muscular) arteries branching from this artery. Forward travelling and reflected waves are in constant interaction and are summed up in a measured pressure wave. The final amplitude and shape of the pressure wave are determined by the amplitude of the forward travelling and the interaction (timing) of the component (forward travelling and reflected) waves. Pressure wave reflection in the arterial system may serve at least two beneficial purposes. When normally timed, the reflected wave returns to the aorta during diastole and therefore enhances diastolic pressure perfusion in the coronary circulation (Nichols & O'Rourke 1998). Partial wave reflection also returns a portion of the pulsatile energy content of the wave form to the central aorta where it is dissipated by viscous damping. Thus, wave reflection limits transmission of pulsatile energy into the periphery where it might otherwise damage the microcirculation (Safar et al. 2003). In health, the heart and the vascular tree are therefore normally tuned for optimal efficiency. Factors determining the timing of the incident and reflected waves include the duration of ventricular ejection, the travelling distance of the pressure and the pulse wave velocity (PWV). Arterial distensibility is inversely related to PWV, and the desirable timing of the component waves is disrupted by increased PWV in relation to arterial stiffening. A stiffening of the central circulation relative to the peripheral circulation results in a reduction in the normal impedance mismatch that exists between these two areas. This may diminish wave reflections (Mitchell 2004) or cause wave reflections to occur during systole rather than during diastole. This leads to an amplification of aortic pressure during systole, increasing

transmission of pulsatile energy into the microcirculation, and reducing diastolic perfusion of the coronary circulation (Benetos et al. 1993).

The Aging Arteries

In a healthy arterial system there is a steep gradient of increasing arterial stiffness moving outward from the heart. However, advancing age is known to drastically alter this profile, and age related increases in central arterial stiffness (2-fold increase from early adulthood to age 80 years) in both healthy and diseased populations have been well described (O'Rourke & Nichols 2005). Using multivariate models, age has been shown to be the major clinical determinant of aortic stiffness (Avolio et al. 1983; Asmar et al. 1995). This age dependent increase in central artery stiffness is independent of MAP or the presence of other risk factors (Relf et al. 1986) and therefore likely reflects intrinsic alterations of the vessel walls. Although not yet completely understood, these alterations may arise as a function of the cumulative cycles of pulsatility that lead to the breakdown of the elastic structure (elastin fibres) in the arterial walls (Nichols et al. 1998). A more dynamic, cellular ionic basis for these changes has also been postulated (O'Rourke & Hashimoto 2007). Although the central, predominantly elastic arteries are known to progressively stiffen with age, the distal, predominantly muscular arteries are thought to exhibit very little change with age (Benetos et al. 1993, Kelly et al. 1989). As a result aortic stiffness may equal or exceed peripheral artery stiffness in the elderly.

As traditional measurements of brachial BP will not reveal the extent to which arterial stiffening increases aortic pressure (and thus LV afterload) the measurement of arterial stiffness/function is of paramount importance. The various techniques used to assess arterial properties are explored in the next section of this review.

Measurement of Arterial Stiffness

Several methods for quantifying arterial mechanical properties have been proposed. These can be divided into techniques which apply propagative models to circulation, which relate to wave speed and/or wave reflection, and those using non-propagative models (Woodman et al. 2005). Propagative models assume that the velocity with which a pulse wave travels along a given artery has a finite value (Laurent et al. 2006). Non-propagative methods include the Windkessel model that likens the circulation to a mechanical device found on old fire engines. A Windkessel pump was designed to transform the intermittent pulsing of a manual water pump into a continuous stream of water from a hose. In its simplest two element form, the Windkessel model describes the circulatory system in terms of parallel resistance and capacitance components. The resistance element corresponds to peripheral vascular resistance, whilst the capacitance component relates to the compliance of the arterial system. Whilst the advantage of the Windkessel model is its simplicity, its value as a comprehensive explanation of arterial function under a range of physiological circumstances is limited. Analysis of the arterial system using solely this model is restricted since it assumes that all pressure changes occur instantaneously, whereas the circulation serves to distribute CO through a series of branching networks and therefore the elastic properties described by the model are not present at just one site but are distributed along the aorta and major arteries. For this reason the Windkessel model is unable to explain important phenomena such as wave reflection (Dart & Kingwell 2001). Furthermore the pressure wave has a finite velocity, and importantly, the amplitude and contour of pressure waveforms differ from central to peripheral arteries (O'Rourke et al. 2002); characteristics that the Windkessel model cannot describe. Considering the arterial system as comprising of a distributed compliance will overcome these limitations to a large extent (Dart & Kingwell 2001).

Inferences about the mechanical properties of arteries can be made by measuring PP or by measuring a variety of 'stiffness indices'. These stiffness indices usually measure one of three possible types of stiffness: systemic stiffness (i.e. a measure of the stiffness of the entire arterial circulation); regional or segmental stiffness (i.e. a measure of the stiffness of a segment of the arterial tree); or local stiffness (i.e. a measure of the stiffness in a small section of the vessel being studied). Such indices can however, provide information about more than one aspect of the circulation and can thus be classified as indices that: quantify pulse transit time, analyse the pulse waveform, or provide direct estimation of vessel stiffness by the simultaneous measurement of arterial diameter and a corresponding distending pressure.

Pulse Pressure

The measurement of PP is the simplest surrogate measure of arterial stiffness. As we have discussed, PP is the consequence of intermittent ventricular ejection and is calculated by subtracting DBP from SBP. It is determined by SV, cardiac contractility and arterial stiffness (Dart & Kingwell 2001), and indicates the degree of impairment of the buffering function of larger arteries. Elevated pulse pressure has been shown to be a predictor for cardiovascular disease in general populations (Panagiotakos et al 2005), healthy individuals (Assmann et al. 2005), treated and untreated hypertensive patients (Millar et al. 1999) and patients with type 2 diabetes mellitus (Cockcroft et al. 2005).

Many authors have reported that PP is more meaningful if measured centrally rather than at the brachial artery as this will more accurately describe the pressures faced by the LV (Dart & Kingwell 2001; Laurent et al. 2006). As we have discussed, the Windkessel model of the circulation is unable to explain the phenomenon pertinent of wave reflection. This is of

particular importance in the understanding of PP and its modification in shape and amplitude along the arterial network. Increased central SBP (and hence PP) over and above that generated by LV ejection alone refers to the superposition of a visible reflected wave onto the forward travelling wave. Peripherally, PP amplification refers to the phenomenon wherein PP measured at the peripheral artery is amplified in comparison to that present at the proximal aorta. Although this amplification is lower for the brachial artery than it is for the femoral artery and lower in older compared to younger age groups, it is important to note that peripheral estimates are not identical to central measured PP (Dart & Kingwell 2001).

Arterial stiffness may be defined as the relationship between changes in pressure and volume. As PP reflects the difference between systolic and diastolic pressure, and SV may be used as substitute measure of volume changes in the arterial system, the ratio of SV to PP has been proposed as a more refined estimate of arterial compliance than PP alone. This ratio has been shown to a predictor of cardiovascular events in the general population (Lind et al. 2004) and in patients with hypertension (de Simone et al. 1999). It is clear from this approximation that an elevation in PP can be secondary to a rise in SV (or increased cardiac contractility) or fall in arterial compliance. It has been suggested that whereas elevation of PP in the young is related to increases in SV, a rise in PP (or SBP) with age relates to a decrease in arterial compliance (Franklin et al. 1997, Alfie et al. 1999). The study by Franklin et al aimed to characterise age-related changes in BP (and infer underlying haemodynamic mechanisms) in a population based cohort from the original Framingham Heart Study. Up to 30 years data from approximately 2,000 normotensive and untreated hypertensive subjects were studied and regressions of BP versus age within subjects produced slope and curve estimates. There was a linear rise in SBP from age 30 to 84 years and concurrent increases in DBP and MAP. After

50 to 60 years age, however, DBP declined and PP rose steeply. The authors concluded that this haemodynamic profile (late fall in DBP after age 60, associated with a continual rise in SBP) is consistent with increased arterial stiffness.

Pulse Wave Velocity

The most hallowed measure of arterial stiffness is pulse wave velocity (PWV). The PWV is the speed at which the forward pressure wave is transmitted from the aorta through the vascular tree. The principal determinants of PWV can be described using the Moens-Korteweg equation that was derived in the 1920's, and relates the velocity of pulse wave travel in a vessel to the distensibility of that vessel:

$PWV = \sqrt{Eh/2R\rho}$, where E is Young's modulus of the arterial wall in the circumferential direction, h is the wall thickness, R is the vessel radius and ρ is the density of fluid.

In a given blood vessel filled with blood of fixed density, PWV is proportional to the square root of the Young's modulus of elasticity of the vessel. More simply, the stiffer the vessel the faster the PWV. PWV is usually obtained by measuring the time taken for a pulse wave to travel a specified distance; the distance being divided by the time to give velocity. Distance is usually measured using a tape measure of the body surface, whilst timing is performed by measuring the interval between points on a pressure or flow waveform using a proximal and distal sensor. Usually the 'foot' of the waveform is used as the reference point since this is the part least affected by wave reflection (Asmar et al. 1995). The time delay between the arrival of the pulse wave at two sites is obtained by simultaneous measurement or by recording the waveforms separately but comparing the time delay at both sites to a defined point on an

ECG. Problems with PWV measurement include the inaccessibility of the central arteries, necessitating compromise by using the nearest superficial arteries. There can also be some difficulty in estimating the actual arterial distance between recording sites using only surface measurements.

Pulse wave velocity varies from vessel to vessel, with PWV generally greater in peripheral rather than central arteries. For a healthy middle-aged adult, a typical velocity in the ascending aorta would be of the order of 4m/s compared with 8m/s in the brachial artery (Latham et al. 1985). This may be explained by the differences in the elastic properties of the various arteries, the varying cross sectional area of the blood vessels and the modifying BP profile along the arterial tree. Briefly, as we descend the arterial tree, there is 1) an increase in SBP and hence PP; 2) the internal cross sectional area of the arteries decreases affecting blood viscosity and density; and 3) the ratio of collagen to elastin within the arterial wall increases.

Pulse Waveform Analysis

The variation in the pulse waveform throughout the arterial tree is also due to the phenomenon of waveform reflection (Nichols & O'Rourke, 1998). As discussed previously, the pressure waveform at any point is a composite of the forward-going incident and the backward travelling reflected wave. As a consequence, aortic SBP may be lower than the brachial artery SBP by more than 20 mmHg (Pauca et al. 1992) and, importantly, the relationship between the two is not fixed (Kelly et al. 1989; Nichols & O'Rourke, 1998). Such differences may be clinically important because it is central aortic, and not brachial pressure that determines left ventricular workload (Westerhof & O'Rourke, 1995). Therefore, measurement of central aortic, rather than brachial artery, pressure may provide a better

prediction of CV risk (McEniery et al. 2008). However, due to the aorta's inaccessibility, until recent methodological advancements, invasive techniques were required for accurate central pressure measurement. It is however easy to obtain a pressure waveform from a peripheral artery such as the radial artery using applanation tonometry and mathematical processes can be applied to waveforms measured peripherally in order to predict the waveform in the more proximal aortic circulation. Such models or 'generalised transfer functions' have been used with some success (Chen et al. 1997) leading to widespread clinical use. The rationale for such functions is that, if the relationship between pressure waveforms in two parts of the circulation is known, the pressure waveform at one site may be extrapolated from data collected at the other site (Hope et al. 2004). If a transfer function is to be useful, it must yield accurate and reproducible data on an individual and group or population basis. Unfortunately, the validity of such transfer functions has been questioned. Recent studies have reported poor agreement between aortic augmentation index (see below) derived by applying transfer functions to the carotid and radial pressure waveforms and it has been proposed that in the case of aortic augmentation index, the use of a transfer function offers little advantage over the useful information provided by the non-transformed radial pulse (Millasseau et al. 2003). In contrast, several studies have observed close relationships between several radial derived and invasively measured central aortic waveform parameters (Chen et al. 1997; Millasseau et al. 2003; Hope et al. 2003). In particular it is increasingly accepted that the use of a radial-to-aortic transfer function improves the evaluation of central SBP and that any inaccuracy probably stems from the error in peripheral calibration pressure rather than the transfer function itself. For example, Chen et al. (1997) found the generalised transfer function to estimate central arterial pressures to $<0.2 \pm 3.6$ mmHg error when compared to aortic pressure measured invasively by central micromanometer catheter.

Augmentation index (AI) is perhaps the most used parameter measured by PWA and is often applied as a surrogate measure of systemic arterial stiffness. The phenomenon of pulse wave reflection is fundamental to understanding the concept of AI. Near the aortic root, the initial rise in pressure following LV ejection is rapidly superimposed with a reflected pressure wave returning from the periphery. The start of this reflected wave is visible on the central pressure waveform as an inflection point, shown on the figure below as P1. The AI is a mathematical expression of the augmentation point in the pressure domain, where the increment in pressure after the first systolic shoulder (P1) to the peak of the aortic pressure (P2) is calculated as a percentage of PP (Kelly et al. 1989). The AI depends on the shape of the forward pressure wave, which is influenced by LV outflow and the elasticity of the aorta, as well as the timing of reflected wave (TR), a factor influenced by gender, height, reflected wave amplitude, and vessel stiffness (Kingwell & Gatzka 2002). Yasmin & Brown (1989) has suggested a modest correlation between AI and aortic PWV ($r = 0.29$) since a higher aortic PWV will result in the faster transmission of pressure waves to the peripheries and, in turn, a faster return of reflected waves that determine AI. The strength of this correlation was greater when each gender was examined separately ($r = 0.42$ in men and 0.56 in women) indicating that gender (at least partly affected by height) is a major confounder.

Figure 1.2

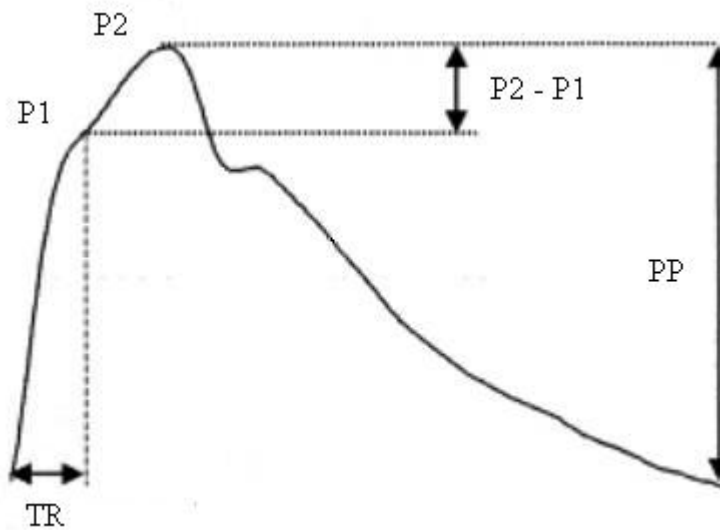


Figure 1.2. PWA and the calculation of Augmentation Index (AI). AI is calculated as the difference between the early systolic shoulder (P1) and the peak aortic pressure (P2) and expressed as a percentage of pulse pressure (PP)

As discussed earlier, exercise BP may be clinically important, and as such a non-invasive means to derive it would be extremely beneficial. With this in mind, Sharman et al. (2006) aimed to test the validity of a generalised transfer function to non-invasively derive central BP during exercise. In 30 subjects, a high correlation ($r = 0.995$) was found between central pressures derived from PWA and those measured by aortic micromanometer catheters during peak exercise. Furthermore, Holland et al. (2008) have observed very good reproducibility of PWA derived measures when these were recorded during cycling exercise on two separate occasions. Intra-visit correlations during exercise (cycling at 50% age predicted max HR) were 0.93 and 0.89 for AI and central PP respectively. PWA should therefore provide a reproducible technique for measurement of central waveform indices during exercise. It must

however be noted that Dawson et al. (2009) recently observed, despite identical bouts of handgrip exercise, differing central haemodynamic responses when PWA was measured in the exercised vs. the non-exercised arm. This study aimed to determine whether exercise-induced alterations in vascular tone would influence measures derived from the Sphygmocor device and its associated transfer function. Subjects performed repeated identical and repeated bouts of incremental handgrip exercise whilst radial waveforms were captured from either the exercising or contra-lateral (non-exercised) limb. Augmentation index was shown to be consistently higher (~10%) when radial waveforms were acquired in the exercised arm and similarly, consistent and significant differences were observed for central BP's derived from waveforms captured from the exercised and non-exercised arms. It was therefore concluded that changes in vascular tone with exercise may therefore compromise the underlying transfer function assumptions used to derive central haemodynamic indices. As such, interventional studies employing this technique and involving haemodynamic alterations should be designed with care.

Local Assessment of the Mechanical Properties of Arteries

Arterial compliance is defined as the change in arterial blood volume for a given change in arterial BP. Therefore by simultaneously measuring the diameter of a blood vessel and the BP in that area, compliance can be directly calculated. Diameter may be measured using ultrasound or magnetic resonance imaging (MRI). Distending pressure is however more difficult to measure since 1) it is difficult to physically place a pressure sensor next to an ultrasound probe; 2) invasive pressure catheters probably influence local flow and are not suitable for routine use; and 3) the use of pressure waveforms taken elsewhere in the circulation can produce pressure errors and phase delay (Meinders & Hoeks 2004). Pressure

waveforms can however be reliably extrapolated from diameter waveforms and thus a measure of compliance can be obtained by tracking the diameter change in a vessel wall. A major advantage of such techniques is that local arterial stiffness is directly determined from the change in pressure driving the change in volume. However, because these measures require a high degree of technical expertise and take longer than measures of PWV or PWA, local measurement of arterial stiffness is only seldom used for epidemiological studies (Laurent et al. 2006).

Regulation of Artery Stiffness

Arterial stiffness is affected by a number of key mechanisms that include structural changes to the vessel wall, the passive influence of arterial BP, and the dynamic control of the endothelial/smooth muscle mechanism.

The Effects of BP and HR on Arterial Stiffness

Because the arterial media consists of both collagen and elastin, the relationship between pressure and vessel diameter is non-linear (Liu et al. 1986). Therefore stiffness can only be quantified at a given level of pressure as tangent to the curve (O'Rourke et al. 2001). Arterial stiffness is increased with a rise in MAP, which influences the entire arterial tree as a passive effect. As distending pressure increases, there is a greater recruitment of relatively inelastic collagen fibres and, consequently, a reduction in elasticity (Armentano et al. 1991).

Animal studies have revealed that when HR is increased by pacing, both carotid and femoral arteries display reduced distensibility (Mangoni et al. 1996). This may suggest that HR may be one of the factors modulating the mechanical properties of arteries. The evidence for HR playing a similar role in humans is however less clear. Although, observational studies have reported a positive link between HR and arterial stiffness (aortic and femoral arteries assessed using echo-tracking techniques, Sa Cunha et al. 1997), studies examining arterial stiffness during HR changes have given conflicting results. Some studies have shown that increased HR using pharmacological agents or pacing resulted in a concomitant increase in PWV in various arterial segments (Lantelme et al. 2002; Giannattasio et al. 2003).

