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Impact of alpha adrenergic and myogenic control on forearm vasomotor properties

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ABSTRACT

We tested the hypotheses that forearm vascular compliance (C) but not resistance (R) would be influenced by myogenic stimuli, and changing (Δ) forearm transmural pressure (TP) would influence the effect of α-adrenergic input on C and R. Continuous forearm blood flow was measured during Norepinephrine (NE; α-agonist) and during concurrent NE and Phentolamine (PH; α-antagonist) infusion with the arm above and below heart level (n=10). C was inversely related to TP ($p<0.05$). NE decreased C and increased R ($p<0.05$). PH abolished these responses. The effect of NE on ΔC was greater with the arm above versus below heart level ($p<0.05$), while ΔR was only observed with the arm below the heart ($p<0.05$). Conclusions: Myogenic changes affect forearm vascular C independent of changes in R. Alpha -adrenergic activation reduces C and increases R. Furthermore, with NE, ΔC requires a high starting value of C, while ΔR occurs under high TP.

Keywords: Myogenic, Alpha-adrenergic, Norepinephrine, Phentolamine, Windkessel, Doppler ultrasound

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BP	Blood pressure (mmHg)
BSL	Baseline
C	Compliance ($mL \cdot mmHg^{-1}$)
ECG	Electrocardiogram
DBP	Diastolic blood pressure (mmHg)
FBF	Forearm blood flow ($mL \cdot min^{-1}$)
FBV	Forearm blood velocity ($cm \cdot sec^{-1}$)
FMAP	Forearm mean arterial pressure (mmHg)
HR	Heart rate (beats per minute)
MAP	Mean arterial pressure (mmHg)
MSNA	Muscle sympathetic nerve activity
NE	Norepinephrine ($100\ ng \cdot kg^{-1}/min$)
PH	Phentolamine ($200\ \mu g \cdot min^{-1}$)
PP	Pulse pressure (mmHg)
Q	Cardiac output ($L \cdot min^{-1}$)
R	Resistance ($mmHg \cdot mL^{-1}/min$)
SBP	Systolic blood pressure (mmHg)
SD	Standard deviation
SV	Stroke volume (mL)
SVC	Systemic vascular conductance ($L \cdot min^{-1}/mmHg$)
SYSC	Systemic compliance (mL/mmHg)

TP	Transmural pressure (mmHg)
TPR	Total peripheral resistance ($mmHg \cdot L^{-1}/min$)
Δ	Changing

Chapter 1: Introduction

1.1 Background

The contractile state of blood vessels serving skeletal muscle is variable and is affected by mechanical, neural and biochemical processes. Specifically, the integration of adrenergic, local metabolic and myogenic determinants have been recognized as important factors in the regulation of basal tone through the regulation of vascular resistance (R) (30; 53; 71; 83), that is, the vessel diameter. Also, pressure and flow within the arterial vasculature are dependent on the structural components that comprise arteries. While much work has focused solely on changes in the diameter of resistance arteries to produce change in flow, recent work has shown that the efficiency of vascular function is dependent on additional physical characteristics such as vascular geometry (including arterial diameter and length), structural properties such as the viscoelasticity (K) of the arterial walls, as well as functional properties such as vascular compliance (C) (94; 111; 144).

Decreases in diameter of resistance arteries, producing an increase in R to blood flow, can adequately explain changes in *steady state* flow that occur over the course of an entire cardiac cycle. However, blood flow is also pulsatile by nature due to the pumping action of the heart and the reflected pressure waves generated by the impedance changes located at multiple branching and narrowing points along the vascular bed. The R variable does not account for this pulsatile behaviour. To account for these functions, further arterial components exist that are subject to adjustment in order to regulate delivery of blood flow more effectively (145). These components include the compliance (C), inertia (L) and K of the system (144; 145).

These additional arterial properties can be estimated indirectly from the relationship between pressure and flow waves in the peripheral vasculature (140; 145). Zamir *et al.* (145) demonstrated that vasomotor R, C, K, and L may each play important discernible roles in the regulation of blood flow to the downstream vascular beds. R, C, K, and L can be derived from a modified lumped Windkessel model to provide the relationship between pressure and flow (145). Nevertheless, exactly how neurogenic, metabolic and myogenic inputs affect these vasomotor properties and whether these vascular properties are altered independently and to different extents is not fully understood.

It has been suggested that myogenic input plays an important role in the regulation of vascular smooth muscle tone (76; 84). The suggested mechanism of the myogenic response resides in the ability of vascular smooth muscle to modify the contractile state in response to mechanical stress or stretch (84). For example, with an increase in intravascular pressure, the smooth muscle contracts, while a decrease in intravascular pressure leads to smooth muscle relaxation. Zamir (145) *et al.* have demonstrated that changing the intravascular pressure in the forearm by repositioning the limb to above or below heart level can affect vascular C independent of changes in vascular R. Moving the arm above the heart will change the hydrostatic component of intravascular pressure (reduction) while keeping the arterial-venous pressure gradient constant; thus, changes in the flow waveform are seen without changes in total flow (121; 128; 137; 145). These changes in waveform, (primarily in the systolic component) result from changes in arterial C without changes in R (145).

Within the autonomic nervous system, the sympathetic branch has been recognized as an important regulator of both peripheral circulation (blood flow) and blood pressure. A rich adventitial plexus of sympathetic never fibres extends throughout the arteriolar network (34; 46; 80). Direct arterial infusion of non-selective α-adrenoceptor antagonists into the forearm of conscious humans have demonstrated an important role of alpha receptors in the regulation of basal vasomotor resistance through sympathetic nerve activity (SNA), with tonic SNA accounting for ~50% resting state constriction (23).

In addition to the independent roles of myogenic and neurogenic vasomotor control, adrenergic input may contribute towards the autoregulation of blood flow by augmenting the myogenic response to increases in transmural pressure (30; 84; 105; 106). The methods by which neurogenic and myogenic inputs interrelate to affect vascular resistance still remain undefined, although some associations have been observed (30; 105). An important modulator appears to be intracellular calcium concentrations within smooth muscle cells, as calcium has been shown to affect both adrenergic and myogenic inputs (30; 105). Yet, the role of these pressure-dependent and neurogenic stimuli on vascular C, and its interaction with R, has not been investigated in the intact vascular bed.

1.2 Purpose

The major purpose of the present study was to examine α-adrenergic receptor contribution to the C and R of the forearm vascular bed and whether this neurogenic input interacted with myogenically-induced changes in vasomotor contractile state.

1.3 Hypotheses

It was hypothesized that changes in vascular C and R would be influenced independently by myogenic stimuli. This hypothesis predicts that a reduction of myogenic influence (reducing transmural pressure) would increase C due to an expected relaxation of vascular smooth muscle but have little impact on R. Conversely, under a higher myogenic load, C would decrease due to constriction of vascular smooth muscle and increase in transmural pressure, without any change in R. Secondly, this study tested the hypothesis that C and R would be affected by an α-adrenergic effect produced by NE infusion, but that this adrenergic effect would depend on the myogenic load. Specifically, the conclusions of Meininger & Faber (84) as well as Ping & Johnson (105) would suggest that C would decrease to a greater extent during NE infusion with the arm lowered below heart level due to combined effects of myogenic loading and α-adrenergic stimulation.

Chapter 2: Literature Review

2.1 Introduction

Blood flow in the intact animal is pulsatile. This pulsatile flow has two components: 1) a steady state or mean flow over a period of time (e.g., a cardiac cycle), and 2) the oscillatory component with the flow rate being greater during systole than diastole. To date, the regulation of blood flow to skeletal muscle is thought of strictly in terms of its steady state component using indices such as the overall flow rate or the R of the vascular bed. This concept of vascular R reflects the calibre or diameter of the vessel in question, or the overall cross-sectional area of the vascular bed. It is this concept that is used almost universally to reflect changes in the vasomotor contractile state in response to, for example, local, circulating or neural factors that constrict or dilate this vascular segment. This steady state component is informative of average conditions but does not satisfy an understanding of the capacitive nature of the vascular bed to store and then eject volume. Recently, the attention of this laboratory has focused on this dynamic oscillatory component of organ blood flow, which is fundamentally understood as an issue of vascular stiffness or C. A major concern is whether the oscillatory component is regulated independently from the steady state component and whether each are affected by sympathetic neurogenic control. This literature review examines 1) the anatomy of the vascular tree, 2) how this anatomy contributes to the mechanical properties of vascular R and C. 3) How this concept of C can be understood in conduit vessels and, more to the context of this study, the forearm vascular bed. 4) How such C is potentially regulated by pressure-dependent (myogenic) and/or sympathetic neurogenic factors and lastly 5) how these myogenic and neural factors interact to potentially affect vascular R and/or C.

2.2 Arterial Structure

The arterial vasculature is specialized to deliver blood throughout the body according to the metabolic needs of the tissue with a regulated amount of quantity and pressure (94). Through both passive and active changes (such as through myogenic and neurogenic inputs, respectively) the vascular walls can accommodate to the changing conditions to which they are exposed (118). The ability to accommodate changes in blood pressure and flow is partially dependent upon the anatomical design of blood vessels.

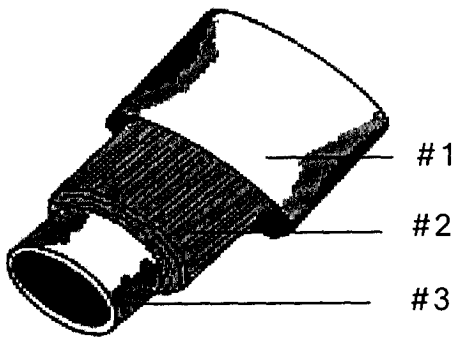


Figure 1 – Structural representation of arterial blood vessel with different concentric zones: the tunica adventitia (#1), the tunica media (#2), and the tunica intima (#3).

Arterial vascular walls are structurally composed of three distinct concentric zones: the tunica intima, media, and adventitia (87; 94; 109; 118) (Figure 1). The tunica intima (Figure 1- #3), the innermost layer, consists of a single layer of endothelial cells bordering the lumen that respond to changes in blood flow (66; 94) through a sensitivity to changes in shear stress. In response to this mechanical shear, and other biochemical stimuli, changes in vascular diameter are influenced by the endothelial layer through compounds that constrict (e.g., endothelin) or dilate (e.g., nitric oxide, prostaglandins) vascular smooth muscle (40).

Adjacent to the intima is the tunica media (Figure 1-#2), the largest constituent of the arterial wall. This layer consists of a matrix of collagen and elastin linked to smooth muscle cells that are circumferentially arranged and that function to withstand changes in

wall stress and influence the mechanical properties of arteries (91). In larger arteries, this tunica media may consist of up to 40 layers of smooth muscle cells, and this number decreases with vessel size (56). Together, smooth muscle, collagen and elastin bear the majority of wall stress. Elastin is a highly extendible fibre and provides arterial wall elasticity and resists deformation, while collagen, a fibre that is markedly stiffer, provides rigid reinforcement to support the arterial walls (4; 43; 87; 109; 118; 142). Not only are all components important in determining the mechanical properties of the vessel, but the way in which these are architecturally arranged is also a significant determinant of vascular function (20; 91).

The tunica adventitia is the outermost layer. It is composed largely of collagen but also elastin fibres and merges with surrounding connective tissue. Its function is to prevent overexpansion of the artery and to loosely tether the vessel to surrounding tissue. The adventitia layer contains small blood vessels called the *vasa vasorum* that supply the walls of larger arteries and veins. Furthermore, nerves course through this layer en route to supply the tunica media (91; 94).

2.3 Arterial Tree

Differences in vascular structure have been observed along the length of the arterial tree, which occurs as a branching network. The larger conduit arteries have a cushioning or a pulse-reducing effect on the flow of blood throughout the vasculature, and distal arteries and arterioles are more involved with the distribution of blood; these functions can be attributed to the structural components of the arterial wall. The distribution of elastin and collagen differs between the central (i.e., carotid, aorta) and the peripheral (i.e., brachial, radial, femoral) arteries. More specifically, within the central

conduit arteries, elastin is the dominant component, while in the peripheral arteries collagen dominates (32; 87; 94). The prevalence of elastin within the central conduit arteries affords these vessels great elasticity, providing the ability to stretch and recoil. For this reason these arteries are referred to as elastic arteries. This feature enables pulsatile blood flow to be converted from the oscillating contractions of the heart into a more continuous flow for the downstream circulation.

The regulation of the contractile state or tone of the peripheral vasculature has important implications for the propagation of flow and pressure waves throughout the cardiovascular system (145). With increasing distance from the heart, the arterial vasculature becomes more muscular and is associated with a progressive decline in luminal diameter (94). Muscular arteries are less distensible, but more capable of active changes in vessel tone and diameter than the more central vessels (29; 94; 110). The increased ability of peripheral versus central arteries to change their calibre or tone can be attributed in part to a greater sympathetic nerve supply to the muscular arteries (75; 122). This will be discussed in detail below (Section 2.6.3).

