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## Ventricular-Vascular Coupling And Central Arterial Pulse Pressure

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# **Ventricular-Vascular Coupling And Central Arterial Pulse Pressure**

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## Abstract

Central pulse pressure (cPP), a product of ventricular-arterial interaction, is an important determinant of cardiovascular outcomes in hypertension. The aim of this thesis is to advance the understanding of pulsatile haemodynamics and to explore mechanisms that may selectively reduce cPP.

The conventional view is that cPP comprises a component determined by the direct interaction of myocardial contraction with the impedance of the proximal arterial tree (closely related to pulse wave velocity, PWV) and a component ‘augmentation pressure’ generated by pressure wave reflections from muscular conduit arteries. Surprisingly little is known regarding regulation of conduit artery tone despite its potential influence on cPP. In the first part of this thesis, muscular large arterial tone was examined using a human forearm blood flow model. Vasoactive substances were infused locally into the brachial artery and vasodilator responses of the radial artery, as a muscular conduit artery, and forearm resistance microvasculature were examined. Nitric oxide donors, in particular, glyceryl trinitrate (GTN) were found to have the most selective action on conduit arteries compared to other vasodilators. In the second part of the thesis, I examined whether the action of GTN to reduce augmentation pressure could be accounted for by this selective dilation of muscular arteries. GTN was given systemically and by intra-coronary infusion in patients undergoing cardiac catheterisation. Invasive aortic blood pressure and flow velocity were analysed in the time domain by wave intensity analysis. This allows separation of pressure into a forward component generated by myocardial contraction and a backward component generated by ‘reflection’ from the peripheral arterial tree. A surprising finding was that changes induced by GTN were mainly attributable to a reduction in forward rather than backward pressure waves. That this resulted from a change in myocardial contractility was confirmed by local intra-

coronary injection of GTN. The final part of the thesis examines the relative contribution of forward and backward pressure waves in hypertension. An elevated cPP in hypertensive compared to normotensive subjects was accounted for primarily by an increased forward pressure wave. That this was due to increased myocardial contractility was confirmed by examining whether the pattern of wave intensity seen in hypertension could be reproduced, in normotensive subjects, by the inotrope dobutamine (when compared to the vasoconstrictor norepinephrine used as a control). This thesis thus provides novel insight into a) regulation of conduit artery tone, and b) pulsatile haemodynamics, highlighting the contribution of left ventricular ejection characteristics in determining pressure augmentation and cPP.

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## List of abbreviations

ACEi	Angiotensin-converting enzyme inhibitor
AIx	Augmentation index
AP	Augmentation pressure
ARB	Angiotensin II receptor blockers
BCW	Backward compression wave
BCW <sub>ls</sub>	Late systolic backward compression wave
bDBP	Brachial diastolic blood pressure
BEW	Backward expansion wave
BEW <sub>ls</sub>	Late systolic backward expansion wave
bPP	Brachial pulse pressure
BPW	Backward pressure wave
bSBP	Brachial systolic blood pressure
c	Wave speed
CO	Cardiac output
Db	Dobutamine
ECG	Electrocardiogram
Einc	Young's elastic modulus
ESH	European society of hypertension
FBF	Forearm blood flow
FCW	Forward compression wave
FEW	Forward expansion wave
FEW <sub>ls</sub>	Late systolic forward expansion wave
FPW	Forward pressure wave

GMP	Guanosine monophosphate
GTF	Generalised transfer function
GTN	Glyceryl Trinitrate
h	Arterial wall thickness
HYD	Hydralazine
ISH	Isolated systolic hypertension
l	Length of blood vessel
MAP	Mean arterial pressure
MMP	Matrix metalloproteinase
NF- $\kappa$ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NE	Norepinephrine
NO	Nitric oxide
NTG	Nitroglycerin
NP	Nitroprusside
NYHA	New York heart association
P	Pressure
$\rho$	Blood density
P1	Pressure at the first systolic shoulder above diastolic blood pressure
P2	Pressure at the second systolic shoulder above diastolic blood pressure
Pd	Pressure difference
pGC	Particulate guanylate cyclase
PHT	Phentolamine
PP	Pulse pressure
PWA	Pulse wave analysis

PWV	Pulse wave velocity
Q	Laminar flow
r	Radius
RAD	Radial artery diameter
sGC	Soluble guanylate cyclase
SV	Stroke volume
t	Time
T <sub>ej</sub>	Ejection duration
TPR	Total peripheral resistance
T <sub>R</sub>	Timing of wave reflection
U	Flow velocity
VER	Verapamil
Z <sub>c</sub>	Characteristic impedance

## **Publications from this thesis**

### **Chapter 3**

Fok H, Jiang B, Clapp B, Chowienczyk P. Regulation of vascular tone and pulse wave velocity in human muscular conduit arteries: selective effects of nitric oxide donors to dilate muscular arteries relative to resistance vessels. *Hypertension* 2012; 60(5): 1220-1225

### **Chapter 4**

Fok H, Guilcher A, Li Y, Brett S, Shah A, Clapp B, Chowienczyk P. Augmentation pressure is influenced by ventricular contractility/relaxation dynamics: novel mechanism of reduction of pulse pressure by nitrates. *Hypertension* 2014; 63(5): 1050-1055

### **Chapter 5**

Fok H, Guilcher A, Li Y, Brett S, Jiang B, Epstein S, Alastruey J, Clapp B, Chowienczyk P. Dominance of the forward compression wave in determining pulsatile components of blood pressure: similarities between inotropic stimulation and essential hypertension. *Hypertension* 2014; 64(5): 1116-1123

## **Statement of conjoint work**

Dr Brian Clapp performed invasive aortic pressure and flow measurements during cardiac catheterisation in chapter 4.

Dr Antoine Guilcher and Dr Sally Brett assisted in data collection in chapter 4.

Dr. Antoine Guilcher wrote the custom software for wave separation and wave intensity analyses in chapter 4 and 5.

Dr Benyu Jiang performed vascular ultrasound and echocardiography measurements in chapter 5.

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# CHAPTER 1: INTRODUCTION

## **1.1 Hypertension**

Hypertension affecting almost 1 in 3 of the population is the major preventable risk factor for premature disability and mortality globally.<sup>1,2</sup> Symptoms are usually absent, at least in the early stages; therefore it is often underdiagnosed and undertreated despite the unequivocal evidence supporting the health and economic benefit for blood pressure lowering in hypertension.<sup>3-8</sup> Other than lifestyle modifications such as reducing salt intake and weight management, the mainstay treatment of hypertension remains pharmacological and most anti-hypertensive drugs are vasodilators.<sup>9-11</sup> Early epidemiological studies confirmed the importance of elevated peripheral resistance as the hallmark haemodynamic change in hypertension<sup>12</sup> and unsurprisingly the diagnostic criteria and therapeutic focus was on diastolic blood pressure and peripheral resistance until relatively recently.<sup>13</sup> Pharmacological research is still mainly centred on the effects of vasodilators on resistance arterioles determining peripheral resistance.<sup>14</sup> Despite a longstanding appreciation of associations between arterial stiffening and hypertension<sup>15</sup>, the importance of aortic stiffness and systolic and pulse pressure in predicting cardiovascular outcome have only become evident from epidemiological studies in the last two decades and specific therapeutic targets for these are currently lacking.<sup>3, 16, 17</sup>

## **1.2 Components of blood pressure**

Non-invasive brachial artery blood pressure measurements form an important part of cardiovascular risk assessment in everyday clinical practice. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) values obtained from these measurements represent the peak and the nadir of the brachial arterial pressure waveform. An alternative



way of characterising the waveform, which may offer more insight into the haemodynamics, is by steady state component, mean arterial pressure (MAP), the pressure averaged over one cardiac cycle and the pulsatile component (PP), the difference between SBP and DBP.<sup>18</sup> SBP and PP are dependent on both MAP and DBP with the exact relation being dependent on the form of the pulse wave but MAP is closer to DBP than SBP, so that SBP is influenced more by the PP, than is DBP. PP represents the pulsatile load exerted on the heart and large arteries and increases along the length of large arteries – a phenomenon termed pulse pressure amplification.<sup>18</sup> Thus in health brachial artery PP (and SBP) may be 20mmHg higher than the aortic SBP.<sup>19</sup> The steady state blood pressure components DBP and MAP, however stay relatively constant with a small fall in MAP of 1-2 mmHg in the peripheral circulation.<sup>20</sup> DBP has traditionally been viewed as the most important clinical parameter in hypertension as it is closely related to total peripheral resistance (TPR), an increase in which is thought to be the hallmark of hypertension.

### **1.3 Effect of ageing on blood pressure components**

The Framingham study showed components of blood pressure change differently with age.<sup>21</sup> SBP increases approximately linearly with age. DBP, however reaches its maximum between 50-60 years and falls thereafter. Consequently pulse pressure rises steeply from this age onwards. The increase in pulsatility with ageing is thought to be principally related to arterial stiffening, in particular aortic stiffening.<sup>18, 21-24</sup> The cyclical mechanical stress exerted on the proximal aorta over time is thought to lead to fracturing of elastin, abnormal deposition of collagen and calcification. This reduces the cushioning function of the aorta to store energy in systole for peripheral run off during diastole. This in part explains the continue rise of SBP and a fall in DBP with ageing.<sup>18, 22</sup> In the presence of cardiovascular risk factors such as

smoking<sup>25</sup>, hypertension<sup>26</sup>, diabetes mellitus<sup>27</sup> and end stage renal failure<sup>28</sup>, age-related aortic stiffening is thought to be accelerated, exacerbating the age-related increase in PP.

#### **1.4 Isolated systolic hypertension**

A number of epidemiological surveys of middle aged and elderly people published since the 1980s have confirmed the importance of brachial PP widening as a strong independent predictor of cardiovascular events and mortality.<sup>29-41</sup> Isolated systolic hypertension (ISH), defined by the European Society of Hypertension (ESH) guidelines as DBP <90mmHg and SBP >140mmHg, is the most common type of hypertension from middle age onwards. In the NHANES III survey (The National Health And Nutrition Examination Survey) 75% of subjects with hypertension were aged over 50 years and 80% of these patients had ISH, demonstrating that widened PP is a highly prevalent in an ageing population with hypertension.<sup>42</sup> Despite this there have been few therapeutic trials addressing the selective reduction in SBP and PP to reduce cardiovascular mortality. This may be due to the lack of molecules targeting specific mechanisms e.g. aortic stiffness and lack of drugs to selectively lower SBP compared to DBP.<sup>3, 43,44</sup> In the context of ISH, there is a theoretical disadvantage in attempting to control systolic pressure with vasodilators, since diastolic pressure will fall thus compromising vital organ perfusion such as the coronary circulation.<sup>45</sup>

#### **1.5 Central blood pressure (CBP)**

Brachial SBP (bSBP) and DBP (bDBP) measurements are the current gold standard for the diagnosis of hypertension.<sup>46</sup> Over the past decade emerging epidemiological data suggests that central measures of blood pressure and other CBP waveform indices taken from either

invasively measured or non-invasively derived aortic blood pressure waveform have an independent predictive value in cardiovascular risk assessment.<sup>47</sup> Whether CBP indices have unquestionable advantages over brachial measurements is less clear. The 2013 ESH guideline does not recommend routine measurement of central haemodynamic indices in clinical practice, except for a potential role in assessing ISH in young patients. However it acknowledges their fundamental role in aiding the understanding of the haemodynamic mechanisms and therapeutics of hypertension.<sup>10</sup> CBP indices are potentially important in the study of pulsatile haemodynamics because central pulse pressure (cPP) is what vital organs such as the brain, the heart and the kidney are exposed to not bPP.<sup>18, 48, 49</sup> Since pulse pressure amplification may vary between subjects the assessment of central pulsatility by bPP may be inaccurate.<sup>48, 50</sup> Furthermore, different classes of anti-hypertensive drugs appear to have differential effects on central and peripheral blood pressure measurements with consequent impact on outcomes favouring regimes that preferentially reduce CBP.<sup>19, 51-55</sup>

## **1.6 Pulse wave analysis**

The clear advantages of using bSBP and bDBP values in clinical practice are their established use and relative ease with which they may be derived using modern oscillometric methods. However bSBP and bDBP values alone provide an incomplete characterisation of haemodynamics as they represent the peak and nadir of the pressure at the brachial artery.<sup>56</sup> The introduction of commercially available high fidelity tonometers has advanced the field of pulse wave analysis (PWA) a great deal as they allow non-invasive recording of an arterial pressure waveform, which can then be calibrated from bSBP and bDBP. Tonometry is usually performed at the radial artery and it has been shown that a generalised transfer function (GFT) can be applied to a radial waveform to obtain a central aortic waveform with

reasonable precision. Alternatively, tonometry can be performed at the carotid artery which, because of its close proximity to the aorta, provides a waveform that closely resembles the aortic waveform. A number of indices to describe the central aortic pulse contour are shown in figure 1.

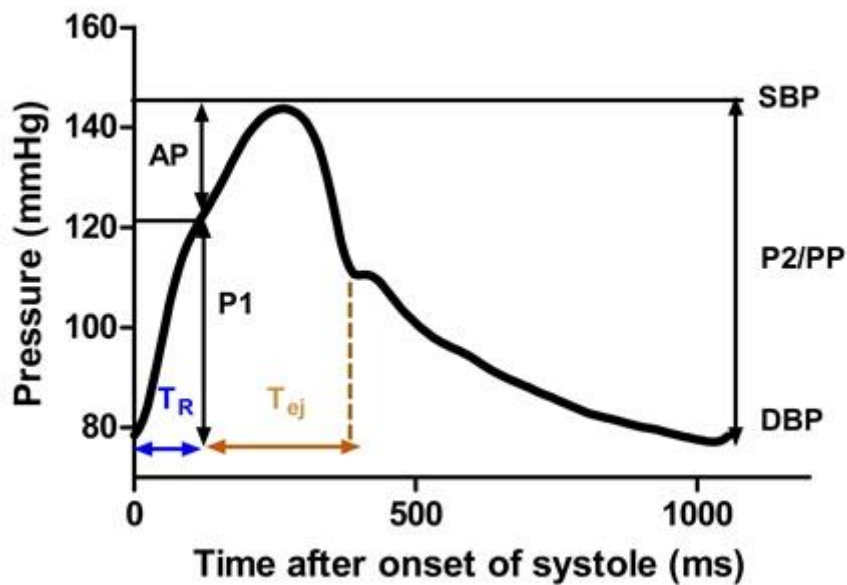


Figure 1: An aortic pressure waveform can be measured invasively or estimated non-invasively by radial tonometry via a transfer function. AP = augmentation pressure, the height above the first systolic shoulder; PP = pulse pressure; P1 = pressure at the first systolic shoulder above diastolic pressure; P2 = pressure at the second systolic shoulder above diastolic pressure;  $T_R$  = Timing of wave reflection;  $T_{ej}$  = Ejection duration.<sup>57</sup>

These indices provide a description of the pressure pulse contour and are thought to relate to ventricular and arterial properties. P1, the first shoulder above the diastolic pressure, is thought to be generated by a forward pressure wave from left ventricular ejection. P1 also

corresponds to peak blood flow velocity within a cardiac cycle and  $T_R$  is thought to be the point at which the forward pressure wave generated by ventricular contraction coincides with a backward pressure wave caused by pressure reflected backwards from the peripheral arterial tree. Augmentation pressure (AP), the difference between pressure at the second shoulder (P2) and P1 and augmentation index (AIx), the percentage ratio of AP to cPP are thought to relate the magnitude and timing of pressure wave reflection.<sup>18</sup> However this classic interpretation of AIx as a marker of pressure wave reflection has been challenged by a number of authors.<sup>58-62</sup> Regardless of the mechanism and perhaps the meaning of AIx, the index itself has been shown in a meta-analysis to have independent predictive value for cardiovascular events and all-cause mortality.<sup>47</sup> In addition the majority of the age-related increase in cPP is accounted for by an increase in AP and thus AP is a potential target for therapeutic intervention.<sup>56, 63</sup> AP and AIx is thought to be influenced by a number of factors which include age, heart rate, height, ethnicity, aortic tapering and the pattern of left ventricular dynamics.<sup>18, 46, 64-71</sup> Crucial to the accurate estimation of AP/AIx is the identification of  $T_R$  or P1. Some investigators use the first systolic shoulder to mark the period of  $T_R$  and P1 where others use the inflection point.<sup>18, 72, 73</sup> The correct identification of  $T_R$ , P1 and AIx is further complicated by physiological variation of the aortic pressure waveform. Murgu et al<sup>72</sup> have classified the contours into Types A – C.

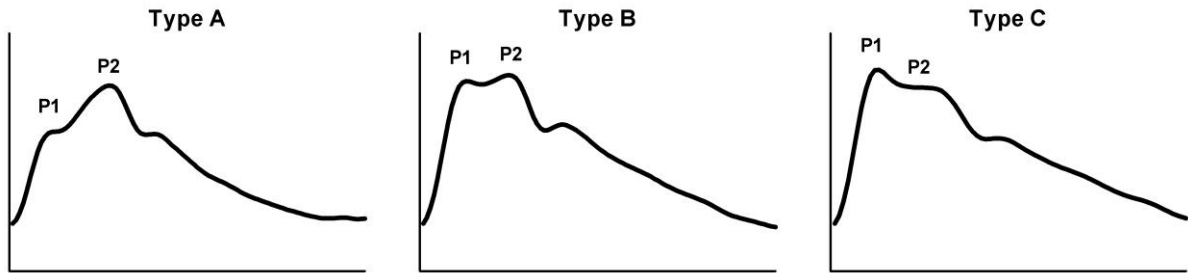


Figure 2: Types of aortic pressure waveform as described by Murgu et al <sup>72</sup>. Both type A and B waveforms are defined by  $P2 > P1$ . Type A waveforms have  $AIx > 12\%$  and Type B have  $AIx 0-12\%$ . Type C waveform is defined by  $P1 > P2$ , therefore  $AIx$  is  $< 0\%$ .

Nichols and colleagues<sup>18</sup> have subsequently added a Type D waveform to this classification. A type D waveform is defined by a pressure pulse in which no inflection point can be identified. Figure 2 illustrates the importance of the methodology used to identify P1 and  $T_R$  as the results can varied widely, especially for Type D waveforms.<sup>18, 61</sup> Type C waveforms are commonly seen in young healthy individuals. With ageing, the contour of the waveforms appear to progress from C to A, which is thought to be principally due to stiffening of large elastic arteries but changes in left ventricular dynamics and/or microvasculature are also potentially important.<sup>22, 57</sup> Type D waveforms are reported in older individuals with wide PP.<sup>18</sup>

### **1.7 Haemodynamic interpretation of the pressure pulse**

It is clear that the pressure pulse contour contains vital information on ventricular and vascular functions and therefore much effort has been devoted to model the pressure pulse in

order to establish its haemodynamic determinants. Currently the wave propagation model<sup>18</sup> is the most widely accepted explanation for the pressure pulse. It is a one dimensional model describing wave travel in the forward and backward plane. A forward pressure wave (FPW), generated by left ventricular contraction travels distally along the aorta and its tributaries at a given speed termed pulse wave velocity (PWV). PWV is closely related to aortic characteristic impedance ( $Z_c$ ) which determines the ratio of forward pressure wave to flow in the absence of pressure wave reflection. This relationship can be represented by a modified water hammer equation<sup>18</sup> as:

$$Z_c = \frac{4\rho \cdot PWV}{\pi \cdot D^2}$$

Where  $\rho$  = blood density and  $D$  = vessel diameter. Since blood density remains constant  $Z_c$  is mainly determined by PWV and vessel diameter ( $D$ ). PWV is directly proportional to the elastic modulus of blood vessels, a measure of stiffness, as demonstrated by the Moens-Korteweg equation<sup>18</sup>:

$$PWV = \sqrt{\frac{E_{inc} \cdot h}{\rho \cdot D}}$$

Where PWV = pulse wave velocity  $E_{inc}$  = Young's elastic modulus of arterial wall,  $h$  = arterial wall thickness,  $\rho$  = blood density,  $D$  = vessel diameter. It is evident from the above equations that both  $Z_c$  and PWV are closely related to the contractile status and the intrinsic stiffness of the arterial wall. As the FPW propagates along the arterial tree, it encounters a backward pressure wave (BPW) generated by wave reflection at sites of impedance mismatch within the vascular tree<sup>18</sup>. Impedance mismatch is due to elastic and geometric tapering of arteries and arterial branching sites.<sup>18, 72, 74</sup> Elastic tapering corresponds to the increase in proportion of vascular smooth muscle and collagen fibres in the extracellular matrix of the

arterial wall when moving distally away along the arterial tree away from the aortic root, thus smaller arteries are physiologically ‘stiffer’ in comparison to elastic large arteries. Geometric tapering is the gradual decrease in arterial diameter further along the arterial tree. Pressure wave reflection, therefore, does not only originate at distinct arterial branching points but is a continuous process along the arterial tree. Multiple BPW are thought to integrate to form a single combined BPW that interacts with the FPW to form the final pattern of the aortic pressure pulse.<sup>18, 48, 72</sup>

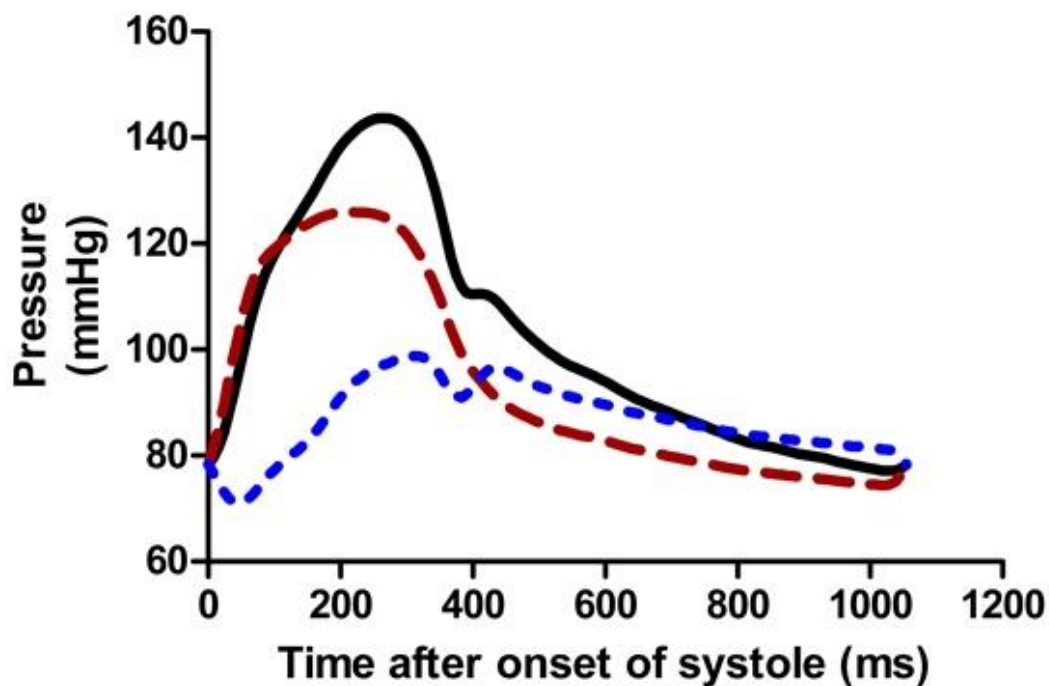


Figure 3: Type A aortic pressure waveform decomposed into forward (red) and backward (blue) pressure components. The forward pressure wave generated from left ventricular contraction integrates with the backward pressure wave at early systole therefore augmenting the pressure wave to form the final aortic pressure pulse (black).