However, Stefanadis et al. (1998) have reported conflicting findings, with data that shows pacing-induced increases in pulse frequency resulting in improved aortic distensibility -

assessed using local invasive measures of aortic diameter and pressure, and a favourable change in the timing of wave reflections (reduced AI). In further contrast, Wilkinson et al. (2002), report that incremental pacing had no effect on aortic stiffness, and therefore conclude that reduced AI with increased HR is simply due to an alteration in the relative timing of the reflected wave rather than a true reduction in arterial stiffness.

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 therefore alter the vessel stiffness (Wilkinson et al. 2004). The muscular arteries have a large sympathetic innervation and because smooth muscle tone is also influenced by circulating and local vasoactive substances; arterial stiffness may be actively regulated. (Wilkinson et al. 2004).

The Endothelium and the Role of NO in Arterial Stiffening

The systemic infusions of drugs that promote or inhibit nitric oxide (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), independently of any effect on BP in healthy subjects, and reduce AI in those with a range of CV risk factors including hypertension and hypercholesterolemia (Wilkinson et al. 2002). NO donors have also been shown to reduce other measures of large artery stiffness such as PWV in hypertensive individuals (Safar et al. 1986), but not always independently from changes in distending pressure.

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 (2002) have shown that systemic infusion of L-NMMA increases AI in healthy normal volunteers. The findings of this study must be interpreted with caution however, since the changes in stiffness observed were invariably accompanied by increases in MAP and reflex reductions in HR.

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 are normally unaffected (Wilkinson et al. 2004). 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 (Wilkinson et al. 2002). Kinlay et al. (2001), also report similar findings in the human brachial artery when elasticity, compliance and PWV are observed. Conversely GTN (Kinlay et al. 2001) or ACE (Ramsey et al. 1995) administrations reduce arterial stiffness in the muscular arteries.

Together these studies suggest that basal, stimulated, 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.

Arterial Stiffness and Sympathetic Nerve Activity

Gerova et al. (1969) 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 (Boutouyrie et al. 1994), and smoking (Failla et al. 1997), i.e. manoeuvres that cause sympathetic activation.

Further evidence for the stiffening effect exerted by neural influences in vessels is provided by Failla et al. (1999) who demonstrated that in humans, anaesthesia of the brachial plexus, lower spinal chord and lumbar sympathectomy caused an increase in the distensibility of the radial or femoral artery respectively. 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 (Tardy et al. 1991), or a reduction in the viscoelastic opposition of the arterial wall to inside pressure when HR is increased (Mangoni et al. 1996). 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 (Boutouyrie et al. 1994) 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 (Mangoni et al. 1997). Further evidence for arterial stiffening influence of sympathetic nerve activity is provided by the hand transplantation studies by Giannattasio et al. (2005) which report that radial deinnervation is accompanied by an increased arterial distensibility.

A few recent studies have provided further valuable insight into the cardiovascular and haemodynamic responses to sympathetic activation by examining the effects of environmental stress on the magnitude and timing of aortic wave reflection and central pressure. For example Edwards et al. (2006) have demonstrated that cold exposure and the resulting peripheral vasoconstriction increase wave reflection and central SBP. Vlachopoulos et al. (2006) have also shown increased aortic stiffness with premature wave reflection during acute mental stress, findings that have recently been supported by Lydakis et al. (2008). Significantly, the responses observed in these studies were not evident from measures of brachial BP.

Ventricular – arterial (V-A) coupling

As we have discussed, left ventricular function is determined by arterial load (Kass et al. 2005), and in turn, arterial properties are influenced by left ventricular performance (Sunagawa et al. 1983). Indeed the interaction of the heart with the systemic vasculature, termed V-A coupling, is a key determinant of cardiac performance and energetics.

The vascular component of LV afterload has historically been assessed in terms of MAP and TPR. As we have seen, these measurements do not take into account the pulsatile characteristics of blood flow in the arteries. Following pioneering work by Sunagawa et al. (1983) the functional properties of the arterial system may now be more accurately characterised by effective arterial elastance (AE). This steady state parameter incorporates the principal elements of total vascular load, and is related to mean vascular resistance as well as pulsatile components due largely to arterial compliance and wave reflections (Sunagawa et al. 1983; Kelly et al. 1992). AE therefore essentially represents a measure of the net arterial load that is imposed on the LV. AE can be determined invasively from pressure volume loops or may be accurately approximated by the ratio of end-systolic pressure (ESP) to SV (Kelly et al. 1992). The ESP/SV ratio has been shown to be nearly identical ($r^2=0.98$) to invasively measured AE over a broad range of altered conditions (Kelly et al. 1992).

AE shares common units with elastance measures of LV function (e.g. end-systolic LV elastance (E_{es}), and their ratio (AE/E_{es}) is a commonly used index of V-A coupling (Sunagawa et al. 1983). A non-invasive method of measuring end-systolic LV elastance has been developed that requires systolic and diastolic BPs, and Doppler echocardiogram derived EF,

SV and pre-ejection period (Chen et al. 2001). This single-beat elastance approach has been validated against invasively measured E_{es} with a correlation coefficient of 0.81 ($P < 0.001$).

Normal V-A coupling ensures optimal cardiac work, power and chamber efficiency, and maintained CO and BP as blood is transferred from LV to the periphery. This is achieved when the properties of the heart and the arterial system are perfectly matched (Starling et al. 1993). However, alterations in these factors may result in fundamental alterations in cardiac reserve (Kass 2002).

Consequences of V-A mismatch

Increased AE and E_{es} (V-A stiffening) will combine to reduce left-side circulatory compliance, resulting in a larger change in LV end-systolic pressure for any given change in ejection volume (Williams and Frenneaux 2007). This means far greater haemodynamic instability and alterations in central and arterial pressures for any given change in central blood volume (Chen et al. 1998). In addition, combined V-A stiffening also limits cardiac reserve mechanisms. A healthy heart will respond to exercise by enhancing CO output through increased HR, increasing LV end-diastolic volume and increasing systolic contractile function (increased E_{es}). However, if basal conditions already include an elevated E_{es} and AE (as seen in age (Najjar et al. 2004) or disease (Kawaguchi et al. 2003)), there is less reserve capacity, or the inability to attain maximal efficiency, as apparent by a lesser reduction in the coupling index. Furthermore, an increase in AE will exacerbate systolic hypertension during exercise, which will impose an energetic demand on the heart whilst limiting its capacity to eject efficiently. Arterial stiffening raises myocardial oxygen consumption for a given SV (Kelly et al. 1992), an effect which will be augmented by ventricular systolic stiffening

(Kawaguchi et al. 2003). Finally, increased V-A stiffening and the consequent rise in systolic pressure during stress can worsen diastolic function (Kawaguchi et al. 2003). Elevated systolic load will delay cardiac relaxation, which may translate into compromised filling and increased end-diastolic pressure (Leite-Moreira et al. 1999).

It has been postulated that abnormal V-A interaction may contribute to the pathophysiology of heart failure with preserved ejection fraction, a classification which incorporates nearly half of those patients with congestive heart failure (Kitzman et al. 2001). As previously discussed in this review, arterial stiffness rises with age and hypertension. LV systolic stiffening also increases with age, and when combined with vascular stiffening, it can greatly amplify the effects of changes in blood volume on arterial pressure and cardiac workload (Chen et al. 1998). By limiting cardiac reserve mechanisms, exacerbating diastolic abnormalities and raising cardiac energy demands, combined stiffening of the heart and vascular may play an important pathophysiological role in this disorder (Kawaguchi et al. 2003).

V-A coupling during exercise

Exercise provides a powerful tool to examine the response of the CV system to stress. During exercise, the goal of the CV system is to prioritise cardiac efficacy over energetic efficiency (Najjar et al. 2004). As discussed earlier in this review, the CV system uses a complex combination of alterations in HR, LV contractility, preload and afterload to ensure adequate blood supply to the tissues. To date only a few studies have examined the changes in V-A coupling during exercise. In healthy human subjects undergoing supine cycling ergometry, Asanoi et al. (1992) observed that AE/E_{es} decreased by 35% and 54% at workloads corresponding to 30% and 30% above the anaerobic threshold respectively; and Chantler et al.

(2008) found that AE/E_{es} decreases approximately 65% from rest to peak exercise. It is generally agreed that there is a substantial increase in E_{es} with exercise and the decrease in the AE/E_{es} may therefore be attributed to a larger increase in E_{es} vs. AE during exercise (Chantler et al. 2008). The change in AE during exercise represents a complex interplay among the changes in BP, HR, TPR and arterial stiffness. At rest, the sensitivity of AE to a change in TPR/HR is approximately three times higher than to a similar change in arterial stiffness (Chemla et al. 2003). In contrast during exercise, arterial stiffness has a progressively and intensity-dependent greater impact on AE than TPR (Otsuki et al. 2006). Arterial responses to exercise are therefore of prime importance when examining V-A coupling during exercise.

Acute arterial responses to exercise

As discussed previously in this review, large arterial distensibility is physiologically important for circulatory efficiency. An artery with lower stiffness may have a higher buffering capacity and the ability to efficiently absorb energy during the systolic component of pulsatile blood flow and reduce energy loss by making the blood flow smooth. During exercise, blood flow will be markedly increased to meet the oxygen demands of active muscle and it may therefore be favourable to increase the buffering capacity of the arterial system. Exercise is an integral part of normal life, yet until recently the acute effects of exercise on the arterial distensibility have received little attention.

Resistance Exercise

Increased arterial stiffness has been observed immediately following acute resistance exercise (DeVan et al. 2005; Heffernan et al. 2007), although this is not a universal finding (Rakobowchuk et al. 2005). When increased stiffness has been observed this has been restricted to the central arteries and has not been observed in the peripheral muscular arteries. Although yet to be fully elucidated, an epiphenomenon of corresponding BP changes, increased sympathetic adrenergic vasoconstrictor tone and/or impaired endothelial function have been proposed as potential mechanisms responsible for the central artery response to resistance exercise (DeVan et al. 2005).

To date, many of the studies examining the effect of acute resistance exercise on the functional properties of the arterial system have employed a whole-body resistance exercise protocol. Therefore, these studies have been unable to elucidate the effects of resistance exercise that specifically targets regional muscular vascular beds on different segments of the

arterial system. Interestingly, a recent study by Heffernan et al. (2006) has shown that acute single-leg resistance exercise decreased femoral artery stiffness in the exercised limb whilst having no effect on the stiffness of the contra-lateral limb or central arteries. The reduction in stiffness in the exercised limb was still observed 25-mins post exercise. The findings from this study suggest that acute resistance exercise-induced regional factors modulate muscular arterial wall properties without having a systemic effect.

A recent study by Lydakis et al. (2008) investigated the effects of isometric fatiguing handgrip exercise on indices of central arterial stiffness. This study clearly demonstrated elevated sympathetic tone (elevated renal vascular resistance index), HR, MAP, central pressures and systemic arterial stiffness (AI) during handgrip exercise. The transit time of the pulse wave (TR) was also decreased indicating elevated aortic PWV and therefore increased central arterial stiffness. A significant reduction in the peripheral PP/central PP ratio also occurred, showing that the pulse pressure increase was more exaggerated centrally than peripherally. These results suggest that during isometric handgrip exercise there is an acute increase in systemic and central arterial stiffness that is likely related to the synergistic effects of increased sympathetic tone and BP.

Peripheral Responses to Aerobic Exercise

Kingwell et al. (1997) have observed elevated whole body compliance, determined non-invasively from simultaneous measurements of aortic flow and carotid pressure, 30-mins after a bout of moderate intensity cycling. Aortic and peripheral PWV were also reduced at this time, indicating decreased stiffness in both the central and peripheral arteries. These elevations in compliance post-exercise occurred without a change in the MAP, suggesting a

true change in arterial wall properties. As the time course of these observations was not consistent with intrinsic changes to the vessel wall structure, the most likely mechanisms relate to alterations in the loading of elastin and collagen, which can occur with changes in vascular smooth muscle tone.

In order to further describe the immediate effects of exercise on peripheral arterial distensibility, Naka et al. (2003) characterised the time course of peripheral PWV changes following maximal treadmill exercise. PWV was measured over upper and lower limb arteries as representative of large arteries supplying non-exercising and exercising muscles, respectively. In the upper limb, the “immediate” (3 min) post-exercise PWV was $\approx 35\%$ higher than its pre-exercise basal level, declining to $\approx 6\%$ below baseline by 10min and toward a near steady level $\approx 10\%$ below baseline by 60min. PWV in the lower limb showed a similar early decrease but from an initial post-exercise value, to a nadir $\approx 23\%$ below baseline at 10min, after which it increased to a near steady level of $\approx 10\%$ below baseline by 60min. These data provide evidence of the previously unreported pattern of changes in PWV immediately following maximal exercise. The difference between the patterns of recovery in the upper and lower limbs highlights the possibility of discriminating between local (e.g., endothelial-derived vasoactive substances, exercise muscle derived metabolites) and systemic (e.g., sympathetic nerve activity, circulating hormones) consequences of exercise.

To test the hypothesis that exercise induced decreases in arterial stiffness, at least in midsized muscular arteries, would be mainly caused by regional factors, Sugawara et al. (2003), performed an acute low intensity, short duration single leg cycle exercise test in healthy young males. The responses of each leg by estimating PWV from the femoral artery to the

right and left ankles were compared. The results demonstrated that exercise induced a significant decrease in the PWV of the exercised but not that of the non-exercised leg. These results further support the claim that the decrease of peripheral arterial stiffness with exercise may be induced mainly by exercise-related regional factors.

Munir et al. (2008) have recently shown that cycling exercise induces marked changes in the morphology of the radial and digital pulse similar to that introduced by nitrovasodilators such as nitroglycerin (NTG), comprising a reduction in late systolic and early diastolic pressure augmentation. In principle, such changes could be attributed to altered HR/ventricular ejection characteristics, central artery stiffness or changes in the tone of muscular arteries influencing pressure wave reflection (Nichols et al. 1998). However changes in waveform morphology were present in recovery when SV and carotid-femoral PWV values were similar to that at baseline. Changes in pressure augmentation were however accompanied by dilation of the femoral arteries and the changes in augmentation observed with exercise were similar to those induced during a NTG infusion trial. These results are therefore consistent with the decrease in PWV after exercise in muscular arteries of the exercising limb described by other investigators. Furthermore, results from this study suggest that vasodilation of the muscular arteries with a reduction in pressure wave reflection from the lower body is an independent mechanism underlying exercise-induced changes in pulse waveform morphology. These changes are likely to form an important part of the functional adaptation to exercise that enhances V-A coupling and reduces load on the LV.

The Role of Nitric Oxide

As hypothesised by those studies discussed above, a potential mechanism explaining the acute peripheral arterial adaptations to exercise may involve locally released vasodilatory substances. The basal release of NO has been shown to have a major influence on regulating the tone of both small peripheral and large central arteries at rest (Wilkinson et al. 2002), and studies performed in vivo have shown NO release to increase arterial distensibility (Kinlay et al. 2001; Wilkinson et al. 2004). It is therefore no surprise that exercise induced NO release has been postulated as primary mechanism contributing to the responses we have described.

Some recent human studies however seem to discount NO as significantly contributing to the increased arterial distensibility observed during or immediately following exercise. Sugawara et al. (2004) examined the effects of systemic NO synthase (NOS) inhibition on changes in PWV in both leg arteries with low-intensity, single leg aerobic exercise. However irrespective of whether systemic NOS inhibition by intravenous administration was carried out, exercise induced a decrease in femoral artery stiffness in the exercised but not in the non-exercised leg. Thus the authors concluded that systemic NOS inhibition appeared to have no effect on the decrease in muscular arterial stiffness with exercise and that the decrease in arterial stiffness in the exercised leg was induced by other exercise-related regional factors. In accordance with these findings, Sharman et al. (2008) have recently shown that inhibition of NO, by intravenous infusion of L-NMMA, had no significant effect on PP amplification or systemic arterial stiffness (augmentation index) during dynamic aerobic exercise. These studies may suggest that discrete mechanisms affect arterial stiffness (and therefore pressure amplification and myocardial afterload) under resting conditions compared with low level exercise. It may also be that NO plays a key role in regulating exercise haemodynamics, but that other

vasodilatory compounds (e.g, adenosine, bradykinin and/or prostaglandins) released during exercise have a stronger influence than NO on large artery function during aerobic exercise. Alternatively, other vasodilatory mechanisms may compensate for NO blockade.

In contrast to the above studies, a recent paper by Campbell et al. (2010) has provided evidence to suggest that NO does play a role in the arterial response to exercise. In this study, although acute NO synthase inhibition with L-NMMA had no effect on the aortic response to exercise (aortic PWV was similarly increased after exercise with either saline or L-NMMA), the normal postexercise decrease in femoral PWV were attenuated with L-NMMA. This data would suggest that NO is an important contributor to reductions in femoral artery stiffness following dynamic exercise. Furthermore, acute inhibition of NO synthase caused an augmented BP increase to sub-maximal exercise but did not lead to an exaggerated BP response to maximal exercise or reduced maximal oxygen consumption.

NO is a key determinant of resting BP and V-A coupling; acting to lessening myocardial afterload by attenuating reflected pressure waveforms (Haynes et al. 1993). As discussed earlier however, evidence is conflicting on the role of NO in the hyperaemic response to exercise, with some studies supporting a significant role (Dyke et al. 1995; Green et al. 2002), whilst others have not (Wilson et al. 1993; Endo et al. 1994). Sharman et al. (2008) argue that when all data is considered, blood flow in the recovery period after exercise is lower with NO synthase inhibition, and that when the change in blood flow from baseline is evaluated, NO synthase inhibition does not have a significant effect on the net dilatory response to exercise (Shoemaker et al. 1997; Joyner & Dietz 2007). Therefore they argue, with support from non-significant data from their study and a recent study by Munir et al. (2007) that the influence of

peripheral vasodilation on central BP indices such as augmentation index, maybe largely unaffected with NO synthase blockade during exercise, but a response following exercise may be observed.

Central vs Peripheral Artery Responses to Dynamic Exercise

Whilst the studies examining peripheral artery responses to exercise have produced concurring results, the central artery response to exercise remains somewhat less clear. To date, a small number of studies, each employing different exercise protocols, have observed indices of central artery stiffness after dynamic exercise. For example, in accordance with the study by Kingwell et al (1997) mentioned above, Heffernan et al. (2007) have shown aortic PWV to be reduced 20-mins post exercise indicating reduced aortic stiffness, although aortic stiffness was unchanged immediately following exercise. However, studies by Sharman et al. (2005) and Lydakis et al. (2008) have shown decreased pulse transit time (TR) during dynamic exercise, indicating increased central arterial stiffness. In the study conducted by Sharman et al. which involved incremental cycling exercise performed up to 80% of maximal HR, a significant rise in PP amplification (the peripheral PP/central PP ratio (PPP/PPP) and a fall in AI were also observed. The recent study by Lydakis et al. did however not observe a significant rise in PPP/PPP or a change in AI. These differences are however likely attributable to the differences in exercise intensity, since this dynamic exercise protocol only involved one-legged exercise which will have only utilised a fraction of the maximal cardiac output.

Overview & Thesis Aims

This review has highlighted that the large arteries may serve as key sites for the distribution of CO and the active regulation/maintenance of BP during exercise. We have seen that arterial stiffness may be actively regulated by both systemic factors as well as local vasodilatory stimuli. During physiological stress increased sympathetic tone and/or BP can cause acute changes to the walls of the central arterial vasculature. It is speculated that in these central arteries, concomitant constriction of the vascular smooth muscle cells transfers the load from the elastin fibres to the stiffer collagen fibres leading to increased central artery stiffness. Following the withdrawal of sympathetic stimulus and the ensuing BP fall, a swift decrease in central arterial stiffness is observed.