2.4 Arterial Mechanics - Blood Flow and Blood Pressure Regulation

Arteries serve a conduit function, delivering an adequate supply of blood to tissues and organs. Blood flow regulation is achieved through a host of factors that impact the mechanical properties of the wall such as sympathetic nerve activity, local myogenic factors, metabolic factors and hormones. The arterial vasculature responds to stimulatory and inhibitory factors by the adjustment of vascular constituents through vasoconstriction, and dilation, as well as through changes in stiffness of the arterial walls. It is worth noting that certain mechanisms can differ and/ or overlap in their effects of the

vasculature. For instance, sympathetic nerve activation and myogenic loading can induce vasoconstriction whereas removal of sympathetic inputs and myogenic unloading will induce vasodilation of the vascular wall. On the other hand, endothelin-1 only functions to constrict and nitric oxide will only function to vasodilate arterial vasculature (39).

The arterial vasculature tapers and branches as it moves further away from the heart. With each subsequent branching point or in areas with decreases in vascular lumen diameter, there is a resulting reflection of a portion of the forward propagating pressure wave that is produced as a result of a mismatch in impedance (59; 62; 64). These reflected waves travel backwards with approximately the same speed as the forward-flowing waves. Reflected waves serve two beneficial functions: 1) reduce the spread of pulsatile energy to the periphery where damage to the lightly thinned-walled microcirculatory beds might occur (112) and 2) enhance the diastolic perfusion pressure in the coronary arteries (94). Through the use of an arterial tree model, Kember *et al.* (62) demonstrated that changes within the downstream vasculature can produce instantaneous effects in the blood flow dynamics at the level of the aorta, as the accumulated effect of backward traveling waves generated by reflections from the branching and narrowing points along the arterial vasculature, were shown to have an important effect on aortic pressure distribution. Thus, dynamic changes in pressure have the ability to be relayed from the peripheral arterial tree and sensed as aortic wall distortions within the aortic inner arch. The elasticity and geometry within the peripheral vasculature are likely to be important factors for proper cardiac control (62).

The mechanical properties of arterial walls are strong determinants of the propagation and reflection rate of pressure waves along the arteries (75). At the level of

the aorta, the incident and reflected waves are traditionally thought to summate (134). Thus, the pressure waveform recorded at any site of the arterial tree is the sum of the forward-outgoing and the backward-incoming (reflected) waves. When arteries are highly compliant, elastic vessels are able to store increased volumes of blood and thereby buffer the pressure swings so that the reflected pressure wave merges with the outgoing wave during late systole or the diastolic phase; accordingly, there is little or no effect on central systolic blood pressure (SBP). In contrast, with decreases in arterial C or increases in arterial stiffness, the inability to store increased volumes of blood increases the pressure oscillations and increases the pulse-wave velocity so that reflected pressure waves return to the central arteries earlier in the cardiac cycle, summing with the outgoing wave. In this situation, the reflected wave arrives in the systolic phase, resulting in an amplification of central SBP (135) (Figure 2).

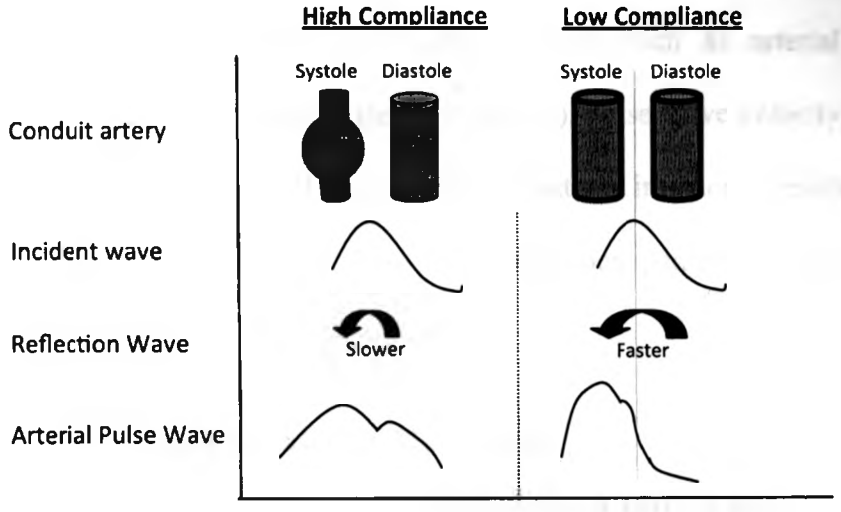


Figure 2- Modified schematic of the effects of arterial compliance on arterial pulse wave. The conduit artery expands during systole and recoils during diastole to provide constant blood flow to the peripheral vasculature. Pressure increases during systole and is maintained during diastole in a normal compliant vessel. Decreases in compliance results in a reduced ability to distend during ventricular ejection, resulting in a greater proportion of the stroke volume being transmitted to distal vessels during systole, and diastolic flow is diminished, leading to a high systolic and low diastolic blood pressure (75).

The current view of blood pressure (BP) regulation through the arterial vascular system is that in addition to cardiac output (Q), BP is mediated by changes in vessel diameter to affect changes in resistance, in order to produce a corresponding change in flow rate. Hemodynamically, mean arterial pressure (MAP) is usually expressed as the product of total peripheral resistance (TPR) and Q. This is often referred to as Ohm's Law as applied to the cardiovascular system: MAP = TPR x Q. However, MAP is the average BP determined over the cardiac cycle and, therefore, this Ohmic

relationship provides no account for the pulsatile nature of blood pressure. Within the vasculature, properties aside from peripheral R, such as arterial compliance (C), viscoelasticity (K), inertia properties of blood (L), pulse wave velocity, and the timing of pulse wave reflections, are all integral factors that can interact to regulate arterial BP (75; 145). It is worth noting that these aforementioned parameters are dependent on the composition makeup of vessel walls (75; 99).

2.4.1 Passive Control of Vascular Mechanics

The changes in R and C that are observed with changing pressure are due to structural modifications within the vascular constituents. For instance, elastin fibres play a major role in determining the mechanical strength at lower pressures. Thus, at low BPs, the arterial wall is highly distensible and has the greatest ability to expand to a rise in force. Conversely, at higher pressures, collagen fibres bear most of the mechanical stress in order to withstand increasing loads (5; 109). However, due to the greater recruitment of collagen, arterial walls are less compliant at higher BP. Thus, the organization of the constituents within the tunica media will determine the mechanical state of the arterial wall to a stressor or stimuli (5; 66). However, it is primarily the smooth muscle cells of the media layer which provide the artery with the ability to actively modify mechanical properties (94). Therefore, modifications within the walls of arterial vasculature are necessary for the maintenance of arterial flow and blood pressure.

2.4.2 Active Control in Vascular Mechanics

Vasomotor function within the arterial wall is affected by the interaction between the different arterial wall components (75). For instance, the distensibility of a blood vessel depends on the proportions and interconnections of different vascular components

such as: endothelial cells, connective tissue, bands of elastin and fibres of collagen (87). An example of the modifications that take place has been observed during smooth muscle relaxation, where stress is transferred from collagen to the more distensible elastin (6). These structural modifications to arterial wall properties occur in response to any given stimulus or stress, such as with exercise, gravitational effects, etc, and for this reason, they are a crucial component within the control of the cardiovascular system (47; 112; 115).

2.5 Modeling: Determining Compliance of a Vascular Bed

At present, direct measures of microvascular properties within skeletal muscle under different pressures and flows cannot be attained in humans. Thus, to accurately determine the dynamics of flow, non-invasive modeling methods have been applied (2; 144-146). To this end, the arterial system has been commonly represented by a Windkessel model (2; 140; 144-146). The use of such models to study blood flow and arterial wall properties has provided a valuable method for the understanding of vascular mechanics through different parts of the arterial tree (17; 140; 144-146). As used in our hands, this Windkessel approach is referred to as a "lumped" model because it provides information on the overall response from the conduit vessel at the point of measurement to the downstream capillaries, as reflected in the pressure and flow waveforms at the entry conduit vessel (Figure 3).

In a lumped model, the complex vascular structures within the arterial network are subsumed under a concept of a single tube having properties representative of the network as a whole (140; 144; 146). These models generally study the vasculature by examining the relationship between BP and blood flow. Since direct and concurrent

measures of both pressure and flow are possible at the conduit or "entry" vessel, both variables are integrated into the model. The output is the calculated factor, as it represents the ultimate function of the entire system measured. Due to the numerous branches within the arterial tree, the output cannot be measured experimentally with enough accuracy. Therefore, the model provides a theoretical means by which one can gather meaningful information about the entire system. It is a meaningful method, as its accuracy can be verified through direct physiological measures from the system; these being the measures of BP and blood flow. It is worth noting that because it is a lumped model, wave transmission and wave travel, as well as differential changes in blood flow through different levels of the arterial tree cannot be identified (140).

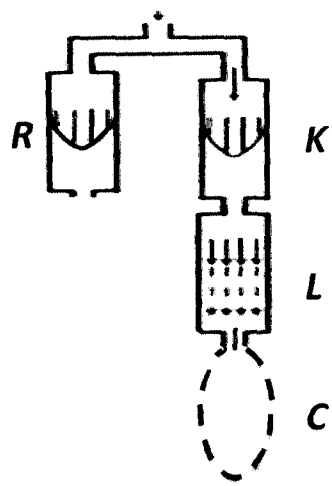


Figure 3- Schematic of the modified windkessel model. This model contains four different elements or vasomotor properties that account for both the resistive and capacitance functions within the vasculature. The parameters are: resistance (R), as well as viscoelasticity (K), inertia (L) and compliance (C). K, L and C are placed in series to each other (144; 145).

Previous research has given emphasis to the parameter of R when dealing with peripheral vasomotor function (18; 50; 140). Vascular R, based on Poiseuille's law, is inversely proportional to blood vessel radius to the fourth power. If the forearm vasculature were to be purely resistant, then according to Poiseuille's principle, blood flow and BP would be directly related. Thus, if one tried to superimpose concurrent BP and blood flow waveforms, one would expect perfect congruency. However, when comparing a blood flow waveform and BP waveform, it is evident that these two are not identical (Figure 4). This is because arterial vessels possess additional parameters apart from resistance. Therefore, any disparity between the two waveforms must then be explained by further arterial parameters.

Figure 4 - Comparison of blood pressure and blood flow waveforms. Blood pressure waveform (dashed line) and blood flow waveform (continuous line). Side by side comparisons shows incongruence between the two measures. This indicates that blood pressure and blood flow are not directly related, but that different vasomotor parameters aside from vascular resistance must be accounted for to reach agreement between the two waveforms.

Earlier Windkessel models related BP and flow with only two parameters: R and C (87; 94; 140). Arterial C, defined as the change in arterial blood volume in response to a change in arterial pressure, is affected by the combination of BP changes and conformational changes within the vascular walls (75; 99; 144). Because the two element models lead to poor prediction of the pressure and flow relationship, later models incorporated further parameters such K and L. The parameter K represents the viscoelastic resistance to stretch as a result of intrinsic properties of the wall (144). Related to C, a higher K indicates that the vascular bed is less able to accommodate to changes in blood volume throughout the cardiac cycle phase. The L parameter also provides a form of resistance to blood flow because it represents inertia of the blood and vessel wall and is most influenced when a change in the driving pressure difference occurs (144). While current understanding of the precise mechanisms behind K and L are not well understood, the inclusion of these parameters in the modeling system is imperative for explaining the relationship between BP and blood flow within different parts of the circulation and under different physiological conditions (140; 144; 145).

The modeling system operates in a very simplistic yet functional manner. It predicts what the blood flow waveform would be for a measured pressure waveform. This predicted blood flow waveform is then contrasted against the measured flow waveform. To reach congruency between the new predicted and the measured blood flow waveforms, different parameter values for C, K and L can be adjusted in order to achieve the closest possible agreement between the two waveforms (Figure 5). The congruency that is reached between the BP and blood flow measures shows evidence for

the validity of the model and demonstrates that the mechanics of blood flow can be influenced by many different parameters.

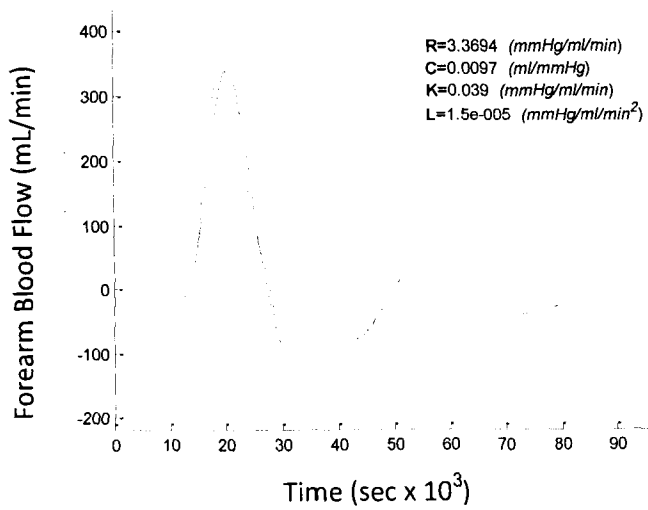


Figure 5 – Measured and calculated forearm blood flow waveforms. Measured (continuous line) and calculated (dotted line) forearm blood flow waveforms. Agreement between the two waveforms is achieved through modification with model parameters: compliance (C), viscoelasticity (K), and inertia (L). Forearm vascular resistance (R) remains consistent.