The overall pressure waveform is the sum of the FPW and BPW and can be decomposed into FPW and BPW if pressure and flow are measured simultaneously as described in chapter 2. The amplitude as well as the timing of the BPW arrival at the proximal aorta is thought to determine the type of pressure waveform.<sup>72</sup> In youth the Type C aortic pressure waveform is attributed to the arrival of BPW at late systole and early diastole, hence supporting coronary perfusion pressure during diastole. An increase in PWV as a result of aortic stiffness, is thought to lead to an earlier return of the BPW in systole. This widens the cPP and changes the morphology of the waveform (from Type C to Type A) resulting in an increase in afterload and a reduction in coronary perfusion pressure.<sup>18, 75, 76</sup> As mentioned previously AP, the pressure above P1 of the pressure pulse, is thought to represent the integration of FPW and BPW, hence AP is thought to be a measure of wave reflection. Moreover, the increase in AP has been shown to drive the increase in cPP with ageing and other pathological processes<sup>46, 71, 77, 78</sup>, which leads to the hypothesis that pressure wave reflection is an important determinant of pulsatility within the cardiovascular system.<sup>18</sup> In the peripheral circulation, pressure wave reflection could also potentially explain the phenomenon of PP amplification.<sup>50</sup> In health the geometric and elastic tapering of the vascular tree leads to a regional variation of PWV (stiffness) with distal arteries 'physiologically stiffer' than proximal arteries. This phenomenon leads to an earlier arrival of BPW in the distal muscular arteries compared to elastic arteries nearer to the heart, which results in a wider PP progressively from the aortic root towards the distal end of the muscular conduit arteries. With aortic stiffening, the magnitude of PP amplification reduces and this has also been shown to be an independent marker of cardiovascular risk in its own right.<sup>50, 79-</sup>

Within the arterial tree, the junction of the muscular conduit arteries – and resistance arteries is proposed to be the main site of pressure wave reflection by O'Rourke and colleagues.<sup>18</sup> This stems from the observation that a rapid rise in peripheral resistance together with a fall in pressure occur over a very short distance within this region of the arterial tree, creating a concentrated area of impedance mismatch. In addition vasodilators acting on these vessels can reduce pressure wave reflection<sup>84-88</sup> further supporting the potential significance of this region of the vascular tree in influencing pressure wave reflection. The main anatomical site for pressure wave reflection however remains elusive. It is thought that the distal aorta could be a discrete site of pressure wave reflection. However mechanistic studies designed to localise the exact anatomical site of pressure wave reflection have so far created conflicting results with no definitive site located.<sup>18, 24, 58, 59, 61, 72, 89-92</sup> This may be because the assumptions applied to the estimation of the timing and distance of effective reflection site may be too simplistic for the human vascular tree.<sup>91</sup>

The location of the site of effective pressure wave reflection is one of the many questions still to be explored regarding our current understanding of pressure wave propagation and its physiological contribution in determining pulsatile haemodynamics.<sup>59</sup> The other major criticism of the wave propagation model is the incomplete explanation of the interaction of the FPW and BPW during diastole and the failure to consider the Windkessel or cushioning effect of large arteries.<sup>93</sup> The traditional two element Windkessel model has been updated since it was first described by Frank to account for the shortcoming in modelling the pressure pulse during systole.<sup>94</sup> However, the major disadvantage of this model is its failure to incorporate wave propagation in the vascular tree and the incorrect assumption of instantaneous propagation of the pulse, which are important aspects of pulsatile haemodynamics.<sup>18, 94-96</sup> A hybrid approach to reconcile different aspects of the wave

propagation and Windkessel models has been proposed by Tyberg and colleagues<sup>97,98</sup> but more experimental and/or epidemiological data will be required to validate this hybrid model.<sup>99-101</sup> Nevertheless it has introduced a different interpretation of pulsatile haemodynamics e.g. AIx, traditionally ascribed to being the proportion of the pressure pulse contributed by pressure wave reflection, could instead be viewed as a function of the arterial Windkessel.<sup>62</sup> The specific drug target for reducing excess pulsatility could therefore potentially be one that can restore optimal timing and amplitude of pressure wave reflection and/or optimise arterial Windkessel function.

### **1.8 Selective reduction of cPP**

Vasodilators are the most widely used treatment for hypertension and heart failure. In hypertension they have been shown to improve cardiovascular outcome due to their blood pressure lowering effects, although specific class effects beyond blood pressure lowering could also be implicated.<sup>102</sup> Their common feature is their effect to reduce peripheral resistance<sup>103-107</sup>, once thought to be the sole haemodynamic culprit leading to hypertension. For the last two decades it has been increasingly recognised that brachial DBP control is often achievable but not SBP and PP, even under clinical trial conditions.<sup>3, 8, 108</sup> Often multiple drugs are required to optimise SBP control<sup>4, 109</sup> and in an attempt to optimise SBP, further resistance arteriolar vasodilatation precipitates a greater than desired fall in DBP thus potentially compromising perfusion pressure to vital organs. This is especially true in the context of ISH, the commonest type of hypertension in middle aged and elderly patients.<sup>42</sup> Beside reduction in peripheral resistance, therefore, the rationale for design of new vasodilators specifically targeting PP reduction should be the ability to manipulate arterial stiffness and/or pressure wave reflection within the cardiovascular system.

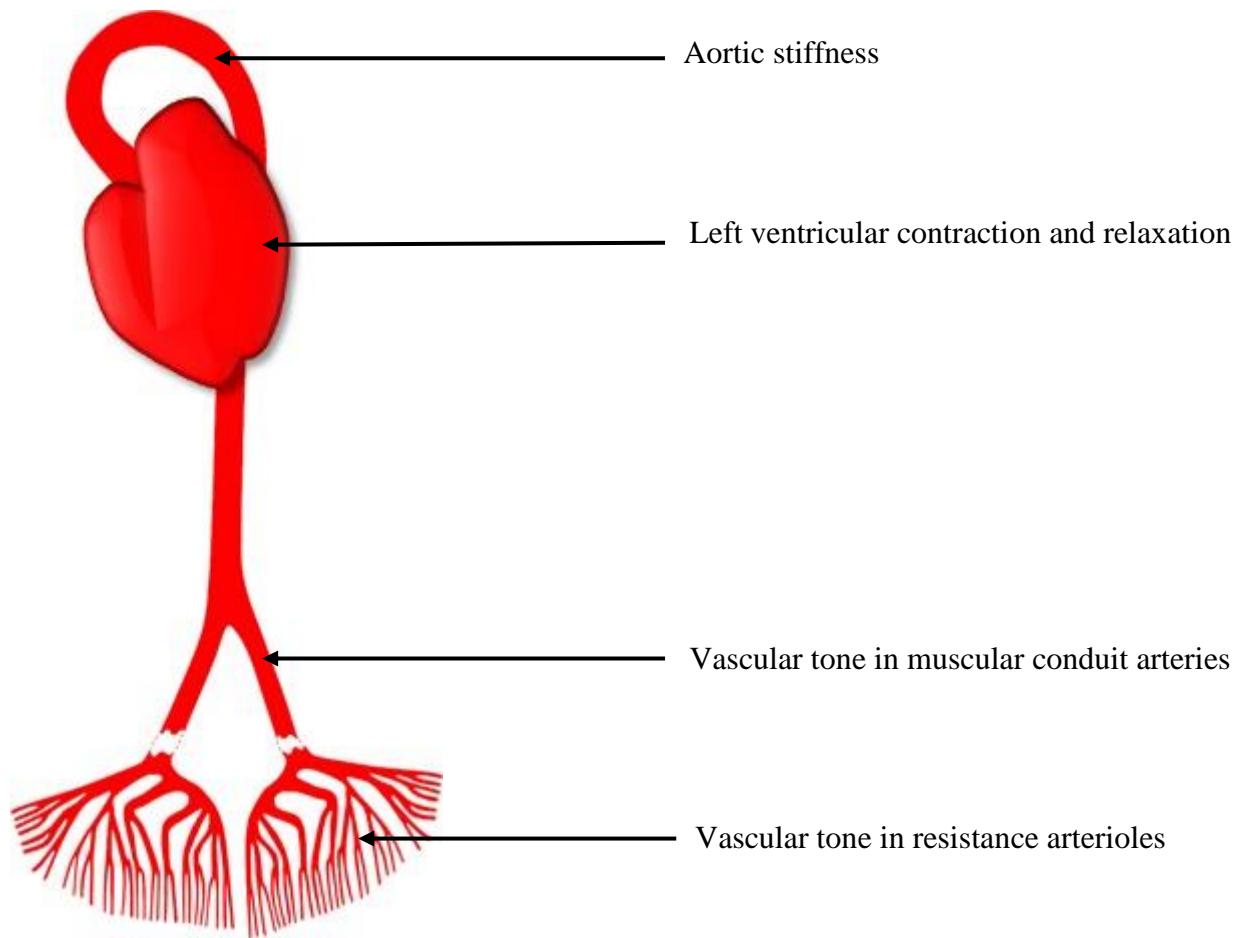


Figure 4: Schematic diagram showing the potential haemodynamic targets for optimisation of ventricular vascular coupling and pulse pressure reduction.

Figure 4 shows the various components within the cardiovascular system that could potentially influence cPP. These include aortic stiffness, muscular arterial tone influencing pressure wave reflection and ventricular contraction and relaxation.

## *Aortic Stiffness*

Arterial stiffening is an ageing process driven by a complex interaction between systemic factors and local haemodynamic forces that activate mechanical and cellular changes within the arterial wall.<sup>18, 110, 111</sup> Large elastic arteries are arbitrarily defined as arteries with internal diameter above 2mm and muscular large arteries with internal diameter between 150µm – 2mm.<sup>22</sup> The structural integrity of the arterial wall, which is a balance of elasticity and tensile strength is determined by the proportion of elastin to collagen in the extracellular matrix and the number of vascular smooth muscle cells (VSMC).<sup>110, 111</sup> From the aortic root down to the distal 5cm of thoracic aorta, elastin predominates over collagen and there are relatively few VSMC within the tunica media. However, beyond this the ratio reverses with more collagen compared to elastin and proportionally more VSMC; this phenomenon continues towards the distal muscular arteries.<sup>22</sup> Arterial stiffening is not uniform: ageing and pathological processes stiffen proximal elastic large arteries more than distal muscular large arteries.<sup>112-115</sup> Since aortic stiffness has been shown to correlate with cPP in epidemiological studies<sup>42, 71</sup>, it is thought to be a potential therapeutic target for cPP. The positive correlation between aortic PWV, a functional measure of aortic stiffness and cPP is thought to be due to the early arrival of the BPW to the proximal aorta and/or an increase in Windkessel pressure. However, the relationship is likely to be more complex as vasodilators can alter cPP without affecting aortic PWV.<sup>52, 63, 88, 116</sup> A further incentive in modifying aortic stiffness is that aortic PWV had been shown to be an independent predictor of cardiovascular outcome<sup>117, 118</sup>, thus it is a subject of much research interest.<sup>44, 119</sup>

Arterial stiffening appears to be a result of interactions between genetic and environmental factors.<sup>120-122</sup> A number of risk factors have been associated with aortic stiffening but age and blood pressure are the two most important factors in predicting the

progression of aortic stiffness.<sup>123</sup> At the microscopic level, changes in the arterial wall occur mainly within the extracellular matrix and the cells it supports, especially VSMC and inflammatory cells. These changes are thought to occur in response to the autocrine, paracrine and neuroendocrine effects of excess salt and glucose and also to pulsatile stress.<sup>22,</sup>  
<sup>110</sup> The roles of the renin-angiotensin system and of angiotensin II and aldosterone have been widely studied. Angiotensin II and aldosterone are thought to contribute to arterial stiffening by stimulating the signalling of multiple inflammatory pathways such as tissue growth factor- $\beta$ , NF- $\kappa$ B and reactive oxygen species production with reduction in nitric oxide (NO) bioavailability.<sup>124-129</sup> The inflammatory effects upset the balance between the production of proteases and their inhibitors and also promote the synthesis of advanced glycation end-products (AGE). Matrix metalloproteinases (MMP) are proteases that are responsible for the accelerated breakdown of elastin and destruction of the molecular folding of collagen. AGE promotes the irreversible cross-linking of collagen, which together with overexpression of MMP, creates a stiff extracellular matrix. The inflammatory response also potentiates proliferation of VSMC and increased VSMC tone.<sup>130-132</sup> Furthermore, it is thought that chronic cyclical stress from ageing leads to elastin fracturing and thinning, which is accelerated in the presence of hypertension.<sup>18</sup> Since production of elastin is negligible in adulthood there is an reversible decline in the ratio of elastin/collagen which stiffens the aorta.<sup>133</sup>

Aortic stiffness can be altered directly through modification of arterial properties or indirectly through change in MAP.<sup>134</sup> Duration of exposure to therapy may also be important as the effects on aortic stiffness may not be apparent in acute or short-term studies. A number of short term, small scale randomised controlled trials investigating the blockade/inhibition of angiotensin II<sup>135-138</sup>, endothelin<sup>139</sup>, aldosterone<sup>140, 141</sup>, phosphodiesterase V<sup>142</sup>, HMG-coA

reductase<sup>143</sup> have shown reductions in PWV in human studies. Since, with the exception of statins these agents mostly reduce MAP, it is difficult to establish whether there is a MAP independent effect on arterial stiffness. The AGE breaker alagebrium is the only drug studied in humans so far that directly targets a molecular mechanism of arterial stiffness. Despite promising initial results, however, alagebrium has not been studied in Phase III trials yet.<sup>144,</sup>

145

### *Selective large artery vasodilators*

The observations that cPP can be selectively lowered without alteration of aortic PWV suggest that there may be more than one mechanism to alter cPP.<sup>52, 63, 88, 116</sup> Since cPP is the result of the interaction between left ventricular dynamics and aortic input impedance, vasodilators with an action on large arteries Windkessel or reservoir properties and/or pressure wave reflection could potentially alter cPP.<sup>146, 147</sup> Furthermore the FPW generated by the left ventricles has been shown in epidemiological and mechanistic studies to influence cPP.<sup>67, 148-150</sup> Therefore vasoactive substances with actions on left ventricular dynamics may also alter cPP.

As discussed, vasodilators with selective actions on larger arteries could theoretically reduce cPP without overt effect on steady components of blood pressure.<sup>151</sup> However, relatively little is known about whether the control of vasomotor tone in large arteries differs from resistance arterioles. Previous studies in rat aortas and mesenteric arteries suggested that nitric oxide (NO) has a greater influence on the vascular tone of large arteries and potassium channels in resistance arterioles.<sup>152</sup> More recently *in vivo* human studies have suggested that both NO and potassium channels contribute to the resting vascular tone of muscular arteries.<sup>153, 154</sup> However the effect of other vasoregulatory pathways that have a major influence on resistance arteriolar tone such as renin-angiotensin-aldosterone, prostaglandin

and endothelin systems on muscular conduit artery tone is unknown. The study of drug effects on large arteries became more refined when techniques of high resolution vascular ultrasound began to mature in the early 1980s. NO donors<sup>147, 155</sup>, Angiotensin receptor enzyme (ACE) inhibitors<sup>156</sup>, vasodilating  $\beta$ -blockers<sup>157</sup> and calcium channel blockers<sup>49</sup> have vasodilatory effects on large arteries. However, the relative effects of these drugs on tone in large and small arteries and whether any are relatively selective for large arteries is unknown. Of particular interest is the haemodynamic effect of low dose glyceryl trinitrate (GTN), which is remarkably effective in reducing cPP mainly through its action on pressure augmentation.<sup>86, 116, 146</sup> In fact it has been shown that in middle aged subjects, administration of sublingual GTN 400  $\mu$ g reduced cPP by almost 30% with little effect on diastolic pressure and MAP.<sup>71</sup> The mechanism of AP reduction is thought to be due to GTN's unique selective action on large muscular arteries.<sup>116, 146</sup> However its direct effects on ventricular dynamics, arterial reservoir and resistance arterioles could also be contributory.<sup>86, 158-160</sup> It is speculated that GTN lacks effects on elastic arteries due to the relatively smaller proportion of VSMC within the wall of elastic arteries compared to large muscular arteries.<sup>86, 88, 111</sup> The understanding of the vasoregulatory mechanism(s) controlling the tone of muscular large arteries could therefore be potentially important.

### *Ventricular dynamics*

Because early studies in hypertension demonstrated cardiac output to be similar in hypertensive and normotensive subjects, it has been assumed that hypertension is a problem relating to the arterial tree. Thus diastolic hypertension is thought to result from an increase in peripheral arteriolar tone and hence in total peripheral resistance that leads to an elevation in MAP and DBP. Similarly an increase in PP is thought to result from increased arterial stiffness and/or pressure wave reflection. However, because SBP occurs early in systole when



only a relatively small proportion of the cardiac output has been ejected it is possible for cardiac output to remain unchanged but for the ventricular dynamics in early systole to influence the pressure pulse. Indeed, the water hammer equation described earlier means that in early systole, when there is no reflection, pressure will be determined by the product of aortic flow velocity (in turn determined by fibre shortening within the ventricle) and aortic PWV. Thus ventricular dynamics may be an important determinant of the pulsatile components of blood pressure.

### **1.9 Aims and hypothesis**

The central hypothesis of this thesis is that vasodilation of muscular conduit arteries will reduce pressure wave reflection and hence selectively reduce AP.

#### Aims

- 1a) To explore the regulation of vascular tone in muscular conduit arteries using vasodilators with known mechanisms of action and to establish drugs that are selective for muscular conduit arteries relative to resistance vessels.
- 1b) To determine whether changes in PWV in muscular conduit arteries are determined by vascular tone.
- 2) Determine whether reduction of AP by a drug selective for muscular conduit arteries is mediated by a reduction in pressure wave reflection.
- 3) Determine to what extent pressure wave reflection contributes to increased pulsatility in essential hypertension.

Local infusion of drugs into the brachial artery with measurement of their effects on the radial artery as an example of a conduit artery and resistance arterioles will be used to characterise the selectivity of drugs for muscular conduit arteries and resistance vessels. Haemodynamic effects of drugs that dilate conduit arteries will be examined using wave intensity analysis and by separating central pressure waveforms into forward and backward waves. Finally, wave separation analysis will be applied to hypertensive and normotensive subjects to determine the contribution of pressure wave reflection to pulsatility in hypertension. Drugs with relatively selective actions on the ventricle and arterial tree will be used to determine to what extent pulsatility may be influenced by ventricular dynamics independent of the arterial tree.

## METHODS

## **2.1 Brachial blood pressure measurements**

Brachial blood pressure was measured using the oscillometric method and according to established guidelines<sup>161</sup> and manufacturer's instructions (Omron 705CP, Omron, Japan or Intellivue MP30, Philips, Netherlands). At least 3 successive measurements were taken and averaged for the calibration of arterial tonometry measurements (see section 2.8) and for haemodynamic analysis.

## **2.2 Venous occlusion plethysmography**

Human resistance arteriolar tone is difficult to measure directly *in vivo*. In research practice the measurement of forearm blood flow (FBF) by venous occlusion plethysmography is used to indirectly infer resistance arteriolar tone. When mean arterial pressure is stable, laminar fluid flow is proportional to the fourth power of vessel radius according to Hagen-Poiseuille's law.

$$Q = \frac{Pd \cdot \pi \cdot r^4}{8\mu l}$$

Where Q = laminar flow, Pd = pressure difference, r = radius,  $\mu$  = viscosity coefficient, l = length of vessel/tube. Therefore changes in resistance arteriolar tone can be inferred from changes in FBF.<sup>162</sup>

The majority of FBF measured by venous occlusion plethysmography is believed to be arterial flow within the skeletal muscles.<sup>162, 163</sup> By occluding venous return at a pressure in the upper arm that does not impede arterial inflow, forearm limb volume increases proportionally with blood flow.<sup>164</sup> Change in forearm limb volume can be measured by

electrically calibrated mercury-in-silastic strain gauges, which have been shown to accurately reflect limb volume.<sup>162, 163, 165, 166</sup>

Several conditions were standardised in order to ensure FBF measurements were accurate and reproducible. Studies were performed in a quiet, temperature-controlled room (24 to 26<sup>0</sup>C). Participants were studied at approximately the same time of day, after a standardised light meal and were asked to avoid caffeine and alcohol on the day of the study. The hand circulation, which has a higher proportion of arterio-venous shunts compared to the forearm circulation, and hence more variable blood flow, was excluded by inflating wrist cuffs to a pressure above systolic blood pressure one minute before and during FBF measurements.<sup>167</sup> Circumference of the widest part of the forearm was measured with an appropriate sized strain gauge (Hokanson, USA) 2-3cm shorter than the measured arm circumference, in accordance with the manufacturer's instructions. Since the relationship between FBF and forearm volume is also dependent on the degree of distension within the venous system, the forearm was elevated above the level of the heart to ensure that the veins were not fully distended and measurements were made over after the first few seconds of upper arm cuff inflation, before the veins became fully distended.

#### *FBF measurement protocol*

Participants lay supine for at least 30 minutes before measurements were initiated. Brachial blood pressure was monitored non-invasively over the left arm throughout the study (Intellivue MP30, Philips, Netherlands). The cuffs were applied to the upper arms and the wrists for venous occlusion and interruption of the hand circulation respectively. Strain gauges were applied to the widest part of the forearm as previously described. One minute before FBF measurements, wrist cuffs were inflated to 180mmHg to allow FBF to stabilize. FBF measurements were taken in both arms simultaneously by inflating the upper arm cuffs

at 40mmHg to achieve venous occlusion without interruption of arterial inflow. Five FBF measurements were taken with the upper arm cuffs inflated at 10 second intervals and with a 5 second deflation period in between. Strain gauge voltage was recorded on an electronic chart recorder (Powerlab, AD instruments, Australia). FBF was calculated from the initial slope of the strain gauge vs time plot over the initial period of upper cuff occlusion when forearm volume increases approximately linearly over time as described by Whitney.<sup>164</sup>

### **2.3 Radial artery diameter measurements**

Simultaneous assessment of radial artery diameter and FBF during brachial artery infusion of vasoactive substances provides a powerful tool to probe the differential vasoregulatory pathways of muscular conduit arteries and resistance arterioles. High resolution vascular ultrasound measurements of the radial artery diameter were obtained during steady state conditions within a few minutes of FBF measurements. In order to avoid a possible influence of a reactive hyperaemic response following deflation of wrist cuff, radial artery diameter measurements were performed before each set of FBF measurements. A 10MHz ultrasound probe (Aspen, Siemens, Germany) was placed 3 to 5cm distal to the point of arterial cannulation to image the right radial artery. Once a good quality two dimensional radial artery image was acquired, the ultrasound probe was placed in a stereotactic holder with micrometer adjustment to allow precise optimisation of image quality throughout the study. End-diastolic (ECG referenced) radial artery diameter (RAD) was obtained from images acquired every 3 seconds over 2 minutes at baseline and when steady state had been achieved during drug infusion. Images were analysed off-line using automated software (Brachial analyser, Medical Imaging Applications, USA) which provided a mean diameter over a length of artery of approximately 10mm. The coefficient of variation of arterial diameter

measurements repeated at baseline and during drug infusion was 4%. The change in radial artery diameter was reported as percentage change relative to baseline.

#### **2.4 Pulse wave velocity measurements of the brachial-radial artery**

Pulse wave velocity (PWV) measurements over the brachial-radial arteries were carried out to investigate the relationship between arterial tone and stiffness of muscular large arteries. PWV was measured over the brachial to radial path from simultaneous pressure cuff recordings at each site using the Vicorder® system (Skidmore Medical, UK). The Vicorder® device uses a modified cross-correlation algorithm for measuring transit times with a resolution of 1.8 milliseconds and the coefficient of variation of readings repeated during the course of one study was 3%. The path distance was determined using a measuring tape taken from the proximal edge of the upper arm cuff to that of the wrist cuff. Three measurements were made following radial artery diameter and FBF measurements and the average PWV was reported. Data was considered acceptable when at least five sequential good quality waveforms were obtained from each cuff.

#### **2.5 Intra-brachial artery drug infusion**

Vasoactive drugs were infused directly into the right brachial artery to probe the regulatory mechanisms of vascular tone in muscular conduit and resistance arteries in the human forearm. When infused locally, physiological or pharmacological concentrations can be achieved in the forearm circulation with negligible systemic drug delivery. This route of drug administration, therefore, avoids a systemic haemodynamic response and consequent activation of baroreceptors and other autonomic responses, which would make interpretation of the local vasodilatory responses difficult. A 27 gauge unmounted steel needle (Cooper's

Needle Works, UK) sealed with dental wax to an epidural catheter (Smiths Medical Portex ®, UK) was inserted into the right brachial artery using  $\leq 1$ ml of 1% lidocaine hydrochloride (Braun Pharmaceuticals) to provide local anaesthesia. Following successful cannulation, either 0.9% saline or drugs dissolved in 0.9% saline vehicle were infused at 1ml/min using a syringe driver (Injectomat, Agilia®, Fresenius Kabi, Germany). Cumulative doses of comparator drugs were infused at each dose until steady state was achieved (typically between 6-12 minutes). Infusion of each drug was preceded by a baseline period, a washout period or studies were performed on different occasions. Brachial cuff blood pressure measurements and FBF were obtained from the non-cannulated arm to ensure there were no significant systemic responses that would influence the interpretation of the results of the FBF response to local infusion of the drugs.