In the peripheral arteries, dynamic exercise has been shown to have the ability to dilate the muscular arteries and cause peripheral artery stiffness to fall. This lowers the amplitude of the reflected wave and reduces central pressure augmentation and thereby increase the PPP/PPP ratio; an effect that will enhance V-A coupling. In contrast, in vascular beds where a local vasodilatory stimulus is not present, increased sympathetic tone may lead to a stiffening of both the central and peripheral arteries.

This thesis sought to further examine the effects of acute physiological and psychological stress on arterial function. Specifically, the following studies examine the interaction of local vasodilatory stimuli and sympathetic vasoconstrictor influences on arterial function during differing forms of stress.

Chapter 2

Methodology

This chapter will discuss the various techniques and methodologies employed throughout the experimental chapters of this thesis. Details of all subsequent data analysis and calculations are also included.

Haemodynamic Measurements

Blood Pressure (Chapters 3, 4, and 5)

Continuous beat-to-beat blood pressure (BP) was assessed using a non-invasive, bio-impedance monitor (Task Force[®] Monitor). Finger BP was measured using the ‘vascular unloading technique’. The monitor calibrated finger BP values using BP measured at the brachial artery in the opposite arm. This method has been shown to measure beat-to-beat BP with accuracy: $\pm 1\text{mmHg}$ (absolute values $\pm 5\text{mmHg}$ (Task Force[®] Monitor)).

Blood Pressure (Chapter 6)

In chapter 6, brachial-cuff blood pressure was measured in the right arm using standard sphygmomanometry. A cuff placed over the brachial artery was attached to a freestanding aneroid sphygmomanometer (Accoson CE 0120) with a pressure range from zero to three hundred. Systolic blood pressure (SBP) was defined as the first “beat” heard during cuff deflation (Korotkoff I) and diastolic blood pressure (DBP) was defined as the last beat heard (Korotkoff V). During all measurements, the sphygmomanometer was level with the subject’s heart and the arm was kept in a relaxed and supported position. BP measurements using this technique were performed by an experienced cardiac technician.

Electrocardiogram

A standard 3-lead electrocardiogram (ECG) was used to measure heart rate continuously throughout each protocol (Task Force[®] Monitor). An ECG uses electrodes on the skin to provide details of the electrical activity in the heart. The ECG will consist of a P wave, a QRS complex and a T wave. The P wave indicates atrial contraction; the QRS complex a marker of ventricular contraction and the T wave a marker for ventricular relaxation. As ventricular contraction signals stroke volume ejection, the QRS complex of the ECG is used to assess the heart rate at any given time. The Task Force monitor sampled at a rate of 1000Hz.

Stroke Volume and Cardiac Output

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 $\frac{DZ}{DT}(t)$ and $Z_0(t)$ new electrodes were designed. The ICG signals $\frac{DZ}{DT}(t)$ and $Z_0(t)$ are used for the detection of stroke volume, whilst a newly developed signal processing tool is used to eliminate the mechanical activity associated with breathing, to detect the maximum $\frac{DZ}{DT}$ signal (C-point), the aortic opening point (B-point) and the aortic closing point (X-point). 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).

To confirm the accuracy and reliability of the haemodynamic measures used, our laboratory has previously conducted a small reproducibility study (ten subjects) during a maximal exercise test on a stationary cycle ergometer. The protocol began with ten minutes of

baseline and then subjects began cycling 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). Similar increases in load and speed were applied in order to achieve three minute intervals at 120, 150 and 180 Watts. The table below outlines the reproducibility (coefficient of variation) of our measures at baseline, exercise (at 60, 120 and 180 Watts) and the first reading following exercise cessation.

Table 2.1. Reproducibility (coefficient of variation) of haemodynamic measurements at rest, during exercise and recovery.

	Baseline	60 Watts	120 Watts	180 Watts	Recovery
Systolic BP	2.8%	3.2%	4.6%	5.4%	3.6%
Diastolic BP	3%	4%	5.2%	5.8%	3.9%
Stroke Volume	5%	7%	8%	8.9%	6.8%
Cardiac Output	4.8%	7.6%	9.2%	11.1%	8.4%

Arterial Function

In summary, peripheral (femoral–tibial) pulse wave velocity was measured using an oscillometric technique, whilst central arterial variables were assessed via pulse wave analysis using the Sphygmocor system.

Femoral-Tibial Pulse Wave Velocity (Chapters 4 and 5)

Femoral-Tibial PWV (FTPWV) was measured simultaneously and non-invasively by oscillometry (time resolution +/- 2ms; QVL P84, SciMed, Bristol, UK) using a method developed to identify waveforms by their early phase in order to improve wave recognition and timing. Non-occlusive cuffs were placed over the mid thigh (femoral) and ankle (tibial), and connected to computerised pressure transducers by non-compliant tubing. The cuffs were inflated to 65-70mmHg and pulse pressure waveforms caused by volume displacement were obtained from each cuff. Waveforms were characterised by a computer programme with respect to time at 30, 40 and 50% of peak pressure along their ascending phase. The programme was designed to discard ectopic beats and abnormally shaped waveforms. The transit time between cuffs was calculated using an average of the three points (30, 40 and 50%), for 15 continuous beats. PWV was derived as the distance between the proximal edges of each cuff (mm) divided by pulse transit time (ms). This technique has been shown to give within day (minute to minute variation) of 2.2% and between day reproducible (10 minute averaged) of 5.6% (Naka et al. 2003).

Pulse Wave Analysis (PWA) (Chapters 3, 5 and 6)

Radial artery pressure waveforms were acquired using applanation tonometry by placing a high-fidelity strain gauge transducer over the radial artery. Applanation tonometry has previously been shown to record a pressure wave that does not differ from waveforms obtained from intra-arterial measurements (Kelly et al. 1989). The radial waveform was calibrated using measures of SBP and DBP and central aortic pressure waveforms were generated using pulse wave analysis by a generalised transfer function with the Sphygmocor Px system (AtCor Medical, Sydney, Australia). 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).

AI is defined as the ratio of reflected wave amplitude (pressure augmentation) and pulse pressure, or $AI = (SBP - P_i)/(SBP - DBP)$, where SBP and DBP are systolic and diastolic pressures respectively, and P_i is the inflection point marking the beginning upstroke of the reflected pressure wave. As HR has been shown to effect augmentation index the Sphygmocor Px system applies a generalised correction factor to establish AI at a heart rate of 75 (AI@7HR5).

Timing of the reflected wave (TR) is a measure (in ms) of the time it takes to for the reflected wave to travel to the periphery and back to the heart and is defined as the time delay from the foot of the pressure wave and the inflection point on the central waveform. A stiffer arterial system results in faster wave travel and quicker return of the reflected wave and thus a lower TR.

The use of a transfer function to approximate the central pressure wave has been validated using intra-arterially (Chen et al. 1997) and non-invasively (Gallagher et al. 2004) obtained radial pressure waves. The largest and most comprehensive study analysing the use of a generalised transfer function was performed by Pauca et al. (2001). This study assessed 62 anaesthetised patients before initiation of cardiopulmonary bypass, both before and during intra-venous infusion of nitro-glycerine (NTG), with fluid filled manometer systems. Sphygmocor derived waveforms were compared with simultaneously intra-arterially recorded aortic waveforms. Correspondence between pressure values from Sphygmocor derived and measured aortic waveforms was excellent. The differences between Sphygmocor derived aortic and measured aortic pressures were as follows: SBP 0.0 ± 4.4 mmHg, DBP 0.6 ± 1.7 mmHg and PP 0.7 ± 4.2 mmHg. Pressure differences remained of the same order during infusion of NTG. Further validation of the use of a transfer function under differing haemodynamic states has been provided by Soderstrom et al. (2002) who assessed responses to NTG infusion, and Sharman et al. (2006) who validated the transfer function during low level aerobic exercise.

A number of studies have been performed to assess the reproducibility of performing Sphygmocor PWA measurements. A high level of reproducibility and repeatability has been shown during each of these studies over a range of patient groups. For example Wilkinson et al. (1998) studied the reproducibility of AI on 33 subjects (5 controls, 12 diabetics and 16 hypertensives) aged between 24 and 67 years. Two investigators performed two readings in random order. The inter-observer difference was $0.23 \pm 0.66\%$ and the intra-observer difference was $0.49 \pm 0.93\%$. Subsequent studies yielding very similar results have been undertaken by Seibenhofer et al. (1999), Filipovsky et al. (2000) and Savage et al. (2002).

Forearm Blood Flow

Forearm blood flow (FBF) was measured using strain gauge plethysmography. Mercury in silastic strain gauges were used for the measurement of FBF (D.E. Hokanson, Inc., Bellevue, Washington), as described previously (Gunaruwan et al., 2002). For each measurement, a cuff placed around the upper arm was inflated to 40mmHg with a rapid cuff inflator (E20, D.E. Hokanson, Inc., Bellevue, Washington) to occlude venous outflow from the extremity. Flow measurements were recorded for approximately 15seconds; 4 readings were obtained for each mean value.

Stress Tests

Inducing reproducible physiological or psychological stress was of paramount importance to the studies carried out during this thesis. The following methods were used:

Isometric Calf Plantar-Flexion (Chapter 4)

Subjects lay supine in a custom built isometric dynamometer designed to measure ankle plantar flexion force. 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. Maximal voluntary force (MVC) was determined as the highest value obtained on three attempts; separated by 2min. Subjects' MVC was used to calculate the force required for each protocol.

Isometric Handgrip Exercise (Chapters 3 and 4)

Isometric handgrip exercise was performed with the right hand on a Lafayette hand dynamometer. As for isometric calf exercise, MVC was determined prior to each protocol.

Arithmetic Stress Test (Chapter 6)

A widely used and validated mental arithmetic stress test was used. 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.

Haemodynamic and Arterial Calculations

The haemodynamic and PWA obtained allowed the calculation of the following indices:

$$\text{Pulse Pressure (PP)} = \text{SBP} - \text{DBP}$$

$$\text{Mean Blood Pressure (MAP)} = \text{DBP} + (\text{PP}/3)$$

$$\text{Total Peripheral Resistance (TPR)} = \text{MAP}/\text{CO}$$

$$\text{Arterial Compliance (AC)} = \text{SV}/\text{PP}$$

$$\text{Arterial Elastance (AE)} = \text{end-systolic pressure (ESP)} \text{ derived from the central waveform}/\text{SV}$$

Ventricular-arterial Coupling (Chapter 6)

Ventricular-arterial (V-A) coupling was assessed using the $\text{AE}/\text{E}_{\text{es}}$ ratio (Sunagawa et al. 1983). LV end-systolic elastance (E_{es}) was calculated using the modified single-beat method of Chen et al. (2001), which has been validated against invasive measures, showing excellent correlation without systematic bias. This method employs brachial blood pressures, echo-Doppler SV, echo-derived EF and an estimated normalised ventricular elastance at arterial end-diastole (E_{nd}). Echocardiograms were performed with subjects supine (Vivid 7 echo machine) using a wide-band frequency-fusion phase-array transducer by an experienced echo-technician with accreditation from the British Society of Echocardiography.

E_{es} was calculated by the following formula: $\text{E}_{\text{es}} = (\text{dBP} - [\text{E}_{\text{nd}} \times \text{sBP}]) / (\text{E}_{\text{nd}} \times \text{SV})$.

Chapter 3 - Study 1

Vascular function and aortic wave reflections during isometric exercise: the role of the muscle metaboreflex.

Abstract

Over recent years there has been an increasing recognition that changes in the tone of the vascular smooth muscle in large arteries influences their distensibility. The aim of this investigation was to determine the acute effects of the sympatho-excitatory muscle metaboreflex on the timing and amplitude of central aortic wave reflection and central BP. Ten healthy subjects performed 2min of either non-ischemic isometric handgrip exercise (HG, control condition) or ischemic handgrip (IHG) at 40% maximal voluntary force. IHG was followed by 2min PECO (post exercise circulatory occlusion) to maintain muscle metaboreflex activation. BP and HR were measured continuously throughout using a bio-impedance monitor. Radial pulse-waveforms were recorded every minute throughout the study by applanation tonometry in the non-exercising arm. Central aortic waveforms were assessed using the Sphygmocor pulse wave analysis system. The central waveform allows central BP, augmentation index (AI) and timing of the reflected wave (TR) to be measured. Handgrip exercise during both trials (HG and IHG) induced significant increases in HR and peripheral and central BP's. Significant increases in AI, and decreased TR were observed during both minutes of HG. Immediately following exercise these variable returned to baseline. IHG exercise evoked vascular responses of the same direction and magnitude as those seen during HG (increased AI and decreased TR). During recovery with PECO, whilst BP's but not HR remained elevated, these measures stayed changed from baseline at the same levels seen during exercise. In conclusion, increased sympathetic outflow during muscle metaboreflex activation is associated with marked increases in systemic arterial stiffness and pulse wave reflections leading to an augmentation of central BP.

Introduction

Until recently large artery function was thought to relate virtually entirely to the structural characteristics of the vessel wall, reduced distensibility with age being a consequence of loss of elastin and collagen cross-linking (Cameron et al. 1995). Over recent years there has been an increasing recognition that changes in the tone of the vascular smooth muscle in large arteries also influences their distensibility (Kingwell et al. 1997; Matsuda et al. 1989; Koutsis et al. 1995). This tone is determined by endothelial, humoral and neural factors (Ramsey et al. 1995). Changes in large artery distensibility may have considerable impact on cardiac performance during exercise. Acute increases in impedance are known to cause an acute impairment of LV active relaxation, and it has been proposed that this may be an important mechanism responsible for the syndrome of Heart Failure with Preserved Left Ventricular Ejection Fraction (Kawaguchi et al. 2003; Kass 2005).

Changes in microvascular tone during exercise and the regulatory mechanisms responsible for these changes have been well described. These mechanisms ensure diversion of blood flow to exercising skeletal muscle (Buckwalter and Clifford 2001). In contrast, until recently the effect of exercise on large artery function has received little attention. Acute alterations in arterial stiffness have been observed following single bouts of exercise, but this response is dependent on the exercise mode. Whilst acute aerobic exercise is associated with regional increases in peripheral arterial distensibility (Kingwell et al. 1997; Naka et al. 2003; Sugawara et al. 2004; Heffernan et al. 2007, Munir et al. 2008), the relationship between resistance exercise and arterial function is less clear (DeVan et al. 2005; Heffernan et al. 2006; Heffernan et al, 2007).

Increased sympathetic drive induced via mental arithmetic (Boutouyrie et al. 1994) and cold exposure (Edwards et al. 2006) has been shown to have a stiffening influence on the arterial system. The skeletal muscle metaboreflex has long been known to induce sympathoexcitation, and to contribute to the vasoconstriction in non-exercising microvascular beds and restrain the microvascular dilation seen in exercising limbs (Hansen et al. 1994). However the impact of this reflex on arterial function is currently unknown.

Alterations in the stiffness of the systemic arterial system during exercise may have dramatic effects on the central pressure wave. Increased arterial stiffness is known to increase central SBP and PP as a result of increased wave reflections and a reduction in the ability of the aorta and large central arteries to buffer and absorb pressure waves from the heart (Nichols & O'Rourke 2005). The muscular arteries also play an important role by altering the speed of pressure wave travel along their length they can determine when reflected waves return back at the heart, whilst the peripheral vasculature and arterioles serve as key reflectance sites.

Reflected wave speed and amplitude affects peak central SBP but not peripheral pressure (Vlachopoulos et al. 2001), and therefore brachial cuff BP will not reveal these effects. Central BP reflects left ventricular (LV) afterload, and is a key determinant of myocardial oxygen consumption and coronary perfusion pressure. Therefore the effects of metaboreflex activation on BP (and myocardial oxygen demand) maybe greater than previously thought. The purpose of this study was therefore to determine the effects of metaboreflex activation on the timing and amplitude of aortic wave reflection and central pressure.

Isometric exercise lends itself to this type of study because the influence of the metaboreflex on cardiovascular function can be readily controlled (Fisher and White 2004). It has long been established (Alam and Smirk 1937) that this reflex can be evaluated using the technique of post exercise circulatory occlusion (PECO), which traps metabolites in a previously exercised muscle and sustains the sympathoexcitatory stimulus at the same levels seen during exercise (Victor and Seals 1989; Fisher et al. 2005). Isometric exercise will also allow measures of central wave reflection to be conducted during exercise, which is not possible during other exercise modes because of inevitable movement artefacts.

It was hypothesised that isometric handgrip exercise would result in an early return of reflected pressure waves from the periphery and in increase in central SBP as result of vasoconstriction throughout the arterial tree. It was also hypothesised that PECO following ischemic exercise would maintain this stiffening effect.

Methods

Subjects

10 subjects (5 male) aged 22 ± 1 yr (mean \pm standard error of the mean, SEM) volunteered to participate in this study. All subjects were non smokers and refrained from caffeine and alcohol for at least three hours before testing. 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).

Experimental Protocol

Each subject underwent two experiments in a randomised manner on separate visits to the laboratory. Prior to each trial maximal voluntary force (MVC) was determined on a Lafayette hand dynamometer using the right hand. After a supine 10min equilibrium period, 10min of baseline recordings were made after which the subject performed 2min of either non-ischemic isometric handgrip (HG, control condition) or ischemic handgrip (IHG) at 40% MVC. IHG was followed by 2min PECO to maintain metaboreflex activation. All measurements were made at baseline, during exercise and for 3min following exercise. A schematic diagram of the experimental protocol is shown in figure 3.1 overleaf.

Figure 3.1:

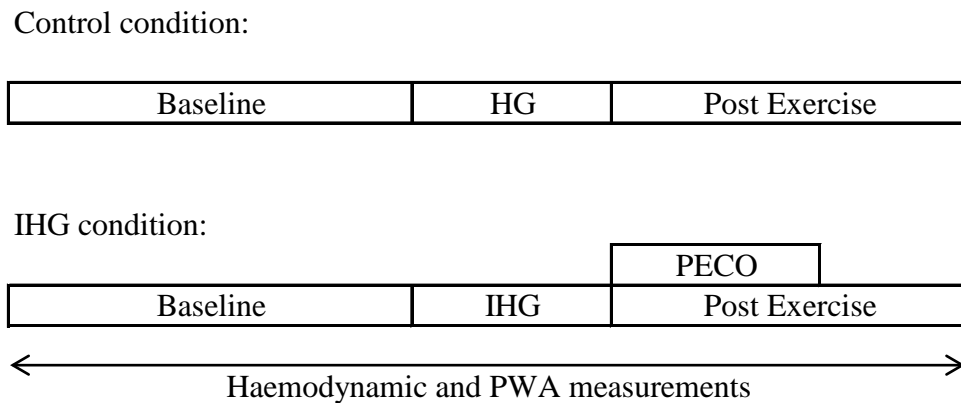


Figure 3.1. Schematic of experimental protocol. HG, handgrip; IHG, ischemic handgrip; PECO, post exercise circulatory occlusion.

Measured Variables

Continuous beat-to-beat BP was assessed using the Task Force® Monitor at the middle finger of the left hand. A standard 3-lead ECG was used to measure HR continuously during the entire protocol.