2.6 Regulation of Vascular Resistance and Compliance:

2.6.1 Myogenic Effects

2.6.1.1 Vasomotor responses to myogenic stimuli

Sir William Bayliss (1860 – 1924) was the first to identify the myogenic response in 1902 (7). The myogenic effect can be described as a feature in local blood flow regulation when arterial smooth muscle is able to constrict or relax in response to increases or decreases in transmural pressure, respectively. It has also been shown, that this response can result independently of endothelial (27; 31) or neural (35; 36) influences. Since Bayliss, several animal and human studies have been conducted to study the changes of different vascular properties by evoking a myogenic stimulus (4; 76; 83; 86; 105; 106; 125; 145; 148). The myogenic response in humans can be elicited in various ways, such as raising and lowering the arm with respect to heart level (125; 145; 148) as well as with a water pressure cuff (4) and an arm pressure tank (4; 76).

An external limb pressure tank has been used as a non-invasive method for changing forearm arterial transmural pressure without affecting the remainder of the systemic circulation (3; 5; 6; 76; 78; 79). As an example of this approach, Lott et *al.* (76) studied the acute effects of increases and decreases in transmural pressure on brachial artery mechanics through changing the forearm arterial pressure by -50 mmHg and +50 mmHg, respectively. The authors reported no change in brachial artery diameters with changes in transmural pressure. Furthermore, it was shown that increases in transmural pressure (negative external pressure; -50 mmHg) resulted in a rapid increase in mean blood velocity and conversely, decreases in transmural pressure (positive external pressure; +50mmHg) resulted in a rapid decrease in mean blood velocity. Since no diameter changes were observed, these results suggest that other variables aside from R

were affecting vasomotor control. However, these results should be viewed with caution, as interpretation of the model remains uncertain. This is because the transmural pressure is altered by a method (compression) that can also restrict blood flow. Thus, vasomotor properties would be affected independently of vascular control due to the mechanical compression of vessels.

Passive movement of an extremity has been commonly used to measure the myogenic response in humans. It has been well documented that movement of the arm above and below heart level, thereby changing the gravitational potential energy, significantly influences arm blood pressure (93; 125). Suzuki *et al.* (125) showed that changes in potential energy are proportional to the vertical distance from the extremity to the level of the heart. Placing the arm at different positions induces a range of intravascular pressures (93; 125). Zheng & Murray (148) used the protocol of adjusting the limb position relative to heart level through a range of angles from 90° to -90° and measured the effect of arm position on concurrent changes in arm pulse wave transit time and arterial volume distensibility. The authors observed that decreases in distensibility were correlated with decreases in propagation time. Furthermore, arterial volume distensibility and arterial pressure showed an inverse non-linear relationship, with the greatest effects observed when arm was at low arterial pressures (higher distensibility measures observed). Pulse wave transit time has been commonly used as an indirect measure of arterial wall stiffness, and is defined as the time taken for the arterial pulse pressure wave to travel from the left ventricle to a peripheral site measured at a distance (37). This is in accordance to the findings of Suzuki *et al.* (125) who observed higher forearm C with the arm above heart level (where the lowest transmural pressure is

observed). Similarly, Zamir *et al.* (145) observed that movement of the arm above heart level had little effect on R, but significantly and consistently increased C.

These studies demonstrate that changes in arterial transmural pressure affecting the myogenic response produce dynamic changes in the background tone of the vessels through its vasomotor properties (21; 76; 112; 145; 148). The studies to date have examined this relationship on local vasculature. The effect of changes in transmural pressure through the downstream vascular beds is still not known.

2.6.1.2 Mechanisms at the Molecular Level

Under baseline conditions, arterioles exhibit a state of partial contraction, or myogenic tone that is dependent on the level of intraluminal pressure (13; 61). These adjustments take place within the arterial wall through the inherent ability of smooth muscle to constrict and dilate in response to changes in pressure. The effect of transmural pressure on the vasomotor contractile state is related to the mechanical effect of smooth muscle membrane potential. For instance, an increase in transmural pressure has been coupled to changes in smooth muscle cell membrane potential through increases in cytosolic calcium concentrations. An increase in calcium concentration causes contraction of vascular smooth muscle (55; 86; 132; 149). Myogenic constriction has been shown to be heavily dependent on intracellular calcium concentrations in smooth muscle (53; 55; 86; 138; 149). For instance, Watanabe *et al.* (138) demonstrated that depletion of intracellular calcium concentrations through the use of ryanodine slowed the rate of pressure induced myogenic contraction. An increase in intracellular calcium concentration brings about smooth muscle cell contraction through calmodulin binding

and activation and subsequent phosphorylation of myosin (149). This phosphorylation of myosin leads to force generation and contraction (149) (Figure 6).

Calcium entry to the cell through voltage-dependent mechanisms has been studied as a possible modulator of the myogenic response (15; 51; 67; 107). Consistent with the role of calcium in the myogenic response, blockers of L-type voltage operated calcium channels including nifedipine, verapamil and nicardipine have been shown to greatly inhibit the myogenic response (67; 107). However, the underlying signalling pathways and molecular identity of mechanosensors in vascular smooth muscle remain to be understood.

In addition to voltage operated calcium channels, arteriolar smooth muscle cells possess ion channels sensitive to cell membrane stretch that may be activated by vessel distension arising from an increase in intraluminal pressure (22; 65; 70). However, the importance of cell stretch alone as a stimulus is uncertain. For instance, Hill *et al.* (54) have shown that increases in transmural pressure with no changes in cell stretch resulted in increases in intracellular calcium concentrations and reductions in arterial diameter of the rat cremaster. This indicates that stretching of smooth muscle walls may not be obligatory for myogenic responses to occur.

Lastly, modulation of the myogenic tone can also be affected by potassium (K^+) channels, as these channels play an important role in the regulation of membrane potential. K^+ channel activation, which leads to K^+ ion efflux from the cell interior leads to vasodilation and hyperpolarization in smooth muscle cells; conversely, inhibition results in depolarization of the cell membrane and vasoconstriction (15; 67; 92).

It is well known that the state of arterial vasoconstriction is based on changes in transmural pressure; however, the mechanisms behind the myogenic response still require further investigation. The myogenic response involves the activation of multiple signalling pathways that have been identified, whether each pathway functions through the interaction of other multiple pathways through cross-talk warrants further investigation (116). For a more complete review, please refer to Schubert *et al.* (116).

2.6.2 Sympathetic Nervous System

2.6.2.1 Mechanisms

The autonomic nervous system is fundamental for the regulation of cardiovascular function, and is composed of the parasympathetic and sympathetic nervous systems. The tonic neurogenic outflow of sympathetic nerve activity results in basal control over the arterial vasculature (9; 129). For this reason, sympathetic effects elicit a constant degree of smooth muscle contraction, referred to as vascular tone accounting for about 50% of the restriction of blood flow under baseline conditions (94). In accordance with this, acute sympathectomy results in a doubling of limb blood flow in the human forearm (25).

Sympathetic fibres affecting the vasculature of skeletal muscle originate in the lateral horns of the thoracic and lumbar regions (T1-L2). Throughout the circulation, sympathetic nerves are heterogeneously distributed (8). Sympathetic axons branch out over the adventitial surface of arteries and arterioles (56) producing a rich perivascular plexus of sympathetic nerve fibres surrounding feed arteries and extending into the arteriolar network of skeletal muscle (56; 74; 118; 128). The distribution of sympathetic nerve fibres increases progressively towards the peripheral arteries, where higher

innervation density is observed compared to the elastic conduit arteries. Thus, the smallest arteries and arterioles are the most richly innervated (95-98; 122).

The sympathetic neural fibres innervating the peripheral vasculature are post-ganglionic. Postganglionic fibres release norepinephrine (NE) as well as co-transmitters such as adenosine triphosphate and neuropeptide-y (NPY). Each of these transmitters can initiate a contractile response of the vascular smooth muscle cell by binding to its corresponding membrane receptor: alpha(α)-adrenergic, purinergic, or Y receptors respectively (24).

Only the adrenergic fibres are ubiquitously distributed throughout the circulation and, because they are tonically active, contribute to the resting arterial contractile state (110). Furthermore, α_1 and α_2 adrenoceptors elicit constriction of both proximal arterioles and feed arteries (29; 111) and this is inhibited by both α_1 and α_2 antagonists (1; 33). Thus, α_1 and α_2 adrenoceptors mediate smooth muscle contraction and are critical mediators of SNS regulated physiological responses (29; 33; 69; 82). Mechanistically, NE is released from nerve terminals and diffuses to vascular smooth muscle. NE then binds to postjunctional G-protein coupled α_1 and α_2 -adrenergic receptors causing arterial smooth muscle constriction. The activation of G-coupled proteins results in the release of calcium from the sarcoplasmic reticulum and subsequent contraction of the smooth muscle cells (56). More specifically, when NE binds to α_1 subtype receptors, this results in increased inositol-1,4,5-triphosphate, inducing calcium release from the sarcoplasmic reticulum and subsequent increase in intracellular calcium concentrations. Binding of NE to α_2 subtype receptors is thought to increase intracellular calcium concentrations through

the inhibition of adenylyl cyclase (Figure 6) (19). Furthermore, α_2 receptors that are situated prejunctionally, work to inhibit NE and NPY release (autoinhibition) (19).

2.6.2.2 Vasomotor responses to Sympathetic nerve activity

Adrenergic receptors are found at all levels of the arterial vasculature; however, the extent of vasoconstriction produced by sympathetic activation has been shown to be variable throughout the arterial tree. While sympathetic activation produces vasoconstriction of terminal arteries and arterioles, the effect on conduit arteries has been equivocal (94) with reports that increases in sympathetic nerve activity can result in the presence (14; 26; 103) and absence (14; 26; 68; 102; 113; 127) of diameter changes across both elastic and muscular arteries. For instance, Tschakovsky & Hughson (127) conducted a study with lower body negative pressure at a suction intensity of -60mmHg concurrent with dynamic handgrip exercise. Although these manoeuvres elicit large increases in MSNA (63; 120; 143) the authors (127) observed no change in brachial artery diameter when participants were undergoing the protocol with lower body negative pressure alone. Similarly, Dyson *et al.* (26) investigated this effect using four different types of protocols to induce sympathetic nerve activation, including lower body negative pressure, cold pressor test, mental arithmetic task, and activation of muscle chemoreflex. In this study, the authors observed no change in brachial artery diameter in the tasks with the exception of the muscle chemoreflex test, in which the diameter decreased. These results show that different physiological stresses may provoke different hemodynamic responses, regardless of increases in sympathetic nerve activity. However, inconsistency in hemodynamic responses has also been observed within studies that use similar protocols. This may be attributed to the use of a single vascular parameter (vascular R)

to infer the effects of sympathetic nerve activity (26; 103). Also, the singular focus of diastolic diameter as the indicator of vasoconstriction ignores the idea that systolic diameter may be affected more than diastolic diameter, an event that, in theory, would be indicated by a change in the stiffness of the vascular segment rather than a change in R.

Recently, focus has been given towards the study of arterial C as a separate variable that affects vascular function independently from R and is affected by sympathetic activation (14; 145). Initial efforts along this line of research focused on the C and R variations in conduit vessels. Specifically, Boutouyrie *et al.* (14) examined the effects of sympathetic activation on C and R of the radial artery. The effects of sympathetic nerve activity were studied through a 2 minute cold pressor test as well as a 2 minute mental stress test. Both stimuli increased sympathetic nerve activity (16; 26; 48). The authors observed a decrease in C with no changes in radial artery diameter, or R (14). Similar results were observed by Salzer and colleagues (113) who assessed the brachial artery during lower body negative pressure and a cold pressor test. These studies are examples that, in addition to changes in diameter (and R), C plays an important role in vascular dynamics and may represent a neural target for reflex cardiovascular control.

More recently, our new modeling approach has facilitated examination of C and R relationships in the forearm vascular bed as a lumped "whole" rather than the local effects on the conduit vessel (145; 146). As mentioned above, the advantage of this approach is that it captures changes that occur downstream in the microvessels rather than just the conduit vessel. Using this approach, Zamir *et al.* (145) studied the effects of the lumped forearm vasculature R and C during two tests: 1) 3-5 minutes of lower body negative pressure at -40 mmHg and 2) 1.5 minutes of a cold pressor test. These authors

observed that vascular C decreased during both protocols, whereas vascular R only increased during the lower body negative pressure test. Furthermore, these authors concluded that arterial C represents a more sensitive marker of vascular response to increases in sympathetic nerve activation. While the effect of sympathetic activation on vasomotor control has been studied previously, the isolated effects of α-adrenergic influence on the downstream vasculature remain to be examined.