## **2.6 Invasive aortic pressure and flow velocity measurements**

Invasive aortic blood pressure and flow velocity measurements were carried out by Dr Brian Clapp during cardiac catheterisation at St Thomas' Hospital cardiothoracic unit in patients undergoing clinically indicated angiography and/or angioplasty for the investigation/treatment of coronary artery disease. Following routine preparations for angiography, vascular access to the right femoral or radial artery was established with a 6Fr haemostatic sheath. A 6Fr-guiding catheter (JL4, Boston Scientific, UK) was advanced to the aortic root over an 0.35" wire using standard techniques and connected to a closed flushing system with a haemostatic valve. All subjects were systemically heparinized with unfractionated heparin (Wockhardt, UK) to reduce the risk of thrombus formation within the catheter. A Combwire pressure and Doppler flow velocity transducer (9500XT,



VolcanoCorp, USA) was passed through the guiding catheter and the tip of the wire was positioned under fluoroscopic guidance in the proximal aortic root to obtain optimal coaxial stable pressure and flow tracings. The transducer was connected to a monitor (Combomap, VolcanoCorp, USA) with an analogue output at a frequency of 1 kHz and the signals were recorded via an analogue to digital converter (Micro 1401, Cambridge electronic design, UK). Aortic root pressure and Doppler flow velocity were obtained over at least 10 cardiac cycles at baseline and following administration of drugs (see below). All measurements were taken before the administration of ionic contrast agents for the subsequent angiogram or angioplasty except for a subset of subjects receiving intra-coronary drug infusions where diagnostic coronary angiography was performed before the research study to ensure the absence of significant left main stem or critical left anterior descending coronary artery disease that could affect the distribution of drug within the left ventricle. The aortic Doppler flow velocity waveforms were integrated over time and multiplied by aortic cross-sectional area (estimated from body surface area) to obtain a measure of stroke volume and hence cardiac output and total systemic vascular resistance.

## **2.7 Non-invasive carotid pressure measurements**

Since, invasive measurements of aortic pressure can only be obtained at the time of cardiac catheterisation, non-invasive measures of carotid pressure were used as a surrogate for aortic pressure in subjects for whom cardiac catheterisation was not clinically indicated. Although the carotid and aortic pressure waveforms are not identical they resemble each other closely both at rest and during dynamic testing with physiological manoeuvres and drugs.<sup>168</sup> Correct calibration of the carotid pulse is important due to pulse pressure amplification and the most

widely used method is to calibrate carotid pressure from brachial mean and diastolic blood pressure on the assumption of equality of these pressures at central and peripheral sites.<sup>20</sup>

Apart from the same variation in waveform morphology other drawbacks of using the carotid pulse as a surrogate for the aortic pulse include the difficulty in obtaining a stable pressure tracing with applanation tonometry, the potential activation of baroreceptors and theoretical adverse effects on undiagnosed carotid plaques. However, the carotid pressure waveform has been widely used as a surrogate for the aortic pressure pulse in epidemiological studies, clinical trials and mechanistic haemodynamic studies.<sup>24, 54, 60, 169-171</sup>

Measurements were performed in a quiet temperature controlled (24-26 °C) room. Subjects were asked to avoid caffeine and alcohol on the day of the study. All pressure measurements were taken supine following 20-30 minutes resting. Radial and carotid pressure waveforms were obtained by applanation tonometry using the SphygmoCor system (AtCor, Australia). Approximately 10 cardiac cycles were obtained and ensemble averaged. Waveforms that did not meet the in-built quality control criteria in the SphygmoCor system were rejected. Brachial blood pressure was measured in triplicate by a validated oscillometric method (Omron 705CP, Omron Health Care, Japan) and used to calibrate radial waveforms to obtain MAP. Carotid waveforms were calibrated from MAP and diastolic brachial blood pressure on the assumption of equality of these pressures at central and peripheral sites. Carotid pressure and aortic Doppler flow velocity (see below) were measured under the same condition less than 5 minutes apart.

## **2.8 Non-invasive aortic flow measurements**

Ultrasound imaging was performed by Dr Benyu Jiang using the Vivid-7 ultrasound platform (General Electric Healthcare, UK). Flow velocity across the aortic valve was recorded using continuous wave Doppler obtained from an apical 5- chamber view. Stroke volume (SV) was calculated from the product of VTI and cross-sectional area of the aortic valve (obtained in the parasternal long axis view). All measurements were averaged over at least 3 cardiac cycles. Cardiac output (CO) was calculated from SV and heart rate and total peripheral resistance (TPR) from mean arterial pressure (MAP) divided by CO.

## **2.9 Waveform Post-processing**

Aortic Doppler flow velocity and aortic/ carotid pressure waveforms were stored and analysed in Matlab (2012b, Mathworks, USA) for wave intensity, wave decomposition and pulse wave analyses. The first systolic shoulder of the aortic/carotid pressure waveform was identified as the first local minimum of the first derivative of the pressure curve (and confirmed by visual inspection) to determine P1, P2 and AP, the difference between P2 and P1. Augmentation Index (AIx) was calculated as the percentage proportion of AP to PP.

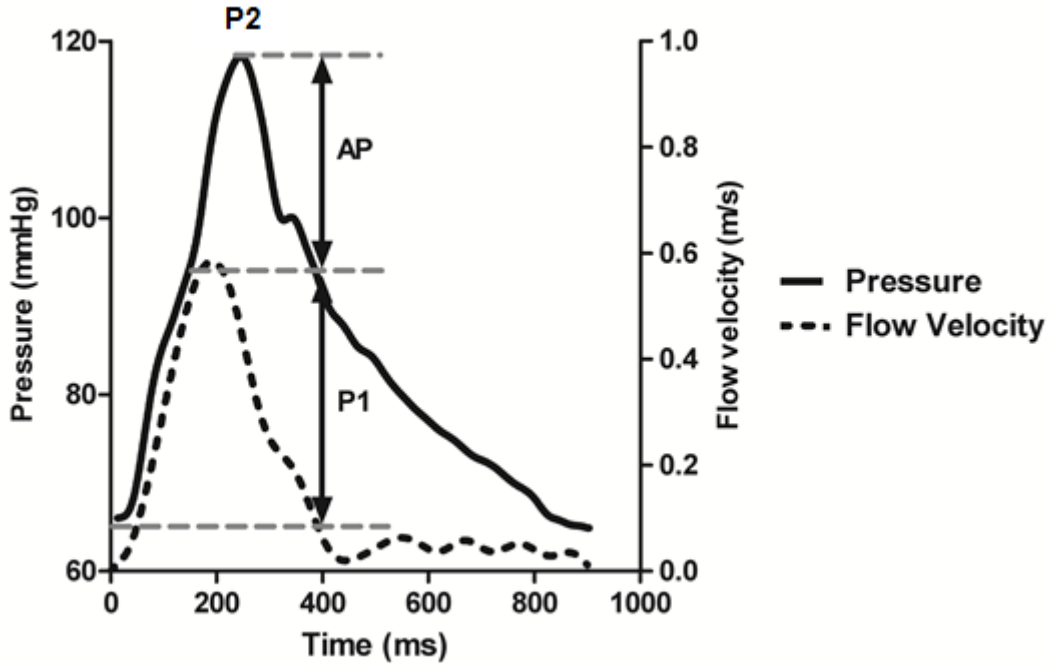


Figure 5: Aortic pressure and flow velocity waveforms and the components of central pulse pressure. P1 is the first shoulder of the waveform above diastolic pressure and AP is the difference between P2 and P1.

Pressure wave decomposition into forward and backward waves was performed using the equations derived by Euler in 1775, based on the conservation of mass and momentum. The aorta was considered as an elastic tube, blood as an incompressible and inviscid fluid and the flow was assumed to be unidimensional. These equations were solved using the method of Parker<sup>172</sup> and the following formulas were obtained for FPW and BPW:

$$FPW = \frac{1}{2} \int_0^T \left( \frac{dP}{dt} + \rho c \cdot \frac{dU}{dt} \right) dt$$

$$BPW = \frac{1}{2} \int_0^T \left( \frac{dP}{dt} - \rho c \cdot \frac{dU}{dt} \right) dt$$

Where  $P$  and  $U$  are the pressure and the flow velocity respectively,  $T$  is the foot to foot duration of a pulse,  $\rho$  is the density of blood and  $c$  the speed of travel of waves equal to the local pulse wave velocity (PWV). The wave speed  $c$  was calculated using the single point method described by Davies et al using the following formula.<sup>173</sup>

$$c = \frac{1}{\rho} \sqrt{\frac{\sum dP^2}{\sum dU^2}}$$

Where  $P$  and  $U$  are the pressure and flow velocity respectively. This method of wave separation produces the same results as that performed in the frequency domain.<sup>174</sup>

Separation of the pressure wave allows the individual contributions of the FPW and BPW to each of the components (e.g. P1, P2 and AP) of the pressure pulse to be determined. Since the BPW is usually regarded as the result of “reflection” of the FPW. The ratio between the peak amplitude of FPW: BPW is often used to obtain a reflection coefficient. This allowed the contribution of the forward wave and reflected wave to AP to be assessed and hence to allow the actions of vasodilator drugs in reducing AP to be examined.

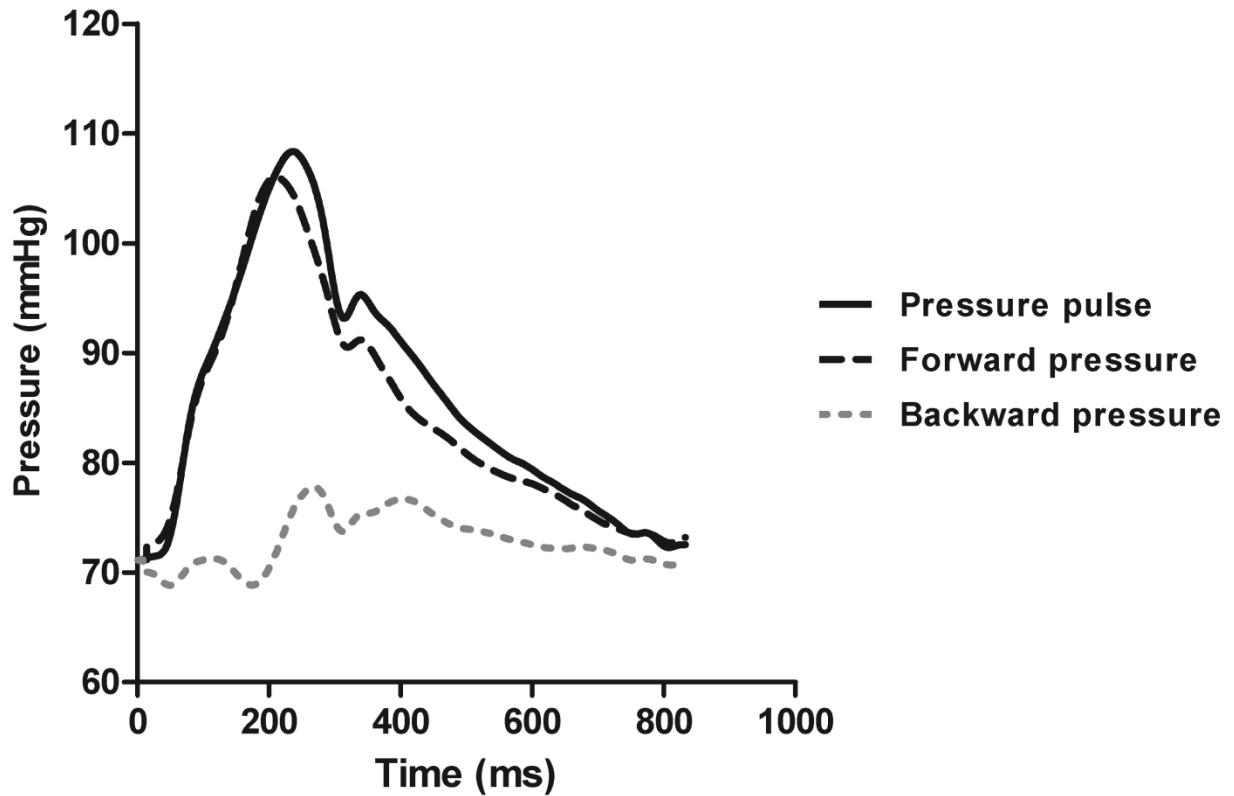


Figure 6: Aortic pressure waveform decomposed into forward and backward pressure components.

#### *Wave intensity analysis of aortic pressure*

Wave intensity is the product of the first derivative of pressure and the first derivative of flow velocity<sup>172</sup>:

$$WI = \frac{dP}{dt} \times \frac{dU}{dt}$$

Where  $P$  and  $U$  are the pressure and the flow velocity respectively. With wave intensity analysis pressure waves can be further categorized as compression or expansion (suction). Thus wave separation and intensity analysis allow waves to be characterised according to their origin (either from the ventricle for a FPW or from the peripheral circulation for a BPW) and whether that are generated by compression or suction at the point

of origin. Thus a forward compression wave (FCW) is thought to be generated by the force of contraction of the left ventricle and a forward expansion wave (FEW) in early systole by the braking effect of the ventricle as it starts to relax. A backward compression wave can be generated by reflections of the FPW wave from the peripheral arterial tree. Other minor waves arise from other ventricular – vascular interactions.<sup>172</sup>

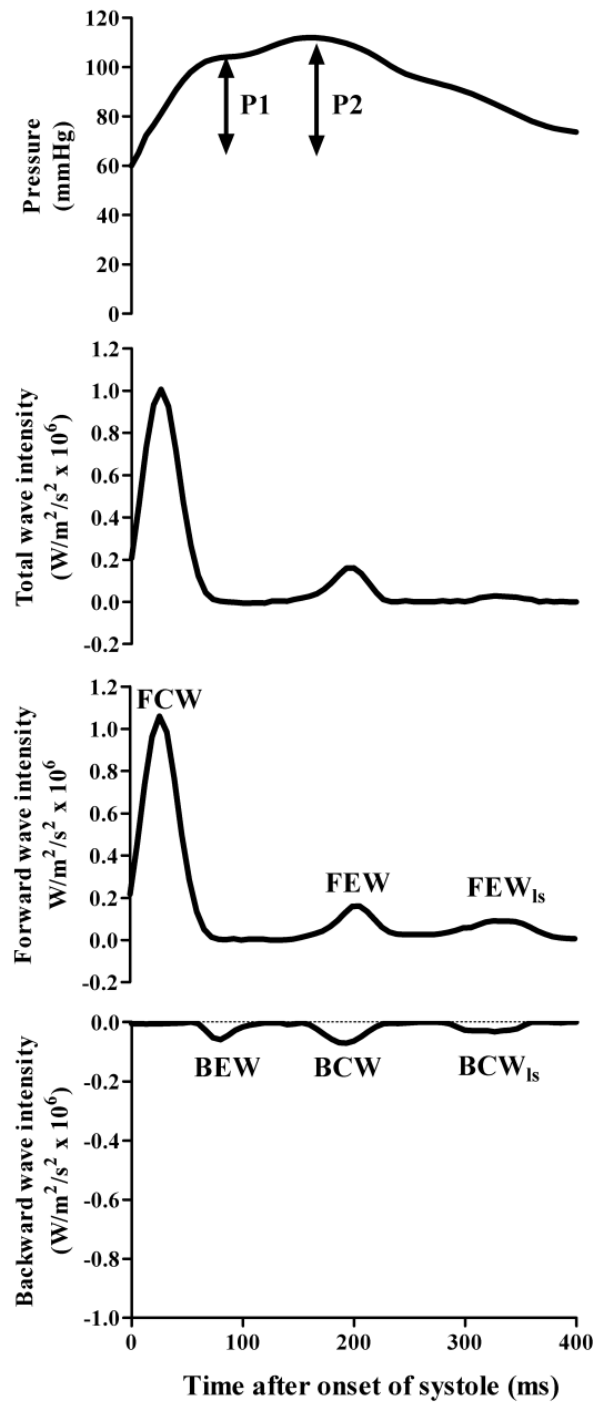


Figure 7: Wave intensity analysis of an aortic pressure waveform in systole. FCW, forward compression wave; FEW, forward expansion wave; FEW<sub>1s</sub>, late systolic forward expansion wave; BEW, backward expansion wave; BCW, backward compression wave; BCW<sub>1s</sub>, late systolic backward compression wave.



## **2.11 Statistics**

Statistical analysis was performed using SPSS version 19. Subject characteristics were summarised as means  $\pm$  SD. Unless where stated results are expressed as means  $\pm$  SE.

Comparison between groups of subjects or response to different drugs was performed using Student's paired *t*-test or by analysis of variance for repeated measures. In the cases of non-normally distributed data, a Wilcoxon signed-rank test was used. All tests were 2-tailed and  $P < 0.05$  was considered significant. The within subject coefficient of variation was used to assess reproducibility where appropriate and calculated by dividing within subject standard deviation by the mean.

REGULATION OF VASCULAR TONE AND PULSE  
WAVE VELOCITY IN HUMAN MUSCULAR  
CONDUIT ARTERIES: SELECTIVE EFFECTS OF  
NITRIC OXIDE DONORS TO DILATE MUSCULAR  
ARTERIES RELATIVE TO RESISTANCE VESSELS

### **3.1 Background**

Human pharmacology of vasodilator drugs has focused almost exclusively on their actions on vascular smooth muscle in “resistance” vessels comprising the arterioles and microvessels less than 100  $\mu\text{m}$  in diameter that determine peripheral vascular resistance and hence mean arterial blood pressure (MAP). The pulsatile component of blood pressure, which is of more prognostic importance than MAP, especially in older subjects<sup>175</sup> is, however, dependent on characteristics of large elastic and muscular conduit arteries.<sup>176, 177</sup> Vasodilator drugs have little effect on large elastic arteries (other than the passive effect due to a change in blood pressure) presumably because these have little smooth muscle.<sup>178</sup> By contrast, conduit arteries are comprised mainly of vascular smooth muscle and, dependent on basal vascular tone, might be expected to be responsive to vasodilators. Conduit arteries influence the pulsatile component of blood pressure through their contribution to total arterial functional compliance or reservoir property and to pressure wave reflections.<sup>179</sup> Pressure wave reflections may be important over and above their contribution to blood pressure, since they are predictive of cardiovascular mortality independently of conventional blood pressure components.<sup>47</sup> The effects of wave reflections may be influenced both by artery diameter and local arterial pulse wave velocity within conduit arteries (PWV), since this PWV influences the timing and functional consequence of reflections in terms of ventricular loading.<sup>179</sup>

The aim of the present study was to firstly compare the effects of different classes of vasodilator drug on arterial tone and vasodilation of the radial artery, as an example of a muscular conduit artery, and on forearm resistance vessels representative of those contributing to total peripheral vascular resistance. A second objective was to examine the relationship of local PWV within the radial artery to vascular tone and diameter. By infusing drugs locally into the brachial artery I was able to study these effects independent of any

change in blood pressure or activation of neurohormonal reflexes. I examined drugs acting through the cyclic guanosine monophosphate (cGMP) pathway: the NO donors nitroglycerin and nitroprusside acting on soluble guanylyl cyclase (sGC) and brain natriuretic peptide (BNP) acting on particulate guanylyl cyclase (pGC) as there is indirect evidence that these may exhibit relative specificity for muscular arteries.<sup>180</sup> I compared these cGMP agents to the alpha-adrenergic antagonist phentolamine that would oppose sympathetically induced tone, the calcium channel antagonist verapamil that may antagonise myogenic tone and hydralazine, a vasodilator acting through multiple pathways.

### **3.2 Methods**

#### *Participants*

Participants were normotensive healthy men aged 19 – 53 years (mean±SD, 27.6±8.4 years). All were asymptomatic with no history of or risk factors for cardiovascular disease (mean serum total cholesterol, 4.3±1.1 mmol/L and blood pressure 126±10/ 73±9 mmHg) and were on no regular medication. Due to the large number of drugs examined and constraints on the duration of each intra-arterial study and number of arterial cannulations performed on each subject not all drugs could be administered to all subjects. However, as far as possible, the allocation of study drug to subjects was randomised to avoid bias. Forty subjects were studied in total with effects of each drug being studied in a minimum of 8 subjects (except as noted below for norepinephrine where n=7) and an average number of 2 drugs studied per subject. There were no significant differences in subject characteristics (age, blood pressure or serum lipids) between subjects receiving the various drugs. The study was approved by the London Westminster Research Ethics Committee, and written informed consent was obtained from all participants.

*Study 1: Effects of vasodilators on muscular conduit arteries and resistant arteries*

Studies were performed in a quiet temperature-controlled room (24-26°C). Subjects were studied at approximately the same time of day, after a standardised light meal and were asked to avoid caffeine and alcohol on the day of the study. Subjects lay supine for at least 30 minutes before measurements were initiated. Brachial blood pressure was monitored non-invasively over the left arm throughout the study (Intellivue MP30, Philips, Netherlands). A 27 gauge unmounted steel needle (Cooper's Needle Works, UK) sealed with dental wax to an epidural catheter (Smiths Medical Portex®, UK) was inserted into the right brachial artery using less than 1ml of 1% lidocaine hydrochloride (Braun Pharmaceuticals) to provide local anaesthesia. Either 0.9% saline or drugs dissolved in 0.9% saline vehicle were infused at 1ml/min using a syringe driver (Injectomat, Agilia®, Fresenius Kabi, Germany). Cumulative doses of comparator drugs: nitroprusside (NP, 0.3, 1, 3 µg/min, n=11); nitroglycerin (NTG, 0.03, 0.1, 0.3, 1 µg/min, n=8); nesiritide (BNP, 0.1, 0.3, 1, 3 µg/min, n=8); phentolamine (PHT, 10, 30, 100 µg/min, n=9); verapamil (VER, 10, 30, 60 µg/min, n=8); hydralazine (HYD, 10, 30, 100 µg/min, n=8) were infused for 12 min (at each dose). With the exception of NP (the infusion of which was followed by a washout period and then infusion of a second drug), the duration of action of drugs was too long to allow for more than one drug to be infused on one visit and therefore each drug was given on separate occasions.

Acquisition of radial artery diameter (RAD) and forearm blood flow measurements were carried out as described in Chapter 2. End-diastolic (ECG referenced) radial artery diameter (RAD) was obtained from images acquired every 3 seconds over 2 minutes and after 7 minutes infusion when steady state had been achieved (before measurement of forearm

blood flow as described below in order to prevent cuff inflation from interfering with ultrasound images).

Following acquisition of ultrasound images, forearm blood flow (FBF) measurements were taken in both arms simultaneously by venous occlusion strain gauge plethysmography (Hokanson, USA) during the final 9-12 min of drug infusion. Wrist cuffs were inflated to 180mmHg. Upper-arm cuffs were inflated to 40mmHg intermittently and five measurements used to calculate mean FBF.

*Study 2: Relationship between pulse wave velocity and muscular conduit artery diameter*

In this study the aim was to examine the relation between RAD and PWV irrespective of FBF. To modulate arterial tone over a wider range, I used norepinephrine (NE, 0.01, 0.02, 0.04 µg/ min, n=7) infused into the brachial artery alone and co-infused with PHT to generate beta-adrenergic mediated vasodilation (beta-adrenergic vasodilation being opposed by alpha-adrenergic mediated vasoconstriction). NTG (0.03, 0.1, 0.3, 1µg/min, n=8) was used to generate an increase in RAD. PWV was measured as described in Chapter 2 using the Vicorder ® system (Skidmore Medical, UK). The path distance was taken from the proximal edge of the upper arm cuff to that of the wrist cuff. RAD and PWV were measured at baseline and after 8 min of infusion of each dose of drug.

Characteristic impedance of the forearm vascular bed ( $Z_c$ ) was calculated from PWV and RAD using the relation:

$$Z_c = \frac{4\rho \cdot PWV}{\pi \cdot RAD^2}$$

derived from the water hammer equation,<sup>181</sup> where  $\rho$  is blood density. Changes in PWV were partitioned into those due to a change in the intrinsic elasticity of the arterial wall as represented by Young's incremental elastic modulus ( $E_{inc}$ ), thickness of the arterial wall ( $h$ ) and RAD using the Moens-Korteweg equation:

$$PWV = \sqrt{\frac{E_{inc} \cdot h}{\rho \cdot RAD}}$$

### *Drugs*

Drugs were obtained from Baxter Healthcare (saline); Hospira Incorporation (NP and NTG); Alliance pharmaceuticals (PHT); Sovereign Medical (HYD); Abbott laboratories (VER); Scios Incorporation (BNP/Nesiritide) and Aguetant Ltd (NE).