Radial artery pressure waveforms were acquired using applanation tonometry (Millar Instruments) and central pressure waveforms were generated using pulse wave analysis (PWA) (Sphygmocor, AtCor Medical). The central waveform yields central aortic pressures, augmentation index (AI), heart rate corrected augmentation index (AI@HR75) and the timing of the reflected wave (TR). 5 waveforms (acquired over 10min), were obtained for mean baseline values, whilst waveforms were acquired every minute during exercise and recovery. Average values for cardiovascular and PWA variables were calculated over the whole 10min baseline period and each successive minute of exercise and recovery periods.

Statistical Analysis

All values are expressed as mean \pm SEM. 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

Control Condition

Table 3.1 displays average haemodynamic data for baseline, exercise and recovery periods in both control and IHG protocols. In the control condition, HR significantly increased during exercise and promptly returned to baseline immediately following exercise cessation. Similarly, handgrip exercise induced significant increases in both central and peripheral BP's ($P < 0.05$ for all), all of which returned to baseline levels within the first minute of recovery. It is clear from figures 3.2 that isometric exercise evoked marked vascular responses. Significant increases in AI, and decreased TR were observed in both minutes of the exercise period ($P < 0.05$ for all). AI@HR75 also significantly increased during both minutes of handgrip exercise ($P < 0.05$). Immediately following exercise, these variables returned to pre-exercise levels.

IHG Condition

Table 3.1 shows that all haemodynamic variables (HR, and both peripheral and central BP's) significantly increased during IHG exercise. During PECO, HR returned to baseline, whilst peripheral and central BP remained significantly elevated. Following PECO, during the 3rd minute of recovery, these variables returned to pre-exercise levels and remained unchanged thereafter. IHG exercise evoked vascular responses of the same direction and magnitude as those seen in the control condition (increased AI and AI@HR75 and decreased TR) as seen in figures 3.2. However, during recovery with PECO, whilst BP's remained elevated, these measures stayed changed from baseline at the same levels seen during exercise. Following PECO, during the 3rd minute of recovery, all measures of vascular function returned to baseline.

Table 3.1. HR and peripheral and central pressure changes during handgrip exercise (control condition) or during ischemic handgrip with PECO (IHG conditions).

	Baseline	Ex 1	Ex 2	Post 1	Post 2	Post 3
HR (bpm)						
Control	75 ± 2	87 ± 3 *	86 ± 4 *	79 ± 3	78 ± 2	75 ± 3
IHG	79 ± 3	87 ± 4 *	93 ± 4 *	76 ± 4	78 ± 4	75 ± 4
SBP (mmHg)						
Control	124 ± 5	131 ± 5 *	134 ± 6 *	126 ± 4	127 ± 4	126 ± 4
IHG	124 ± 4	138 ± 6 * †	149 ± 7 * †	148 ± 7 * †	145 ± 7 * †	127 ± 5
DBP (mmHg)						
Control	73 ± 4	78 ± 4 *	83 ± 5 *	73 ± 4	72 ± 4	73 ± 4
IHG	70 ± 3	84 ± 3 *	93 ± 4 * †	88 ± 3 * †	87 ± 4 * †	74 ± 3
cSBP (mmHg)						
Control	104 ± 4	113 ± 3 *	115 ± 4 *	107 ± 4	105 ± 4	105 ± 3
IHG	101 ± 3	115 ± 4 *	124 ± 6 *	121 ± 6 * †	119 ± 6 * †	105 ± 3
cDBP (mmHg)						
Control	74 ± 4	81 ± 3 *	83 ± 4 *	75 ± 4	75 ± 4	75 ± 3
IHG	72 ± 4	85 ± 4 *	92 ± 4 *	86 ± 4 * †	85 ± 4 * †	76 ± 2

HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; * indicates significantly different from respective baseline value. † indicates difference between control and IHG.

Figure 3.2:

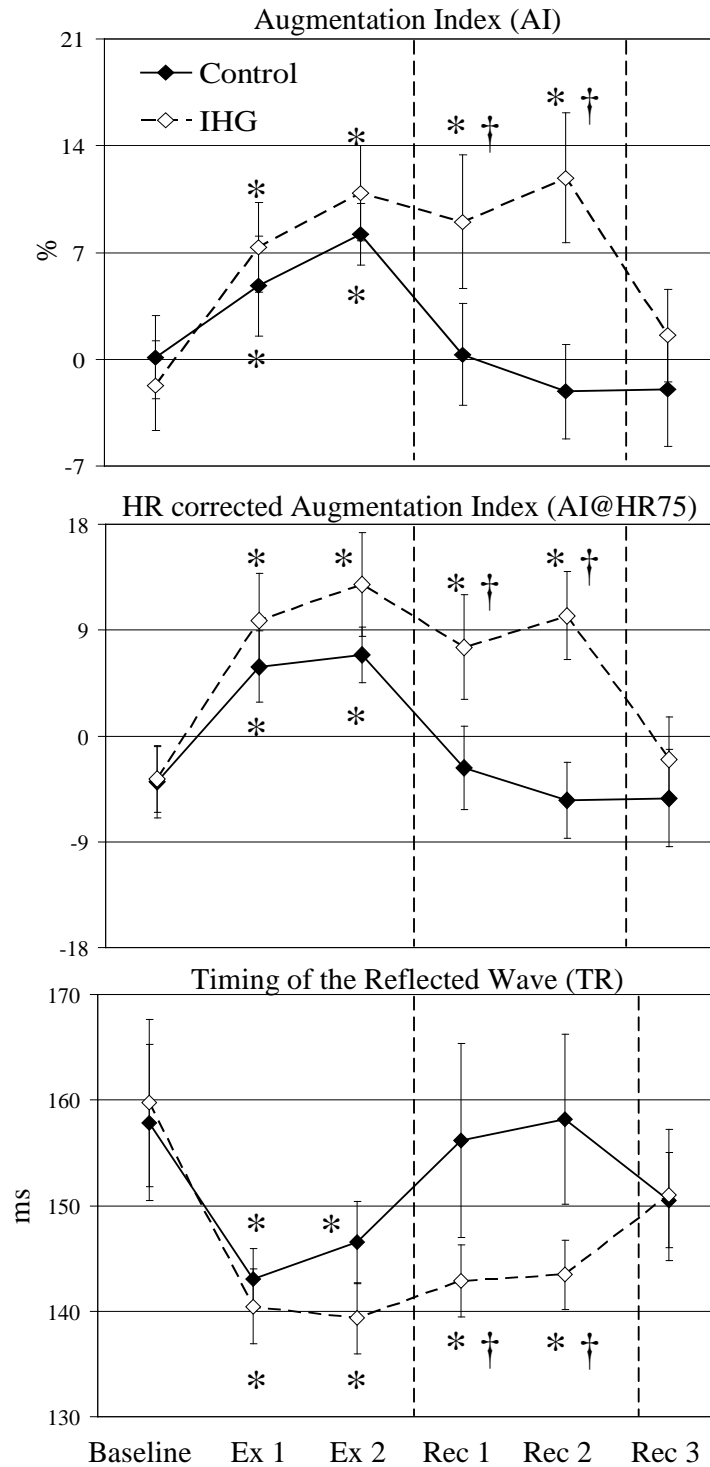


Figure 3.2. AI and TR at baseline, during handgrip exercise (control or IHG), and in recovery with (IHG) or without (control) PECO. Dashed vertical lines indicate PECO period. * = different from respective baseline value. † = difference between control and IHG.

Discussion

The present study investigated the acute effects of isometric exercise on large artery function and pulse wave reflections. PWA was performed during isometric exercise and during PECO (following the IHG trial) to ascertain the effects of sustained sympathoexcitation on arterial function and central BP. The major findings demonstrate that AI, a measure of wave reflection and an indicator of systemic arterial stiffness, was significantly elevated during both isometric handgrip trials and during PECO following the IHG trial. Additionally, TR was substantially reduced during exercise and PECO, indicating increased aortic stiffness and premature return of reflected waves to the heart. Both brachial cuff and derived central pressures were significantly increased during handgrip and metaboreflex activation. Increases in central pressure can be attributed to metaboreflex induced increases in pulse wave reflections and systemic stiffening of the arterial system.

Vascular Responses to Isometric Exercise

Both isometric exercise trials evoked marked vascular responses. Although Lydakis et al. (2008) have recently published findings from a similar experimental protocol to the present study, to our knowledge we were the first to examine central artery function and pulse wave reflections during isometric exercise. The results from the present study reveal that isometric exercise is associated with marked increases in systemic arterial stiffness and pulse wave reflections (increased AI and decreased TR). This may have important clinical implications as reductions in arterial distensibility, as seen here, result in increased amplitude and premature return of reflected arterial pressures waves that lead to the observed augmentation of central BP. Increased central aortic BP may have a deleterious effect on LV workload, myocardial oxygen consumption and coronary perfusion pressure. These effects would not be seen using

traditional brachial cuff BP measurements. Representative radial and aortic pressure waves demonstrating these effects are presented in figure 3.3 below:

Figure 3.3:

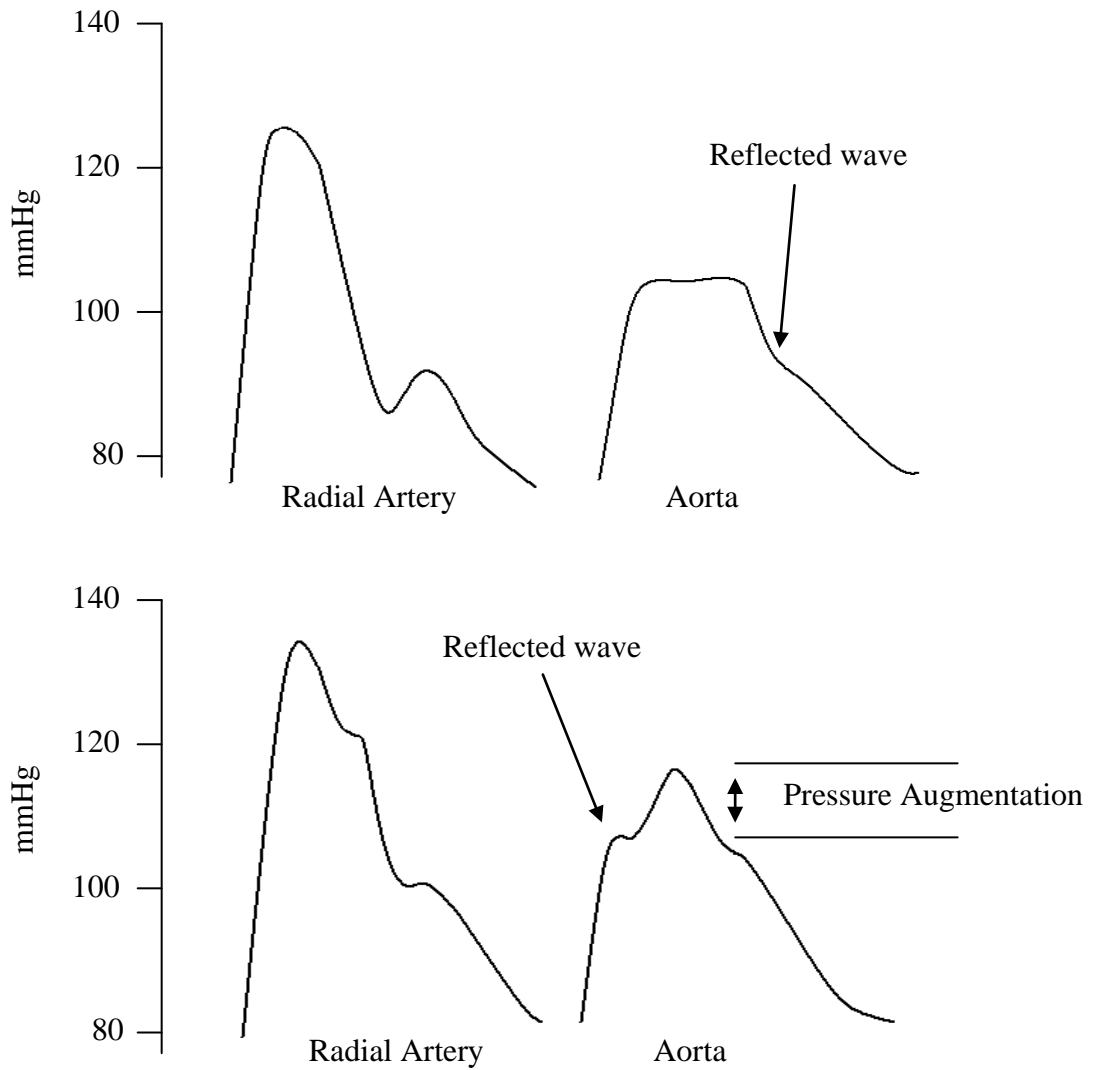


Figure 3.3. Representative radial and central aortic pressure waveforms at baseline (top) and during handgrip exercise/metaboreflex activation during PECO (bottom).

The Effects of Metaboreflex Activation

Sympathetic nerve activity has been reported to exert a tonic stiffening influence on the arterial walls (Gerova 1969). Arterial 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 (Boutouyrie et al. 1994), and smoking (Failla et al. 1997), i.e. manoeuvres that cause sympathetic activation. In addition, Edwards et al. recently (2006) demonstrated that cold exposure and the resulting sympathetic activation and peripheral vasoconstriction increased wave reflections and central systolic BP.

A period of circulatory occlusion following isometric exercise has been widely used to maintain muscle metaboreflex activation and cause sympathoexcitation (Wallin et al, 1989). PECO entraps the metabolic results of exercise and therefore sustains afferent feedback from the exercised muscle (Alam and Smirk 1937). Muscle metaboreflex activation typically evokes parallel sympathetic activation in exercising and resting human skeletal muscle (Hansen et al. 1994) that maintains or elevates arterial pressure (Lind et al. 1964). In the present study, maintained muscle metaboreflex activation during PECO resulted in increased systemic arterial stiffness and wave reflections that were similar to those seen during exercise. As BP was elevated at this time, these results are consistent with muscle metaboreflex-mediated vasoconstriction. Since the peripheral muscular arteries are under greater sympathetic innervation than central elastic arteries it is likely that this vasoconstriction lead to a decrease in diameter of the muscular arteries and arterioles, increasing pressure wave velocity and potentially altering the site of wave reflection.

The Potential Influence of Heart Rate and Blood Pressure

Although previous research has demonstrated an inverse, linear relationship between HR and AI, HR can probably be discounted as influencing the central pressure waveform in the present study. HR was indeed increased during exercise in both trials when significant increases in AI were observed. However during PECO in the IHG trial, AI remained above pre-exercise values despite HR returning to baseline.

There was a significant decrease in TR from rest to exercise and during PECO following IHG. It is well known that arterial compliance is reduced with a rise in MAP (Liu et al. 1986). Therefore changes in arterial compliance occurring in the presence of MAP changes may simply be a consequence of the nonlinearity of the vascular pressure-volume relationship rather than an intrinsic change in vessel wall properties (Liu et al. 1986). It is therefore difficult to exclude the possibility that the rise in MAP seen during exercise and PECO will account for any increases in arterial stiffness. Indeed, Sharman et al. (2005) have demonstrated that aerobic exercise induced reductions in TR are strongly correlated to increases in MAP. As MAP increases there is a progressive recruitment of collagen fibres within vessel walls (Armentano et al. 1991), which will effectively stiffen the large central elastic arteries, resulting in increased PWV and decreased TR (Sharman et al. 2005).

Lydakakis et al. (2008) recently evaluated acute central (large artery) haemodynamic changes by measuring the wave reflections in two different haemodynamic models. The first model (isometric exercise) representing a state of elevated sympathetic tone with increased HR & BP, produced results similar to the present study. The second model (lower body negative pressure – simulating orthostasis) was used to represent a state of increased sympathetic drive

and HR, but without changes in BP. As measures of arterial stiffness were unchanged throughout the second protocol the authors concluded that central arterial function and haemodynamics were linked to BP rather than sympathetic tone per se. Given these findings, it is therefore difficult to determine whether the stiffening observed in the present study was due to altered smooth muscle tone or to changes in distending BP. Using local assessments of arterial wall properties (such as the ultrasound measures described earlier in this thesis) allows arterial compliance to be directly calculated and the use of such techniques would therefore enable the direct effects of sympathetic activation on the large arteries to be distinguished from the indirect influence of elevated BP.

The effects of the handgrip exercise (and arterial occlusion during PECO) on the site of wave reflection must also be considered. The sites of major wave reflection are determined by the impedance mismatch between the large central arteries, such as the aorta, and the smaller conduit (muscular) arteries branching from this artery. Handgrip exercise and maintained occlusion may alter the state of such arteries and it is therefore possible that the apparent stiffening observed here was due to a proximal shifting of the wave reflection point.

Conclusions

In conclusion, in the present study we used PWA to assess the effects of acute isometric exercise and sustained sympathetic outflow during PECO on parameters of central haemodynamics. Our findings were that AI, wave speed (decreased TR) and central BP were increased during metaboreflex activation.

Chapter 4 - study 2

Conduit artery responses to isometric exercise:
the interaction between local vasodilatory and systemic
vasoconstrictor factors

Abstract

The effects of exercise on the distensibility of large and medium-sized arteries are poorly understood, but can be attributed to a combination of local vasodilator effects of exercise opposed by sympathetic vasoconstrictor tone. This study sought to examine this relationship at the conduit artery level, with particular reference to the role of the sympatho-excitatory muscle metaboreflex. The effect of maintained muscle metaboreflex activation on a previously passive or exercised limb femoral artery was investigated. Ten healthy volunteers performed 2min of isometric ankle plantar-flexion at 40% MVC (maximal voluntary force), in conjunction with 2min of either non-ischemic isometric HG (handgrip; control condition) or IHG (ischemic HG) at 40% MVC. IHG was followed by 2min of PECO (post-exercise circulatory occlusion) to maintain muscle metaboreflex activation. FTPWV (femoral–tibial PWV (pulse wave velocity) was measured in the exercised or contralateral limb at baseline and immediately following calf exercise. BP and HR were measured continuously throughout. In the HG condition, BP and HR returned promptly to baseline post-exercise, whereas exercised leg FTPWV was decreased (less stiff) and the non-exercised leg PWV was not changed from baseline. PECO caused a sustained increase in BP, but not HR, in the IHG condition. Contralateral leg PWV increased (stiffened), whereas exercised limb FTPWV was not changed from baseline. In conclusion, muscle metaboreflex activation causes a systemic stiffening of the arterial tree, which can overcome local exercise-induced decreases in arterial PWV.

Introduction

Previous research has suggested that local rather than systemic factors modulate the peripheral arterial response to exercise (Kingwell et al. 1997; Naka et al. 2003; Sugawara et al. 2004; Heffernan et al. 2007). The results from Study 1, however demonstrate that isometric handgrip exercise induced increases in systemic arterial stiffness and wave reflections that caused a substantial augmentation of central BP. Furthermore, this effect is maintained during sustained sympathoexcitatory stimulus using post exercise circulatory occlusion (PECO). These findings are likely attributable to a stiffening of the muscular arteries and arterioles, speeding pressure wave travel and altering the site of wave reflection.