2.6.3 Myogenic and adrenergic interaction

2.6.3.1 Vasomotor response to the interaction of the myogenic and adrenergic response

It has been proposed that control through the interaction of both myogenic and neurogenic mechanisms may optimize the intrinsic and extrinsic regulation of peripheral vasculature (30). For this reason, many studies have examined the interaction between myogenic and adrenergic inputs on vasomotor function in animals and humans (6; 30; 73; 84; 105; 106; 125).

In animals, isolated tissue preparations have been used to examine myogenic responsiveness in arterioles during stimulation of α receptors to measure the effects on arteriolar diameters (30; 84; 105; 106). To test the interaction in animals, both Ping & Johnson (105; 106) as well as Meininger & Faber (30; 84) used protocols that increase transmural pressure and then combined these with either sympathetic nerve stimulation (106) or NE infusion (84; 105). For instance, Meininger & Faber (84) examined the myogenic response, which was evoked by increases in the intravascular pressure, during varying infusion dosages of NE in rat cremaster muscles. The authors observed an enhanced myogenic response when arterioles were pre-constricted by NE and similarly

found that increased intravascular pressures augmented the sensitivity to NE. Similar results were observed by Ping & Johnson (106) who also demonstrated an increase in the autoregulation of arterial vasculature under a combined adrenergic and myogenic influence.

The animal work of both Faber & Meininger (30; 84) as well as Ping & Johnson (105; 106) point towards a directional change between pressure and arterial vasoconstriction. More specifically, increases in transmural pressure produced greater vasoconstriction when combined with a neural stimulant. It is worth noting that the methods employed to measure the interaction of sympathetic and myogenic inputs in evoking arterial vasoconstriction differed between these two groups. For instance, Faber & Meininger (30; 84) measured the interaction through infusion of NE with concurrent increases in transmural pressure. The authors observed enhanced arteriolar constriction to increases in transmural pressure when α-agonists were infused indicating a positive directional effect. Thus, in this case, the authors were able to 1) measure whether the interaction of these two inputs produced combined constrictive effects and 2) identify whether these effects reached a threshold limit. On the other hand, in the study by Ping & Johnson (105), the myogenic effect to sympathetic activation was measured by first inducing a sympathetic response and subsequently lowering the intravascular pressure (from 110 to 60 mmHg). The authors measured the interaction with opposing stimulants (decreased transmural pressure with concurrent increases in sympathetic activation). Nonetheless, under both models, it has been suggested that there may be an optimal range of tone under which this interaction is most prevalent.

In humans, protocols have also been designed to measure the combined effect of both the myogenic and adrenergic inputs on vasomotor properties (6; 125; 145). However, these studies have focused solely on conduit vessel responses. For instance, Suzuki *et al.* (125) studied the myogenic response of the brachial artery by raising and lowering the arm above and below heart level (40cm), and the sympathetic input by immersion of the contra-lateral finger in cold water, in seven male participants. While it was shown that at all arm positions, cold stimulation reduced C, a greater fall in brachial artery C occurred when the arm was above heart level compared to below heart level. These results stand in contrast to those of Meininger above. The reason for this difference could be due to an initial vasodilation in vascular smooth muscle due to the decreased transmural pressure, or as the authors mentioned, as a result of a shift in arteriolar position on the length-tension curve away from its optimal point, or the point where arterioles would show the greatest response to NE. Bank *et al.* (6) also investigated this interaction in the brachial artery of eight male participants. In this study, NE was infused during changes in transmural pressure through a pressurized cuff. NE was infused at a dose that would induce a vasoconstriction effect on smooth muscle without inducing significant systemic effects in blood pressure or heart rate (HR). By preventing systemic effects, the authors were able to measure solely the direct effects of the adrenergic infusion to those of changes in transmural pressure at the arm. Similar to the results from Suzuki *et al.* (125), it was observed that NE infusion produced a greater absolute change in brachial artery C under decreasing transmural pressures. These data can be interpreted to indicate that there is an interaction between the myogenic and neurogenic stimuli. However, the evidence that a greater NE-induced constriction

occurred when myogenic "tone" was minimized could also mean that the starting point of contractile state is important and will influence the ability to observe a constrictor response to NE. If this is the case, then a high level of C would be required in order to observe a reduction in C with NE infusion.

Similar to the animal studies using isolated arterial vasculature (30; 84; 105; 106), human studies (6; 125) also have pointed towards an interaction of myogenic and adrenergic inputs on isolated vascular function. Rather than examining the effects of arterial constriction, the current work examining this interaction in humans (6; 125; 145) has done so by examining the effects of vascular C on isolated arteries. Unlike arterial vasoconstriction in rats that show a positive relationship to both increases in NE and transmural pressure, human work has consistently observed a negative directional effect between C and neurogenic inputs (6; 145). However, like arterial vasoconstriction (84), there appears to be an optimal range at which the interaction for evoking the greatest changes in C is most prominent (6).

Thus, the integration of adrenergic and myogenic inputs make up essential determinants of dynamic (oscillatory) adjustments within the arterial vasculature that are necessary for the regulation of blood flow from the heart through to the vascular beds. The manner in which these factors interrelate to affect vasomotor function remains an important question. To date, examination of myogenic and sympathetic interactions in human studies has been limited to local conduit vessels, while those on animals have emphasized isolated tissues. So far, the effect of vasomotor control within the whole downstream microvasculature remains unknown. Furthermore, while arterial vasoconstriction (R) as well as C have been identified as important local arterial

properties, the role of each property in the control of vascular function requires further investigation. As well, an understanding of how these mechanisms produce independent and interactive effects on the downstream vascular circulation remains untested.

2.6.3.2 Mechanisms at the Molecular Level

A possible mechanism behind this interaction of myogenic and adrenergic inputs appears to be intracellular calcium concentrations (30; 73) (Figure 6). Myogenically, it has been shown that stretch causes depolarization and potentiation of stretch-operated calcium channels. Neurogenically, α_1 and α_2 adrenoceptor stimulation elicits contraction within arterial smooth muscle through intracellular release of calcium into the smooth muscle cell (30). Thus, myogenic mechanisms may add to the calcium-mediated contractile behaviour. For example, Liu *et al.* (73) suggested that both voltage operated calcium channels and protein kinase C mediated events act conjunctively facilitating adrenergic effects during increased myogenic activity. These authors showed that the infusion of calcium agonist BAY K 8644 significantly increased the extent of NE induced constriction as well as the NE induced myogenic reactivity within the primary arterioles. This is in accordance with results from Faber & Meininger (30) who also demonstrated the possibility of calcium coupling as the basis for the interaction between adrenergic and myogenic inputs through the effects of BAY K 8644. Lastly, with infusion of nifedipine, a calcium antagonist, NE induced myogenic responsiveness was abolished (73). Thus, an interaction between NE and myogenic inputs has been established. It is proposed that increases in NE results in increased sensitivity of the contractile apparatus to calcium and as a result, the effects of calcium entry and or release due to the myogenic response are augmented (84).

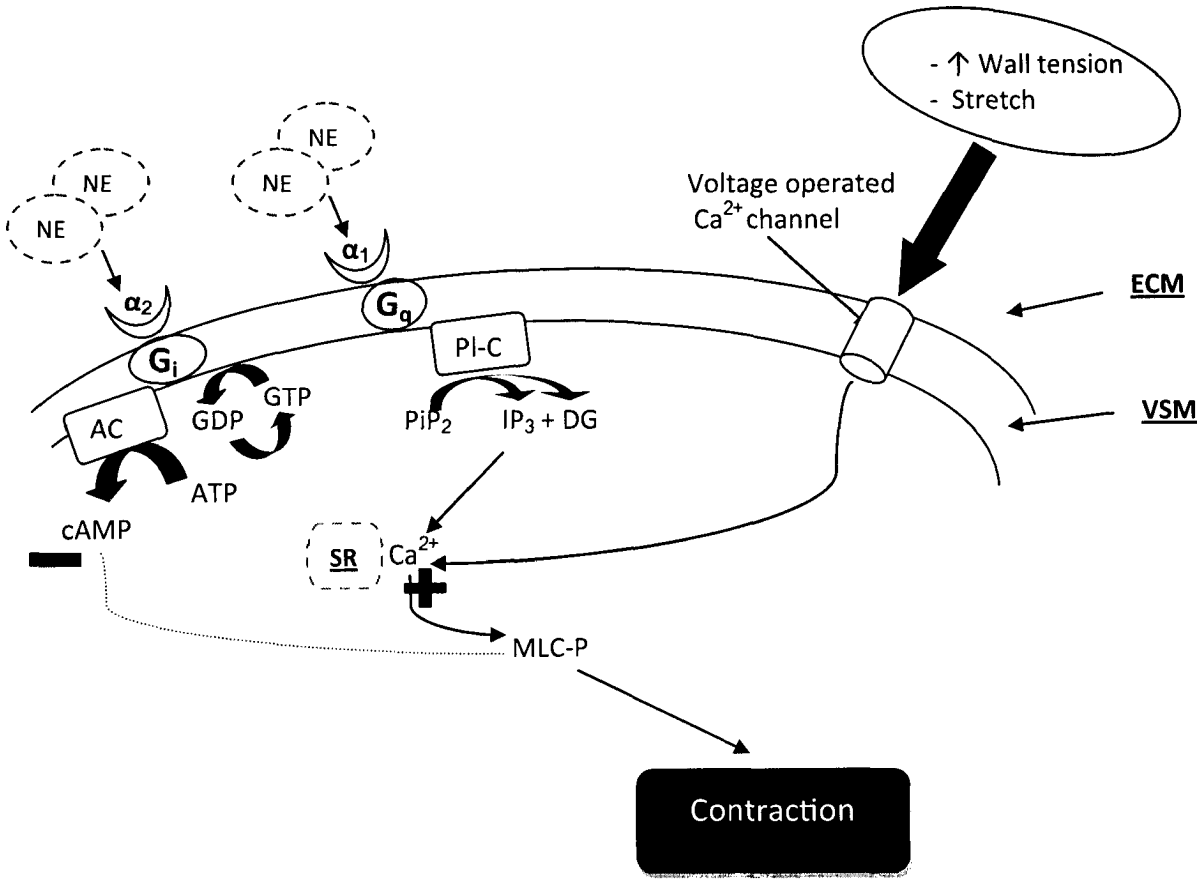


Figure 6 - Simplified schematic of the interaction between α-adrenergic and myogenic inputs in constricting vascular smooth muscle cells.

Both myogenic and adrenergic inputs may be activated together producing a response of greater magnitude than a single stimulus alone (30; 73; 140).

Myogenically, stretch can cause depolarization and potentiation of stretch-operated Calcium (Ca^{2+}) channels thereby increasing intracellular Ca^{2+} (19; 88; 89).

Both adrenergic receptors initiate signals through activation of G-proteins. Specifically, norepinephrine (NE) can bind to alpha $(\alpha)_1$- adrenoceptors and activate $G_{q/11}$ proteins to increase intracellular Ca^{2+} concentrations. NE can also bind to α_2 adrenoreceptors and activate G_i proteins that subsequently inhibit Adenylyl cyclase (AC) and reduce the levels of intracellular cyclic adenosine monophosphate (cAMP) leading to an increase in Ca^{2+} concentrations (88).

2.7 Tools and Techniques

To study the effects of α-adrenergic and myogenic inputs on vasomotor parameters requires the accurate collection of diameter and pressure from an identical vascular location. To achieve this, Doppler ultrasound was used to attain blood flow velocity and diameter measures while the Finometer™ was used to collect BP measures. These methods provided non-invasive assessment of pulsatile changes in artery diameter and pressure for the different trials within the protocol.

2.7.1 Ultrasonic Imaging

The use of ultrasound imaging is commonly used as a non-invasive test for the visualization and assessment of different tissues such as skeletal muscle, arteries and veins. The system operates through the use of sound waves to produce images. This process occurs by the transmission of high-frequency sound waves through a transducer to the region of interest. Once the transmitted waves reach the target region in which there is a difference in density, such as a blood vessel, some energy is deflected off the arterial wall back towards the transducer and detected as an echo. This returning electrical energy can be then processed electronically to discern the signal, in order to create a visual picture of the anatomical structures. The transducer listens to echoes, and the amount of time it takes for the echo to return to the probe is used to calculate the depth of the tissue. The strength of the echo is also an important determinant in visualization. The returning waves arrive back toward the transducer at different wave amplitudes, and in turn, the pixels composing the waves are assigned values according to their brightness (strong echoes are brightest). These pixels are then combined to form a

gray-scale two-dimensional image. Images can be viewed in different modes such as B-mode whereby scans through a plane of the body appear as a two-dimensional image (2-D), and M-mode, which shows movement over time. The transducer is an important component of the system as it controls the frequency at which the system operates. It is defined as a device that converts one form of energy into another; in this case, electric energy is converted into acoustic energy during transmission and from acoustic energy back to electric energy during reception. A linear array transducer is typically used for vascular imaging.