### *Statistical analysis*

Results are summarised as means $\pm$ SEM. In order to compare effects of drugs on RAD for the same degree of action on resistance vessels, dose response curves were extrapolated, for each subject, to obtain a RAD representative of fixed absolute increments in FBF of 1, 2 and 3 ml/min/100ml. Differences between means were evaluated for statistical significance by analysis of variance ANOVA (for repeated measures whereas appropriate). SPSS version 16 was used for all statistical testing and statistical significance was considered when  $P < 0.05$ .

## **3.3 Results**

### *Study 1*

Local intra-arterial infusion of the vasodilator drugs produced a dose-dependent increase in FBF in the infused arm with an approximate 2-3 fold increase in FBF at the highest dose

(table 1). There were minor or non-significant changes in FBF in the non-infused control arm (table 1) indicating that local effects in the infused arm were unlikely to be influenced by changes in systemic haemodynamics.



**Table 1 Forearm blood flow (FBF) in infused and non-infused arms (con) and radial artery diameter (RAD) in infused arm during brachial artery infusion of vasodilator drugs**

<b>Vasodilators</b>	<b>FBF<sub>infused</sub></b> <b>(ml/min/100ml)</b>	<b>FBF<sub>con</sub></b> <b>(ml/min/100ml)</b>	<b>RAD<sub>infused</sub></b> <b>(mm)</b>
<b>Phentolamine (n= 9)</b>			
Vehicle	3.56±0.43	1.91±0.18	2.52±0.08
10 µg/min	5.21±0.39†	2.34±0.26	2.59±0.07
30 µg/min	6.26±0.58†	2.16±0.28	2.60±0.06
100 µg/min	6.91±0.57†	2.21±0.2	2.61±0.07
<b>Verapamil (n= 8)</b>			
Vehicle	2.66±0.35†	2.56±0.28	2.64±0.21
10 µg/min	5.23±0.58†	2.60±0.25	2.81±0.22†
30 µg/min	7.65±1.01†	2.83±0.32	2.80±0.22
60 µg/min	9.46±1.01†	3.3±0.43†	2.88±0.23*
<b>BNP (n= 8)</b>			
Vehicle	3.29±0.50	3.33± 0.58	2.49±0.11
0.1 µg/min	3.53±0.70	2.83±0.15	2.60±0.10
0.3 µg/min	4.39±0.57*	2.73±0.15	2.69±0.10*
1 µg/min	6.26±0.86†	2.78±0.3	2.75±0.10*
3 µg/min	7.56±1.00†	2.56±0.15	2.81±0.11*
<b>Hydralazine (n= 8)</b>			
Vehicle	3.42±0.61	2.21±0.25	2.33±0.14
10 µg/min	4.72±0.62†	2.26±0.28	2.48±0.16*
30 µg/min	7.39±0.87†	2.46±0.33	2.64±0.17†
100 µg/min	13.2±1.66†	3.26±0.56*	2.76±0.19‡
<b>Nitroprusside (n= 11)</b>			
Vehicle	3.25±0.24	2.74±0.37	2.41±0.11
0.3 µg/min	5.22±0.46†	2.56±0.2	2.78±0.10
1 µg/min	7.30±1.00†	2.89±0.29	2.89±0.10*
3 µg/min	9.31±1.05†	2.78±0.31	2.96±0.11†
<b>Nitroglycerin (n= 8)</b>			
Vehicle	3.10±0.37	2.84±0.44	2.59±0.07
0.03 µg/min	3.66±0.45*	2.43±0.29	2.95±0.67†
0.1 µg/min	4.38±0.48*	2.48±0.43	3.11±0.09†
0.3 µg/min	4.50±0.43†	1.94±2.41	3.22±0.09‡
1 µg/min	6.29±0.55‡	2.5±0.27	3.28±0.09‡

\* P < 0.05, † P<0.01, ‡ P<0.0001 compared to vehicle.

With the exception of PHT, all vasodilators produced a significant increase in RAD in the infused arm. However, effects on RAD differed between the drugs. This was more marked when effects on RAD were compared for a given increase in FBF (figure 1). PHT, VER and BNP produced relatively little dilation (mean dilation < 9%). NTG and to a lesser extent NP produced greater dilation (figure 1,  $P < 0.01$  and  $P < 0.05$  compared to PHT). For the lowest increase in FBF of 1 ml/min/100 ml, dilation was  $1.5 \pm 1.0$ ,  $7.8 \pm 1.6$  and  $15.8 \pm 3.2\%$  for PHT, SNP and NTG respectively ( $P < 0.01$  for NTG vs. PHT) and at the highest increase in FBF of 3 ml/min/100ml,  $3.4 \pm 1.4$ ,  $16.8 \pm 2.6$  and  $26.0 \pm 3.5\%$  for PHT, NP NTG respectively ( $P < 0.0001$  for NTG vs. PHT and  $P < 0.001$  for NP vs. PHT). HYD produced an intermediate degree of dilation and the order of efficacy to dilate the radial artery was: NTG>NP>HYD>BNP>VER>PHT, irrespective of the increase in FBF at which actions on RAD were compared (figure 8).

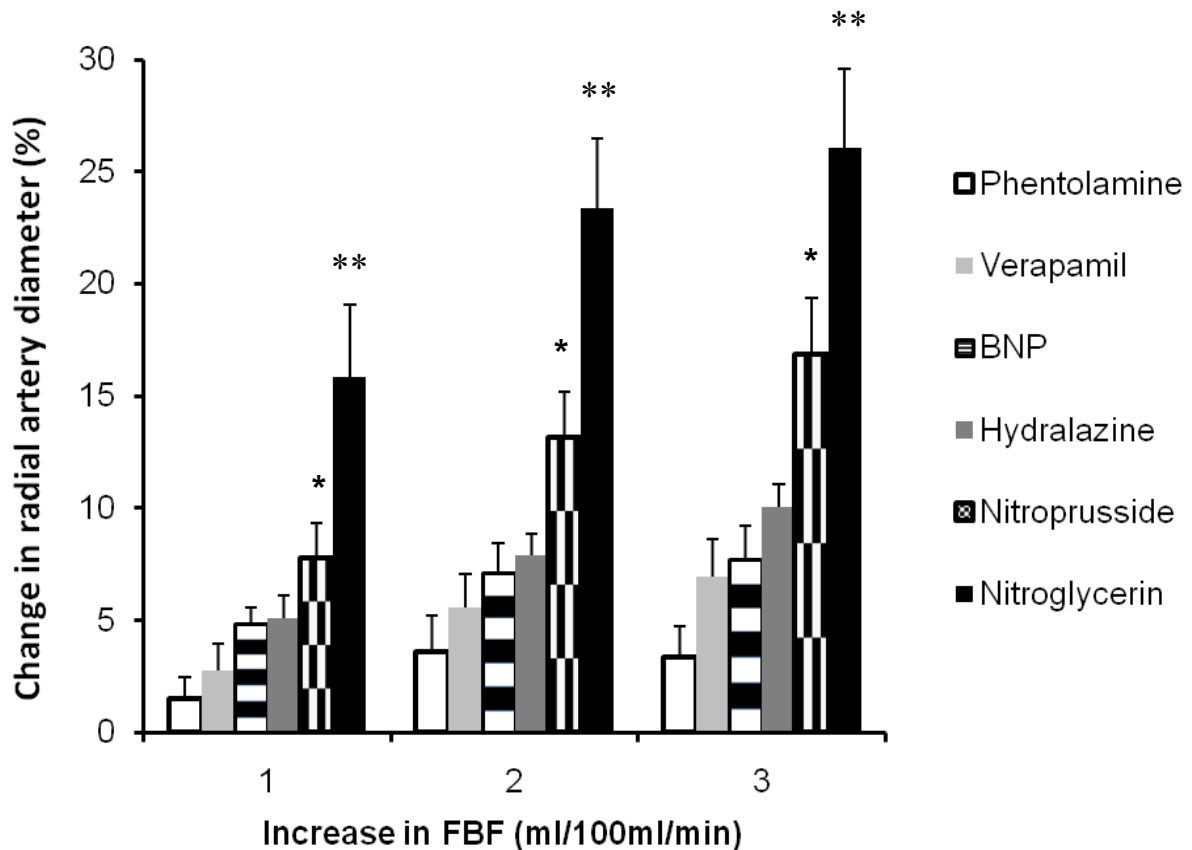


Figure 8

Increase in radial artery diameter compared at doses of drugs that produce the same increase in forearm blood flow (FBF). \*\*  $P < 0.01$  compared to phentolamine, verapamil and BNP. \*  $P < 0.05$  compared to phentolamine. Bars represent mean values; error bars are SEM,  $n = 9, 8, 8, 8, 11$  and  $8$  for phentolamine, verapamil, BNP, hydralazine, nitroprusside and nitroglycerin respectively.

### Study 2

NE ( $0.04 \mu\text{g}/\text{min}$ ) reduced RAD by  $11.4 \pm 4.1\%$  but when co-infused with PHT ( $100 \mu\text{g}/\text{min}$ ) increased RAD by  $5.8 \pm 3.5\%$ . NTG ( $1 \mu\text{g}/\text{min}$ ) increased RAD by  $32.1 \pm 5.4\%$ . Over this range of modulation of vasodilator tone, changes in PWV and Zc were closely related to those in RAD. Changes in RAD of 20% equated to changes in PWV and Zc of  $\sim 5\%$  and  $\sim 50\%$  respectively (figure 2,  $R = -0.89$ ,  $P < 0.05$  for PWV;  $R = -0.99$ ,  $P < 0.001$  for Zc).

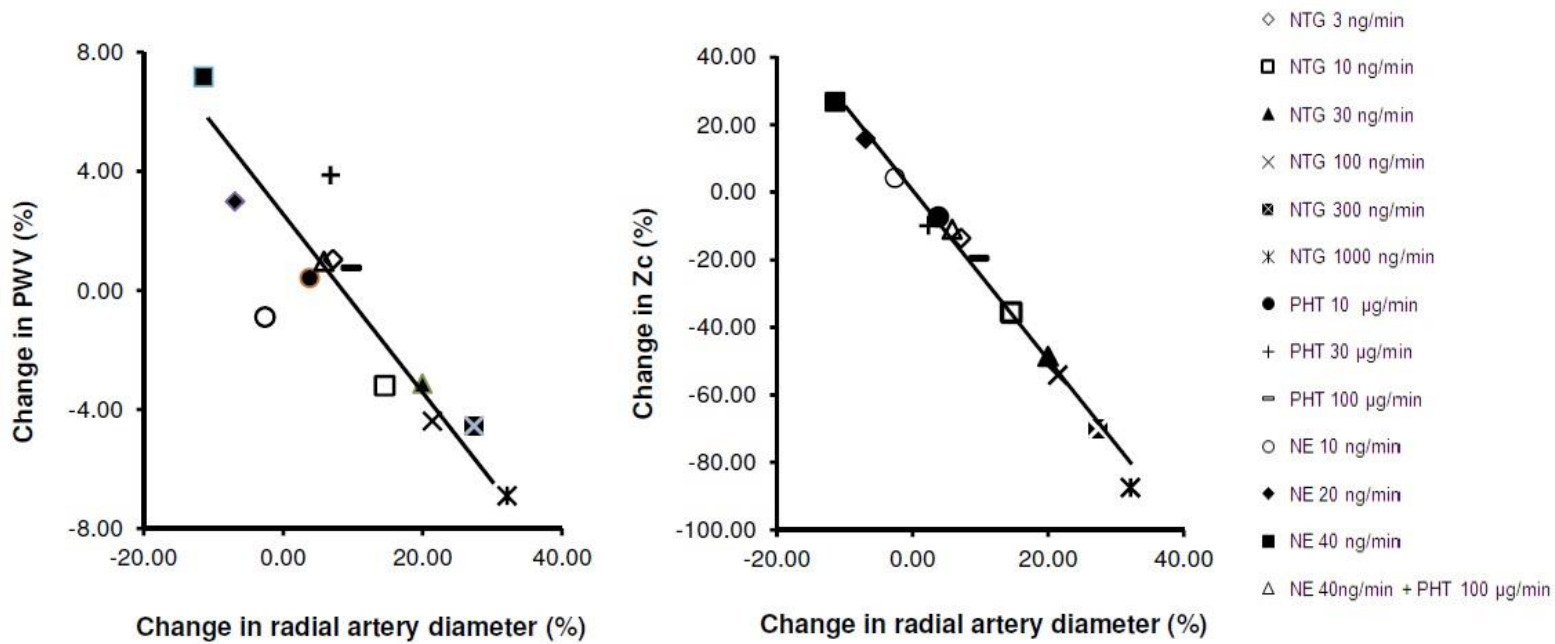


Figure 9

- Relation between changes in radial artery pulse wave velocity (PWV) and diameter during modulation of arterial tone with nitroglycerin (NTG, n=8), phentolamine (PHT, n=8), norepinephrine (NE, n=7) alone and with PHT (n=7). Each symbol represents mean values for each dose of drug. The solid line is the line of best fit by linear regression; Pearson correlation coefficient,  $R=-0.89$ ,  $P<0.05$ .
- Relation between change in local characteristic impedance ( $Z_c$ ) and radial artery diameter during modulation of arterial tone as in (a).  $R= -0.99$ ,  $P<0.001$ .

### 3.4 Discussion

To my knowledge, this is the first study to systematically examine the effects of different classes of vasodilator drugs on both muscular arteries and resistance vessels. For the same degree of forearm resistance vessel dilation, the vasodilator drugs studied differed widely in their effects on RAD. The NO-donors NTG and NP acting through sGC had marked vasodilator effects on the muscular artery with NTG significantly more effective than NP. NTG produced a  $> 20\%$  increase in RAD. The functional consequences of such an increase in diameter result from the change in local  $Z_c$  influencing the local dynamic pressure/flow relation with greater initial flow and hence shear stress for a given pressure and a reduction in

pressure wave reflection. Assuming no change in intrinsic arterial elasticity,  $Z_c$  is proportional to  $RAD^{-2.5}$  and therefore a 20% increase in  $RAD$  will produce an approximate 50% reduction in  $Z_c$ . By contrast to NTG and NP, BNP, acting through pGC rather than sGC, had less effect than either NTG or NP. Differential effects of the NO-donors could relate to variation in the NO species released (e.g. different redox states and/or thiol intermediates) with potential actions on potassium channels and mechanism other than cGMP. Differences between the sGC and pGC activators could be due to spatial compartmentalisation of cGMP production - whereby a higher concentration of cGMP accumulates close to the sarcolemma when activated by sGC in the cytosol as opposed to membrane bound isoform pGC.<sup>182, 183</sup>

The marked vasodilator effects of NTG demonstrate that there is considerable resting tone within muscular arteries. This could be sympathetically mediated (as in resistance vessels), due to an intrinsic “myogenic” response (not necessarily causing vasoconstriction in response to an increase in transmural pressure but an increase in tone) or another mechanism. Despite producing similar effects on resistance vasculature to NTG and reversing constriction produced by the alpha-adrenergic agonist NE, the alpha-adrenergic antagonist PHT had relatively little effect on muscular artery diameter. This suggests there is little sympathetically mediated resting tone within these muscular arteries. The myogenic response is thought to be mediated in part through calcium channels and, in some vessels, is inhibited by the L-type calcium channel blocker VER.<sup>184</sup> In the present study, however, I found that whilst VER dilated resistance vessels with similar efficacy to PHT, it had only minor effects on the muscular artery. Thus if a myogenic response is implicated in resting tone within muscular arteries it is likely that this is mediated through a mechanism other than L-type calcium channels but which is functionally or directly antagonised by an NO donor. In this regard it is notable that NO-donors reduce myogenic tone in rat arteries by a mechanism thought to involve activation of calcium-dependent potassium channels.<sup>185</sup>

A second objective of my study was to examine the relationship between arterial vasodilator tone and local PWV in the radial artery. Previous studies have demonstrated that PWV is influenced by NO, with PWV increased or reduced by inhibition or stimulation of NO synthase respectively<sup>186</sup> and decreased by administration of an exogenous NO donor.<sup>187</sup> However, it is unlikely that these findings relate to a specific effect of NO on arterial elasticity and PWV may simply relate to arterial smooth muscle tone irrespective of the signalling pathway through which this is modulated. In the present study I altered arterial vasodilator tone over a wide range, using NE to constrict the artery, PHT to antagonise the actions of NE on alpha-adrenergic receptors and thus dilate the artery through its unopposed vasodilator actions on beta-adrenergic receptors and NTG as an NO donor and dilator. Changes in PWV were closely related to those in arterial diameter, irrespective of the vasoactive drug used to modulate arterial tone. This suggests that, under physiological conditions, PWV in muscular arteries is determined by smooth muscle tone rather than being influenced by a specific signalling pathway. Since PWV is known to be dependent on transmural pressure,<sup>187</sup> this finding is consistent with arterial tone in muscular arteries being generated mainly as an intrinsic 'myogenic' response to transmural pressure. Although a measure of 'arterial stiffness', PWV may be influenced both by arterial diameter and the intrinsic elasticity of the arterial wall. The Moens-Korteweg equation allows change in PWV to be partitioned into that due to the change in  $E_{inc} \cdot h$  and RAD, with the percentage change in PWV predicted to be 50% of that arising from change in RAD if  $E_{inc} \cdot h$  remains constant. Figure 2 shows that the percentage change in PWV was ~25% of that in RAD suggesting that  $E_{inc} \cdot h$  increases. Since an increase in RAD necessarily implies a reduction in  $h$  as the wall is stretched, my results suggest a paradoxical increase in  $E_{inc}$ . This behaviour exemplifies the complex relations between local geometry, wall stiffness and PWV.<sup>187, 188</sup>

The clinical relevance of my findings relates to the potential to selectively reduce tone of muscular arteries, with the resultant vasodilatation and decrease in PWV reducing pressure wave reflection and delaying the time of its arrival in systole; actions that will reduce systolic blood pressure and pulse pressure.<sup>86</sup> Selective vasodilatation of muscular as opposed to resistance vessels means that diastolic pressure will be relatively unaffected. Such haemodynamic effects are likely to be of particular benefit in subjects with isolated systolic hypertension in whom myocardial wall stress and oxygen consumption are elevated due to raised systolic pressure but in whom a reduction of diastolic pressure in parallel with systolic pressure may compromise myocardial perfusion during diastole.<sup>189</sup> It is notable that NTG is remarkably effective at reducing systolic pressure (particularly central systolic pressure<sup>19</sup>) with little effect on diastolic pressure.<sup>63, 190, 191</sup> The present results suggest that, of currently available vasodilator agents, NO donors show the greatest selectivity for muscular arteries. Furthermore the heterogeneity between NO donors suggests that this is dependent on the exact NO-signalling pathway and that there may be potential to exploit this to enhance the selectivity of these agents to dilate muscular arteries. In this regard it is notable that the NO donor sinitrodil can increase compliance of the brachial artery without effects on systemic vascular resistance.<sup>147</sup> It should be noted that the present study provides insight into local regulation of arterial tone and PWV in muscular arteries in the absence of changes in systemic haemodynamics or neurohormonal activation. Such changes, such as a reduction in mean arterial pressure, for example, may result in a secondary reduction in arterial diameter. There is, therefore, no substitute for systemic studies to assess overall haemodynamic effects of vasoactive drugs acting on muscular arteries.

### **3.5 Conclusion**

Whilst the regulation of vascular tone in resistance vasculature has been studied extensively, relatively little is known regarding that in muscular conduit arteries. Such tone in muscular arteries is likely to be important since it influences systemic haemodynamics through effects on the functional compliance of the arterial tree and on pressure wave reflection. The present study suggests that, under resting conditions, the tone of muscular arteries is largely independent of sympathetic outflow acting on alpha-adrenergic receptors but is generated as a myogenic response to transmural pressure. NO donors appear highly effective at antagonising such resting tone with greater action on muscular arteries relative to resistance vessels than calcium-channel antagonists. However, there is considerable heterogeneity between drugs acting through the NO-cGMP pathway in their selectivity for muscular arteries. This suggests that there is the potential to design drugs acting through this pathway which dilate muscular arteries but have minimal effects on resistance vasculature and which would be useful in treating systolic hypertension.

In conclusion, the smooth muscle tone of conduit arteries that determines arterial diameter and PWV is likely to be generated by an intrinsic myogenic response to transmural pressure and, for an equivalent action on resistance vessels, is functionally or directly antagonised by NO-donors more effectively than by alpha-adrenergic or calcium channel blockade.



AUGMENTATION PRESSURE IS INFLUENCED  
BY VENTRICULAR CONTRACTILITY/  
RELAXATION DYNAMICS: NOVEL MECHANISM  
OF REDUCTION OF PULSE PRESSURE BY  
NITRATES

## **4.1 Background**

Aortic or central pulse pressure (cPP) determines the pulsatile load on the left ventricle, coronary and carotid arteries and is a major risk factor for adverse cardiovascular events, with at least as strong a relation to such events as peripheral pulse pressure.<sup>47, 51, 192</sup> cPP is widely assumed to be determined by the interaction of an outgoing forward pressure wave (FPW) generated by ventricular contraction and a backward going pressure wave (BPW) due to reflections from the periphery of the circulation.<sup>18, 193</sup> These components of cPP are separated by the first systolic shoulder of the aortic pulse (figure 10). The height of this above diastolic blood pressure (P1) is attributed to the FPW and the height above P1, the augmentation pressure (AP), to the BPW.<sup>194</sup> Augmentation index (AIx), the ratio of AP to cPP is widely used as a measure of “pressure wave reflection”. This interpretation has, however, been criticised because of the poor relationship between the magnitude and timing of reflected waves with respect to AP.<sup>60, 61</sup> Nitroglycerin (NTG) has a particularly marked action to reduce AP and hence cPP attributed to a reduction in timing or amplitude of pressure wave reflections<sup>19, 86, 146</sup> However, an alternative explanation relates to an influence of nitrovasodilation on contraction-relaxation dynamics of the myocardium, effects observed in isolated myocytes and in vivo.<sup>158, 159</sup>

The aim of the present study was to determine the mechanism by which AP is selectively reduced by nitroglycerin (NTG). I made simultaneous measurements of aortic pressure and flow and used wave intensity analysis to separate pressure waves into FPW and BPW components.<sup>172</sup> Pressure waves were further categorized as compression or expansion (suction). A primary forward compression wave (FCW) arises from the “push” of the ventricle against the arterial tree and a systolic backward compression wave (BCW) from the push of the arterial tree against the ventricle. A late systolic forward expansion wave (FEW)

arises from the braking effect of the ventricle in late systole.<sup>195</sup> These measurements together with cardiac output and the impedance of the arterial tree were obtained at baseline and after systemic administration of NTG. To further distinguish between effects of NTG related to an action on the myocardium and arterial tree, I examined effects of intracoronary infusion of NTG at a dose below the threshold required to produce systemic effects.