It is increasingly recognised that large artery distensibility is influenced by vascular smooth muscle tone, which is determined by endothelial, humoral and neural factors (Ramsey et al. 1995). This study aimed to investigate the interaction between local exercise induced dilatory factors and the systemic vasoconstrictor influence of sympathoexcitation following isometric exercise. Using PECO to manipulate systemic sympathoexcitation in a controlled fashion, whilst local dilator factors are still active in a previously exercised limb (Kingwell et al. 1997; Naka et al. 2003; Sugawara et al. 2003; Heffernan et al. 2007, Munir et al. 2008) allows their respective contributions to conduit artery function to be revealed. Additionally isometric exercise allows assessment of conduit artery function to be made immediately post-exercise (within 1 minute) using non invasive, pulse wave velocity (PWV) measures. This rapid assessment is not possible following dynamic exercise because of inevitable temporal delays and movement artefacts upon cessation of this exercise mode (Kingwell et al. 1997; Naka et al. 2003; Sugawara et al. 2003).

In this study subjects performed a standardised level of ischemic handgrip exercise to evoke a reproducible level of systemic sympathetic vasoconstrictor drive and this was sustained with subsequent forearm PECO (Victor et al. 1988; Fadel et al. 2003). We investigated the effect of simultaneous one legged isometric calf plantar flexor exercise on arterial stiffness immediately following the combined handgrip and leg exercise and during recovery with PECO. This was achieved by measuring PWV in the femoral-tibial arterial segment of the exercised and contralateral limbs. As a control, these PWV measures were repeated while subjects recovered from non-ischemic handgrip without forearm PECO.

It was hypothesised that in the control condition calf exercise would cause local arterial vasodilatation and therefore decrease femoral-tibial PWV (FTPWV) in the active limb but not in the inactive limb. With the addition of sympathoexcitation, caused by forearm PECO, it was expected that passive limb FTPWV would increase above resting levels. Finally, it was hypothesised that this systemic sympathoexcitation would override local vasodilator responses in the active limb and attenuate the decrease in PWV seen following calf exercise.

Methods

Subjects

10 subjects (5 male) aged 22 ± 1 yr (mean \pm standard error of the mean, SEM) volunteered to participate in this study. None smoked, were hypertensive (blood pressure $>140/80$ mmHg), 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 three hours preceding the experiments.

Experimental Protocol

Each subject underwent four experiments in a randomised manner on separate study days. Subjects lay supine in a custom built isometric dynamometer designed to measure ankle plantar flexion force. Isometric handgrip exercise was performed with the right hand on a Lafayette hand dynamometer. Maximal voluntary force (MVC) was determined prior to each study, with each exercise mode.

Before each trial, the subject rested for 10min in the supine position to establish a stable baseline. Each protocol began with 10min of baseline recordings after which the subject performed 2min of isometric ankle plantar flexion at 40% MVC, in conjunction with 2min of either non-ischemic isometric handgrip (HG, control condition) or ischemic handgrip (IHG) at 40% MVC. IHG was followed by 2min PECO to maintain metaboreflex activation. PWV in the exercised or contralateral femoral-tibial arterial segment was measured at baseline and for 5mins immediately following calf exercise. For each of the control and IHG protocols, PWV was measured in either the exercised or contralateral limb on separate study visits (4 study

protocols on total). A schematic diagram of the experimental protocol is shown in figure 4.1 below:

Figure 4.1:

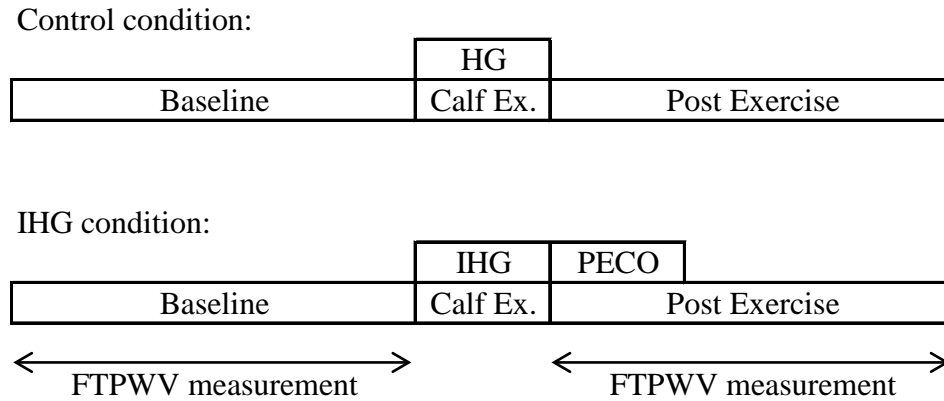


Figure 4.1. Schematic of experimental protocol. HG, handgrip; IHG, ischemic handgrip; PECO, post exercise circulatory occlusion.

Measured Variables

Continuous beat-to-beat BP was assessed using a non-invasive, bio-impedance monitor (Task Force® Monitor) at the middle finger of the left hand. A standard 3-lead electrocardiogram (ECG) was used to measure heart rate continuously during baseline, exercise and recovery periods. The monitor sampled at a rate of 1000Hz.

FTPWV was measured simultaneously and non-invasively by oscillometry (time resolution +/- 2ms; QVL P84, SciMed, Bristol, UK) as described in chapter 2. It is important to note that neither cuff nor leg was allowed to move during either protocol. HR and BP during

corresponding periods of PWV measurement (1min) were calculated from the Task Force data.

Average values for cardiovascular variables and PWV were calculated over the whole 10 minute baseline period and each successive minute of the 5 minute recovery period. In addition average values for blood pressure and heart rate at the end of the exercise period (last 10 seconds of exercise) were also calculated.

Statistical Analysis

All values are expressed as mean \pm SEM. 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

Statistical analysis (repeated measures ANOVA) revealed main effects for PWV and haemodynamic variables (HR and BP) over time but did not indicate interaction effects between these variables. Subsequent *post hoc* analysis was focused on analysis within trials, i.e. post exercise time points versus respective baseline data.

Control Condition

Table 4.1 shows average HR and BP values for the baseline, end exercise, and recovery phases of the trials performed to examine PWV in the previously exercised and contralateral limbs. HR and BP increased markedly during exercise, and in both trials HR and BP returned promptly to baseline levels within the first minute of recovery and remained at these levels thereafter.

FTPWV data is shown in Figure 4.2. It is clear that immediately following combined calf and handgrip exercise, when blood pressure had recovered to baseline, FTPWV in the previously exercised leg had declined ($P < 0.05$) by 0.6m/s, 0.6m/s and 0.5m/s relative to baseline, during minutes 1, 2 and 3 respectively. By minute 4 of recovery FTPWV had returned to baseline levels. In contrast, in the contralateral leg FTPWV was not significantly changed from baseline after the combined calf and handgrip exercise.

IHG Condition

During exercise, heart rate and blood pressure increased in both trials. During PECO, heart rate returned to baseline, whilst blood pressure remained significantly elevated in both IHG

trials. Following PECO, during the 3rd minute of recovery, blood pressure returned to baseline and in both trials remained unchanged thereafter.

During the two minutes of PECO which followed combined calf and ischemic handgrip exercise and when blood pressure was markedly elevated above baseline, FTPWV in the exercised limb was not significantly changed from baseline ($P=0.1$) Upon release of PECO and recovery of blood pressure to resting levels, FTPWV remained statistically indistinguishable from baseline.

In the contralateral limb, FTPWV was initially increased during PECO ($P<0.05$) by 0.8m/s and 0.9m/s, in the first and second minutes, respectively, of recovery. It then returned to baseline upon release of the occlusion. The increase in FTPWV in this limb during PECO was in contrast to the unchanged FTPWV values seen in the exercised leg during PECO (responses post exercise vs. respective values were different). It also contrasts with unaltered FTPWV response in the contralateral limb ($p=ns$ vs. baseline) in the absence of PECO in the control condition.

Table 4.1. Blood pressure and heart rate changes following combined calf and handgrip exercise (control conditions) or following calf and ischemic handgrip with PECO (IHG conditions).

	Baseline	End Ex	Post 1	Post 2	Post 3	Post 4	Post 5
SBP (mmHg)							
Exercised	116 ± 4	148 ± 6*	123 ± 4	117 ± 4	115 ± 5	113 ± 5	112 ± 5
Contralateral	119 ± 3	149 ± 9*	130 ± 8	126 ± 6	125 ± 4	125 ± 3	126 ± 4
Contralateral (IHG)	121 ± 3	152 ± 7*	138 ± 5*	135 ± 4*	125 ± 3	121 ± 3	121 ± 3
Exercised (IHG)	120 ± 3	163 ± 7*	146 ± 6*	144 ± 6*	132±6	127 ± 6	124 ± 5
DBP (mmHg)							
Exercised	67 ± 2	92 ± 3*	66 ± 3	63 ± 2	61 ± 3	59 ± 3	59 ± 3
Contralateral	65 ± 2	89 ± 3*	71 ± 3	69 ± 3	68 ± 2	68 ± 3	70 ± 3
Contralateral (IHG)	70 ± 2	96 ± 3*	79 ± 3*	80 ± 2*	70 ± 2	67 ± 2	67 ± 2
Exercised (IHG)	65 ± 2	99 ± 7*	80 ± 4*	81 ± 4*	70 ± 5	66 ± 4	65 ± 4
HR (bpm)							
Exercised	64 ± 3	87 ± 4*	71 ± 3	62 ± 3	61 ± 3	60 ± 3	60 ± 3
Contralateral	64 ± 5	86 ± 6*	72 ± 5	62 ± 5	60 ± 5	61 ± 5	61 ± 4
Contralateral (IHG)	65 ± 4	90 ± 4*	71 ± 5	62 ± 4	62 ± 3	60 ± 3	60 ± 3
Exercised (IHG)	64 ± 3	88 ± 4*	72 ± 4	65 ± 4	63 ± 4	62 ± 3	59 ± 3

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; * indicates significantly different from respective baseline value.

Figure 4.2:

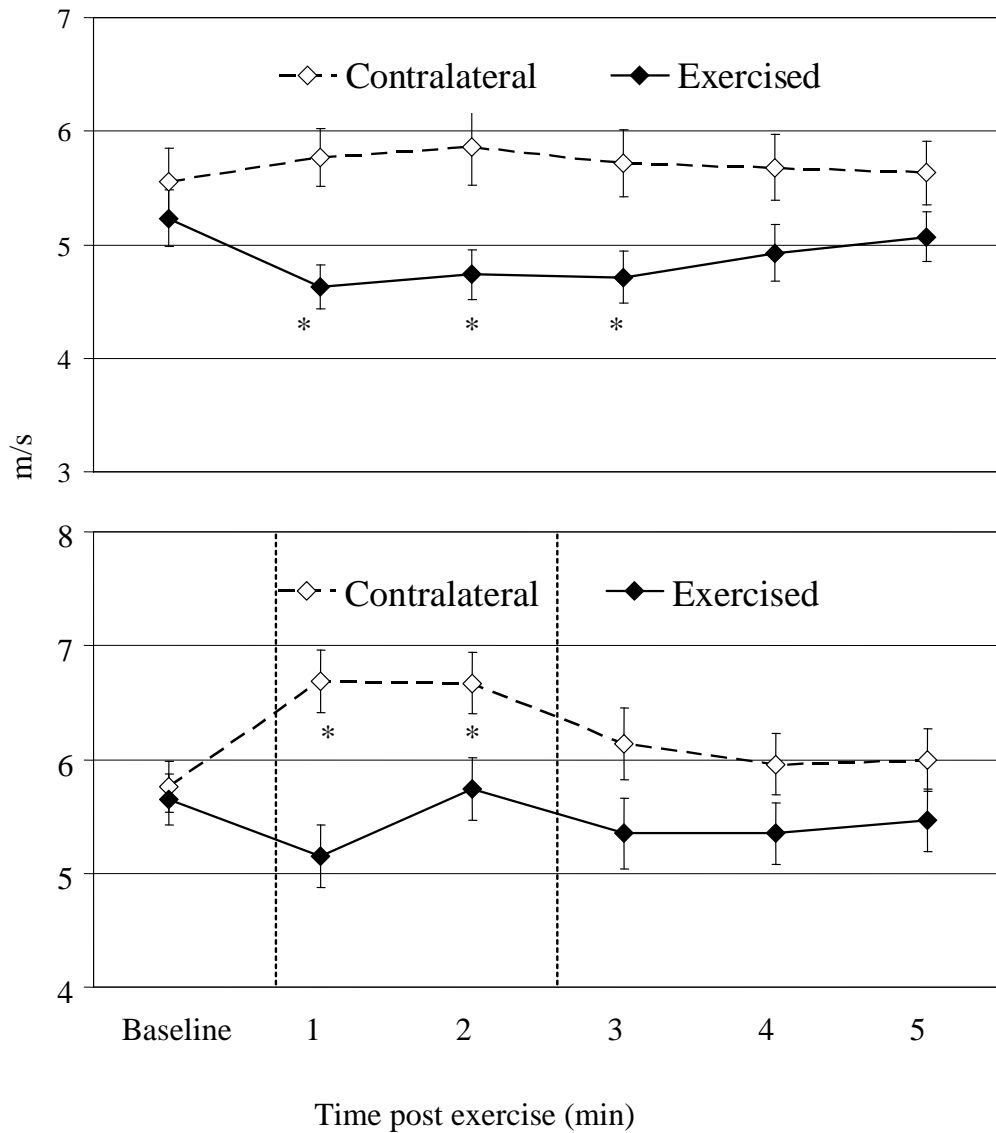


Figure 4.2. FTPWV in exercised and contralateral limbs following calf and handgrip exercise (top) or following calf and ischemic handgrip exercise with PECO (IHG) (bottom). Dashed vertical lines indicate PECO period; * indicates significantly different from respective baseline value.

Discussion

The present study investigated the acute effects of isometric exercise on large (conduit) artery distensibility. Immediately after exercise PWV was measured in the contralateral or exercised femoral artery to examine the influence of systemic and local factors on arterial function. In addition, the ability of exercise induced sympathoexcitation, mediated by muscle metaboreflex activation, to alter these responses was investigated.

Femoral Artery Responses to Isometric Calf Exercise

In the absence of PECO, required to sustain sympathoexcitation and elevated BP, arterial distensibility of the exercised limb was augmented for three minutes following calf exercise. This data is in accordance with all aerobic exercise studies to date (Kingwell et al. 1997; Naka et al. 2003; Sugawara et al. 2003; Heffernan et al. 2007, Munir et al. 2008). One idea used to explain the increase in arterial distensibility post exercise is that it is caused by exercise-related regional factors. 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 limb (Kingwell et al. 1997; Naka et al. 2003; Sugawara et al. 2003; Heffernan et al. 2007, Munir et al. 2008). Once local intramuscular vasodilatation has occurred then flow in the femoral artery will increase and cyclic circumferential stretch and shear stress on its endothelium will follow. This is a well known vasodilator stimulus (Pyke et al. 2005) which would facilitate the increased muscle blood flow. Therefore the observed increase in distensibility immediately post exercise in the control condition 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 (see below). Certainly

systemic sympathoexcitation caused by muscle metaboreflex activation during the handgrip and calf exercise would be expected to oppose vasodilatation during exercise (Hansen et al. 1994; Victor et al. 1988; Fadel et al. 2003). 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 recovery of BP in the control experiments. Finally, the fact that no change was seen in FTPWV in the contralateral limb following exercise, even though it would have experienced the same increased level of sympathetic drive during exercise as the exercise limb (Hansen et al. 1994), provides further support for the idea that the decrease in PWV in the exercise limb is likely dominated by local factors.

The Effects of Metaboreflex Activation

In accordance with the results from study 1, the present study reveals that elevated sympathetic outflow was associated with a stiffening of the arterial tree, as observed in the increased FTPWV of the contralateral limb femoral artery during PECO. The decrease in femoral artery distensibility in the contralateral limb during PECO, mirroring the BP rise at this time, is consistent with sustained muscle metaboreflex-mediated vasoconstriction. This data, to our knowledge, is the first to show a stiffening of the large conduit arteries during muscle metaboreflex activation. As the muscles were relaxed and there was no intention to perform exercise, we can rule out any contribution from muscle mechanoreflex or central command.

Local Dilatory Mechanisms vs. Increased Sympathetic Outflow

The interaction between functional vasodilation and sympathetic vasoconstriction and the phenomenon of “functional sympatholysis” has long driven studies of blood flow control (Buckwalter & Clifford 2001; Remensnyder et al. 1962; Hansen et al. 1996; Tschakovsky et al. 2002; Dinunno & Joyner 2003). Of particular interest to the present study are recent findings that the interaction between sympathetic nerve activity and vasodilatory stimuli seems to vary with vessel branch order (Haug & Segal 2005). The present study measures femoral-tibial arterial PWV between the mid thigh and ankle. As subjects performed isolated calf exercise these measures will provide information about the artery passing through the exercising muscle and also the conduit artery (mid femoral) proximal to the exercising muscle itself.

The ability of muscular contractions to limit the amount of vasoconstriction appears to be a local regulatory mechanism, particularly arising in the arterioles embedded within active muscle fibres, to ensure adequate blood flow and oxygen delivery to the contracting muscle (Anderson & Faber 1991; VanTeefelen & Segal 2003). Further upstream however, vasoconstriction is sustained, as sympathetic nerve activity seems able to impair ascending vasodilation of the feed arteries (Haug & Segal 2005; Anderson & Faber 1991; VanTeefelen & Segal 2003; Folkow 1971). Although ascending vasodilation in the conduit arteries is a controversial idea, data from the present study suggests that some interaction between local vasodilator metabolites and sympathetic tone may occur at this level of the branching network. Following exercise with PECO, the normal increase in arterial distensibility was not observed and this may be attributed to metaboreflex activation related sympathetic tone

abolishing this response. Alternatively, this observation may be a result of increased BP during this period (see below).

The Potential Influence of Blood Pressure Changes on PWV

It is well established that distending BP is a primary determinant of arterial distensibility. In this study the BP response curves are often closely related to the PWV changes observed. It is therefore difficult to exclude the possibility that the rise in MAP seen during PECO will account for any increases seen in PWV during this period. However, MAP increased during both IHG protocols yet the PWV responses of exercised and contralateral femoral arteries were markedly different. It is therefore unlikely that the arterial responses observed were entirely BP dependent. Furthermore in the control condition, exercised leg FTPWV significantly decreased despite unchanged BP during the recovery period. Changing pressure cannot thus be wholly responsible for the observed changes in FTPWV seen during these experiments. The PWV responses thus appear to reflect acute changes in vascular tone, which occur during recovery from exercise.

Method of PWV Measurement

As discussed in chapter 2 (methods section), PWV measured with the present technique has been shown to be sensitive to acute changes of vascular tone, independently of any associated change in blood pressure. PWV in the upper limb, for example, is decreased or increased ~10% by local intra-arterial acetylcholine or *NG*-monomethyl-L-arginine, whereas lower limb PWV, heart rate, and blood pressure are unchanged (Ramsey et al. 1995; Kinlay et al. 2001; Naka et al. 2000). In the present study PWV was measured over the same defined sections of the femoral-tibial arterial segment before and immediately after exercise. The results therefore

provide evidence of relative arterial distensibility changes, and as such the technique provides an objective, sensitive but robust and reproducible measure of acute changes in arterial distensibility as influenced by changes in vascular tone (Naka et al. 2003).