The source of ultrasonic energy comes from the vibration of piezoelectric crystals (38). These vibrate when connected to a source of electrical energy and are emitted as ultrasound waves into the tissue. The longitudinal sound waves that are transmitted into the target tissue are exposed to mechanical energy once the waves reach the target tissue. Subsequently, these waves become a source of electrical energy and are reflected back to the transducer (44). The depth of the tissue can be determined by calculating the time it takes for the waves to reach back (44; 131).

The sound waves used by the Doppler ultrasound are above the audible range of the human ear (20 kHz). Common diagnostic ultrasound frequencies range from 2 to 15 MHz (94). The range in frequencies enables the measurement of tissues at different depths. While higher frequencies provide the clearest resolution since it is inversely proportional to wavelength, the signal attenuates exponentially as it passes through tissue. Thus, as sound waves pass through there is loss of energy in the signal and thereby a decrease in the intensity and amplitude of the signal. For this reason, highest frequencies are best utilized in superficial tissue such as superficial arterial vasculature (i.e. carotid,

brachial arteries). Conversely, lowest frequencies (2-4 MHz) provide optimal penetration and are therefore best utilized for deep tissue and deep lying vessels (i.e. mesenteric artery, abdominal aorta) (94; 126). It is worth noting that the attenuation of the signal is also dependent on the homogeneity of the ultrasound beam, the absorptive features of the medium, as well as the uniformity between different boundaries (38).

2.7.1.1 Physics of Ultrasound

The longitudinal sound waves emitted by the Doppler consist of the same properties as other electromagnetic waves, including: frequency (number of cycles per second; Hz), wavelength (length of peak to peak of subsequent waveforms), amplitude (magnitude of oscillation) and speed (the product of wavelength and frequency). The speed and distance of a sound wave will depend on the density of the medium through which the sound wave passes. For instance, sound waves can travel through blood at a speed of 1570 m/s and through air at a speed of 330 m/s (41; 141). Since sound waves travel so poorly through air, a layer of water-based gel is used as a facilitating medium between the probe and the skin.

2.7.1.2 The Doppler Effect

Blood flow velocity measures are governed by the Doppler Effect principle. This principle refers to the change in sound wave frequency that occurs when a transmitted wave is reflected off of a moving object, or a moving receiver. In the case of blood flow, when the reflection of red blood cells is towards the stationary Doppler transducer, the

signal of the approaching wave will have a higher frequency than the one emitted from the transducer. Conversely, reflection off of red blood cells in blood flowing away from the transducer will result in lower frequency returning sound waves. This change in frequency is known as the Doppler shift (94). This can be determined by knowing the original frequency transmitted, speed of the scatterer, and the direction of motion of the relative wave (94).

With the known frequency from the ultrasound probe, the Doppler Effect can be determined mathematically as:

$$\Delta f_d = (2 f_t \cdot v \cdot \cos\theta) \cdot c^{-1}$$

Where Δf_d represents the difference in frequency between the transmitted and received frequency (Doppler shift, MHz), f_t represents the frequency of the transmitted wave by the ultrasound, v represents the velocity of the target, θ represents the angle between the ultrasound beam and the direction of motion and lastly c represents the speed of ultrasound energy through the tissue (in blood ~1570 m/s) (94). Thus, through the rearrangement of the equation, placing v as the unknown, the velocity of blood can be determined. As observed in the equation, the Doppler shift is proportional to the speed of the moving object. It is the Doppler shift that the transducer detects. However, it is the speed of motion or flow of blood that is of interest.

The inclusion of θ within this equation indicates the importance of having an adequate angle of insonation (Doppler angle) for accurate blood flow velocity measures. For most accurate measures, the angle of insonation should be maintained between 30° and 60° (94). Above this, the detection of flow velocity becomes compromised.

Increasing the angle results in a decrease within the Doppler shift and can fall below the threshold for detection when at an angle of 90°. When this occurs, it appears as if there is no flow in the vessel as both positive and negative Doppler shifts may be detected equally above and below baseline, making it more difficult to discriminate between forward and reverse flow (90; 126). While blood velocity is best detected at 0°, this angle of insonation is very difficult to implement in peripheral arteries and there may be difficulties in obtaining signals at low angles due to total reflection of sound waves at the vessel walls (38; 94). Thus, to ensure proper imaging and blood velocity detection, an angle of 30° to 60° is typically used (94).

2.7.1.3 Measurement of Blood Flow Velocity with Doppler Ultrasound

Doppler frequency shifts occur at a frequency that is audible to the human ear, and therefore, blood flow characteristics can also be identified by sound in addition to displayed as a frequency spectrum of the returning signal over time (94). Blood flow velocity may be measured through pulsed or continuous settings. For the purpose of imaging, pulsed ultrasound is used; whereas for the detection of blood velocity, continuous or pulsed ultrasound can be used.

With the pulsed system, a series of waveform cycles are sent from a crystal sound source, this same crystal then listens for the returning echo. As a result, there is a transmitting phase (on phase) and a receiving phase (off phase) (38; 90). Thus, the received signal is 'gated' so that the time elapsed between the transmission of the pulse and the opening of the gate determines the depth of the velocity measurement. Furthermore, by controlling when the gate is opened, one can control the size and

location of the sample volume. For instance, if the gate is open soon after the release of the ultrasound burst, signals close to the arterial wall are admitted, while delay in the opening of the gate allows for the admittance of signals from distant targets such as the centre of the arterial vessel (38). Being able to control the sample volume, or gate time enables greater control over what measures are taken, in order to gather the optimal reflecting signal source and preventing interference from adjacent tissue (38).

Continuous wave ultrasound contains two crystals, one for sending and one for receiving. Thus, there is only one phase. This method cannot be used for imaging as it is unable to determine the specific velocities within the beam. Furthermore, the sample volume is generally large, resulting in poor spatial resolution, especially when depth is a factor (94). While this method is advantageous for avoiding aliasing under high velocities (38), it presents many challenges. For instance, one cannot select a desired signal, particularly when vessels lie close to each other (such is the case with some arteries and veins). Secondly, it does not permit the analysis of the velocity profile.

For optimal use of pulsed Doppler, combined use with an imaging system such as a duplex machine is crucial (94). The 2-dimensional system allows for real-time, B-mode scanning. B-Mode (brightness mode) refers to the reflected echo that is reflected as a dot with the brightness representing the echo amplitudes (38). These dots are then built up to produce a 2-dimensional image.

2.7.1.4 Accuracy of Doppler Measures

The use of Doppler ultrasound has become a standard tool for the measure of blood flow velocity throughout different vasculature. Studies examining the accuracy of

this tool have concluded that this method is capable of producing systemic errors of 6% or less; however, greater susceptibility for errors was expressed when measuring cross-sectional areas. Repeated measures have been suggested in order to minimize random errors (41).

2.7.2 Brachial Artery Pressure

The Finometer™ (Biomedical Instrumentation, Amsterdam, Netherlands) is currently the hemodynamic monitor device of choice to continuously measure BP due to its practicality and accuracy. Continuous measures of brachial artery BP can be measured non-invasively with a Finometer™. The Finometer™ utilizes the combination of plethysmography and the "volume-clamp method" to directly determine finger blood pressure. Briefly, with this method, the finger is wrapped around a cuff so that the diameter of the artery under it is kept at a constant (clamped) diameter or set-point. As arterial pressure changes occur throughout a cardiac cycle, changes in diameter are detected by means of an infrared photo-plethysmograph built into a finger cuff. The diameter of the finger cuff can make rapid adjustments in diameter if increases in arterial diameter are sensed during systole, thereby preventing collapse of the finger artery (12; 58). Through transfer function equations, the brachial artery pressure waveform is reconstructed and also continuously measured alongside the finger measures.

Thus, the Finometer™ provides continuous measures of arterial pressure at the finger and arm through direct and calculated measures, respectively. Because brachial artery BP measures are a calculation derived from finger measures, there has been concern over the consistency and accuracy of the measures taken in terms of the pulse wave shape and size.

To examine the validity of the system, Imholz *et al.* (57) assessed the accuracy and within subject variability of 48 participants (20 normotensives and 28 hypertensives), of 18-65 years of age. Intra-brachial blood pressure measures at heart level from the non-dominant arm were compared to measures from the Finapres™. The authors observed underestimation of the Finapres™, compared to different intra-arterial levels but concluded the Finapres™ was found to be suitable for tracking changes in blood pressure within each individual, as the accuracy for within-subject precision was high (57). Furthermore, Guelen *et al.*(49) demonstrated that greater accuracy within the reconstruction of the brachial pressure waveform from finger pressure measures by the Finometer™ can be reached through the use of return to flow calibrations. In addition to this, tools such as waveform filtering, level correction and calibration have all been incorporated into the Finometer™ and have been shown to improve the accuracy within these measures (11). Lastly, to address this concern in our laboratory, we have taken concurrent measures of Finometer™ brachial BP waveforms alongside the Millar measurements, and have shown a strong correlation between pulse pressure and waveform shape (145).

The Finometer™ also provides additional features such as the calculation of Q and stroke volume, as well as a hydrostatic height correction for changes in hand position relative to heart level. Q for instance is derived from the arterial pressure waveforms through complex algorithms that account for differential vascular properties throughout the arterial tree. The reliability of these measures has been previously tested and confirmed (104; 117).

2.7.3 Adrenergic Agents

Pharmacological interventions are commonly used to study cardiovascular adaptations (6; 72; 73; 84). Alpha-adrenoceptors have been a commonly used target of drug intervention due to the ubiquitous nature of α-adrenoceptors, as well as the strong support for sympathetically induced vascular smooth muscle constriction via transmission of NE from post-ganglionic fibres to α-receptors (19; 23; 89; 94; 110). Studies examining the interaction of α-adrenergic and myogenic inputs on isolated muscle tissue of rats and cats (73; 106) have shown similar results in vasoconstriction with either NE infusion (73) or direct sympathetic nerve stimulation (106). In the same way, Ping & Johnson (105) compared the effects of direct sympathetic nerve stimulation in the cat Sartorius muscle, to NE infusion, and observed similar vasoconstriction responses for both stimuli.

The study of sympathetic nerve activation on vasomotor function has shown both increases in vascular R and decreases in C (6; 84; 105; 125; 145); however, different tests that evoke sympathetic nerve activation result in differential responses within vasculature adaptation. This could be due to the release of additional neurotransmitters aside from NE. For this reason, the sole effects of single neurotransmitters are still not well defined. Local infusion of NE on local or isolated arterial vasculature has shown vasoconstriction upon direct application (6). That being said, the effects of NE on the differential properties of R and C within the forearm lumped vasculature and the effects of NE under different transmural pressures have not been addressed.

Like NE, phentolamine (PH) has also been commonly used as a non-selective α-adrenergic blocker for neurovascular research (6; 139). The use of PH has been shown to effectively block α_1 and α_2 mediated constrictions with direct NE infusion, and to

attenuate the pressor response to physiological stresses that involve a rise in sympathetic nerve activation (28; 81). Furthermore, Eklund & Kaijser (28) observed attenuation in the rise of forearm vascular R during 60s of handgrip exercise with PH infusion.

2.7.4 Myogenic Response

The myogenic effect was a technique used to alter the transmural pressure within the arterial vasculature in order to determine its effect on vasomotor properties. A common and simple procedure to do this is through the creation of a hydrostatic pressure gradient (144) (See Chapters 1 and 5 for further description)

2.8 Summary

Pressure and flow within the arterial vasculature are dependent on the structural components of the arterial system. Consequently, vascular properties are dynamic to allow for changes in the delivery of blood to the capillaries with adequate quantity and pressure in order to match tissue demand (94). While much work has focused solely on changes in the diameter (R) of arteries to produce changes in flow, recent work has shown that additional vasomotor parameters such as vascular C should be measured, given the dynamic responsiveness of the arterial vasculature and the idea that flow remains pulsatile towards downstream vessels (111; 144). Neurogenic and myogenic inputs represent two stimuli that affect vascular mechanics. The combined effects of these inputs on forearm C and R in terms of which vascular property may be more sensitive to the stimuli, is not fully understood.

It has been proposed that adrenergic input may enhance the ability of the arterial vasculature to regulate blood flow by augmenting the myogenic response to increases in transmural pressure (30; 84; 105; 106; 125). The methods by which neurogenic and myogenic inputs interrelate still remain inconclusive, although some associations have been observed on isolated animal vessels, and local human measures (6; 30; 105; 125). However, the role of independent vascular parameters: R, and C, under the influence of myogenic and adrenergic inputs over the human brachial artery and the downstream vascular beds remains to be investigated.

2.8.1 Purpose

The major purpose of the present study was to examine α-adrenergic receptor contribution to the compliance of the forearm vascular bed and whether this interacted with myogenically-induced changes in vasomotor contractile state.