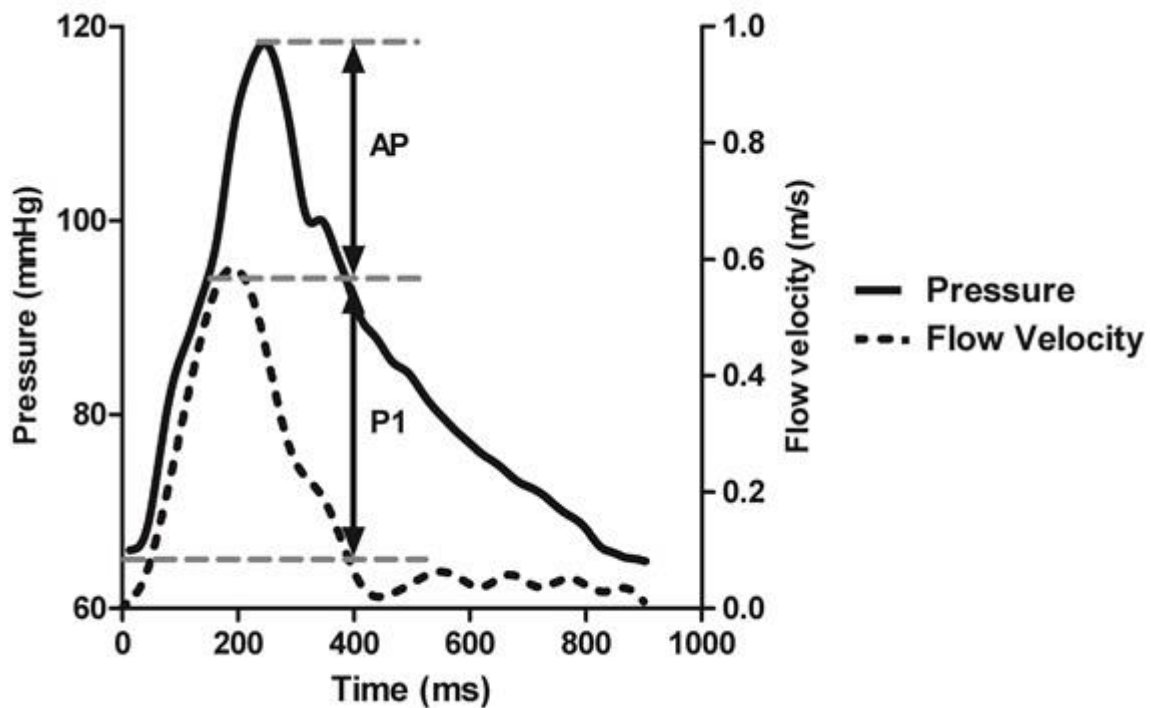


Figure 10

Central aortic pressure and flow waveforms showing components of central pulse pressure (cPP), height above diastolic pressure of the first shoulder of the aortic pressure waveform (P1) and augmentation pressure (AP).

#### **4.2 Methods**

Subjects (n=30, 11 women, mean  $\pm$  SD age  $61 \pm 13$  years) were recruited from those attending Guy's and St Thomas' cardiothoracic unit for diagnostic angiography or elective percutaneous coronary angioplasty. Subjects with acute coronary syndromes, those with

significant valvular disease, heart failure of severity greater than NYHA grade 2 and rhythm other than sinus rhythm were excluded. Subject characteristics including medication are shown in table 2.

**Table 2 Subject characteristics**

Characteristic	Study 1 (n = 20)	Study 2 (n = 10)
Age (years)	63±11	57±17
Sex (men/women)	10/10	9/1
Diabetes mellitus (%)	30	40
Aortic systolic blood pressure (mmHg)	136±23	139±39
Baseline diastolic blood pressure (mmHg)	73±13	69±8.3
Body mass index (kg/m <sup>2</sup> )	28±8.6	28±3.1
Total cholesterol (mmol/L)	4.5±1.2	5.0±0.2
High density lipoprotein (mmol/L)	1.2±0.5	1.0±0.3
Drug treatment		
ACEI/ARB (%)	45	70
β-blocker use (%)	45	40
CCB (%)	30	50
Nitrate (%)	20	10

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker.

*Study 1 Central haemodynamics and response to systemic nitroglycerin*

Invasive blood pressure and flow measurements were carried out as described in chapter 2. Baseline measurements of aortic root pressure and Doppler flow velocity were obtained over at least 10 cardiac cycles. Repeat aortic pressure and Doppler flow velocity measurements were taken following administration of GTN at a sublingual dose of 800µg. Repeat haemodynamic data were taken at the same position at the proximal aortic root, at least 2 minutes after GTN administration and when haemodynamic responses were stable. All

measurements were taken before the administration of ionic contrast agents for the subsequent angiogram or angioplasty.

*Study 2 Central haemodynamic response to intracoronary nitroglycerin*

Preparation and measurements of central haemodynamic response to intracoronary nitroglycerin were carried out as described in chapter 2. A diagnostic coronary angiogram was performed prior to the research study to ensure the absence of significant left main stem or critical left anterior descending coronary artery disease that could affect the distribution of nitroglycerin within the left ventricle. Baseline pressure and flow measurements were obtained from the Combowire in the aortic root as described above. The guiding catheter was then advanced to the left coronary ostium for NTG (Hospira Incorporation) infusion at 1µg/min. This dose was determined by previous studies that found no significant systemic effects when NTG 1µg/min was infused locally into the brachial artery<sup>196</sup> and by dose ranging studies where intravenous NTG infusion at 3µg/min was found to be the threshold dose above which systemic effects on aortic waveform indices (table 3). After a 7 minute infusion of NTG, the guiding catheter was pulled back into the proximal aortic root and Combowire re-positioned in the aortic root with fluoroscopic screening used to ensure position of the Combowire was similar to that at baseline (this taking approximately 1 minute). Measurements of pressure and flow were then repeated as previously described. In 4 subjects following the placement of Combowire in the proximal aortic root for continuous pressure/ Doppler flow velocity measurements, a separate guiding catheter was inserted via a second vascular access in the contra-lateral femoral artery to simultaneously infuse NTG over 7 minutes into the left coronary ostium.

**Table 3 Haemodynamic response to intravenous NTG 1 µg/min at systemic dose ranging studies**

Measure	Baseline	Nitroglycerin 1 µg/min	P value
Heart Rate (min <sup>-1</sup> )	63±4	68±3	0.29
cSBP (mmHg)	139±12	137±11	0.91
DBP (mmHg)	69±3	74±3	0.23
MAP (mmHg)	97±5	100±5	0.70
AIx (%)	31±6	22±5	0.41

cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; MAP, mean arterial pressure; AIx, augmentation index obtained by carotid tonometry.

#### *Waveform Post-processing*

This was carried out as described in chapter 2 (Section 2.11). Aortic flow velocity waveforms were integrated over time and multiplied by aortic cross-sectional area (estimated from body surface area) to obtain a measure of stroke volume and hence cardiac output and total systemic vascular resistance.

#### *Statistics*

Unless where stated results are expressed as means ± SE. Changes from baseline in haemodynamic measures after NTG were compared using Student's paired *t*-test or by analysis of variance for repeated measures. In the case of non-normally distributed data (heart rate, pulse wave velocity and FEW), a Wilcoxon signed rank test was used. All tests were 2-tailed and P<0.05 was considered significant.

### **4.3 Results**

#### *Contributions of FPW and BPW to AP*

Decomposition of the aortic pressure wave demonstrated that P1 was almost entirely due to the FPW and that the BPW contributed only a minor component to P1 (mean contributions at baseline of  $37.1 \pm 2.8$  and  $1.6 \pm 1.1$  mmHg respectively, figure 11). The FPW also provided the major contribution to AP, although the BPW also provided a significant contribution (mean contributions of  $15.6 \pm 2.9$  and  $11.0 \pm 2.8$  mmHg for the FPW and BPW respectively, figure 11).

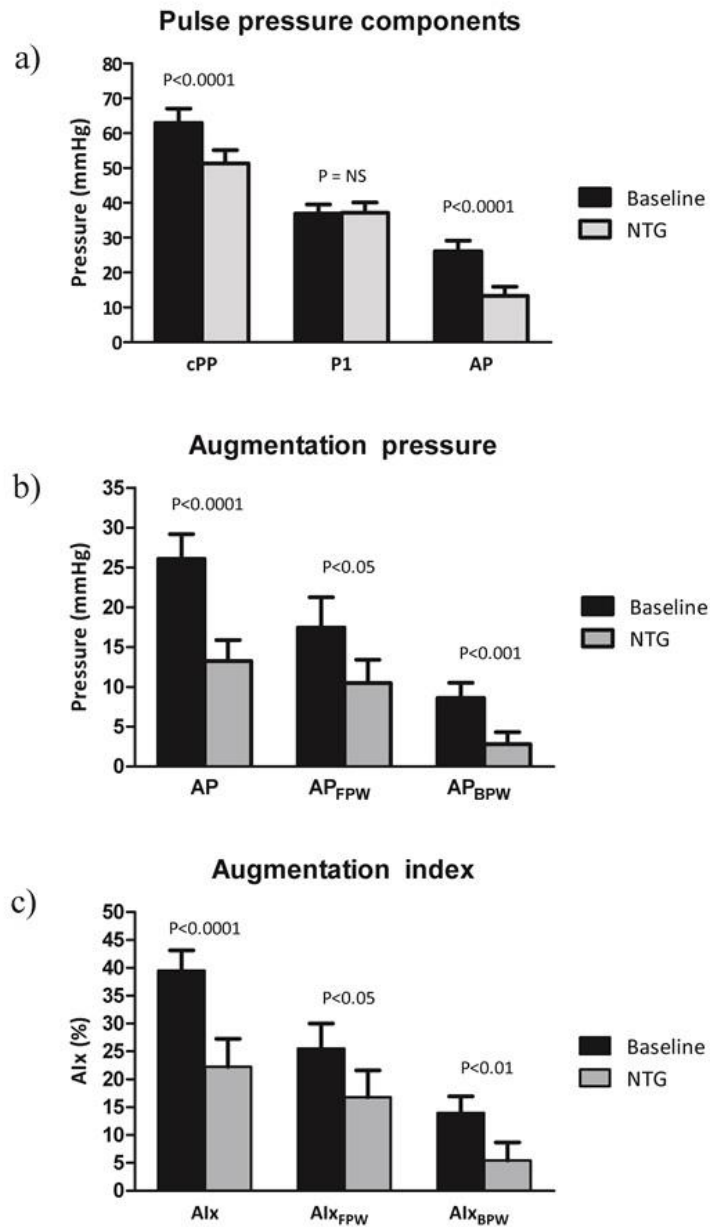


Figure 11

a) Central (aortic) pulse pressure (cPP) and the components of cPP: height of the first systolic shoulder above diastolic blood pressure (P1) and augmentation pressure (AP), the increment of peak aortic pressure above P1 before and after sublingual nitroglycerin (NTG, n=20).



b) AP and AP decomposed into components attributable to the forward pressure wave ( $AP_{FPW}$ ) and backward pressure wave ( $AP_{BPW}$ ) before and after sublingual nitroglycerin (NTG, n=20).

c) Augmentation index (AIx, the ratio of AP to cPP) and AIx decomposed into components attributable to the forward pressure wave ( $AIx_{FPW}$ ) and backward pressure wave ( $AIx_{BPW}$ ) before and after nitroglycerin (NTG, n=20).

#### *Effects of systemic NTG on haemodynamics and pulse pressure components*

Systemic administration of NTG had no significant effect on P1 but reduced AP by  $12.8 \pm 3.1$  mmHg (from  $26.1 \pm 3.1$  to  $13.3 \pm 2.6$  mmHg,  $P < 0.0001$ , figure 11) and reduced cSBP and cPP by similar amounts (from  $136 \pm 5$  to  $127 \pm 6$  mmHg and from  $63.0 \pm 4.0$  to  $51.3 \pm 3.9$  mmHg respectively, each  $P < 0.01$ ). AIx decreased by  $17.3 \pm 3.0\%$  (from  $39.5 \pm 3.6$  to  $22.2 \pm 5.0\%$ ,  $P < 0.0001$ ). By contrast, NTG had no significant effect on mean arterial blood pressure, total peripheral resistance, or on aortic PWV (table 4). There was a non-significant trend to an increase in heart rate of 4 beats/min and decrease in stroke volume of borderline significance (table 4). The phase and modulus of the aortic input impedance did not change significantly after NTG but there was a trend for a phase shift to the left (figure 12).

**Table 4 Haemodynamic changes after sublingual nitroglycerin**

Measure	Baseline	NTG	Difference	95%CI	P value
Heart rate (min <sup>-1</sup> )	64±2	68±3	4.33± 1.43	1.34 to 7.32	0.06
Blood pressures					
cSBP (mmHg)	136±5	127±6	-8.31±2.47	-13.5 to -3.14	<0.01
cDBP(mmHg)	73±3	76±3	3.35±1.93	-0.686 to 7.39	0.10
MAP(mmHg)	99±3	98±4	-0.67±2.26	-5.39 to 4.05	0.77
Pulse pressures					
P1 (mmHg)	36.9±2.6	37.2±2.9	0.28±1.77	-3.43 to 3.99	0.21
AP (mmHg)	26.1±3.1	14.1±2.4	-12.8±1.93	-16.9 to -8.77	<0.0001
cPP (mmHg)	63.0±4.0	51.3±3.9	-11.7±1.50	-14.8 to -8.52	<0.0001
Augmentation Index (%)	39.5±3.6	22.2±5.0	-17.3±3.00	-23.6 to -11.0	<0.0001
Aortic PWV (m.s <sup>-1</sup> )	11.6±1.1	10.6±0.9	0.95±0.55	-2.11 to 0.203	0.57
Stroke volume* (ml)	88.5±5.3	81.0±4.5	-7.44±3.56	-14.9 to 0.004	0.05
Cardiac output* (L.min <sup>-1</sup> )	5.6±0.3	5.4±0.3	-0.17±0.26	-0.708 to 0.361	0.51
TPR* (mmHg.L <sup>-1</sup> .min)	18.4±0.96	18.5±0.82	0.03±0.76	-1.56 to 1.63	0.96

cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; MAP, mean arterial pressure; P1, pressure at the first systolic shoulder of pressure pulse; AP, augmentation pressure; cPP, central pulse pressure; PWV, pulse wave velocity; TPR, total peripheral resistance. \*These values were computed from an estimate of aortic root area assumed to remain constant after NTG

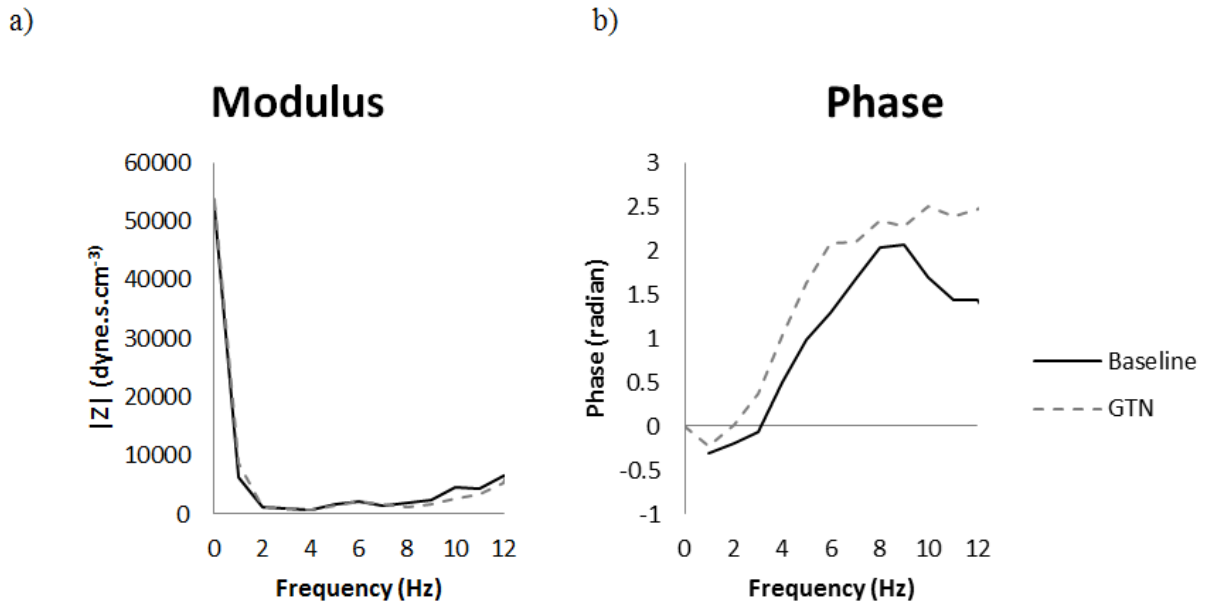


Figure 12

Average modulus a) and phase b) of aortic input impedance at baseline and after sublingual nitroglycerin (n=20).

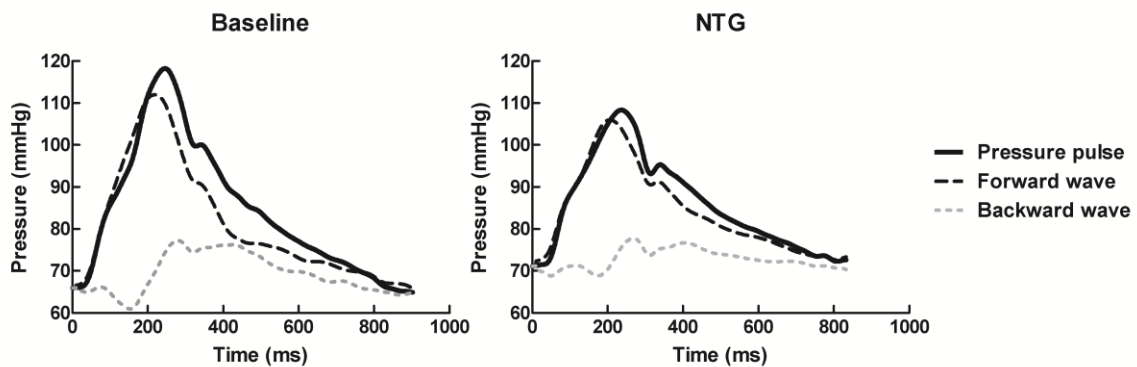


Figure 13

Typical aortic pressure waveforms decomposed into forward and backward pressure waves at baseline and after nitroglycerin (NTG). The major effect of NTG is to reduce the forward wave after the first systolic shoulder, the time of peak systolic stress. Corresponding flow wave is shown in figure 10.

*Effects of systemic NTG on FPW and BPW components of AP and on pressure wave intensity amplitudes, timings and energies*

The reduction in AP after NTG was explained by a reduction of  $7.0 \pm 2.4$  mmHg (from  $17.4 \pm 3.8$  to  $10.4 \pm 2.9$  mmHg,  $P < 0.02$ ) in the FPW and a reduction in the BPW of  $5.8 \pm 1.3$  mmHg (from  $8.6 \pm 1.9$  to  $2.8 \pm 1.5$  mmHg,  $P < 0.01$ , figures 11 and 13). The reflection coefficient as defined as the ratio of the maximum amplitude of the FPW to BPW did not change significantly after NTG ( $0.28 \pm 0.02$  to  $0.27 \pm 0.02$ ,  $P = 0.410$ ). An example of a wave intensity plot before and after NTG is shown in figure 14. Average values of maximum wave intensities, timings (of maximum intensities) and wave energies for the FCW, BCW and FEW before and after NTG are shown in table 4. Amplitude of the BCW but not the FCW decreased after NTG whereas energy of both the FCW and BCW were reduced after NTG and the ratio of BCW/FCW energy was unaltered. There was a small but significant reduction in time to the FEW after NTG but timings of other waves remained similar before and after NTG (table 5).

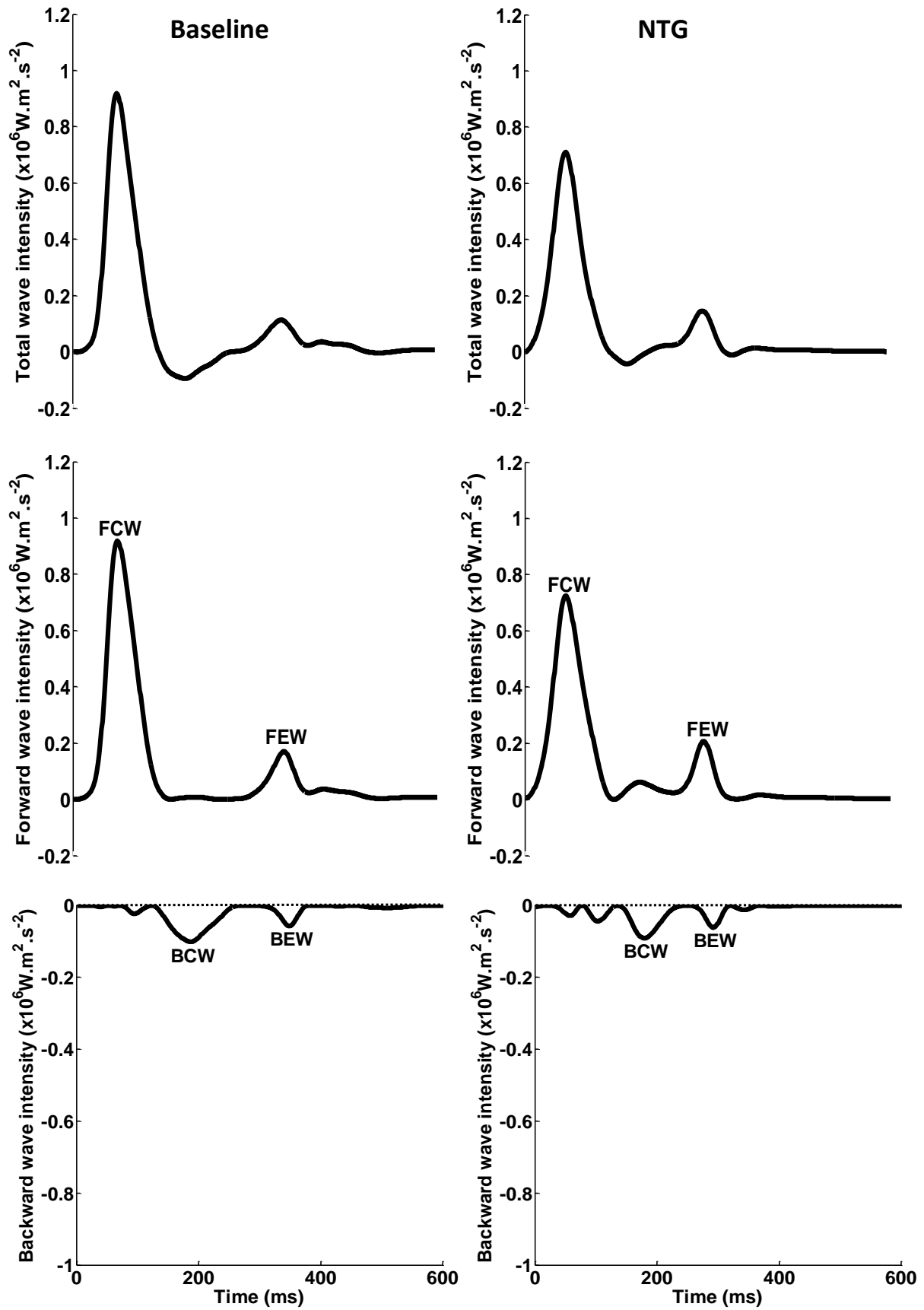


Figure 14

Typical wave intensity plots at baseline and after NTG.