Conclusions

Isometric exercise induced an acute, local augmentation in large artery distensibility. Increased sympathetic outflow during muscle metaboreflex activation causes a systemic stiffening of the arterial tree. This sympathetically induced stiffening is able to overcome the local increase in distensibility in an exercised artery.

Chapter 5 – study 3

Vascular responses to acute mental stress and the role of Nitric Oxide

Abstract

The vascular and haemodynamic mechanisms underlying the BP response to acute mental stress (AMS) are not completely understood. This study sought to concurrently examine local and systemic changes in vascular resistance, arterial compliance and arterial wave reflection during AMS. In a second study, the role of changes in Nitric Oxide (NO) bioactivity in these responses was examined. AMS decreased total peripheral resistance (TPR), but increased femoral-tibial PWV, augmentation index (AI@HR75), arterial elastance (AE), and shortened the timing of the reflected wave (TR) indicating increased conduit and central arterial stiffness. To our knowledge, these results provide the first concurrent measurements of differential vascular responses to AMS and suggest that the pulsatile component of aortic BP is the major contributor to the increased BP observed during AMS. NO synthesis inhibition with L-NMMA significantly increased peripheral and central BP, TPR, AI@HR75, AE and FTPWV, whilst decreasing HR and forearm blood flow (FBF). However, L-NMMA had no significant effect on the magnitude of haemodynamic and vascular changes during AMS, that is, the haemodynamic and vascular response to AMS was preserved with NO synthesis blockade. We therefore conclude that NO has a significant basal role but changes in NO bioactivity do not significantly contribute to the haemodynamic and vascular responses to AMS.

Introduction

Central aortic BP reflects LV 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 MAP, is accurately described as a function of CO and TPR (St John 2000). The pulsatile component, estimated by PP, is affected by the patterns of left ventricular ejection, large artery stiffness and arterial pulse wave reflection (McVeigh et al. 1996). Therefore, it is clear that for a given cardiac performance, aortic BP is influenced by the entire vascular tree.

Chronic mental stress is increasingly recognised as a novel risk factor for coronary artery disease (Rozanski et al. 1999) left ventricular dysfunction (Wittstein et al. 2005), and sudden cardiac death (Leor & Kloner 1996). Furthermore, there is a clustering of cardiovascular events following stresses such as earthquakes or bereavement (Leor & Kloner 1996; Mittleman et al. 1995; Strike et al. 2006). The mechanistic link between stress and increased CV risk however, remains controversial. The haemodynamic response to AMS, which is partly mediated by sympathetic nervous system activation, includes increased CO and BP. AMS also induces endothelial dysfunction (Ghiadoni et al. 2000), 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 (Noll et al. 1996) and angiotensin II (Kosunen 1977). Cortisol may also be involved in provoking these changes (Broadley et al. 2006). 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 (Ghiadoni et al. 2000).

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 (Blair et al. 1959; Barcroft et al. 1960; Rusch et al. 1981; Halliwill et al. 1997), whilst Broadley et al. (2005) demonstrated that a brief episode of mental stress can induce a rapid impairment of the brachial artery response to flow-mediated dilation. To our knowledge, the only previous study looking at central arterial function and AMS has shown an increase in aortic stiffness and pulse wave reflections (Vlachopoulos et al. 2006). 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 (Halliwill et al. 1997), beta-adrenergic vasodilation (Halliwill et al. 1997; Lindqvist et al. 1997) and in particular flow-induced NO release (Cardillo et al. 1997; Dietz et al. 1994) may be of prime importance. Vascular NO, known to be a key regulator of basal vascular tone (Vallance et al. 1989), significantly contributes to circulatory modulation during various physiological stimuli, including forearm exercise hyperaemia (Dyke et al. 1995). Furthermore, as discussed in the literature review of this thesis, basal, stimulated or exogenous NO has been shown to act to reduce arterial stiffness *in vivo*, independently from

any changes in BP, highlighting the importance of the vascular endothelium in the functional regulation of arterial stiffness. 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 (Dietz et al. 1994), its role in the responses of other vasculature to AMS is yet to be determined.

The aim of this study was to investigate the haemodynamic and vascular responses underlying the BP response to AMS. We therefore sought to concurrently examine local and systemic indices of peripheral resistance, arterial compliance and wave reflections during an acute bout of mental stress. In a second study we aimed to examine the role of NO in modulating these responses. It was hypothesised that AMS would result in differential vascular responses, with a decrease in indices of peripheral resistance but an increase in the implied stiffness of the large and central arteries. Inhibition of NO synthase was expected to blunt the peripheral vasodilatory response to AMS.

Methods

Subjects

A total of 14 subjects (9 male) aged 27 ± 1.3 yr (mean \pm standard error of the mean, SEM) volunteered to participate in this study. None smoked, were hypertensive (BP $<140/80$ mmHg), 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 three hours preceding the experiments.

Experimental Protocol

In Study 1, we conducted PWA and measured CO, HR and BP responses, and femoral-tibial pulse wave velocity (FTPWV) during 6min of mental arithmetic in the prone position. During mental arithmetic subjects continuously subtracted the number 7 from a 4 digit number. Subjects answered verbally and were encouraged by an investigator to subtract as fast as possible. Each trial began with a 10min acclimation period and 10min of baseline measurements.

In study 2, the role of NO in the vascular response to AMS was examined in 10 subjects who had completed study 1 (7 men) using the same protocol as study 1. In addition to the measures obtained in study 1, measures of forearm blood flow (FBF) were also included. However due to methodological difficulties (a free arm is needed for both techniques) it was not possible to measure FTPWV and FBF simultaneously. Therefore, FBF was measured in 5 subjects and limb PWV measurements were conducted in the remaining 5 subjects. All other measurements were conducted in all 10 subjects. 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 NO 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. In study 2, each trial began with a 10min acclimation period and 10min of baseline measurements. This was followed by a 5min infusion period and then 10min of post infusion measurements before the AMS test measurement period.

The L-NMMA dosage used in this investigation is similar to that used in several previous studies performed both at rest (Haynes et al. 1993; Stamler et al. 1994) and during exercise (Sugawara et al. 2004; Sharman et al. 2008). This dose has been shown to significantly reduce serum concentrations of cGMP (Bech et al. 1996) and NO (Stamler et al. 1996), inhibit vasodilatation by acetylcholine (Dyke et al. 1995), and induce endothelial dysfunction (Doshi et al. 2001).

Measurements

SV, beat-to-beat BP and HR were measured continuously throughout both studies using a non-invasive, bio-impedance monitor.

Radial artery pressure waveforms were acquired using applanation tonometry (Millar Instruments) and central pressure waveforms were generated using PWA (Sphygmocor, AtCor Medical). The pulse wave analysis transfer function has been validated under differing haemodynamic states (Chen et al. 1997) and recently during supine, low level exercise (Sharman et al. 2006). The central waveform yields central aortic pressures (CBP), HR

corrected augmentation index (AI@HR75) and the timing of the reflected wave (TR). 5 measurements (acquired over 10min), were obtained for mean baseline values, whilst 6 measurements were acquired during AMS to obtain mean stress values.

FTPWV was measured simultaneously and non-invasively by oscillometry (time resolution +/- 2ms; QVL P84, SciMed, Bristol, UK) as described in chapter 2. Measurements were obtained every minute during acquisition periods.

FBF was measured using strain gauge plethysmography. Mercury in silastic strain gauges were used for the measurement of FBF (D.E. Hokanson, Inc., Bellevue, Washington), as described previously (Gunaruwan et al., 2002). For each measurement, a cuff placed around the upper arm was inflated to 40mmHg with a rapid cuff inflator (E20, D.E. Hokanson, Inc., Bellevue, Washington) to occlude venous outflow from the extremity. Flow measurements were recorded for approximately 15seconds; 4 readings were obtained for each mean value.

Data analysis

All values are expressed as mean \pm SEM. For study 1, data was analysed as the mean baseline and stress measurement periods. For study 2, data is expressed as mean baseline, mean post infusion baseline (PIB) and mean stress measurements periods. This data is also expressed as the as the change (delta) from post infusion to stress 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

Study 1: Central and peripheral vascular responses to AMS

Table 5.1 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 DBP ($P < 0.05$ for both).

As seen in figures 5.1, 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).

Table 5.1. Haemodynamic responses to AMS.

	Baseline	AMS		Base	AMS
HR (bpm)	67 ± 4	82 ± 4*	CO (l/min)	6.3 ± 0.5	7.7 ± 0.5*
SBP (mmHg)	121 ± 3	133 ± 4*	cSBP (mmHg)	106 ± 4	116 ± 3 *
DBP (mmHg)	75 ± 3	86 ± 3 *	cDBP (mmHg)	76 ± 5	87 ± 7 *
SV (ml)	95 ± 6	95 ± 8	ESP (mmHg)	96 ± 3	105 ± 4*

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.

Figure 5.1:

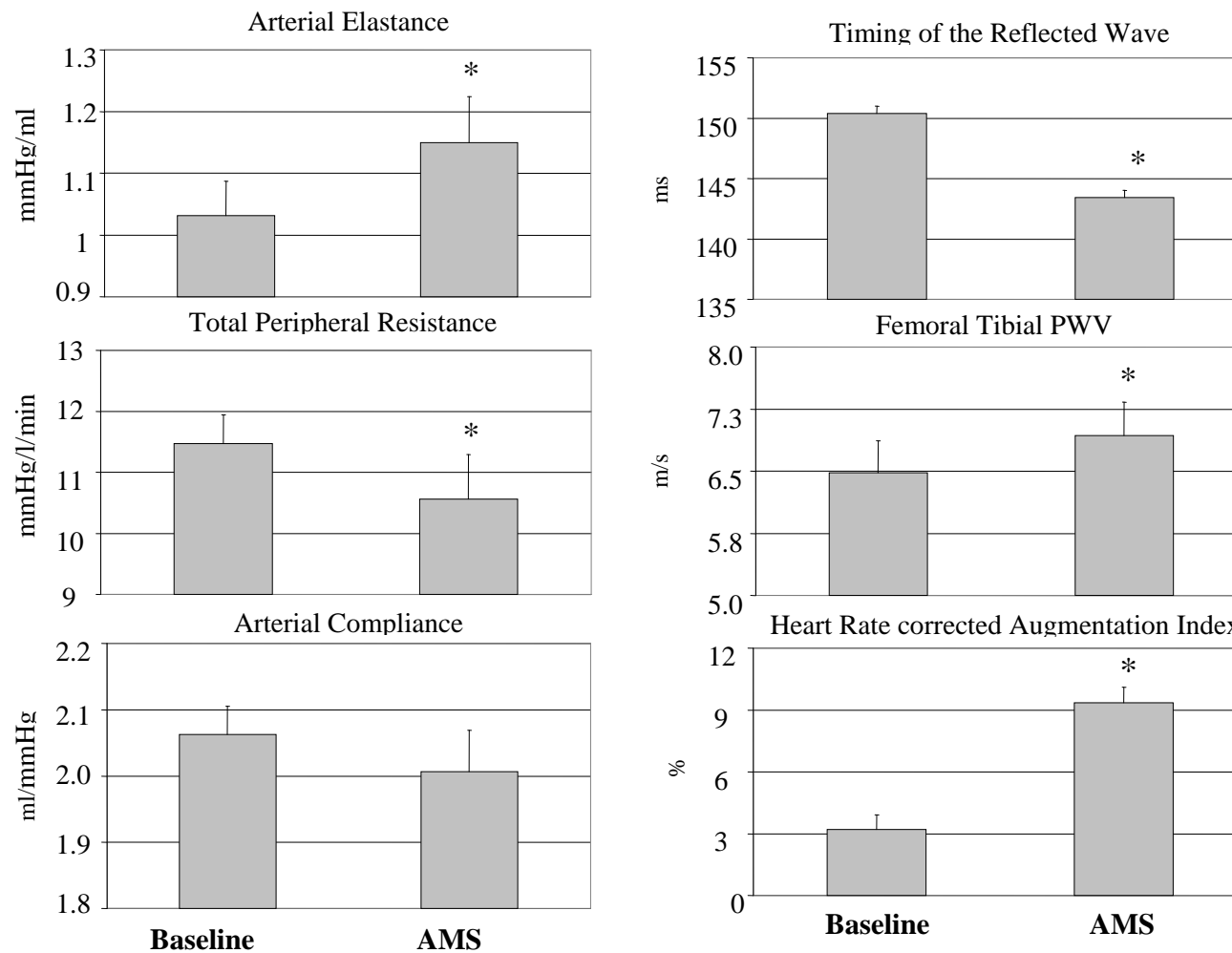


Figure 5.1. Arterial responses to AMS. * = different from respective baseline value.

Study 2: The role of Nitric Oxide

L-NMMA infusion significantly increased central and peripheral BP's, whilst decreasing HR, SV and therefore CO (all $P < 0.05$). These variables responded to AMS in a similar manner (as described in the first study) regardless of saline or L-NMMA infusion (Table 5.2).

At rest NO synthase blockade reduced FBF (Table 5.2) and TR (Figures 5.2), whilst inducing increases in AI@HR75, AE, FTPWV and TPR ($P < 0.05$ for all) (Figures 5.2).

During both saline and L-NMMA infusion, AMS increased AI@HR75, AE, and FTPWV, whilst decreasing FBF, TPR and TR ($p < 0.05$ for all) (Figures 5.2). 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 5.3).

Table 5.2. Haemodynamic responses to AMS following saline or L-NMMA infusion.

	Baseline		Post Infusion		AMS	
	Saline	L-NMMA	Baseline		Saline	L-NMMA
			Saline	L-NMMA		
HR (bpm)	55 ± 2	61 ± 2	56 ± 3	52 ± 2*	73 ± 4*‡	66 ± 3*‡‡
SBP (mmHg)	120 ± 2	117 ± 2	118 ± 3	124 ± 3*	127 ± 2*‡	133 ± 3*‡‡
DBP (mmHg)	77 ± 2	70 ± 3	76 ± 2	80 ± 2*	84 ± 2*‡	88 ± 3*‡
SV (ml)	97 ± 2	101 ± 6	98 ± 3	94 ± 5*	98 ± 5	96 ± 6
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*‡
cSBP (mmHg)	105 ± 2	99 ± 2	104 ± 3	110 ± 3*	112 ± 2*‡	118 ± 2*‡‡
cDBP (mmHg)	77 ± 1	72 ± 3	76 ± 2	81 ± 3*	85 ± 2*‡	88 ± 2*‡
ESP (mmHg)	95 ± 1	88 ± 3	96 ± 2	100 ± 3 *	103 ± 2*‡	106 ± 2*‡
FBF (ml/l/min)	33 ± 5	38 ± 8	28 ± 4	33 ± 9	51 ± 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 at given time point; ‡ indicates significant difference from respective post infusion baseline value.

Figure 5.2:

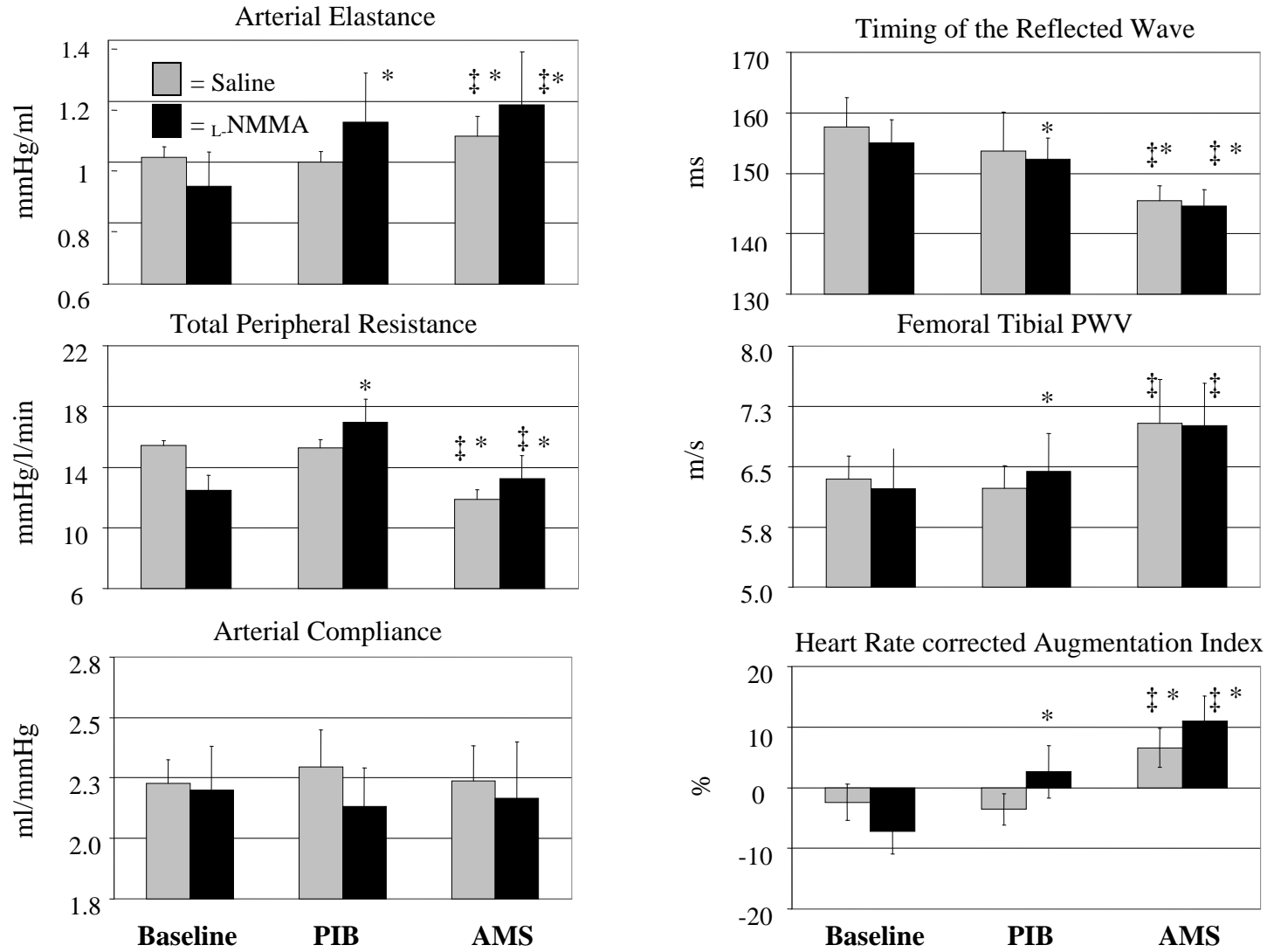


Figure 5.2. Arterial responses to AMS following saline or L-NMMA infusion. * = difference between saline and L-NMMA. † = difference from respective post infusion baseline (PIB).