2.8.2 Hypotheses

First, it was hypothesized that changes in vascular resistance and vascular compliance would be influenced independently by myogenic stimuli. This hypothesis predicts that a reduction of myogenic influence would increase C, due to an expected relaxation of vascular smooth muscle and reduction in transmural pressure but have little impact on R. Conversely, under a higher myogenic load, C would decrease due to constriction of vascular smooth muscle and increase in transmural pressure but R should not change. Secondly, this study tested the hypothesis that C and R would be affected by an α-adrenergic effect but that this adrenergic effect, produced by NE infusion, would depend on the myogenic load. Specifically, the conclusions of Meininger & Faber (84)

and Ping & Johnson (105) would suggest that C would decrease to a greater extent during NE infusion with the arm lowered below heart level due to combined effects of myogenic loading and alpha adrenergic stimulation.

Chapter 3: Methods

All participants provided informed written. The protocol was approved by The University of Western Ontario Ethics Committee for Research on Human Subjects (Review #- 12977; Appendix 1).

3.1 Participants

Ten healthy, normotensive participants (6 males, 4 females; age range was 23 to 31 years; Table 1) volunteered. The participants were asked to abstain from alcohol, nicotine and caffeine as well as physical activity for 24 hours prior to testing. Based on responses to a health questionnaire, all participants were free of cardiovascular or neurological disease, allergies and consumption of medications contraindicative to participation. For the female participants, there was no standardization for the timing of the measurements relative to the menstrual cycle or the use of oral contraceptives.

3.2 Protocol Design

This protocol was designed to evaluate the effects of myogenic and adrenergic inputs on forearm arterial properties. Participants arrived 20 minutes prior to experimentation in order to be acclimatized and properly acquainted with the protocol. While supine, six small adhesive electrodes were placed on the participants' skin in the Lead II electrode position, in order to collect heart rate (HR; electrocardiogram) measures. This was followed by venous catheterization in the antecubital region of the left arm for infusion of NE and PH (see below). Lastly, a finger and brachial cuff were placed on the right arm for the continuous collection of arterial blood pressure waveforms

(Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands). Participants rested in the supine position for 10 minutes prior to data collection.

The overall experimental approach (shown schematically in Figure 7) was to examine R and C and other hemodynamic variables during NE infusion with two different myogenic stimuli on the forearm vasculature. The myogenic stimulus was modified by positioning the arm relative to the heart producing a hydrostatically-mediated change in vascular pressure of about 20 mmHg (see below). Thus, the protocol consisted of two trials: a Control trial and a PH trial with two randomly assigned arm positions in each trial. The Control trial consisted of a baseline period of five minutes, followed by NE infusion for five minutes and then eight minutes of recovery to enable hemodynamic variables to return to baseline levels. This was repeated in each arm position. The PH trial followed the same protocol as the Control trial but in this case PH was infused throughout the trial (Figure 7) with five minute periods of NE infusion against this background of α-blockade. The order of arm position was randomized for each of the NE and PH trials; however, the NE infusion trial was conducted first in all participants due to the lengthy (~20 mins) half-life of PH.

Protocol Design

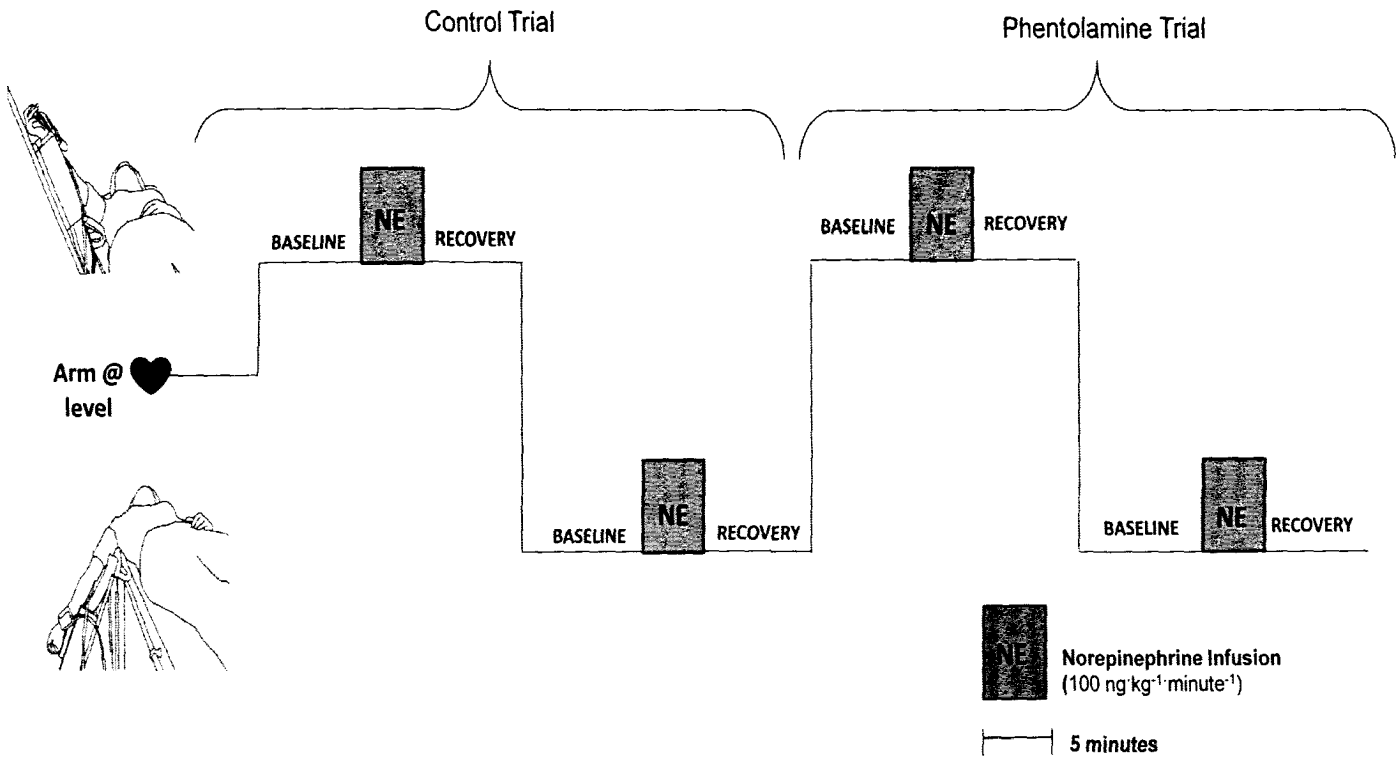


Figure 7 - Representation of protocol design. Norepinephrine (NE) was infused for five minutes following five minutes of baseline at a rate of 100 $ng \cdot kg^{-1}$/min with the arm elevated above heart level, and lowered below heart level during a control session and a session with concurrent phentolamine (PH) infusion throughout.

3.3 Experimental Methods

3.3.1 Myogenic Influence

To study the myogenic influence, forearm arterial transmural pressure was changed through the repositioning of the arm either above heart level (Above) or below heart level (Below) (137; 145). Care was taken to keep the magnitude of change in arm position constant within the trials and participants. Across participants, the arm was raised 18 ± 3 cm in the Above position, reducing vascular pressure by ~13 mmHg relative to heart level. In the Below position, the arm was lowered by 10 ± 2 cm, increasing vascular pressure by ~7 mmHg.

3.3.2 Neurogenic Influence

3.3.2.1 Adrenergic Stimulation

To measure the effects of α-adrenergic influence over forearm vascular control, NE was infused for five minutes at a constant dose of 100 $ng \cdot kg^{-1}$/minute (Harvard infusion pump Model# PHD 22/2000). This was completed once for each arm position for the control trial (NE Infusion) and again during the PH trial to test the effectiveness of the α-adrenergic blockade.

3.3.2.2 Adrenergic Blockade Control

To test the effect of chronic α-adrenergic activation on forearm R and C, PH was infused at a rate of 200$\mu g \cdot min^{-1}$ for the duration of this condition (30 minutes). As indicated above, the effectiveness of the blockade was tested by a subsequent concurrent infusion of NE and PH.

3.4 Data Acquisition

Heart rate was calculated from R-R intervals of a standard electrocardiogram (ECG). Continuous beat-by-beat collection of brachial artery blood pressure and beat-by-beat Q was achieved via recalculation from directly recorded finger pressure values of the non-catheterized arm (Finometer^{TM}, Finapres Medical Systems BV Amsterdam, The Netherlands) (11; 12; 117; 144). Placing one end of the height sensor on the finger cuff and the other affixed at heart level, a height correction option in the Finometer^{TM} was used to determine the hydrostatic difference (in mmHg) between the arm and heart level. Manual blood pressure measures were also obtained during baseline, NE infusion and recovery phases of each condition to ensure accuracy of the Finometer^{TM}. These manual BP measures were obtained from the contralateral catheterized arm that was maintained at heart level at all times.

Continuous measures of the forearm blood flow velocity (FBV; brachial artery) waveform were collected (Doppler ultrasound; GE/Vingmed System Five, 4.7 MHz) for the duration of the study from the right brachial artery. Brachial artery diameters were measured with 2-dimensional B-mode echo Doppler imaging (10MHz). Images for these measures were taken during the fourth minute of both baseline and NE infusion for each trial. The location at which the measures were taken were marked prior to beginning testing to ensure consistency across trials. All measures were taken during diastole. Furthermore, special care was taken to record the timing of each image acquisition. This was to ensure congruency for the time of image acquisition to time of hemodynamic measures to guarantee accurate mean blood flow calculations.

All analog data were sampled at 1000 Hz and collected online using the PowerLab data acquisition system (PowerLab, ADInstruments, Castle Hill, NSW, Australia).

3.5 Data Analysis

Measures of Q were provided by the Finometer™. Briefly, the Finometer uses a validated transfer function to calculate aortic pressure waveforms. From this, stroke volume is estimated by the relationship between systemic compliance (assumed) and aortic pulse pressure. Thus, if the Finometer gets aortic PP from its calculated aortic waveform, and assumes a particular systemic compliance, it can then calculate an estimate of stroke volume. This, with its own heart rate determination, allows it to calculate Q for every heart beat. Total peripheral resistance (TPR) was calculated as MAP/Q. Systemic vascular conductance (SVC) was calculated as Q/MAP. Systemic compliance (SYSC) was calculated as SV/Pulse Pressure (PP). Forearm blood flow (FBF) was calculated as the cross-sectional area of the brachial artery during diastole x FBV x 60. Thirty to sixty seconds of representative data were used to calculate an average for each condition for all hemodynamic variables at baseline and during the 4th minute of NE infusion.

Forearm vascular C was assessed using a modified lumped Windkessel model recently adapted for the forearm vascular bed in our laboratory and for which the methodological details have been published previously (145). Briefly, this model uses the oscillatory pressure and blood flow waves within the brachial artery. Using the 10 dominant frequency harmonics the waves are reconstructed so that the blood pressure waveform matches the flow waveform. Once a calculated flow waveform is created, it is

matched against the measured waveform with MATLAB software and enables values to be set for R, C, K and L, in order to obtain agreement between the calculated and the measured waveforms (Figure 8). At least two different selections of 10 consecutive cardiac cycle waves were used for each condition. It was ensured that the average forearm blood flow and blood pressure within these 10 waves represented the average mean for that minute of data collected.

3.6 Statistical Analysis

Statistical analysis of differences between drug and arm position was completed with Statistical Analysis System (SAS) v. 9.1 using a repeated measures two-way analysis of variance (ANOVA) approach. The effect of NE and arm position on baseline variables was assessed with repeated measures two ways ANOVA separately for control and PH trials. The effect of arm position and PH on hemodynamic responses to NE infusion (i.e. ΔR) was assessed using repeated measures two ways ANOVA. Significant main effects and interactions were assessed further using Tukey's post hoc analysis. Regression analysis was used to study the relationship between forearm vascular C and Δ in C for the arm above and below heart level during the control trial.

The level of statistical significance was set at $P<0.05$. Data are expressed as means ± standard deviation (SD).