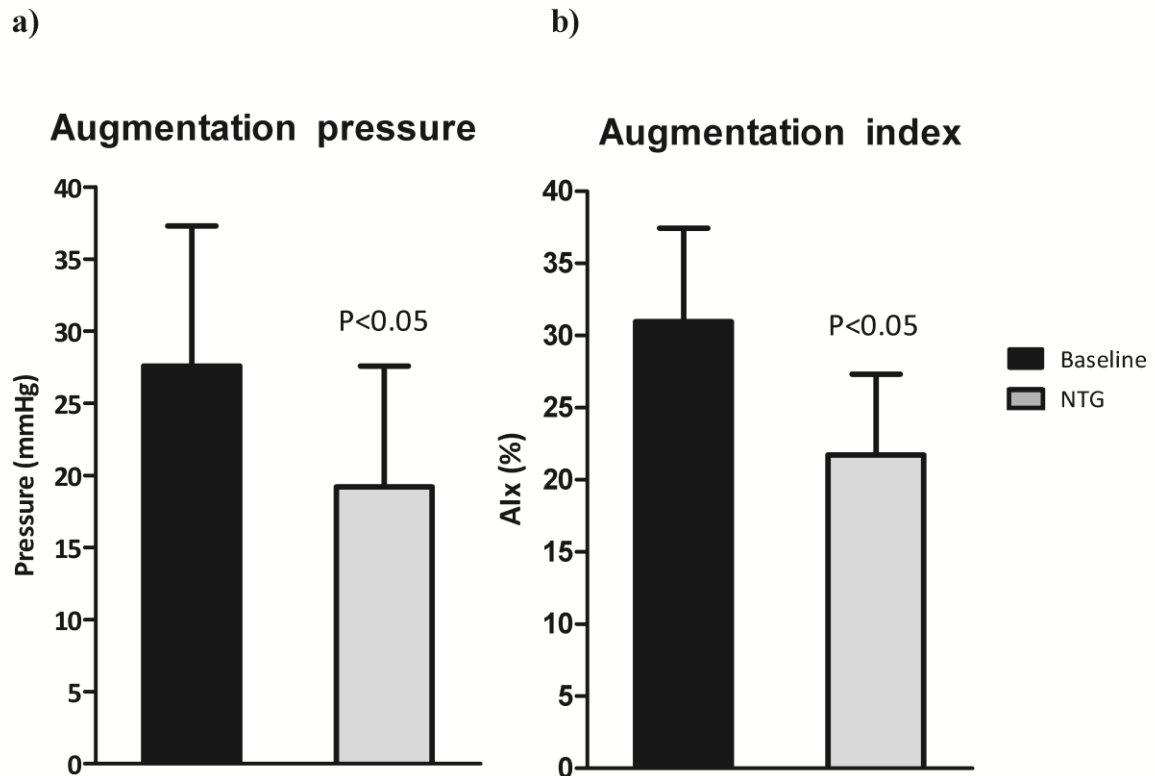
**Table 5 Wave intensity analysis**

Measure	Baseline	NTG	P value
Wave Intensity ( $\text{W}\cdot\text{m}^{-2}\cdot\text{s}^{-2}\times 10^6$ )			
FCW	0.50±0.066	0.48±0.063	0.58
FEW	0.17±0.025	0.16±0.030	0.95
BCW	0.084±0.013	0.060±0.0091	<0.005
BCW:FCW	0.19±0.026	0.14±0.018	<0.01
Wave Timing (ms)			
FCW	59.0±7.43	56.5±7.07	0.33
FEW	314±14.0	304±9.24	0.03
BCW	181±16.2	175±19.0	0.62
Wave Energy ( $\text{J}\cdot\text{m}^{-2}\times 10^6$ )			
FCW	0.045±0.0043	0.039±0.0041	<0.001
BCW	0.011±0.0012	0.0084±0.00094	<0.005
BCW:FCW	0.25±0.021	0.22±0.23	0.25

FCW, forward compression wave; FEW, forward expansion wave; BCW, backward compression wave

#### *Effects of intra-coronary NTG on AP*

There was no significant change in cSBP, MAP, P1 or heart rate after intra-coronary NTG (1  $\mu\text{g}/\text{min}$  for 7 min, data not shown). However intra-coronary infusion of NTG reduced AP by  $8.3\pm 3.1$  mmHg (from  $27.6 \pm 9.7$  to  $19.2 \pm 8.4$  mmHg,  $P<0.05$ , figure 15) and reduced AIx by 9.3% (from  $31.0\pm 6.5$  to  $21.7 \pm 5.6\%$ ,  $P<0.05$ ). Effects of intra-coronary NTG on FPW and BPW components of AP and AIx did not reach statistical significance but there was a trend for a reduction in the FPW components of AP and AIx ( $P=0.17$ , and  $P=0.08$ , respectively) but not the BPW components of AP and AIx ( $P=0.50$ , and  $P=0.96$ ). Thus the reduction in AP and AIx by intra-coronary NTG was potentially explained by an effect on the FPW alone.



**Figure 15**

Augmentation pressure and augmentation index before and after intra-coronary infusion of NTG (1  $\mu$ g/min, n=10).

#### **4.4 Discussion**

AP accounts for the much of the inter-subject variability in cPP<sup>71</sup> and an increase in AP is the major cause of the age-related increase in cPP.<sup>63</sup> AP has, hitherto, been attributed to augmentation of the FPW (which determines P1) by the BPW, the BPW being attributed to wave reflection. However, the interpretation of AP as an index of reflection assumes that the FPW plateaus at P1 and has been criticised because of the poor relationship between the magnitude and timing of reflected waves with respect to AP.<sup>60, 61</sup> Although I observed a significant contribution of the BPW to the AP, the major contribution to AP derived from the FPW with the contribution of the FPW to AP being approximately twice that of the BPW.

This suggests that AP is in large part independent of processes such as pressure wave reflection or other haemodynamic effects that may influence the BPW.

NTG is markedly effective in reducing AP, an effect that has been attributed to dilation of conduit arteries and a change in magnitude and/or timing of wave reflections,<sup>86</sup> In the present study, systemic administration of NTG at a dose that dilates conduit arteries by between 10 to 20%<sup>63</sup> reduced AP by more than 46%. The aortic pressure wave is determined by the interaction of ventricular contraction with the input impedance of the aorta and more distal circulation as “seen” at the aortic root (the point of pressure recording). Thus a reduction in AP could, in principle, result from a change in myocardial contraction/relaxation and/or change in input impedance. With the relatively low dose of NTG that was used, I observed no significant change in the modulus or phase of the impedance but there was a trend to a shift to the left of the phase of the impedance in keeping with previous observations which have been attributed to a delay in reflections.<sup>197</sup> To probe the haemodynamic determinants of this effect of NTG on AP further, I performed waveform decomposition before and after NTG. The reduction in AP was only partially explained by the BPW with the reduction in the FPW component of AP contributing proportionately more than that of the BPW to the total reduction in AP. Furthermore, the ratio of the BPW to the FPW, whether measured as the ratio of the maximum amplitudes of the waves or as ratio of wave energy in FCW and BCW, did not change significantly after NTG suggesting that most of the reduction in the BPW was secondary to that in the FPW.

Since P1 occurs at the time of peak systolic stress (i.e. peak contraction in individual myocytes) in the myocardium,<sup>198</sup> the reduction of the FPW component of AP but not of P1 by NTG implies an earlier or more pronounced relaxation of the myocardium after peak stress. The observation that wave maximal wave intensity of the FCW which occurs before P1 is



unaltered but FCW energy which includes components after P1 during augmentation is reduced is consistent with a reduction of the forward wave during pressure augmentation. Earlier onset of the FEW is consistent with earlier onset of relaxation of the ventricle. It is notable that sodium nitroprusside, a NO donor thought to act through the same downstream cGMP effector pathway as NTG delivered locally into the coronary circulation with negligible systemic delivery to the peripheral circulation, hastens the onset of relaxation of the ventricle<sup>159, 199</sup> and a similar effect is seen in isolated myocytes exposed to NO<sup>158</sup> or to cGMP mimetics acting downstream of the NO-soluble guanylyl cyclase pathway.<sup>199</sup>

To test the possibility that the effect of NTG to reduce the FPW component of AP relates to myocardial contraction/relaxation, NTG was injected directly into the left coronary circulation at a dose below that observed to generate systemic effects when given intravenously and approximately 50 fold lower than that used to achieve systemic effects in the present study (assuming bioavailability of sublingual NTG to be approximate 35%<sup>67, 200</sup>). I observed a significant, albeit less pronounced, effect on AP. This is consistent with an influence of NTG on the FPW component of AP through a direct action on the myocardium to hasten the onset of relaxation after P1. However, delivery of NTG through the myocardial circulation to the central venous return (via the coronary sinus) may result in a relatively greater concentration in pulmonary vessels than achieved by a systemic venous infusion and, as I did not measure left ventricular end-diastolic pressure or volume, I cannot be certain that preload was not altered by intracoronary NTG. Similarly, the reduction in pulse pressure at the baroreceptors brought about by NTG could result in an increase in sympathetic activity. Thus although my intra-coronary studies identify an action of NTG on ventricular contraction/relaxation dynamics independent of an action on the peripheral arterial tree, this could be due to direct and/or indirect actions on the myocardium.

Irrespective of the mechanism through which NTG acts on the myocardium, my results challenge the conventional view that effects of NTG to reduce AP and cPP are mediated mainly by a reduction in wave reflection. Instead they identify a novel mechanism whereby AP and cPP is influenced predominantly by the FPW and hence the contractility/relaxation dynamics of the myocardium. Interestingly Schultz et al have reached a similar conclusion regarding dominance of the FPW as responsible for change in the central pulse during exercise.<sup>67, 201</sup> My results have implications for the age-related increase in pulse pressure, incidence of isolated systolic hypertension (ISH) and impaired ventricular-vascular coupling. Ventricular relaxation is known to decrease with age and there is a strong correlation between ventricular relaxation and AP.<sup>201, 202</sup> This has been assumed to be due to the additional afterload imposed by increased AP.<sup>201, 202</sup> My results raise the possibility of an opposite direction of causality, that changes in myocardial contraction/relaxation drive those in AP. Thus increased AP, cPP and ISH could result, in part, from an age-related decline in myocardial function with a delayed onset of relaxation. This in turn, could be due to an age-related decline in the availability of endogenous NO from endothelial or neuronal NO synthase.<sup>202, 203</sup> Further interventional studies will be required to explore this possibility.

My study is subject to several limitations. By necessity the study was limited to subjects that had clinical indications for coronary angiography and a substantial proportion of these had established coronary artery disease. Thus I cannot exclude the possibility that results in subjects without coronary disease might differ and the results of our study cannot necessarily be extrapolated to younger subjects. The interpretation of my study relies, in part, on wave separation theory. However, this theory relies on relatively few assumptions other than conservation of mass and momentum and results obtained using differing mathematical techniques are similar.<sup>174</sup> Furthermore, the main finding of my study that AP is influenced by

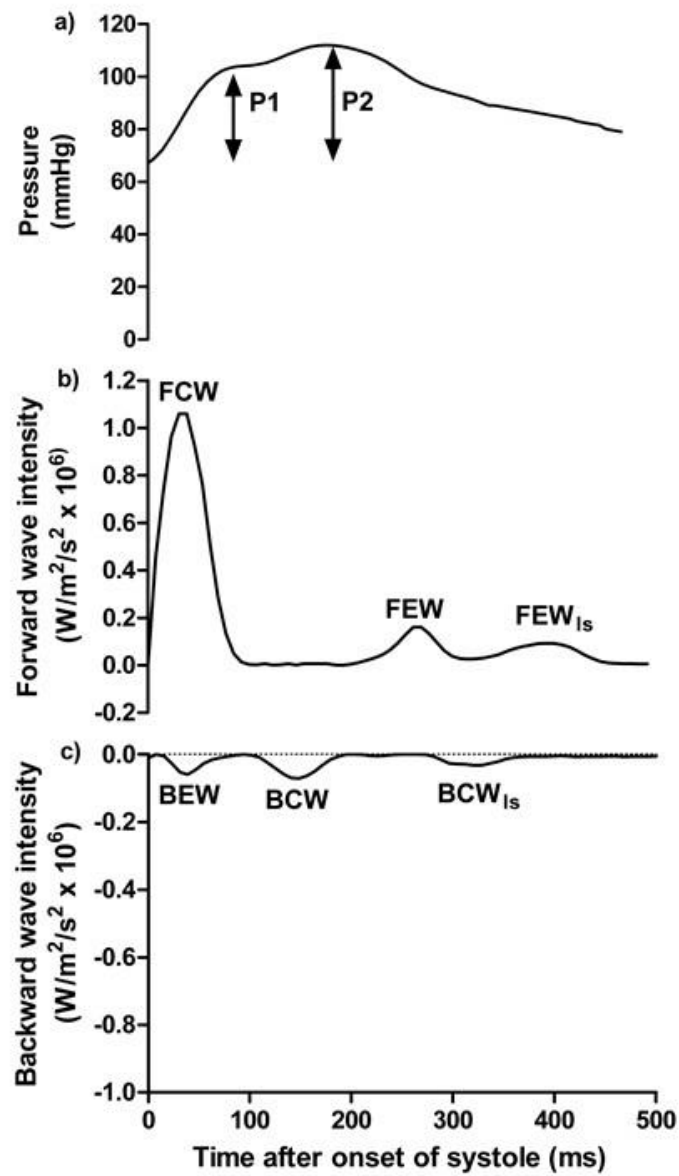
ventricular dynamics is borne out by our intra-coronary study independent of wave separation analysis. Accuracy of CO and TPR measurements was limited by the estimation of aortic cross-sectional area but this would not affect the relative change before and after NTG.

In conclusion, AP is determined in large part by the FPW and ventricular contraction/relaxation dynamics. NTG reduces AP by a reduction in the FPW resulting from earlier onset and/or more pronounced ventricular relaxation. The NO-pathway may present a novel target to reduce AP, systolic blood pressure and improve ventricular-vascular coupling.

CHAPTER 5: DOMINANCE OF THE FORWARD  
COMPRESSION WAVE IN DETERMINING  
PULSATILE COMPONENTS OF BLOOD  
PRESSURE: SIMILARITIES BETWEEN  
INOTROPIC STIMULATION AND ESSENTIAL  
HYPERTENSION

## **5.1 Background**

Pulsatile components of the aortic blood pressure waveform such as the amplitudes at the first (P1) and second shoulders (P2) of the waveform (figure 16) are important determinants of cardiovascular events.<sup>40, 175, 204</sup> The extent to which they are determined by ventricular contraction, Windkessel/reservoir properties or other components of the impedance of the proximal arterial tree and reflections from the peripheral arterial tree is unclear.<sup>23, 62, 179</sup> Wave intensity and wave separation analysis allows pressure to be separated into a forward component travelling from the left ventricle towards the periphery of the arterial tree and a backward component travelling from the arterial tree towards the ventricle.<sup>172</sup> Pressure waves can be further categorized as compression or expansion (suction). A primary forward compression wave (FCW) arises from the “push” of the ventricle against the arterial tree and a systolic backward compression wave (BCW) from the push of the arterial tree against the ventricle. A mid systolic forward expansion wave (FEW) arises from the braking effect of the ventricle in late systole and other minor waves arise from other ventricular – vascular interactions (figure 16).<sup>195</sup> The objective of the present study was to determine the relative contributions of forward and backward waves attributable to pressures generated primarily from the ventricle and arterial tree respectively to 1) changes in pulse pressure during modulation of cardiac contractility and arterial tone by dobutamine and norepinephrine.<sup>205</sup> 2) elevated pulse pressure in subjects with essential hypertension.



**Figure 16**

- a) The central arterial pressure pulse with pulsatile components P1 and P2 determined by the heights of first and second shoulders respectively.
- b) Forward wave intensities showing the forward compression wave (FCW), mid-systolic forward expansion wave (FEW) and late systolic forward expansion wave (FEW<sub>1s</sub>)
- c) Backward wave intensities showing backward expansion wave (BEW), mid-systolic backward compression wave (BCW) and late systolic backward compression wave (BCW<sub>1s</sub>)

## 5.2 Methods

### *Study 1: Effects of dobutamine and norepinephrine on haemodynamics*

Healthy volunteers (n=10, age 35-63 years, 8 male) took part in this cross-over study to investigate the change in pulsatile haemodynamics following inotropic/vasopressor stimulation. They received cumulative doses of dobutamine (Db) and norepinephrine (NE) on two separate visits at least 7 days apart. Measurements were performed in a quiet temperature controlled (24-26 °C) vascular laboratory and subjects were asked to avoid caffeine and alcohol on the day of the study. On arrival in the vascular laboratory a peripheral venous catheter was inserted into the left antecubital fossa through which 0.9% saline (Baxter Healthcare) vehicle or drugs dissolved in saline were infused at 1ml/minute using a syringe driver (Injectomat, Agilia®, Fresenius Kabi, Germany). After 30 minutes resting supine during infusion of saline vehicle, baseline haemodynamic measurements (carotid artery tonometry and aortic Doppler flow velocity measurements) were made as described in chapter 2. Dobutamine (2.5, 5 and 7.5 µg/Kg/min, Hameln Pharmaceuticals, UK) or norepinephrine (12.5, 25, 50 ng/Kg/min, Aguetant Ltd, UK) dissolved in 0.9% saline vehicle were then infused at 1 ml/min and haemodynamic measurements repeated at each drug dose after 7 minutes of infusion when steady state was achieved.

### *Study 2 Comparison of haemodynamic measures in patients with untreated hypertension and normotensive controls*

Patients with untreated essential hypertension or hypertension inadequately controlled on treatment were recruited from the hypertension clinics at Guy's and St Thomas' Hospital.

Hypertension was diagnosed as an office blood pressure  $>140/90$  mmHg on at least 3 occasions and/or ambulatory blood pressure  $> 130/85$  mmHg. Patients on treatment for hypertension were included if office blood pressure on treatment was  $> 140/90$  mmHg. Exclusion criterion included: intercurrent illness, pregnancy, significant systemic disease other than mild hypertensive nephropathy, history of ischaemic heart disease, valvular heart disease, echocardiographic evidence of left ventricular ejection fraction  $<50\%$ , regional wall motion abnormality, left ventricular outflow tract obstruction, pulmonary hypertension and poor transthoracic acoustic window. Healthy volunteers on no regular medication of a similar age and sex distribution to hypertensive subjects were recruited by advertisement from the local community. Subject characteristics are shown in table 6. Subjects were asked to avoid caffeine and alcohol on the day of the study. Haemodynamic measurements were performed as chapter 2 Both study 1 and 2 were approved by the London Westminster Research Ethics Committee and written informed consents were obtained from all participants.



**Table 6 Baseline characteristics**

Baseline characteristics	Normotensive controls (n=20)	Hypertensive patients (n=20)
Age (years)	52.2±12.6	48.8±11.3
Men (%)	85	75
Systolic blood pressure (mmHg)	120±14.2	165±26.6 <sup>‡</sup>
Diastolic blood pressure (mmHg)	71.1±8.20	98.7±14.2 <sup>‡</sup>
Body mass index (kg/m <sup>2</sup> )	24.1±2.5	29.6±5.4 <sup>†</sup>
Total cholesterol (mmol/L)	5.4±0.8	5.0±1.2
High density lipoprotein (mmol/L)	1.7±0.6	1.5±0.5
HbA1c (%)	5.5±0.2	5.8±0.5
Drug therapy		
ACEI (%)	-	25
ARB (%)	-	25
β-blocker (%)	-	15
Calcium channel blocker (%)	-	65
Diuretic (%)	-	50
α-blocker (%)	-	30
Statins (%)	-	60
Median number of antihypertensive medications	-	2.5

Data are means±SD or %. ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker. †P<0.01 ‡P<0.005 compared to normotensive controls

### *Haemodynamic measurements*

Radial and carotid pressure waveforms were obtained by applanation tonometry performed by an experienced operator (HF) using the SphygmoCor system (AtCor, Australia).

Approximately 10 cardiac cycles were obtained and ensemble averaged. Waveforms which did not meet the in-built quality control criteria in the SphygmoCor system were rejected.

Brachial blood pressure was measured in triplicate by a validated oscillometric method

(Omron 705CP, Omron Health Care, Japan) and used to calibrate radial waveforms to obtain a mean arterial pressure (MAP). Carotid waveforms were calibrated from MAP and diastolic brachial blood pressure on the assumption of equality of these pressures at central and peripheral sites.<sup>206</sup> The primary analysis was performed on non-transformed carotid waveforms and a secondary analysis on waveforms transformed to aortic waveforms using the inbuilt transfer function in the SphygmoCor system.

Ultrasound imaging was performed by an experienced operator (BJ) using the Vivid-7 ultrasound platform (General Electric Healthcare, UK). Velocity across the aortic valve was recorded using continuous wave Doppler obtained from an apical 5-chamber view. Stroke volume (SV) was calculated from the product of velocity time integral (VTI) and cross-sectional area of the aortic valve (obtained in the parasternal long axis view). All measurements were averaged over at least 3 cardiac cycles. Cardiac output (CO) was calculated from SV and heart rate and total peripheral resistance (TPR) from mean arterial pressure (MAP) divided by CO.

#### *Waveform post-processing*

This was carried out as described in chapter 2.

#### *Statistical Analysis*

Subject characteristics are summarized as means $\pm$ SD and results as means $\pm$ SEM. Differences between haemodynamic measures in hypertensive and normotensive subjects were examined using Student's unpaired *t*-test. Responses to the vasoactive drugs relative to baseline were

sought by repeated-measures ANOVA.  $P < 0.05$  was considered significant, and all tests were 2 tailed. Analysis was performed using SPSS version 19.

### **5.3 Results**

#### *Effects of dobutamine and norepinephrine on pressure wave components and wave intensities*

The major effect of dobutamine was to increase CO and pulsatility (particularly P1), this being associated with a trend to an increase in MAP and decrease in TPR (table 7).

Norepinephrine increased TPR and MAP and also P2 and AP but not P1 (table 8). There was a small but significant fall in CO after norepinephrine (table 8).

**Table 7 Haemodynamic changes during infusion of dobutamine**

Measure	Baseline	Dobutamine ( $\mu\text{g}/\text{kg}/\text{min}$ )		
		2.5	5	7.5
pSBP (mmHg)	117 $\pm$ 5.25	125 $\pm$ 3.94	138 $\pm$ 4.29	145 $\pm$ 4.15 <sup>‡</sup>
pDBP (mmHg)	65.9 $\pm$ 2.88	65.5 $\pm$ 2.57	66.2 $\pm$ 2.28	67.2 $\pm$ 1.98
MAP (mmHg)	80.3 $\pm$ 3.48	82.0 $\pm$ 3.25	85.1 $\pm$ 2.57	87.9 $\pm$ 2.04
HR (bpm)	64.7 $\pm$ 3.51	66.7 $\pm$ 4.51	69.0 $\pm$ 4.74	73.0 $\pm$ 5.24 <sup>†</sup>
cSBP (mmHg)	103 $\pm$ 5.13	110 $\pm$ 4.86	119 $\pm$ 3.56	127 $\pm$ 2.44 <sup>‡</sup>
P1 (mmHg)	36.8 $\pm$ 3.74	43.7 $\pm$ 3.23	51.3 $\pm$ 3.76	59.0 $\pm$ 3.41 <sup>‡</sup>
P2 (mmHg)	32.5 $\pm$ 3.02	38.0 $\pm$ 2.35	41.4 $\pm$ 2.47	47.2 $\pm$ 3.17 <sup>†</sup>
AP (mmHg)	-4.29 $\pm$ 2.46	-5.73 $\pm$ 2.84	-9.91 $\pm$ 3.82	-11.8 $\pm$ 4.48
CO (L/min)	6.92 $\pm$ 0.341	7.45 $\pm$ 0.482	8.21 $\pm$ 0.576	9.11 $\pm$ 0.741 <sup>‡</sup>
TPR(mmHg·min/L)	12.0 $\pm$ 0.925	11.4 $\pm$ 0.928	10.7 $\pm$ 0.694	10.2 $\pm$ 0.999
PWV (m/s)	4.07 $\pm$ 0.402	4.52 $\pm$ 0.371	5.10 $\pm$ 0.391	5.58 $\pm$ 0.31 <sup>†</sup>
Wave Intensity ( $\text{W}/\text{m}^2/\text{s}^2 \times 10^6$ )				
FCW	1.42 $\pm$ 0.226	2.17 $\pm$ 0.267	3.05 $\pm$ 0.348	3.65 $\pm$ 0.434 <sup>‡</sup>
FEW	0.137 $\pm$ 0.0452	0.140 $\pm$ 0.0452	0.193 $\pm$ 0.0399	0.243 $\pm$ 0.0614
FEW <sub>ls</sub>	0.128 $\pm$ 0.0235	0.181 $\pm$ 0.0357	0.168 $\pm$ 0.0273	0.339 $\pm$ 0.0955 <sup>*</sup>
BEW	-0.0285 $\pm$ 0.0745	-0.0499 $\pm$ 0.0198	-0.0715 $\pm$ 0.0146	-0.0921 $\pm$ 0.0227 <sup>†</sup>
BCW	-0.0200 $\pm$ 0.00505	-0.0409 $\pm$ 0.00835	-0.0468 $\pm$ 0.00833	-0.0399 $\pm$ 0.0136
BCW <sub>ls</sub>	-0.0472 $\pm$ 0.0120	-0.0471 $\pm$ 0.0133	-0.0982 $\pm$ 0.0267	-0.164 $\pm$ 0.0390 <sup>†</sup>
BCW:FCW	0.0188 $\pm$ 0.00632	0.0201 $\pm$ 0.00380	0.0171 $\pm$ 0.00402	0.0161 $\pm$ 0.00468
Wave Timing (ms)				
FCW	34.4 $\pm$ 1.28	33.6 $\pm$ 1.67	30.5 $\pm$ 0.781	30.5 $\pm$ 0.781 <sup>†</sup>
FEW	199 $\pm$ 30.5	195 $\pm$ 28.4	190 $\pm$ 23.4	152 $\pm$ 28.4
FEW <sub>ls</sub>	332 $\pm$ 21.3	326 $\pm$ 24.8	298 $\pm$ 17.2	281 $\pm$ 20.4
BEW	71.1 $\pm$ 13.0	91.4 $\pm$ 7.09	82.8 $\pm$ 11.3	67.2 $\pm$ 12.0
BCW	206 $\pm$ 19.7	224 $\pm$ 21.9	195 $\pm$ 10.8	118 $\pm$ 29.8 <sup>‡</sup>
BCW <sub>ls</sub>	334 $\pm$ 22.7	340 $\pm$ 17.6	297 $\pm$ 16.6	257 $\pm$ 32.9

Data are expressed as means  $\pm$  SEM; AP, augmentation pressure; BCW, mid systolic backward compression wave; BCW<sub>ls</sub>, late systolic backward compression wave; BEW, backward expansion wave; CO, cardiac output; cSBP, central systolic blood pressure; FCW, forward compression wave; FEW, mid systolic forward expansion wave; FEW<sub>ls</sub>, late systolic forward expansion wave; HR, heart rate; MAP, mean arterial pressure; P1, pressure at first systolic shoulder; P2, pressure at second systolic peak; pSBP, peripheral systolic blood pressure; pDBP, peripheral diastolic blood pressure; PWV, pulse wave velocity; TPR, total peripheral resistance. \*P<0.05, †P<0.01, ‡P<0.005 repeated measures ANOVA for dose response curve.