Figure 5.3:

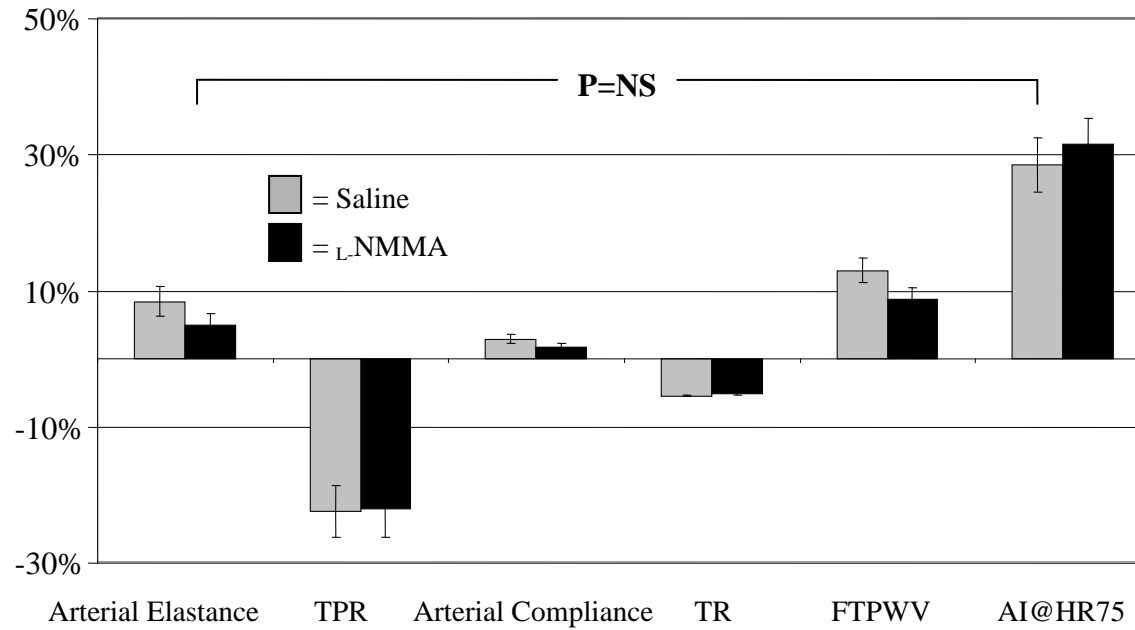


Figure 5.3. Magnitude of response to AMS with saline or L-NMMA infusion. TPR = total peripheral resistance, TR = timing of the reflected wave, FTPWV = femoral - tibial pulse wave velocity, AI@HR75 = heart rate adjusted augmentation index.

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 and 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 reductions in NO bioactivity do 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 (Lindqvist et al. 2004; Vlachopoulos et al. 2006), 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 (Barcroft et al., 1960; Blair et al. 1959; Halliwill et al. 1997; Rusch et al. 1981), whilst recently AMS has been shown to result in a prolonged increase in aortic stiffness with premature wave reflections (Vlachopoulos et al. 2006). 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 (Vallance et al., 1989), and has been shown in two studies to significantly contribute to the forearm vasodilator response to AMS, via cholinergic stimulation of the vascular endothelium (Cardillo et al. 1997; Dietz et al. 1994).

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 (Lindqvist et al. 2004). 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 (Cardillo et al. 1997; Dietz et al. 1994). The different influences of local and systemic (present study and Cardillo et al. 1997; Lindqvist et al. 2004)) 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 (Lindqvist et al. 2004). 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 is not yet known and is often controversial, previous studies have suggested that sympathetic withdrawal (Halliwill et al. 1997) and circulating adrenaline (Lindqvist et al. 1996) may also be of prime importance. The fact that large artery stiffness is increased by AMS 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 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 HR and arterial pressure induced by AMS are mediated; in part by sympathetic activation (Noll et al. 1996) and substantial catecholamine (i.e. adrenaline and noradrenaline) release (Tidgren & Hjemdahl 1989). Circulating catecholamines are widely considered to cause vasoconstriction (Kjeldsen et al. 1993). Indeed, noradrenaline infusion has been shown to result in increases in peripheral and central PP and an increase in AI and aortic stiffness (Wilkinson et al. 2001). 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 (Kjeldsen et al. 1993; Lindqvist et al. 1996).

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 α 1-receptors for the control of arterial BP (Wray et al. 2004), whilst the arterioles contain α 2-receptors for the fine control of tissue perfusion (Anderson & Faber 1991; McGillivray-Anderson & Faber 1990). Recent studies have also demonstrated that the interaction between sympathetic nerve activity and local vasodilatory stimuli appears to vary with vessel branch order (Haug et al. 2003). Alpha 2-mediated vasoconstriction in the arterioles appears more sensitive to metabolic inhibition than α 1-mediated vasoconstriction further upstream where sympathetic nerve activity seems able to impair ascending vasodilation (Anderson & Faber 1991; Haug & Segal 2005; VanTeeffelen & Segal 2003; Wray et al., 2004). Results from the present study may be in accordance with this concept, where local dilation in the forearm is able to override α 2-mediated constriction, but α 1-vasoconstriction upstream in the conduit and large arteries is maintained during AMS.

The passive effects of distending pressure on indices of arterial function must also be considered. In this experiment there was increased BP with AMS despite decreased TPR and increased FBF, suggesting that peripheral vessel function is dissociated from BP in this experiment. However, the rise in BP is associated with a rise in the stiffness indices, and therefore distending pressure may be a key determinant of the observed artery stiffness changes.

Clinical Implications

Chronic stress is increasingly recognised as a risk factor for coronary artery disease (Rozanski et al. 1999) LV dysfunction (Wittstein et al. 2005), and sudden cardiac death (Leor & Kloner 1996), and there is also a clustering of cardiovascular events after acute stressful episodes (Strike & Steptoe 2005; Strike et al. 2006). AMS can act as a trigger for acute cardiac events in susceptible individuals, with a vulnerable period of a few hours (Strike & Steptoe 2005; Strike et al. 2006). 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 (Mittleman et al. 1995; Moller et al. 2005). 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 (Monaco et al. 2005). It is suggested that heightened platelet activation and increased haemodynamic shear stress may play a key role in this process (von Kanel et al. 2001), 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 (Strike et al. 2006).

From a practical perspective this study highlights the importance of adequate rest before assessment of large artery function. In situations such as open access risk factor clinics and

clinical trials it is therefore essential to allow a minimum of a 15min baseline period to allow for the effects of stress on the large arteries.

Conclusions

AMS 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 6 - Study 4

Ventricular-arterial coupling during low intensity aerobic exercise

Abstract

The interaction of the heart and vasculature, termed ventricular-arterial (V-A) coupling, is a key determinant of cardiac performance, SV and CO. This study aimed to investigate the effects of graded sub-maximal exercise on V-A coupling and sought to dissect the components of arterial load that may underlie changes in V-A coupling during exertion. Subjects performed a graded cycling exercise protocol whilst haemodynamic, vascular and LV function indices were measured. Radial pulse-waveforms were recorded every minute throughout the study by applanation tonometry. Central aortic waveforms were assessed using the Sphygmocor pulse wave analysis system. The central waveform allows central BP, augmentation index (AI) the timing of the reflected wave (TR) and arterial elastance to be measured. LV end-systolic function was calculated using the modified single-beat method. V-A coupling was assessed using the ratio of arterial elastance to LV end-systolic function. Data from six healthy subjects was successfully captured. The major findings of the study demonstrate that there was a reduction in arterial compliance but also a decrease in peripheral resistance during exercise. The net effect of these changes was a decrease in total arterial load during exertion. LV end-systolic elastance remained unchanged at the lowest exercise intensity, but showed a significant increase as exercise intensity increased. These alterations in arterial load and LV elastance enabled the V-A coupling ratio to decrease at each exercise intensity, reflecting increasing augmentation of LV pump efficiency with graded aerobic exercise.

Introduction

The interaction of the heart with systemic vasculature, termed ventricular-arterial (V-A) coupling, is a key determinant of cardiac performance (e.g. ejection fraction (EF), SV, CO). The vascular component of left ventricular (LV) afterload is usually assessed in terms of MAP and TPR. However, these measurements do not take into account the pulsatile characteristics of blood flow in the arteries. The functional properties of the arterial system may be more accurately characterised by effective arterial elastance (AE); a steady state parameter that incorporates the principal elements of total vascular load, and is related to mean vascular resistance as well as pulsatile components due largely to arterial compliance and wave reflections (Sunagawa et al. 1983; Kelly et al. 1992). AE shares common units with elastance measures of LV function (e.g. end-systolic LV elastance (E_{es})) and their ratio (AE/E_{es}) is a commonly used index of V-A coupling (Sunagawa et al. 1983). Normal V-A coupling ensures maximal cardiac work, power and chamber efficiency and maintained CO and BP. This is achieved when the properties of the heart and the arterial system are perfectly matched (Starling et al. 1993). However, alterations in these factors may result in fundamental alterations in cardiac reserve (Kass et al. 2002).

V-A coupling during exercise has been examined in only a handful of studies (Asanoi et al. 1992; Cohen-Solal et al. 1998; Najjar et al. 2004). This small body of research suggests that in healthy subjects, V-A coupling during exertion is characterised by a decrease in AE/E_{es} , indicating an augmentation of pump efficiency. This will allow an increase (by up to 30%) in EF during exercise (Fleg et al. 1995). Whilst it is agreed that there is a substantial increase in LV elastance with exercise, the AE response remains controversial. Changes in AE with exercise reflect a

complex physiological response, which includes increased CO, BP, HR, and decreased TPR. Small reductions in AE have been reported following sub-maximal exercise (Asanoi et al. 1992), whilst increased AE has been observed with maximal and high level exercise (Cohen-Solal et al 1998; Najjar et al. 2004). AE may be approximated by end-systolic pressure (ESP)/SV or by the ratio of total arterial resistances to cardiac cycle length (Sunagawa et al. 1983). Elevated AE with exercise may therefore reflect the fact that the reduction in arterial resistance (which is already near maximal during sub-maximal exercise) is overridden by the increases in HR which are observed with graded exercise (Cohen-Solal et al. 1998). It has also been suggested that the contribution of systemic arterial compliance (AC) to AE increases with increasing exercise intensity, and that increased arterial load during exertion is mainly driven by a reduction in AC (Otsuki et al. 2006).

Arterial distensibility is the primary determinant of AC (Seals 2003; Tanaka & Safar 2005). During exercise, alterations in arterial distensibility may be attributed to local vasodilatory stimuli, and/or changes in vasoconstrictor tone, BP or HR. Aerobic exercise is associated with an increase in peripheral artery distensibility post exercise (Kingwell et al. 1997; Naka et al. 2003; Sugawara et al. 2004; Heffernan et al. 2007). Central artery responses to aerobic exercise are however less well understood. Heffernan et al. (2007) report no change in central artery stiffness or wave reflection time following maximal exercise, whilst Kingwell et al. (1997) observed acute reductions in central artery stiffness post submaximal cycling. In further contrast, Sharman et al. (2005) have shown increased aortic stiffness (estimated using the timing of the reflected wave), with decreased systemic arterial stiffness (decreased augmentation index) during incremental cycling exercise.

This study aimed to 1) investigate the effects of graded sub-maximal exercise on V-A coupling, and 2) to examine indices of vascular function and central aortic wave reflections in order to dissect the components of AE that may underlie any V-A coupling changes during exertion. It was hypothesised that LV elastance will increase with exercise. Furthermore it was expected that decreased peripheral resistance and increased distensibility of the conduit arteries during exercise will allow total arterial load to decrease. This will enable the V-A coupling ratio to decrease thereby augmenting pump efficiency.

Methods

Subjects

9 healthy men volunteered to participate in this study. However, due to methodological difficulties (see study limitations in the discussion) only data from 6 subjects (age 24 ± 2 yr) was deemed of a suitable standard to be presented. All subjects were normotensive, non smokers and refrained from caffeine and exercise for at least 3h before testing. All procedures were reviewed and approved by the local ethics committee confirmed to the Declaration of Helsinki (2002).

Experimental Protocol

Subjects initially rested in the supine position for 10min before resting echocardiograms, and vascular and haemodynamic measures were made. Participants then underwent a graded exercise protocol performed on a semi-supine cycle ergometer. The exercise protocol consisted of cycling at 50 r.p.m at 15% and 30% of HR reserve (calculated as resting HR subtracted from maximum HR ($220 - \text{age}$)). Resistance on the cycle ergometer was adjusted in order for each subject to maintain a steady state HR for 5min at each exercise intensity.

Measured Variables

SBP and DBP were measured at the brachial artery by an experienced technician using standard sphygmomanometry. A standard 3-lead electrocardiogram (ECG) was used to measure heart rate continuously throughout the experiment (Vivid 7 echo machine).

LV end-systolic elastance (E_{es}) was calculated using the modified single-beat method of Chen et al. (2001), which has been validated against invasive measures, showing excellent correlation without systematic bias. This method employs brachial BP's, echo-Doppler SV, echo-derived EF and an estimated normalised ventricular elastance at arterial end-diastole (E_{nd}). Echocardiograms were performed (Vivid 7 echo machine) using a wide-band frequency-fusion phase-array transducer by an experienced echo-technician with accreditation from the British Society of Echocardiography.

E_{es} was calculated by the following formula: $E_{es} = (dBP - [E_{nd} \times sBP]) / (E_{nd} \times SV)$.

Radial artery pressure waveforms were acquired using applanation tonometry (Millar Instruments) and central pressure waveforms were generated by PWA (Sphygmocor, AtCor Medical), using a generalised transfer function that has recently been validated during submaximal exercise (Sharman et al. 2006). The central waveform yields central aortic pressures, augmentation index (AI) heart rate corrected AI (AI@HR75) and the timing of the reflected wave (TR). PP amplification was calculated as the ratio of peripheral PP to derived central PP.

AE was calculated as end-systolic pressure (derived from the central pressure waveform) divided by SV (Sunagawa et al. 1983; Kelly et al. 1992). TPR was calculated as MAP divided by CO, and AC as the ratio of SV to PP (Chemla et al. 1998). V-A coupling was assessed using the AE/ E_{es} ratio (Sunagawa et al. 1983).

Statistical Analysis

All measures were made in triplicate at rest and during each exercise stage of the protocol. Average values for each period were used for analysis. All values are expressed as mean \pm SEM. Statistical analysis was performed using one-way ANOVA and *post hoc* analysis using paired t-tests with Bonferroni correction. Significance levels were set at $P < 0.05$.

Results

The haemodynamic effects of exercise are presented in table 6.1.

HR, and peripheral and central SBP and PP exhibited significant increases from baseline to exercise intensity 1, and further increases as the protocol progressed to 30%HR reserve. Peripheral and central DBP remained unchanged throughout. There were initial increases in SV and CO with exercise intensity 1. Whilst SV showed no further change as exercise intensity increased, there was a modest HR mediated increase in CO from exercise intensity 1 to intensity 2. EF remained unchanged at the lowest intensity of exercise before increasing at 30% HR reserve.

Arterial responses to exercise are also presented in figure 6.1.

Figure 1 shows that there were decreases in TR and AI with exercise intensity 1. AI exhibited a further decrease as exercise intensity increased to 30% HR reserve. There was however no difference between TR at the two exercise intensities. HR corrected AI (AI@HR75) showed no change from baseline throughout the protocol.

AC and TPR responses to exercise are shown in figure 6.2.

It is clear that exercise at 15% HR reserve induced a decrease in AC and TPR. Further decreases in these parameters were observed between exercise intensity 1 (15% HR reserve) and exercise intensity 2 (30% HR reserve).

AE, E_{es} and the AE/E_{es} ratio is presented in figure 6.3.

AE decreased from baseline to exercise at 15% HR reserve, but showed no further decrease as exercise progressed to 30% HR reserve. At exercise intensity 1, E_{es} remained unchanged from baseline. There was however a substantial augmentation in E_{es} at exercise intensity 2.

From rest to cycling exercise at 15% HR reserve there was a decrease in the AE/E_{es} ratio. Additionally, there was a further decrease in this value between exercise at 15% and 30% HR reserve.

Table 6.1. Haemodynamic responses to exercise at 15% and 30% HR reserve.

	Baseline	15% HR reserve	30% HR reserve
HR (bpm)	60 ± 3	82 ± 4 *	106 ± 3 * †
SBP (mmHg)	120 ± 2	132 ± 3 *	145 ± 2 * †
DBP (mmHg)	73 ± 2	70 ± 1	72 ± 3
MAP (mmHg)	84 ± 1	91 ± 1 *	96 ± 3 * †
PP (mmHg)	46 ± 3	63 ± 2 *	73 ± 3 * †
SV (ml)	89 ± 4	103 ± 4 *	100 ± 5 *
CO (l/min)	6 ± 0.2	8 ± 0.3 *	11 ± 0.4 * †
EF (%)	64 ± 3	64 ± 2	71 ± 4 * †
cSBP (mmHg)	102 ± 1	108 ± 1 *	116 ± 2 * †
cDBP (mmHg)	74 ± 1	72 ± 1	75 ± 2
cMAP (mmHg)	83 ± 1	84 ± 2	88 ± 2 * †
cPP (mmHg)	28 ± 2	36 ± 1 *	41 ± 1 * †
PP amplification (ratio)	1.6 ± 0.3	1.7 ± 0.4	1.8 ± 0.3
ESP (mmHg)	93 ± 2	90 ± 1	92 ± 3

HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; SV, stroke volume; CO, cardiac output; EF, ejection fraction; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; cMAP, central mean arterial pressure; cPP, central pulse pressure. * indicates significantly different from respective baseline value; † indicates significant difference between exercise intensities.

Figure 6.1:

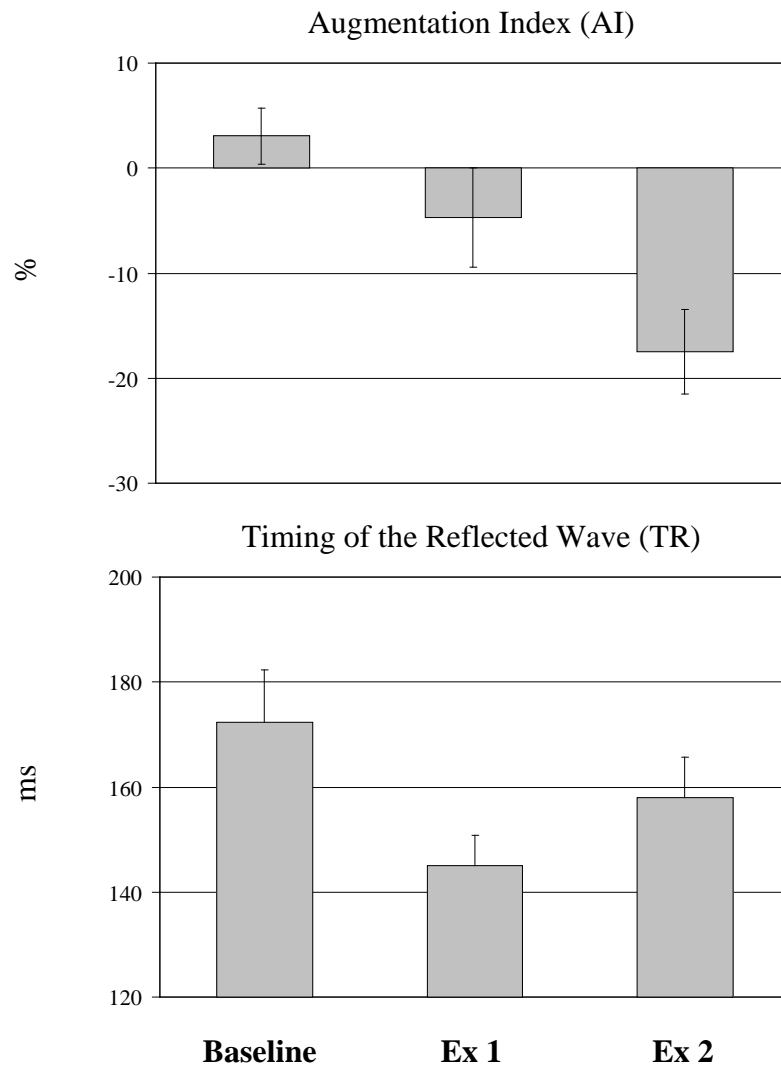


Figure 6.1. AI and TR at baseline and exercise at 15% (Ex 1) and 30% HR reserve (Ex 2).