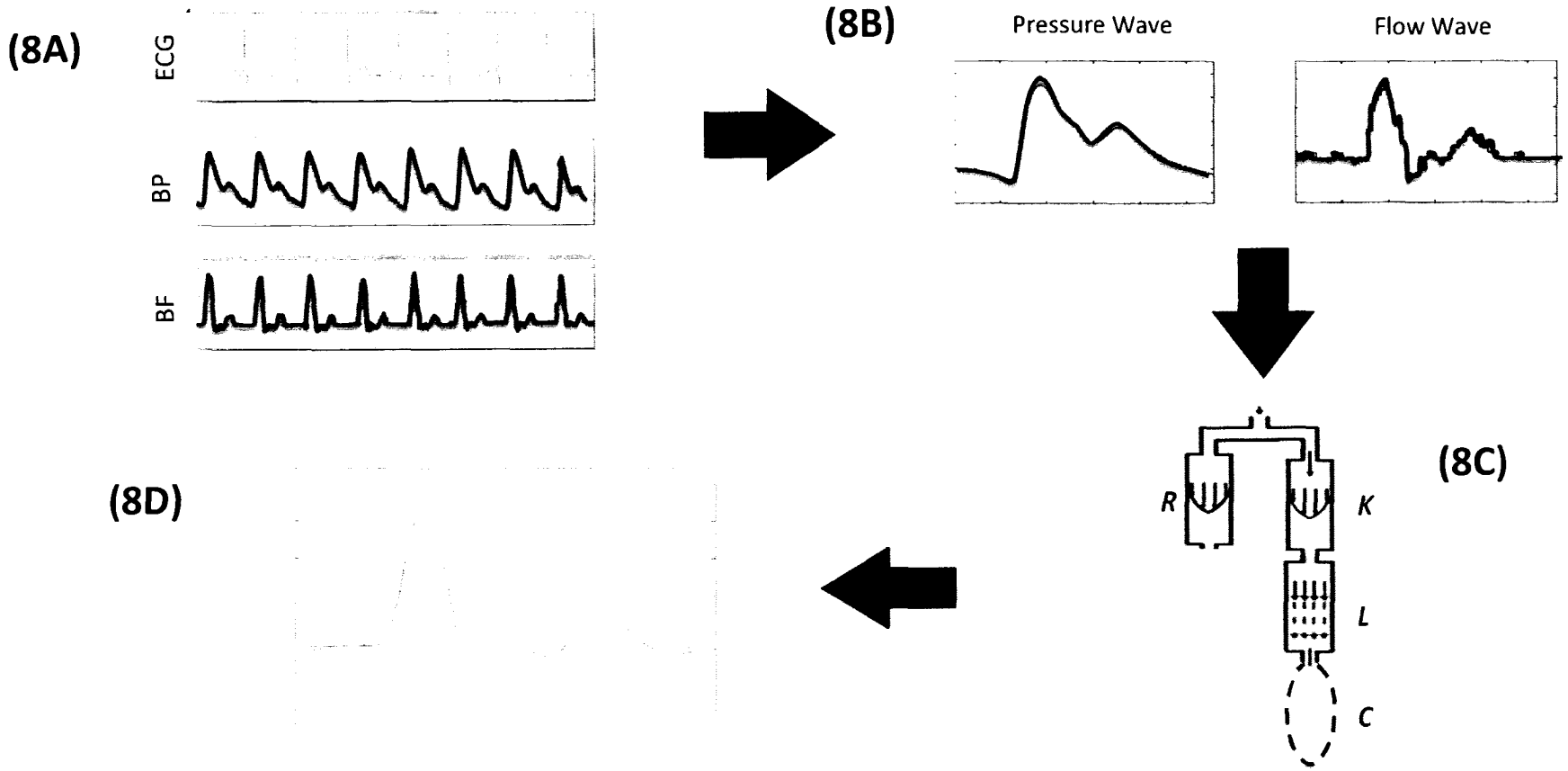


Figure 8 - Representation of analysis design. A selection of heart rate (ECG), blood pressure (BP) and blood flow (BF) waves (8A) were analyzed through MATLAB. The measured blood pressure waveforms (8B) were imported through the Windkessel method that incorporates measures for resistance (R), compliance (C), viscoelasticity (K) and inertia (L) (8C) and a calculated flow waveform was produced (8D). This calculated flow waveform was then matched against the measured flow waveforms through modifications of variables C, K and L.

Chapter 4: Results

4.1 Participant Characteristics

All participants were healthy young individuals (Table 1).

4.2 Systemic Hemodyanmics

4.2.1 Effect of Arm Position

During both Control and PH trials, moving the arm from below to above the heart (or vice versa) had no effect on any central hemodynamic variable (Table 2).

4.2.2 Effect of Norepinephrine Infusion

In the Control trial, HR decreased with NE infusion compared with baseline in both arm positions ($P<0.05$; Table 2). This effect on HR was abolished with concurrent infusion on PH.

In the Control trial, NE infusion increased systemic MAP compared with the baseline period for both the Above (~19 mmHg) and Below (~15 mmHg) arm positions (Main effect of NE, $P<0.05$) (Table 2, Figure 9). This pressor response was abolished when PH was infused; thus, PH blocked the α-adrenergic mediated pressor response in both arm positions. The same pattern of changes were observed for both SBP and DBP with pressor responses observed during NE infusion but not when infused with PH (Table 2).

Table 1 - Participant characteristics (n=10).

	Mean	± SD
Age (years)	27	3
Height (cm)	172	7
Weight (kg)	70	13
HR (bpm)	56	8
MAP (mmHg)	85	6
SBP (mmHg)	117	7
DBP (mmHg)	68	6
Q (L/min)	4.36	1.00

Values are mean ± standard deviation (SD). HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; Q, Cardiac Output.

Table 2- Effect of arm position and norepinephrine (NE) infusion with and without phentolamine (PH) infusion on systemic hemodynamics during baseline and during the fourth minute of NE infusion.

	Control Trial				Phentolamine Trial			
Arm Position	Above		Below		Above		Below	
	Baseline	NE	Baseline	NE	Baseline	NE	Baseline	NE
HR	55 ± 8	48 ± 7*	55 ± 8	48 ± 8*	59 ± 8	59 ± 8	62 ± 9†	61 ± 9
MAP	101 ± 6	120 ± 5*	102 ± 6	117 ± 9*	99 ± 8	106 ± 11	101 ± 5	106 ± 8
SBP	114± 7	139 ± 7*	116 ± 8	135 ± 12*	114 ± 9	123 ± 13	114 ± 7	123 ± 10
DBP	76 ± 5	83 ± 5*	74 ± 7	80 ± 6*	71 ± 8	74 ± 7	75± 9	75 ± 6
PP	38 ± 6	55 ± 8*	41 ± 7	55 ± 11	43 ± 5	49 ± 11	40 ± 6	48 ± 8
Q	4.40 ± 0.91	4.10 ± 0.95	5.22 ± 0.95	4.77 ± 1.34	4.82 ± 1.38	5.36 ± 1.63	5.87 ± 1.50	6.21 ± 1.7
TPR	20± 5	25 ± 6‡	19 ± 5	27± 7*	19 ± 5	19 ± 5	18 ± 6	19 ± 5
SVC	0.05 ± 0.01	0.04 ± 0.01*	0.06 ± 0.01	0.05 ± 0.01*	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02
SYSC	0.09 ± 0.02	0.07 ± 0.02	0.09 ±0.02	0.07 ± 0.02	0.10 ± 0.02	0.10 ± 0.03	0.09 ± 0.03	0.09 ± 0.03

Values are mean ± standard deviation. Heart rate (HR; beats/minute); mean arterial pressure (MAP; mmHg); systolic blood pressure (SBP; mmHg); diastolic blood pressure (DBP; mmHg); pulse pressure (PP; mmHg); cardiac output (Q; $L \cdot min^{-1}$); total peripheral resistance (TPR; $mmHg \cdot L^{-1}/min$); systemic vascular conductance (SVC; $L \cdot min^{-1}/mmHg$); systemic compliance (SYS; mL/mmHg).

* Indicates $P<0.05$ for baseline vs. norepinephrine (NE) sessions during the same arm position; ‡$P=0.08$,

† Indicates $P<0.05$ vs. Control Baseline

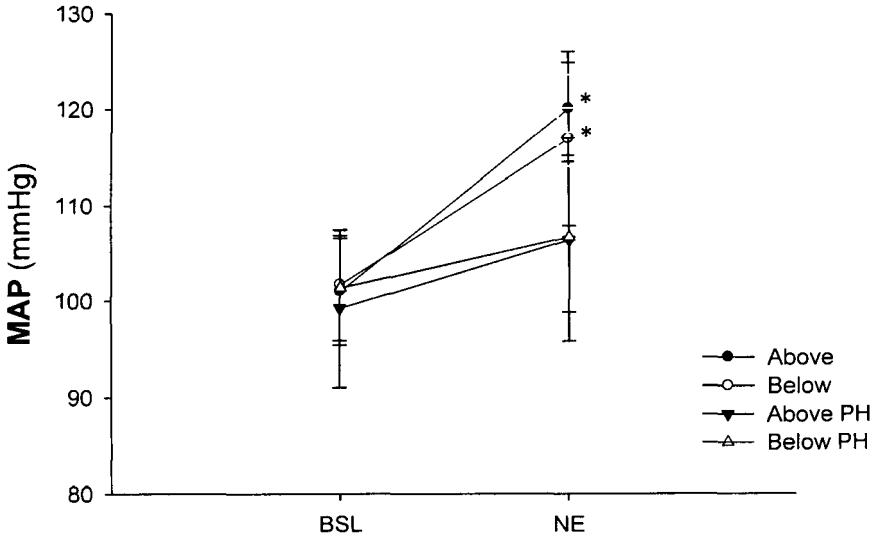


Figure 9 - Changes in mean arterial pressure (MAP; mmHg) with norepinephrine (NE) infusion of 100ng/kg*min during baseline (BSL), and during the 4th minute of NE infusion for the four conditions: arm above heart level (Above), arm above with phentolamine (Above PH) infusion, arm below heart level (Below), and arm below with PH infusion (Below PH).

- * Indicates $P<0.01$ vs. BSL

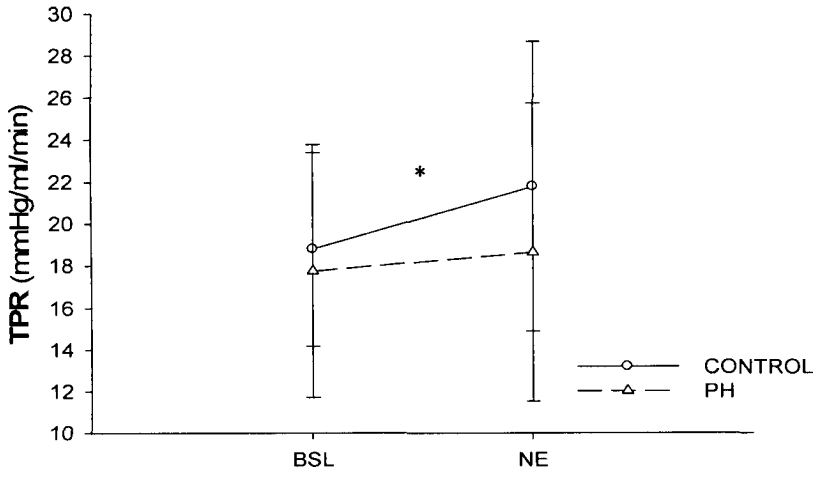


Figure 10 - Changes in total peripheral resistance (TPR; mmHg/L/min) during baseline (BSL) and during the 4th minute of norepinephrine (NE) infusion of 100ng/kg*min during the Control and phentolamine (PH) trials.

- Trial effect (P <0.001), NE effect (P<0.01), Trial·NE effect (P<0.05).
- * Denotes $P<0.05$ vs. BSL.

4.3 Forearm vascular properties

4.3.1 Effect of arm position change on forearm vascular pressure.

Compared with Below, calculated MAP in the brachial artery was reduced by positioning the arm above the heart (Figure 11) $P<0.05$. However, this change in MAP did not result in changes in brachial artery diameter or total blood flow (Table 3).

Forearm MAP increased with NE infusion in both conditions for the control trial. In the Above trial forearm MAP was raised from 89 ± 6 to 105 ± 5 mmHg and in the Below trial it was raised from 97 ± 10 to 111 ± 8 mmHg from baseline to the fourth minute of NE infusion, respectively ($P<0.01$). This response was abolished during the PH trial; thus, PH blocked α-adrenergic mediated increase in forearm pressure in both arm positions (Figure 5).

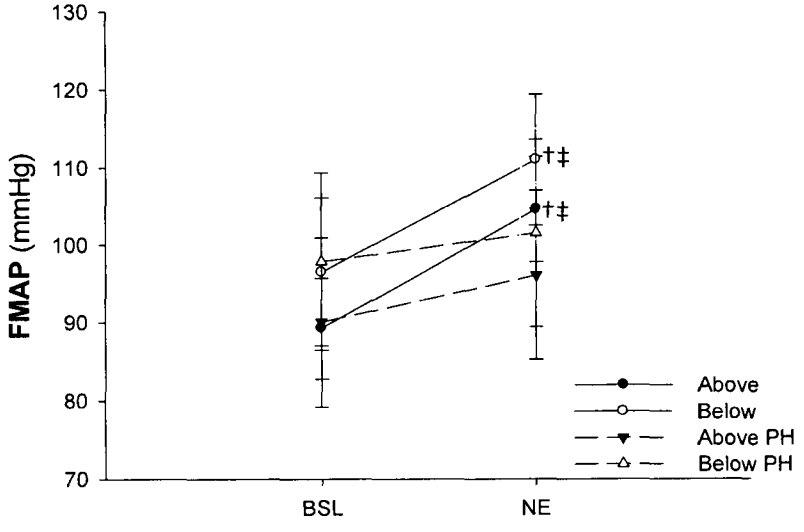


Figure 11 - Changes in forearm mean arterial pressure (FMAP; mmHg) during baseline (BSL) and during the 4th minute of norepinephrine (NE) infusion of 100ng/kg*min, at four conditions: arm above heart level (Above), arm above with phentolamine (Above PH) infusion, arm below heart level (Below), and arm below with PH infusion (Below PH).