**Table 8 Haemodynamic changes during infusion of norepinephrine**

Measure	Baseline	Norepinephrine (ng/kg/min)		
		12.5	25	50
pSBP (mmHg)	114 ± 4.43	117 ± 4.56	120 ± 3.97	126 ± 4.01 <sup>†</sup>
pDBP (mmHg)	67.1 ± 2.31	69.1 ± 2.62	73.4 ± 2.82	78.4 ± 2.84 <sup>‡</sup>
MAP (mmHg)	80.3 ± 3.48	82.0 ± 3.25	85.1 ± 2.57	94.3 ± 3.22 <sup>‡</sup>
HR (bpm)	61.5 ± 2.89	57.7 ± 2.91	55.2 ± 2.81	52.7 ± 2.63 <sup>†</sup>
cSBP (mmHg)	103 ± 4.90	109 ± 5.01	111 ± 5.05	123 ± 6.29 <sup>‡</sup>
P1 (mmHg)	35.5 ± 1.67	36.5 ± 2.05	33.1 ± 2.72	36.8 ± 2.84
P2 (mmHg)	33.0 ± 2.66	35.8 ± 3.18	37.3 ± 2.72	43.7 ± 4.31 <sup>‡</sup>
AP (mmHg)	-2.45 ± 2.39	-0.664 ± 2.43	4.26 ± 2.19	6.92 ± 3.29 <sup>‡</sup>
CO (L/min)	6.36 ± 0.328	5.99 ± 0.327	5.64 ± 0.152	5.13 ± 0.240 <sup>‡</sup>
TPR(mmHg·min/L)	13.2 ± 0.908	14.6 ± 1.13	16.0 ± 0.606	19.2 ± 1.38 <sup>‡</sup>
PWV (m/s)	4.35 ± 0.286	4.71 ± 0.464	4.68 ± 0.163	5.55 ± 0.598
Wave Intensity (W/m <sup>2</sup> /s <sup>2</sup> ×10 <sup>6</sup> )				
FCW	1.03 ± 0.104	1.01 ± 0.0729	0.803 ± 0.104	0.867 ± 0.114
FEW	0.0990 ± 0.0327	0.149 ± 0.0315	0.105 ± 0.0216	0.162 ± 0.0396
FEW <sub>ls</sub>	0.115 ± 0.0404	0.115 ± 0.0282	0.123 ± 0.0343	0.0971 ± 0.0182
BEW	-0.0215 ± 0.00550	-0.0410 ± 0.0107	-0.0357 ± 0.0104	-0.0363 ± 0.00642
BCW	-0.0187 ± 0.00417	-0.0310 ± 0.00633	-0.0308 ± 0.00661	-0.0357 ± 0.00827 <sup>*</sup>
BCW <sub>ls</sub>	-0.0547 ± 0.0171	-0.0540 ± 0.0103	-0.0543 ± 0.0133	-0.0465 ± 0.00956
BCW:FCW	0.0191 ± 0.00272	0.0317 ± 0.00662	0.0383 ± 0.00901	0.0428 ± 0.00887 <sup>*</sup>
Wave Timing (ms)				
FCW	37.5 ± 1.04	35.9 ± 1.28	37.5 ± 1.04	38.3 ± 0.781
FEW	202 ± 32.6	256 ± 25.5	243 ± 35.5	304 ± 19.7
FEW <sub>ls</sub>	333 ± 24.9	381 ± 18.9	381 ± 17.9	427 ± 21.2 <sup>†</sup>
BEW	93.0 ± 12.4	105 ± 8.25	96.9 ± 8.09	93.0 ± 7.22
BCW	192 ± 30.6	224 ± 18.0	234 ± 18.8	234 ± 21.7
BCW <sub>ls</sub>	307 ± 38.6	334 ± 23.1	387 ± 13.0	402 ± 29.1

Data are expressed as means ±SEM; AP, augmentation pressure; BCW, mid systolic backward compression wave; BCW<sub>ls</sub>, late systolic backward compression wave; BEW, backward expansion wave; CO, cardiac output; cSBP, central systolic blood pressure; FCW, forward compression wave; FEW, mid systolic forward expansion wave; FEW<sub>ls</sub>, late systolic forward expansion wave; HR, heart rate; MAP, mean arterial pressure; P1, pressure at first systolic shoulder; P2, pressure at second systolic peak; pSBP, peripheral systolic blood pressure; pDBP, peripheral diastolic blood pressure; PWV, pulse wave velocity; TPR, total peripheral resistance. \*P<0.05, †P<0.01, ‡P<0.005 repeated measures ANOVA for dose response curve.

Whilst dobutamine increased pulsatility, increasing both P1 and P2 it did so by increasing the forward pressure wave, with no significant contribution of the backward wave to the increase in P2 (figures 17, 18 and 19). Dobutamine increased the FCW and also the late systolic FEW, BEW and late systolic BCW. However there was no significant change in the ratios of the secondary waves to the FCW. Norepinephrine tended to increase the backward component of P2, particularly in subjects with a positive AP (figures 17, 18 and 19) and, by contrast to dobutamine, increased the mid systolic BCW both in absolute terms and as a proportion of the FCW (tables 7 and 8).

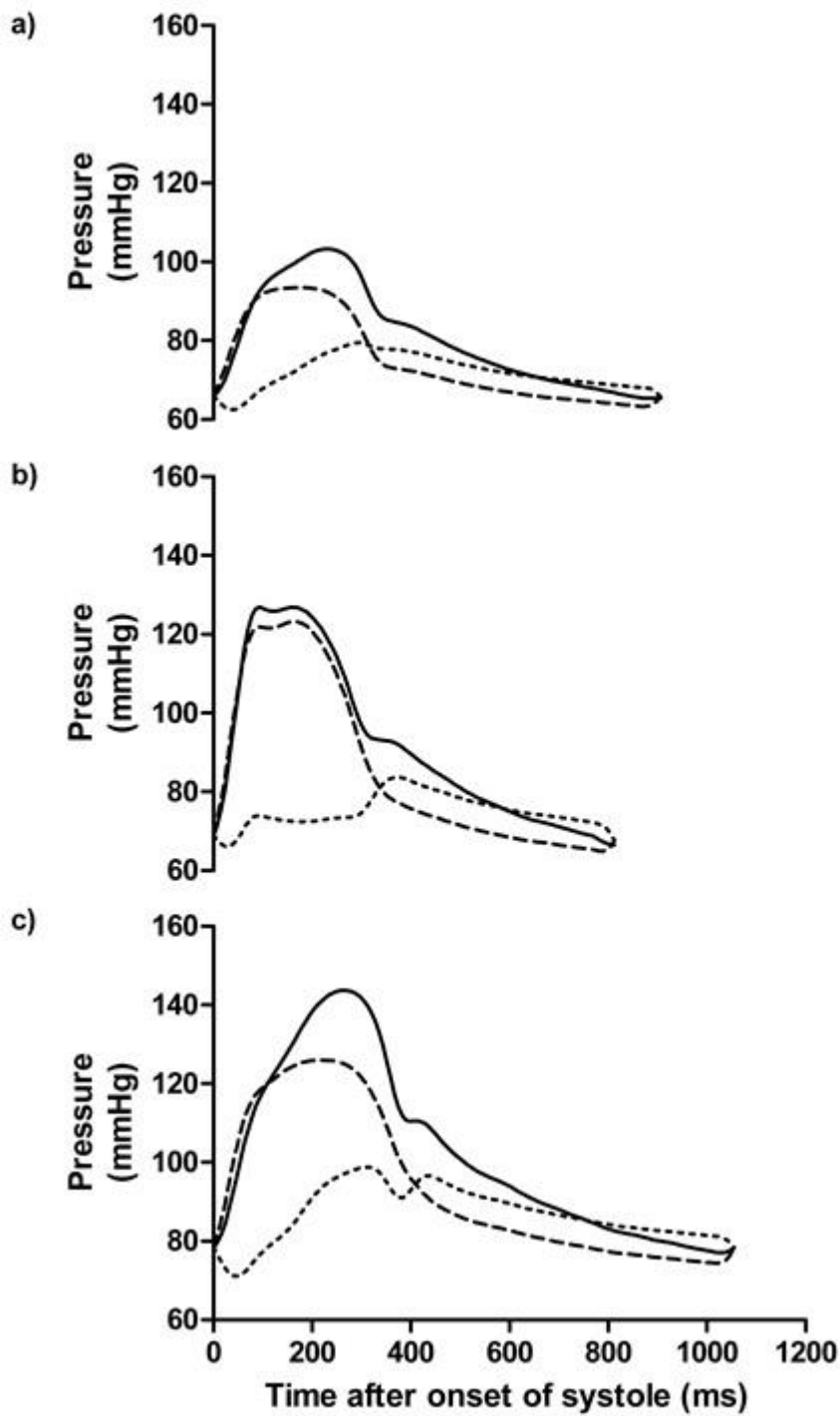


Figure 17

Typical pressure pulses decomposed into forward (dashed) and backward (dotted) components: a) baseline, b) after dobutamine and c) after norepinephrine.

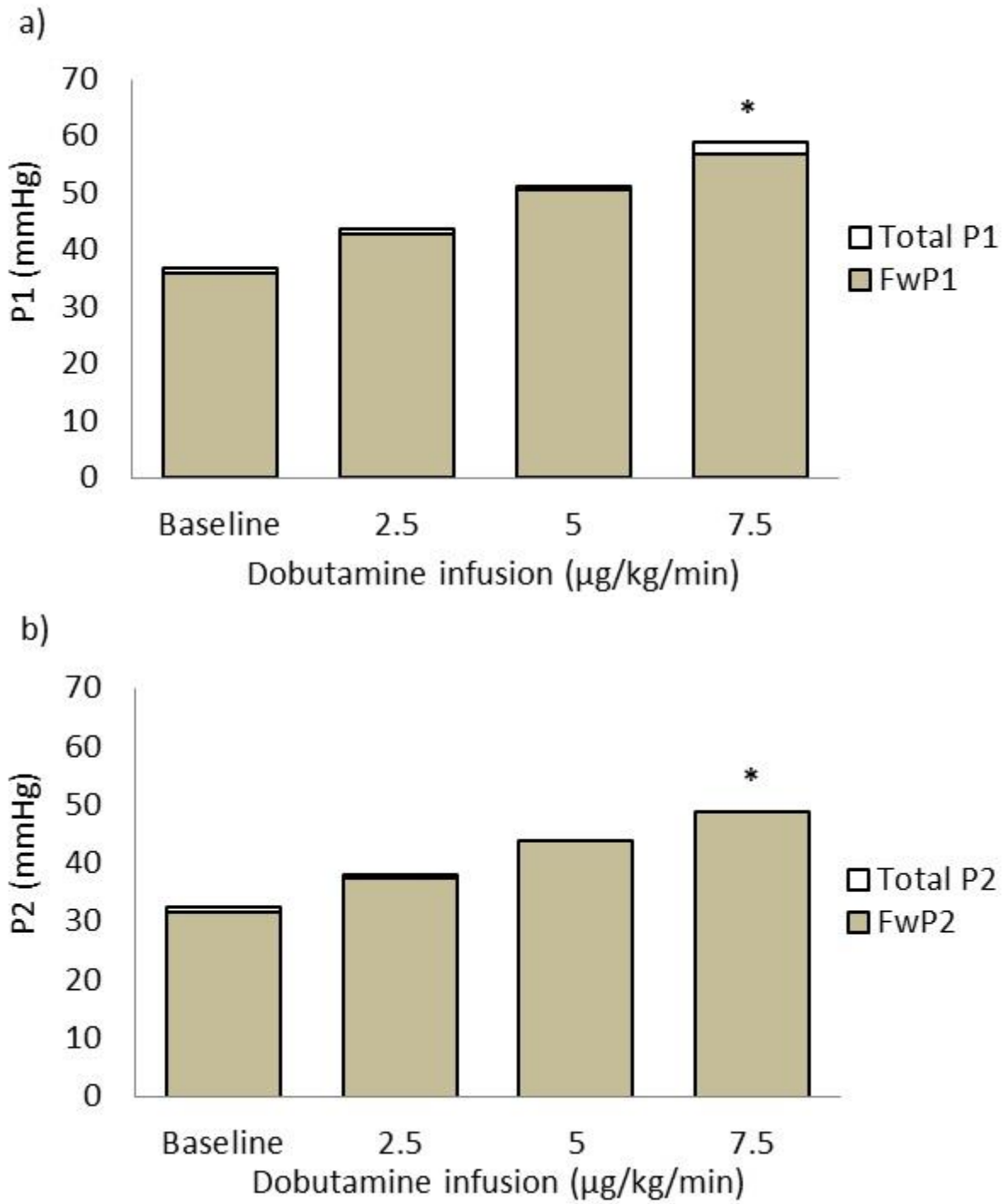


Figure 18

Pressure pulse components showing total pressure pulse and forward (Fw) component of pressure pulse for a) P1 and b) P2 during dobutamine infusion in normotensive subjects.



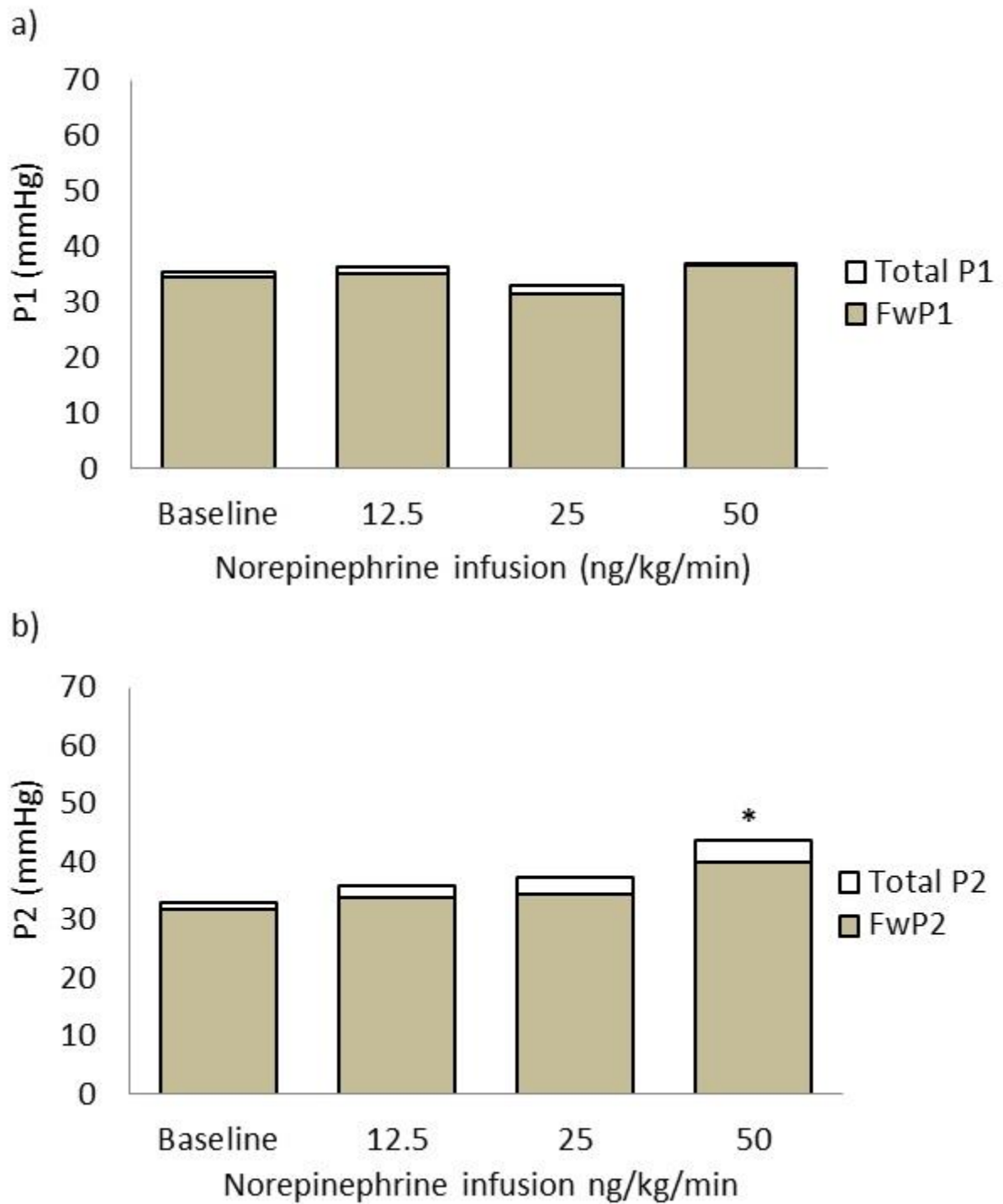


Figure 19

Pressure pulse components showing total pressure pulse and forward (Fw) component of pressure pulse for a) P1 and b) P2 during norepinephrine infusion in normotensive subjects.

However, the forward wave remained the major component of both P1 and P2 during stimulation with norepinephrine. When using transformed carotid waveforms, there were modest but significant differences in absolute pressures from those obtained from the non-transformed waveform (table 9). However, the main findings that majority of the pulse pressure components during stimulation with both dobutamine and norepinephrine derive from the forward pressure wave remained unchanged.

**Table 9: Forward and backward pulse pressure components determined using non-transformed and transformed carotid waveforms**

Measure	Baseline	Db 7.5µg/kg/min	Baseline	NE 50ng/kg/min
<b>Non-transformed</b>				
P1(mmHg)	36.8±3.74	59.0±3.41	35.5±1.67	36.8±2.84
P1f (mmHg)	36.1±3.81	56.9±3.14	34.5±1.61	36.5±3.05
P1b (mmHg)	0.670±0.233	2.08±0.50	0.989±0.509	0.237±0.982
P1f (%)	98.0±0.709	96.6±0.797	97.3±1.31	99.4±2.40
P1b (%)	2.00±0.709	3.36±0.797	2.69±1.31	0.588±2.40
P2 (mmHg)	32.5±3.02	47.2±3.17	33.0±2.66	43.7±4.31
P2f (mmHg)	31.6±3.35	48.9±3.24	31.7±2.75	39.9±3.41
P2b (mmHg)	0.88±1.52	-1.66±2.08	1.27±1.54	3.82±2.10
P2f (%)	97.3±3.65	105±5.55	96.1±4.18	92.4±4.22
P2b (%)	2.74±3.65	-4.62±5.55	3.93±4.18	7.63±4.22
<b>Transformed</b>				
P1(mmHg)	29.9±3.05 <sup>†</sup>	48.3±2.95 <sup>†</sup>	29.0±1.37 <sup>†</sup>	30.1±2.35 <sup>†</sup>
P1f (mmHg)	29.7±3.09 <sup>†</sup>	46.2±2.94 <sup>†</sup>	28.5±1.33 <sup>†</sup>	30.8±2.53 <sup>†</sup>
P1b (mmHg)	0.219±0.182 <sup>†</sup>	2.11±0.895	0.498±0.498	-0.757±0.865
P1f (%)	99.2±0.662 <sup>†</sup>	95.7±2.20 <sup>†</sup>	98.4±1.60	103±2.68
P1b (%)	0.789±0.662 <sup>†</sup>	4.33±2.70 <sup>†</sup>	1.56±1.60	-2.89±2.68
P2 (mmHg)	33.0±2.89	45.7±2.52	33.3±2.41	43.0±4.04
P2f (mmHg)	29.6±2.94 <sup>†</sup>	43.8±2.82 <sup>†</sup>	29.9±2.13	36.5±3.11
P2b (mmHg)	3.40±1.25 <sup>†</sup>	1.91±1.74 <sup>†</sup>	3.33±1.20	6.53±1.74
P2f (%)	89.7±3.13 <sup>†</sup>	96.1±4.38 <sup>†</sup>	90.2±2.79	85.4±3.08 <sup>†</sup>
P2b (%)	10.3±3.13 <sup>†</sup>	3.90±4.38 <sup>†</sup>	9.78±2.79	14.6±3.08 <sup>†</sup>

Data are means ±SEM; P1, pressure at first systolic shoulder; P1f forward pressure wave at P1; P1b backward pressure wave at P1; P2, pressure at second systolic peak; P2f forward pressure wave at P2; P2b backward pressure wave at P2; \*P<0.05, †P<0.01 for comparison between wave separation analysis indices using carotid pressure pulse versus synthesized aortic pressure pulse.

*Comparison of haemodynamic measures in patients with untreated hypertension and normotensive controls*

Compared to normotensive subjects, hypertensive subjects were characterized by increased MAP and increased central aortic pulsatility with P1, P2 and AP and increased by  $11.8 \pm 2.8$ ,  $24.3 \pm 4.8$  and  $12.5 \pm 3.8$  mmHg respectively compared to normotensive subjects. P1 and P2 were of approximately equal magnitude in normotensive subjects so that AP was close to zero. However, in hypertensive subjects, P2 exceeded P1 giving an AP of  $12.6 \pm 3.5$  mmHg. TPR was greater in hypertensive compared to normotensive subjects but CO was similar in the two groups (table 10).

**Table 10**

Measure	Normotensive controls	Hypertensive patients
pSBP (mmHg)	120 ± 14.2	165 ± 26.6 <sup>‡</sup>
pDBP (mmHg)	71.1 ± 8.20	98.7 ± 14.2 <sup>‡</sup>
MAP (mmHg)	87.3 ± 1.97	122 ± 3.38 <sup>‡</sup>
HR (bpm)	63.0 ± 1.66	66.2 ± 2.14
cSBP (mmHg)	109 ± 2.67	159 ± 5.06 <sup>‡</sup>
P1 (mmHg)	33.9 ± 1.67	45.7 ± 2.26 <sup>‡</sup>
P2 (mmHg)	34.1 ± 2.13	58.4 ± 4.35 <sup>‡</sup>
T1 (ms)	106 ± 2.97	99.6 ± 3.34
T2 (ms)	221 ± 4.02	211 ± 5.79
AP (mmHg)	0.133 ± 1.58	12.6 ± 3.49 <sup>‡</sup>
CO (L/min)	5.30 ± 0.306	5.98 ± 0.404
TPR (mmHg·min/L)	17.9 ± 1.41	22.7 ± 1.95*
PWV (m/s)	4.49 ± 0.294	5.74 ± 0.345 <sup>‡</sup>
Wave Intensity (W/m <sup>2</sup> /s <sup>2</sup> ×10 <sup>6</sup> )		
FCW	0.92 ± 0.0779	1.50 ± 0.118*
FEW	0.143 ± 0.0171	0.267 ± 0.0343 <sup>‡</sup>
FEW <sub>ls</sub>	0.0860 ± 0.0151	0.163 ± 0.0192 <sup>†</sup>
BEW	-0.0403 ± 0.00666	-0.0562 ± 0.00938
BCW	-0.0242 ± 0.00478	-0.0904 ± 0.0226*
BCW <sub>ls</sub>	-0.0460 ± 0.00942	-0.0575 ± 0.0112
BCW:FCW	0.0296 ± 0.00659	0.0573 ± 0.00765 <sup>‡</sup>
Wave Timing (ms)		
FCW	36.3 ± 1.03	39.5 ± 1.20
FEW	255 ± 18.4	257 ± 13.5
FEW <sub>ls</sub>	389.5 ± 13.9	374 ± 16.0
BEW	96.5 ± 4.98	81.6 ± 5.8
BCW	209 ± 16.7	175 ± 9.68
BCW <sub>ls</sub>	353 ± 22.3	346 ± 12.2

Data are expressed as means ±SEM; AP, augmentation pressure; BCW, mid systolic backward compression wave; BCW<sub>ls</sub>, late systolic backward compression wave; BEW, backward expansion wave; CO, cardiac output; cSBP, central systolic blood pressure; FCW, forward compression wave; FEW, mid systolic forward expansion wave; FEW<sub>ls</sub>, late systolic forward expansion wave; HR, heart rate; MAP, mean arterial pressure; P1, pressure at first systolic shoulder; P2, pressure at second systolic shoulder; pSBP, peripheral systolic blood pressure; pDBP, peripheral diastolic blood pressure; PWV, pulse wave velocity; TPR, total peripheral resistance; T1, time of P1 after onset of systole; T2, time of P2 after onset of systole. \*P<0.05, †P<0.01, ‡P<0.005 compared to normotensive controls.