Figure 6.2:

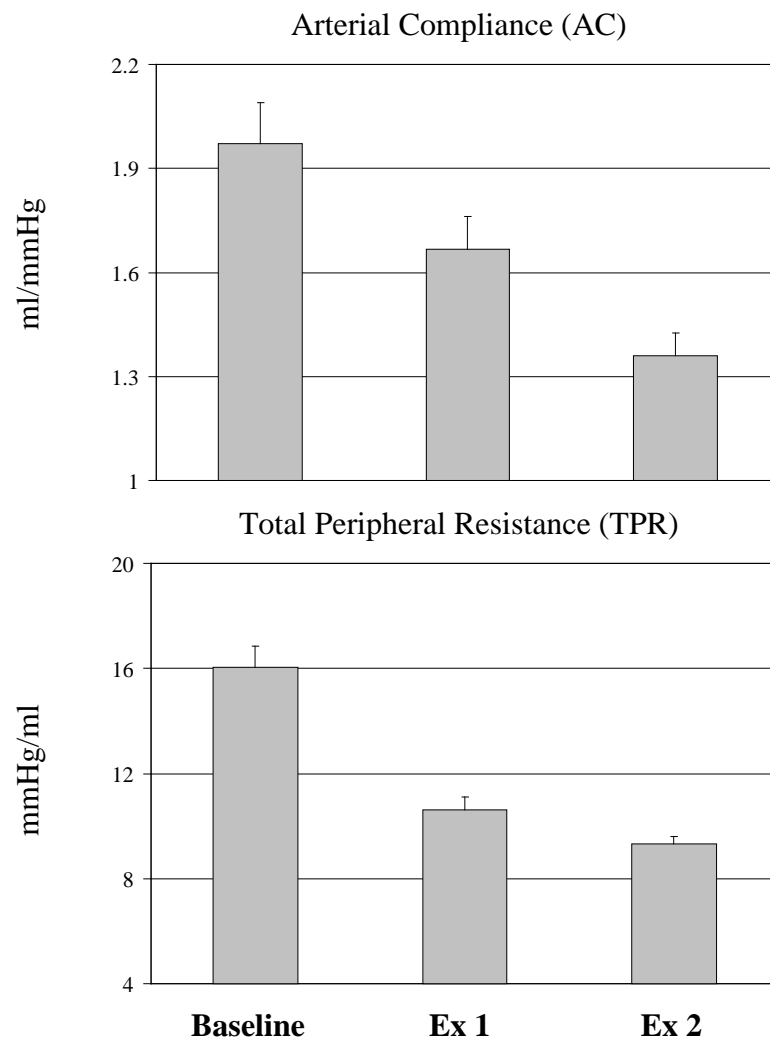


Figure 6.2. AC and TPR at baseline and exercise at 15% (Ex 1) and 30% (Ex2) HR reserve.

Figure 6.3:

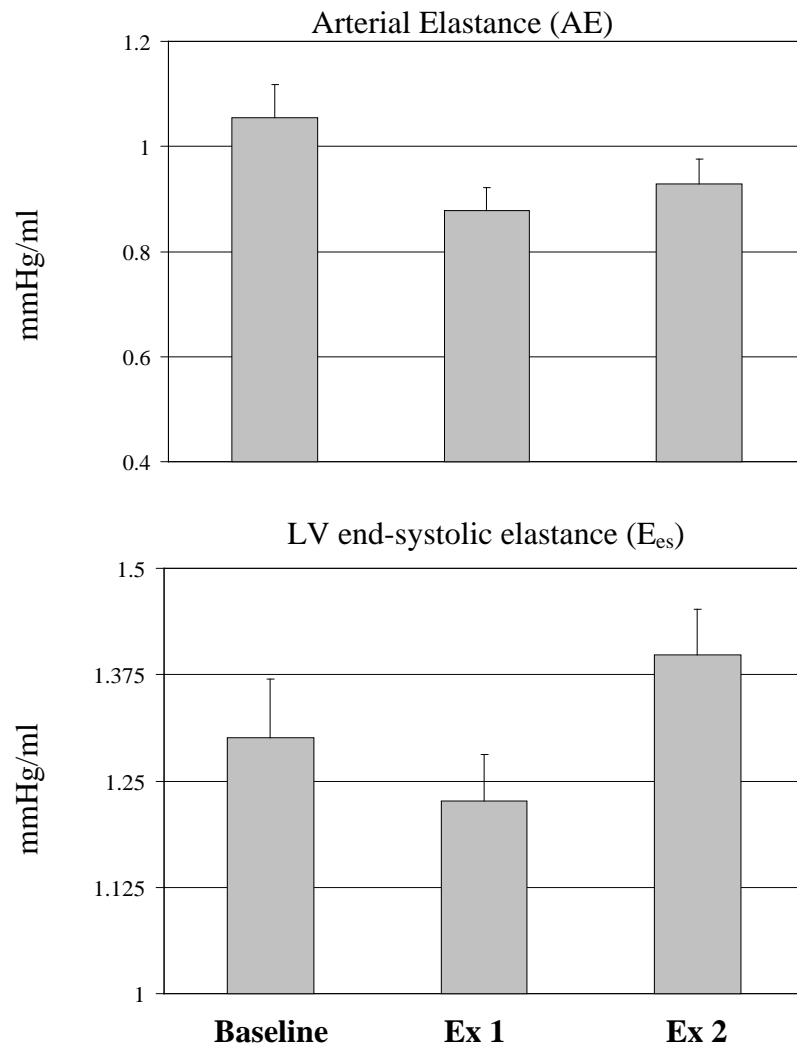


Figure 6.3. AE, and E_{es} at baseline and exercise at 15% (Ex 1) and 30% (Ex2) HR reserve.

Figure 6.4:

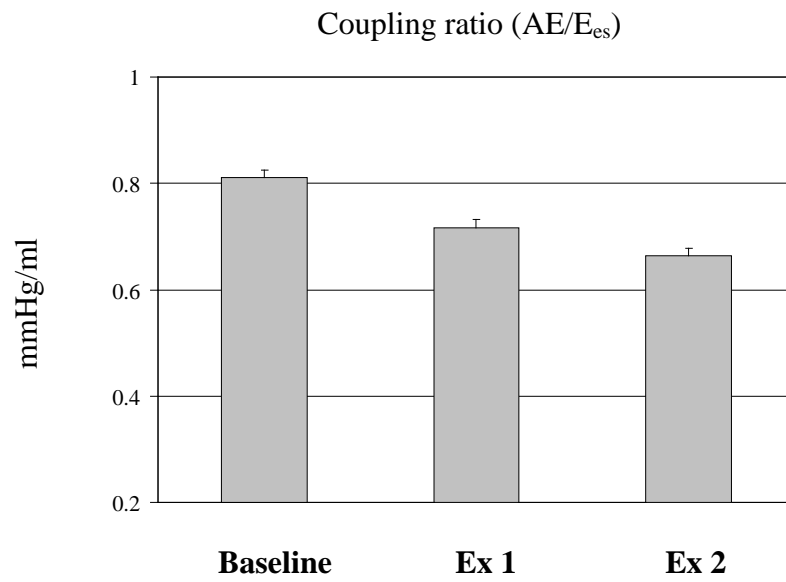


Figure 6.4. Coupling ratio (AE/E_{es}) at baseline and exercise at 15% (Ex 1) and 30% (Ex2) HR reserve.

Discussion

The purpose of this study was to assess vascular function and V-A coupling during low intensity aerobic exercise using non-invasive measures. The major findings of the study demonstrate that there was a reduction in AC but also a decrease in peripheral resistance with exercise. The net effect of these changes was a decrease in arterial elastance (AE), indicating reduced arterial load with exercise. LV end-systolic elastance (E_{es}), remained unchanged at exercise intensity 1, and showed a significant increase at exercise intensity 2. These alterations in AE and E_{es} enabled the AE/ E_{es} coupling ratio to decrease at each exercise intensity, reflecting increasing augmentation of LV pump efficiency with graded aerobic exercise.

Central and Peripheral Pressures during Exercise

In agreement with previous work (Kroeker & Wood 1955; Sharman et al. 2005) pulse pressure amplification between the ascending aorta and the brachial artery was significantly increased during exercise. This may be attributed to a higher relative increase in peripheral than central systolic BP. This may be a result of increased HR during exercise. As HR increases the relative duration of systole is shortened, thus forcing the reflected wave into diastole. Because the reflected wave contributes to central but not peripheral systolic pressure, the net effect will be to attenuate any increase in central SBP, thereby amplifying the difference between peripheral and central systolic pressures (Wilkinson et al. 2000; Sharman et al. 2005).

Vascular Responses to Exercise

The results from this study suggest a differential response of the peripheral and central vasculature to exercise. Exercise caused an acute decrease in AI, whereas the timing of the reflected wave decreased. A reduction in AC with exercise was also observed. Total peripheral resistance decreased during the protocol indicating a dilation of the peripheral vasculature.

AI is a composite of the intensity/magnitude and timing of reflected pressure waves from the periphery, and can be influenced by several factors including HR, aortic stiffness, peripheral muscular artery stiffness, arteriolar vasomotion, and LV ejection (Nichols et al. 2005). Since there was no change seen in HR-corrected AI with exercise, and there is an inverse relationship between HR and AI (Wilkinson et al. 2000), decreased AI in the present study may be related to the significant increase in HR during exercise. Vasodilation in the muscular arteries of the legs during exercise (Kingwell et al. 1997; Naka et al. 2003; Sugawara et al. 2003; Heffernan et al. 2007), may also have contributed to reduced wave reflection and thus a fall in AI during exercise (Sharman et al. 2005).

There was a significant decrease in TR from rest to exercise. This however, may be entirely BP related since Sharman et al. (2005) have demonstrated that exercise induced reductions in TR are strongly correlated to increases in mean arterial pressure. As mean pressure increases there is a progressive recruitment of collagen fibres within vessel walls (Armentano et al. 1991), which will effectively stiffen the large central elastic arteries, resulting in increased pulse wave velocity and thus decreased TR. The effect of HR on PWV and thus TR can also not be ignored. Although

conflicting findings have been reported, some authors have shown a concomitant increase in PWV with elevations in HR (Lantelme et al. 2002; Giannattasio et al. 2003).

Effective arterial elastance (AE), an index of arterial load, has been reported to increase with elevations in LV elastance to maximise the efficiency of LV stroke work during exertion (Cohen-Solal et al 1998; Najjar et al. 2004; Otsuki et al. 2006). AC and TPR are the primary components to AE, and it has been reported that TPR plays a greater role in determining AE at rest (Segers et al. 2002; Chemla et al. 2003). Low level aerobic exercise in the present study resulted in reduced arterial load (decreased AE), which reflects a reduction in LV afterload. Since AC was decreased during exercise, this may be primarily attributed to the observed decrease in peripheral resistance. Reductions in TPR are known to be near maximal at sub-maximal exercise, whereas AC has been shown to markedly decrease during exercise in an exercise-intensity dependent manner. It has therefore been proposed that the contribution of AC to AE will increase during graded exercise and that changes in AE observed at higher levels of exercise are driven by reduced AC (Otsuki et al. 2006).

V-A Coupling During Exercise

At rest V-A coupling is typically maintained between 0.7 and 1.0; the range that maximises the efficiency of the heart (Asanoi et al. 1989; Starling et al. 1993). Prior studies in animal models (Little & Cheng 1993) and humans (Cohen-Solal et al. 1998; Najjar et al. 2004) have shown a decrease in AE/E_{es} from rest to peak exercise. These studies reported an AE increase with high intensity exercise, and the decrease in the coupling index was attributed to a greater relative increase in ventricular contractility than arterial load (Najjar et al. 2004). It is hypothesised that at

peak exercise in health, energetic efficiency is sacrificed in favour of improved cardiac function (i.e. an increase in EF) (Najjar et al. 2004). Asanoi et al. (1992) also reported a decrease (35%) in AE/E_{es} with aerobic exercise. However in contrast to the studies above, no change in E_{es} was seen, and the decrease in the coupling index was attributed to a 30% reduction in AE. During anaerobic exercise in the same study, AE remained the same as during aerobic exercise, but a substantial (89%) increase in E_{es} was seen. This caused a further decrease in the AE/E_{es} coupling index (-54%). Our findings are in accordance with the data of Asanoi et al. At exercise intensity 1, there was a slight (non significant) decrease in E_{es} , which actually indicates a small attenuation of ventricular pump efficiency. This however was offset by a greater relative decrease in AE, which allowed the coupling index to decrease. As exercise intensity increased to 30% HR reserve no further change in AE was observed. The coupling index was however reduced from the previous exercise intensity, which can be attributed to a substantial increase in E_{es} .

In health, V-A coupling during exercise is characterised by a decrease in AE/E_{es} . It seems that during low level aerobic exercise this may be primarily mediated by changes in loading conditions (decreased AE). As exercise intensity increases, there may be an increase in arterial load. In this case, enhanced ventricular contractility (increased E_{es}) may play a more important role in decreasing the coupling ratio.

Consequences of V-A mismatch

Increased AE and E_{es} (V-A stiffening) will combine to reduce left-side circulatory compliance, resulting in a larger change in LV end-systolic pressure for any given change in ejection volume (Williams and Frenneaux 2007). This means far greater haemodynamic instability and alterations

in central and arterial pressures for any given change in central blood volume (Chen et al. 1998). In addition, combined V-stiffening also limits cardiac reserve mechanisms. A healthy heart will respond to exercise by enhancing CO through increased HR, increasing LV end-diastolic volume and increasing systolic contractile function (increased E_{es}). However, if basal conditions already include an elevated E_{es} and AE (as seen in age (Najjar et al. 2004) or disease (Kawaguchi et al. 2003)), there is less reserve capacity, or the inability to attain maximal efficiency, as apparent by a lesser reduction in the coupling index. Furthermore, an increase in AE will exacerbate systolic hypertension during exercise, which will impose an energetic demand on the heart whilst limiting its capacity to eject efficiently. Arterial stiffening raises myocardial oxygen consumption for a given SV (Kelly et al. 1992), an effect which will be augmented by ventricular systolic stiffening (Kawaguchi et al. 2003). Finally, increased V-A stiffening and the consequent rise in systolic pressure during stress can worsen diastolic function (Kawaguchi et al. 2003). Elevated systolic load will delay cardiac relaxation, which may translate into compromised filling and increased end-diastolic pressure (Leite-Moreira et al. 1999).

Study Limitations

The first limitation of this study was that data from 3 subjects from the initial study population had to be excluded for methodological issues. Therefore, only data from 6 subjects was deemed sufficient for analysis. Data was omitted if 1) the captured radial PWA waveforms had a quality index of below 60, and 2) if the echo-technician (who was blind to aims of the study) deemed the echocardiogram images of an unsuitable standard. Unfortunately these issues arose due to the inevitable movement artefacts which occurred when subjects attempted to raise their HR to the required levels.

A further limitation of the study could be the use of PWA and the generalised transfer function to generate central waveforms for the estimation of central pressures. Although this technique is relatively new, reproducibility of AI using this method has been tested and considered to be good – even at low pressures (Papaioannou et al. 2004). Inter observer difference for AI was 0.10 +/- 5.82%. The validity of this technique during exercise has been tested in 30 patients undergoing diagnostic coronary angiography with simultaneous direct aortic pressure measurements during catheterisation. The correlation between non-invasive and invasive techniques was high for the estimation of central BP ($r=0.99$, $P<0.001$) (Sharman et al. 2006).

Conclusions

In conclusion, exercise caused a decrease in the compliance of the arterial system. However, since there was also a substantial reduction in TPR, total afterload (AE) was decreased at the lowest exercise intensity. This lead to a decrease in the AE/E_{es} coupling ratio. As exercise intensity increased there was a significant augmentation of LV contractility, which is likely to play a more important role in enhancing pump efficiency in the face of unchanged or even increased afterload at higher exercise intensities.

Chapter 7 – Conclusions

This thesis sought to examine the effects of acute physiological and psychological stress on arterial function. Studies 1 & 2 demonstrated that elevated sympathetic outflow induced by muscle metaboreflex activation is associated with systemic stiffening of the arterial tree. This may have important clinical implications as reductions in arterial distensibility, as observed in these studies, results in increased amplitude and premature return of arterial pressure waves that leads to an augmentation in central BP and an elevation in LV workload.

Study 2 in this thesis, was to our knowledge the first to show a stiffening of the large conduit arteries during metaboreflex activation. This study also demonstrated that in the absence of sustained sympathoexcitation, conduit arterial distensibility of a previously exercised limb was augmented immediately following isometric exercise. As metaboreflex activation was able to override local dilatory mechanisms and abolish increases in distensibility of the exercised artery, data from this study suggests that the conduit arteries serve as key sites for regulating the distribution of CO and the maintenance of arterial BP.

The effects of acute mental stress on the peripheral and central vascular were examined in study 3. This investigation clearly demonstrated differential responses of the peripheral and central vascular to acute mental stress. From these results we can conclude that systemic arterial stiffening and the resulting premature return of reflected pressure waves from the periphery, override decreased peripheral resistance to increase arterial BP during mental stress. This may provide an important mechanistic link between mental stress and cardiac disease. Although nitric oxide was shown to be a key regulator of basal vascular tone, responses of the central and peripheral vasculature to mental stress were unchanged with nitric oxide synthase blockade. This

suggests that impaired nitric oxide bioavailability plays little or no part in the overall vascular response to acute mental stress and that other mechanisms underlie the adverse effects of stress on vascular function.

The final study in this thesis aimed to investigate the effects of graded sub-maximal exercise on ventricular-arterial coupling. This study also sought to examine numerous indices of vascular function and wave reflection in order to dissect the components of arterial load that may underlie ventricular-arterial coupling changes during exercise in healthy humans. The major findings demonstrate that there was a reduction in arterial compliance but also a decrease in peripheral resistance during exercise. At low level exercise, the net effect of these changes was a decrease in total arterial load which enables optimal ventricular-arterial coupling and augmented left ventricular pump efficiency. However, as exercise intensity increases reduced arterial compliance appears to have a progressively greater contribution on total arterial load and as a result enhanced ventricular contractility is required to maintain optimal coupling of the heart and vasculature. With further work and validation, this study may prove a valuable model that will enable ventricular-arterial interaction during exercise to be characterised in health and disease.

These studies provide novel insights into the CV response to physiological and environmental stress. A common theme throughout the experimental chapters of this thesis is that the peripheral and central vasculature may respond to stress in different ways. In two of the studies, arterial stiffening was observed despite decreased peripheral vasculature resistance. Although the passive effects of increased BP on arterial stiffness in these experiments cannot be ignored, we may speculate that the arterial response to during stress may be a key determinant of the net load faced

by the LV. In this regard, arterial stiffening during stress may increase central BP's and therefore adversely affect V-A interaction. Future research should be aimed at examining the response of aging and/or CAD populations, to determine clinical significance.

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