*Relative contribution of forward and backward waves to pulsatile components of blood pressure in normotensive and hypertensive subjects*

Wave separation analysis demonstrated that, in normotensive subjects, both P1 and P2 were determined almost exclusively by forward components of the pressure wave, although in subjects with a positive AP, the backward wave did contribute to P2. In hypertensive subjects (in whom AP was positive), P1 was also determined by the forward wave and the forward wave provided the major contribution to P2. However, the backward wave provided a greater contribution to P2 than in normotensive subjects ( $7.3 \pm 1.9$  mmHg compared to  $1.0 \pm 0.7$  mmHg in normotensive subjects,  $P < 0.01$ , figure 20) and contributed  $7.1 \pm 2.1$  mmHg to the total AP of  $12.6 \pm 3.5$  mmHg in hypertensive subjects.

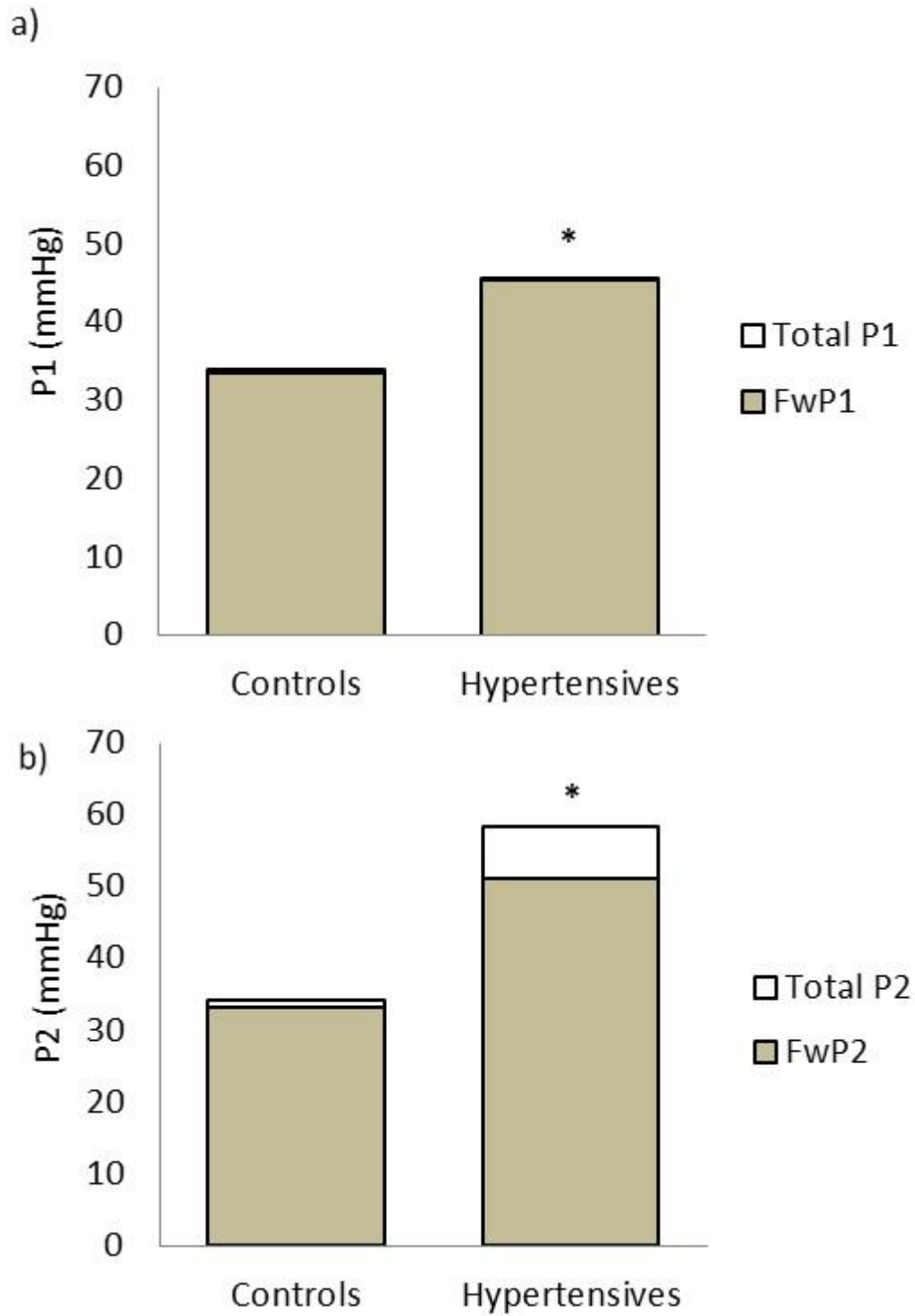


Figure 20

Pressure pulse components showing total pressure pulse and forward (Fw) component of pressure pulse for a) P1 and b) P2 in normotensive controls and hypertensive subjects. \*P < 0.05.

### *Wave intensities in normotensive and hypertensive subjects*

The FCW was greater in hypertensive compared to normotensive subjects (table 10). The mid systolic BCW was also greater in hypertensive compared to normotensive subjects, both in absolute terms and as a proportion of the FCW and tended to arrive earlier in systole in hypertensive compared to normotensive subjects, although the difference in timing was not statistically significant (table 10). The FEW were also greater in hypertensive compared to normotensive subjects but the FEW/FCW ratios were not significantly different in the two groups.

## **5.4 Discussion**

Temporal variation of blood pressure during systole offers not only an insight into ventricular-vascular interaction but contributes to the risk of future cardiovascular events. At any age, pulse pressure provides additional prognostic information over MAP, and in older subjects, is the single most important component of blood pressure in determining outcome.<sup>175</sup> Central (aortic or carotid) pressures generated at the interface between the ventricle and arterial tree propagate along the arterial tree to determine peripheral blood pressure and appear to be at least as important a determinant of outcome as peripheral pressures.<sup>47</sup> In addition to central pulse pressure, the difference between P1 and P2 as expressed by AP or augmentation index may be an independent determinant of outcome.<sup>47</sup> Despite the importance of P1 and P2, the properties of the ventricle and arterial tree that generate increased pulsatility are still poorly understood. Previous studies have focused on central pulse pressure (equal to P2 in older subjects and P1 in younger subjects) and the established view is that an elevated pulse pressure in hypertension results from increased

augmentation of the forward pressure wave by the backward wave, this in turn arising from increased amplitude and/or earlier return of the backward wave.<sup>18, 179</sup> An alternative view is that increased pulse pressure results from increased aortic characteristic impedance (which has greater dependence than PWV on aortic cross-sectional area) and relates, in part, to an effective decrease in aortic diameter.<sup>181</sup>

To my knowledge, this is the first study to use wave separation analysis to make direct estimates of the contributions of the forward and backward waves to pulsatile components of central blood pressure at rest and during modulation of pulsatility using dobutamine and norepinephrine, drugs that both have inotropic properties but which have divergent actions on the peripheral arterial tree and produce markedly different effects on pulse pressure and peripheral resistance. At rest, in normotensive individuals, both P1 and P2 pulsatile components were determined almost exclusively by the forward pressure wave. Dobutamine produced a marked increase in both P1 and P2 that was again almost exclusively due to effects on the forward wave, as would be expected from an inotrope increasing contractility of the ventricle from which the forward wave derives. By comparison to dobutamine, norepinephrine produced a greater increase in MAP but only a modest increase in pulse pressure (determined by an increase in P2) for which forward and backward components were approximately equal. These results suggest, therefore, that the contribution of the backward wave to pulsatile components of blood pressure in normotensive subjects at rest and during inotropic and vasopressor stimulation is minor in comparison to that of the forward wave. Wave intensity analysis provides further insight into the haemodynamic origin of forward and backward waves. This analysis confirms that the increased forward pressure wave components of P1 and P2 generated by dobutamine acting to increase myocardial contractility result from an increased FCW which also occurs earlier in systole. Interestingly



Schultz et al have reached a similar conclusion regarding dominance of the FPW as responsible for change in the central pulse during exercise<sup>67</sup>, a physiological stimulus to increased myocardial contractility. Changes in other waves (backward expansion, late systolic FEW and late systolic BCW) could be secondary to the greater intensity and earlier timing of the FCW.

By contrast to dobutamine, norepinephrine did not increase the FCW. Although the intrinsic action of norepinephrine on the myocardium is to increase contractility, its action on the peripheral vascular tree to increase peripheral resistance will depend on basal sympathetic tone and can result in a reflex decrease in sympathetic output, myocardial contractility and a decrease in CO (as seen in the present study).<sup>207</sup> Thus the lack of effect of norepinephrine on the FCW is expected. Norepinephrine did increase the mid systolic BCW both in absolute terms and as a proportion of the FCW, an action consistent with increased reflection that would be expected to increase the backward pressure wave. However, quantitative analysis of the relative contributions of forward and backward waves shows the contribution of the latter to increased pulsatility is minor.

In hypertensive subjects, both P1 and P2 components were greater than in normotensive subjects. As in normotensive subjects, P1 was determined exclusively by the forward wave component. P2 was determined by both the forward and backward waves. However, the forward wave accounted for the majority of P2 and approximately two thirds of the total difference in P2 between hypertensive and normotensive subjects. The backward wave was a significant component of AP in hypertensive subjects, but even for AP, the backward wave only accounted for approximately 50% of AP. Wave intensity analysis confirmed a greater FCW and mid systolic BCW in hypertension compared to normotensive

subjects consistent with the relative contribution of forward and backward waves to pulsatility. Comparison of the difference between hypertensive and normotensive subjects with the results of inotropic and vasopressor stimulation in normotensive subjects, show that increased pulsatility in hypertension most closely resembles that simulated with dobutamine in normotensives. These results, therefore, challenge the view that increased pulsatility in essential hypertension results from increased wave reflection. Instead they underline the dominance of the forward wave generated by ventricular contraction. Increased sympathetic activation is invariably present in essential hypertension and either alone or in combination with increased pre-load may explain increased ventricular ejection early in systole and hence increased forward wave components of pulsatility in hypertension.<sup>208</sup>

This study is subject to a number of limitations. The majority of the hypertensive subjects I studied were on treatment with diuretics and/or vasodilator drugs which would tend to increase sympathetic activity. Thus, although sympathetic activation is thought to be an early event in the pathogenesis of hypertension<sup>208</sup> I cannot be certain that these results apply to untreated hypertension. It is likely that the relative contribution of FCW and BCW components to increased pulsatility differ across the spectrum of essential hypertension and further studies will be required to determine the relative importance of these components according to age, neuro-endocrine profile and blood pressure treatment. Errors in our analysis could relate to non-invasive derivation of pressure and flow and the use of carotid pressure as a surrogate for aortic pressure. However, scaling errors relating to the exact point at which flow velocity is measured do not influence wave intensity values or wave separation analysis. We also obtained similar findings relating to the dominance of the forward pressure wave irrespective of whether we used a carotid-to-aortic transfer function. Furthermore, the use of the inotropic and vasopressor drugs to verify that the FCW arises primarily from myocardial

contractility and the BCW from vasoconstriction of the arterial tree strongly support the conclusions from these analyses.

## **5.5 Conclusion**

An increase in pulse pressure is the major haemodynamic change contributing to hypertension in an ageing population. It could result from either an increase in a forward compression wave generated by the ventricle or backward compression wave generated from the arterial tree. The present results suggest that the former dominates both during inotropic stimulation with dobutamine and in subjects with treated essential hypertension. Even when arterial tone is increased with norepinephrine, the contribution of the backward wave to increased pulsatility is relatively modest. The results highlight the potential importance of ventricular dynamics as well as arterial stiffness in the genesis of systolic hypertension and suggest interventions that might modify this may be particularly effective in reducing pulse pressure and systolic hypertension. In conclusion, wave separation and intensity analysis suggest increased pulsatile components of blood pressure in essential hypertension derive predominantly from a forward pressure wave generated by increased myocardial contractility. An increased backward wave does contribute to increased central pulse pressure in hypertension but its contribution is less than predicted from augmentation pressure which is influenced both by forward and backward waves.

## DISCUSSIONS

The first part of this thesis systematically examined the effects of vasodilators on large muscular arteries and resistance arterioles in the human forearm circulation. The relationship between arterial diameter and PWV was also explored in order to examine whether modulation of PWV is due to a change in arterial diameter or is determined by a specific molecular pathway. For a given change in resistance arteriolar vascular tone, the NO donors GTN and NP acting on the NO-sGC-cGMP pathway were shown to have the most selective action on the radial artery, an example of a muscular large artery. By contrast vasodilators acting on the L-type voltage-dependent calcium channels,  $\alpha$ -adrenoceptors and BNP-pGC pathways had relatively little effect on the radial artery suggesting that their contribution to resting vascular tone of muscular large arteries is minimal. Both sGC and pGC produce cGMP in VSMC, so it is unclear why BNP and NO donors should have a differential effect on VSMC in large muscular arteries and resistance arterioles. One possible explanation is the spatial compartmentalization of cGMP production within VSMC where sGC is thought to produce cGMP nearer to the sarcolemma compared to cGMP produced by pGC.<sup>182, 183</sup> It is also possible that cGMP is not the only mechanism producing VSMC relaxation in muscular large arteries. This is supported by the observation that GTN and NP, both acting on the sGC-cGMP pathway, have different effects on large muscular arteries and resistance arterioles. An intermediate metabolite of GTN and NP and/or activation of other mechanisms such as endothelial hyperpolarizing factors could potentially explain the differential effects of NO donors on large muscular arteries.<sup>154</sup>

One of the limitations of this *in vivo* human forearm circulation model is the difficulty to control for local exposure to vasoactive substances as a result of dynamic changes to vascular tone and blood flow, which may have confounding effects on the results of these studies. At present there is no obvious solution to this other than to confirm these findings

with *in vitro* experiments. Within the limits of this however, the same human forearm circulation model could be used to further elucidate the regulation of vascular tone in muscular arteries. Firstly co-infusion of NO donors and phosphodiesterase V inhibitors/sGC activators could be used to examine the importance of cGMP in the vascular tone of muscular large arteries and resistance arterioles. Secondly, the contribution of other known signaling pathway such as endothelin, angiotensin II, prostaglandin and potassium channels could be explored systematically by local infusion of agonist/antagonists. The regulation of vascular tone in hypertensive subjects may differ from that in healthy normotensive volunteers and this should be explored. A further finding from chapter 3 was the close relationship between arterial diameter and PWV, irrespective of the vasoactive substance used to modulate arterial tone. This suggested that short-term alteration in PWV is mostly dependent on change in vascular tone rather than dependent on a specific signaling pathway. Partitioning the components of PWV using the Moens-Korteweg equation demonstrated the complex interactions between structural proteins and VSMC in determining arterial wall stiffness and PWV. The paradoxical increase in elastic modulus with vasodilatation is likely an important property in the maintenance of arterial wall structural integrity over a wide range of vascular tone and pressure.

GTN, the drug found to be the most selective dilator of large muscular arteries in chapter 3, is also the drug thought to be most specific in reducing AP and hence cPP. This is entirely consistent with an action to dilate muscular arteries and hence reduce pressure wave reflection from peripheral arteries – the generally accepted explanation for this action. However, the effect could also be due to an action on the myocardium. In chapter 4 I explored central haemodynamic effects of GTN during simultaneous invasive measurements of aortic pressure and flow velocity. This allows wave separation and wave intensity analyses

to be applied to test the hypothesis that reduction in the amplitude and delay in timing of reflected pressure waves is the main mechanism through which GTN reduces AP and cPP. In the first part of this study GTN was administered systemically via the sublingual route and in the second part of this study GTN was infused locally into the coronary arteries in order to examine the contribution of left ventricular dynamics on AP. At baseline wave separation analysis showed that the FPW provided a greater contribution to AP relative to the BPW than previously thought. Following sublingual administration of GTN, cPP and AP but not MAP or DBP were reduced, consistent with previous data in the literature.<sup>86, 116, 146</sup> Application of wave separation and wave intensity analyses further revealed the reduction in AP was due to a reduction in both FPW and BPW and the ratio of BPW:FPW and BCW:FCW energy were unaltered suggesting most of the changes in BPW were secondary to a change in FPW. This surprising finding is not consistent with a reduction in wave reflection as the mechanism by which GTN reduces AP. Further analysis of the FPW by wave intensity analysis suggested an earlier and more pronounced relaxation of the left ventricle following administration of sublingual GTN. Firstly, the timing of FEW was shortened, which is consistent with an earlier onset of the 'braking effect' or relaxation of the left ventricle. Secondly, the maximal FCW wave intensity, which occurred in early systole at peak myocardial stress, remained relatively constant but FCW energy, which is an integral of wave peak that also includes waves at late systole, was significantly reduced indicating that FCW was diminished at the time of pressure augmentation.

In the second part of the study infusion of intracoronary GTN at a dose that does not have systemic effects was used to confirm that reduction of AP by GTN is mediated, at least in part, by an action on the myocardium. Intracoronary GTN reduced AP despite no significant change in heart rate, DBP or MAP. Taken together these observations suggest that

at doses of GTN where DBP and MAP are unaltered, the reduction in AP and cPP is secondary to a direct lusitropic effect on the left ventricle. This challenges the established view that vasodilatation of large muscular arteries and consequent reduction of pressure wave reflection is the main mechanism by which GTN reduces AP and cPP. Furthermore, it adds to the body of evidence against the conventional view that AP and AIX are measures of pressure wave reflection.<sup>61, 68, 98</sup> Results from this study suggest that AP is more closely related to left ventricular contraction/relaxation dynamics rather than the arterial load imposed on the left ventricle. The sensitivity of AP to GTN suggests that endogenous production of NO within the myocardium may be an important determinant of ventricular relaxation.<sup>202, 203</sup> Further studies are required to probe the relationship between lusitropy and AP. In addition dose ranging studies with intracoronary GTN infusion with the inclusion of left ventricular pressure-volume loop analysis will be helpful to elucidate the role of pre-load and to confirm the threshold for which GTN exerts a direct effect on the myocardium. Furthermore local intracoronary infusion of comparator drugs that have opposing effects to GTN on the vasculature and the left ventricle such as phentolamine or esmolol may be helpful in determining how different stimuli affect AP and also to investigate whether lusitropy associated with AP reduction is determined by a specific signaling pathway(s).<sup>159, 209, 210</sup>

With the unexpected finding in chapter 4 that AP relates predominantly to the FPW, rather than the BPW, I questioned the established view that increased pulse pressure in hypertension relates primarily to increased arterial stiffness and increased pressure wave reflection. The objective of chapter 5 was to explore the relative contribution of FPW and BPW to cPP and its components in healthy volunteers under pharmacological stress, and also in subjects with hypertension and elevated pulse pressure. Wave separation and wave intensity analyses were again applied to examine in detail the contribution of FPW and BPW



to cPP and its components. Dobutamine markedly increased pulsatility, increasing cPP and its components, P1 and P2 without any significant effect on MAP. Most of this increase in pulsatility was attributable to an increase in FPW. Moreover the magnitude of increase was greater for P1 than in P2 resulting in a trend for a reduction in AP. Wave intensity analysis showed that FCW intensity increased and occurred earlier in systole with incremental doses of dobutamine but the BCW: FCW ratio did not change significantly, which is consistent with the findings that inotropic stimulation with dobutamine determines the FPW in both P1 and P2, which in turn increases cPP. By contrast norepinephrine also increased cPP but to a smaller magnitude in comparison to dobutamine and with an increase in MAP. Increase in pulsatility was attributed to an increase in P2 but not P1, resulting in a significant rise in AP. Despite a significant increase in BPW with incremental doses of norepinephrine the FPW remained the dominant contributor to P1, P2 and cPP. In the second part of this study, cPP and its components were compared in hypertensive subjects and age-matched healthy normotensive volunteers. For both groups of subjects wave separation analysis illustrated that the FPW provided the dominant contribution to both P1 and P2, but in subjects with hypertension, the relative contribution of the BPW to P2 was higher in comparison to normotensive volunteers. Approximately 50% of AP was accounted for by the FPW. Although CO was similar in the two groups, wave intensity analysis showed that both FCW and FEW were significantly higher in subjects with hypertension. In addition, the BCW and BCW:FCW ratio were also significantly increased suggesting that in subjects with hypertension and elevated pulse pressure both FPW and BPW contributed to pulsatility but the former predominates. In comparison to inotropic and vasopressor stimulation by dobutamine and norepinephrine, the increase in pulsatility in hypertension resembled the combined effect of both drugs but the contribution of BPW to pulsatility is less than previously thought.

Taken together, the results of chapters 4 and 5 represent a paradigm shift in the haemodynamic genesis of hypertension. Because CO is generally similar in hypertensive and normotensive subjects, hypertension has traditionally been regarded as an arterial problem, with diastolic hypertension being due to an increase in peripheral resistance and an increased pulsatility and hence systolic pressure being due to increased arterial stiffness and/or increased pressure wave reflection. The findings from chapters 4 and 5 suggest that hypertension results in large part from altered ventricular dynamics. Chapter 4 highlights a novel potential therapeutic mechanism for reducing the ventricular generated FPW and hence pulse pressure via an action on lusitropy. This might be a particularly important therapeutic approach in subjects with isolated systolic hypertension but relatively normal arterial stiffness. In these subjects conventional vasodilator therapy will reduce systolic pressure in parallel with diastolic pressure reducing myocardial perfusion pressure and, especially in the presence of obstructive coronary disease, jeopardizing myocardial perfusion. By contrast, a drug such as GTN with preferential effects to reduce systolic but not diastolic pressure through a lusitropic action may confer greater benefit for a given degree of systolic blood pressure reduction. This is of course a hypothesis and would need to be tested through a clinical trial.

### **Perspective**

The major findings from this thesis challenge the conventional view that widened pulse pressure is predominantly driven by pressure wave reflection resulting from increased arterial stiffness. Instead experimental results from both chapters 4 and 5 of this thesis showed that left ventricular contraction/relaxation dynamics has a major influence on pulsatility. This has

a strong translational potential for the therapeutic development for ISH and perhaps also for heart failure with preserved ejection fraction. The application of wave intensity analysis to studies using pharmacological probes, in addition to left ventricular pressure-volume loop analysis could further our understanding between left ventricular contraction/relaxation dynamics and pulse pressure. In this regard the nitric oxide donors appear to be the most promising but a number of important questions pertaining to their effects on preload and the contribution of other known signaling pathway such as the renin-angiotensin-aldosterone system should be systematically explored. Furthermore the haemodynamic changes induced by pharmacological probes in the presence of left ventricular hypertrophy and diastolic dysfunction will be of much interest as it will shed light on the haemodynamic effect of ventricular stiffening. Patients with hypertrophic cardiomyopathy in the absence of coronary artery disease and hypertension would be an interesting group of subjects to investigate for this matter. Finally the differential vasoregulatory mechanisms of muscular conduit arteries and resistance arterioles should be explored further in hypertensive patients to determine the effect of arterial disease on the resting tone of muscular conduit arteries.

## **Conclusion**

This thesis was set out to determine the mechanism by which vasodilators reduce AP and cPP. The major findings of this thesis were (i) NO donors have a selective action to dilate large muscular arteries relative to resistance arterioles. (ii) Despite this action on large muscular arteries, the major action of GTN to reduce AP and cPP is mediated by a novel direct lusitropic mechanism on the left ventricle, rather than a reduction in pressure wave reflection. (iii) Both FPW and BPW contribute to the increase in AP and cPP in hypertension. However most of the increase in pulsatility is mediated through altered ventricular

contractility. Further research is required to investigate the relationship between ventricular dynamics, lusitropy in particular, and pulse pressure and the signaling pathway(s) that modulates these properties.

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