- Arm position effect ($P<0.01$), NE effect ($P<0.01$), Arm position·NE ($P< 0.01$).
- † Denotes $P<0.001$ vs. BSL
- ‡ Denotes $P<0.01$ for Control vs. PH trial.

Table 3 - Forearm blood flow (FBF) and brachial artery diameter responses to norepinephrine (NE) infusion measures during arm above (Above) and below (Below) heart level with and without phentolamine (PH)

	Control Trial				Phentolamine Trial			
Arm Position	Above		Below		Above		Below	
	Baseline	NE	Baseline	NE	Baseline	NE	Baseline	NE
FBF	22 ± 9	22 ± 9	26 ± 10	19 ± 6	27 ± 13	29 ± 18	30 ± 23	28 ± 20
Dia	3.73 ± 0.07	3.66 ± 0.08	3.63 ± 0.07	3.49 ± 0.08	3.62 ± 0.08	3.59 ± 0.07	3.59 ± 0.08	3.51 ± 0.08
R	4.75 ± 2.38	5.64± 2.36§	4.53 ± 2.10	6.68± 3.13§	4.19 ± 2.38	4.72 ±2.44	5.07±2.99	5.27 ± 2.70
C	0.0068 ± 0.0034	0.0042 ± 0.0019§	0.0041 ±0.0014*	0.0027 ±0.0011§	0.0083 ± 0.0036	0.0072 ± 0.0036§	0.0045 ± 0.0023*	0.0036 ± 0.0015§

Values are mean ± standard deviation. FBF, forearm blood flow (ml/min); Dia, Brachial artery diameter (cm). R, forearm vascular resistance ($mmHg \cdot mL^{-1}/min$); C, forearm vascular compliance ($mL \cdot mmHg^{-1}$), (n=7).

* Denotes significant difference from Above Baseline ($P<0.05$); §, Significantly different from Baseline within each arm position ($P<0.05$);

Forearm Vascular Resistance: An effect of arm position and of NE infusion was observed for forearm R ($P<0.01$; Figure 12). NE caused an overall increase in R. However, the effect of NE on forearm R was greater in the Below position. In detail, R changed by 0.89 ± 0.70 mmHg·ml^{-1}/min in the Above condition compared to an increase of 2.15 ± 1.33 mmHg·ml^{-1}/min in the Below condition (NE * Arm Position Interaction; $P<0.05$). The increase in R was not observed when PH was infused regardless of arm position.

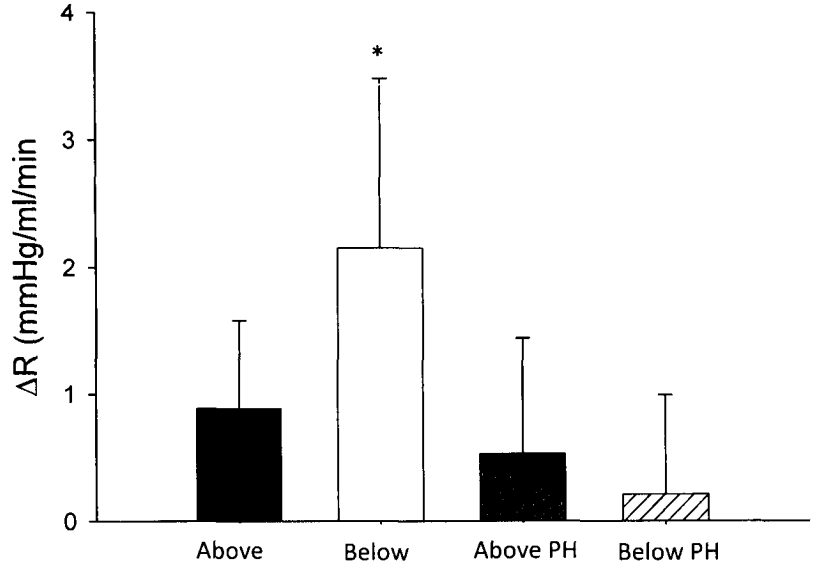


Figure 12 - Changes in forearm vascular resistance (ΔR; mmHg/ml/min) during baseline (BSL) and during the 4th minute of norepinephrine (NE) infusion of 100ng/kg*min, for the four conditions: arm above heart level (Above), arm above with phentolamine (Above PH) infusion, arm below heart level (Below), and arm below with PH infusion (Below PH).

- Arm position effect (P <0.01), NE effect (P<0.01), Arm position * NE effect (P< 0.05).
- * Denotes P<0.05 to BSL

Forearm Vascular Compliance: Forearm C was greater in the Above compared with the Below position (Main effect; $P<0.05$). During the Control trial, Baseline C was 0.0068± 0.0034 ml·mmHg^{-1} in the Above condition and 0.0041± 0.0014 ml·mmHg^{-1} in the Below condition. Similarly, during the PH trial, Baseline C ranged from 0.0083± 0.0036 ml·mmHg^{-1} in the Above PH condition and 0.0045 ± 0.0023 ml·mmHg^{-1} in the Below PH condition (Table 3).

Forearm C was reduced with NE infusion ($P<0.01$) in each arm position during the Control trial (Figure 13). However, an arm position · NE interaction was observed ($P<0.05$) whereby the effect of NE to reduce C was greater in the Above versus Below position. The changes measured during NE infusion were -0.0027 ± 0.0018 and -0.0013 ± 0.0009 ml·mmHg^{-1} for the Above and Below conditions, respectively. PH minimized the NE-induced changes in C.

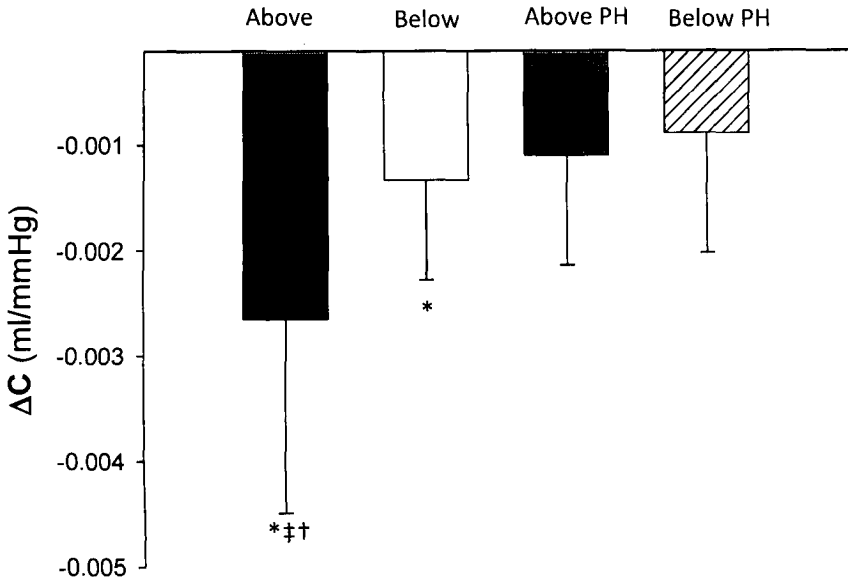


Figure 13 - Changes in forearm vascular compliance (ΔC; ml/mmHg) from baseline (BSL) to the 4th minute of norepinephrine (NE) infusion of 100 ng/kg/min, for the four conditions: arm above heart level (Above), arm above with phentolamine (Above PH) infusion, arm below heart level (Below), and arm below with PH infusion (Below PH).

- Arm position effect (P <0.01), NE effect (P<0.01), Arm position · NE effect (P<0.05).
- * Denotes P< 0.05 to BSL.
- ‡ Denotes P< 0.05 for Control trial vs. PH trial.
- † Denotes P< 0.05 for Above vs. Below

The influence of myogenic increases in C with manipulations in adrenergic receptor activation was further assessed by comparing the change in C on moving from the Below to Above position during Baseline, NE, and PH conditions (Figure 14). It was observed that with arm position alone, vascular C increased on moving from the arm below to the arm above heart level. This was not affected by PH alone. However, NE reduced this myogenic increase in C with the change in arm position, an effect that was abolished by PH.

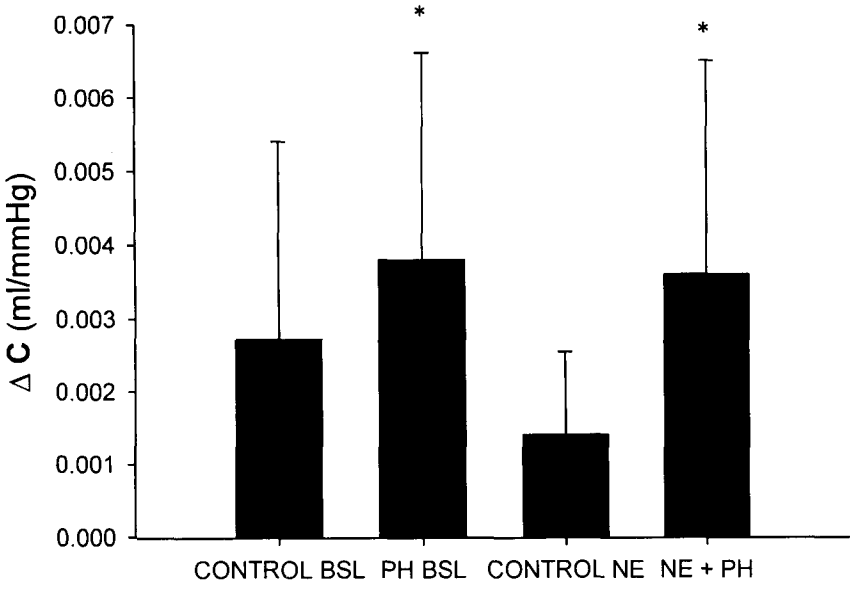
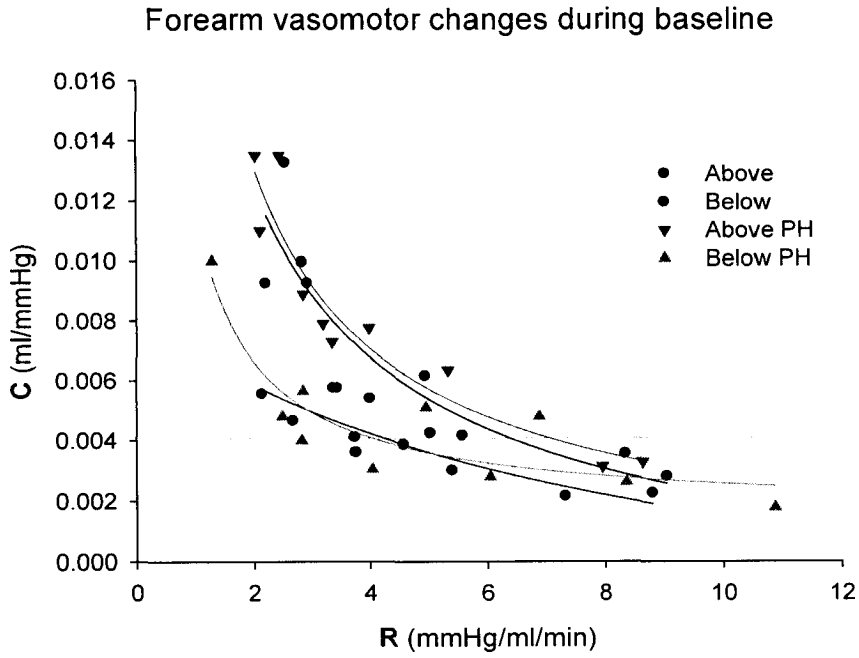


Figure 14 - Changes in forearm vascular compliance from arm below to above heart level. Change in compliance (ΔC; ml/mmHg) across all individuals during changes during movement in arm position from below to above heart level (Below to Above) during a period of baseline for the control and the phentolamine (PH) trials, and during the 4th minute of norepinephrine (NE) infusion at 100ng/kg/min for the control and the PH trials.

*Denotes $P< 0.05$ vs. Control trial with NE

Due to the large inter-individual variability within R and C in different individuals, Zamir *et al.* (146) demonstrated recently that the R and C values cannot be used as absolute independent markers of the state of a vascular bed but can be investigated together to address how these factors respond independently and collectively to adapt to a physiological stimulus. These variables, although mathematically independent, produce a distinct hyperbolic relationship (Figures 15a & b). While changes were observed, this hyperbolic pattern was prevalent throughout the different trials. Specifically, in the Above position, the curve shifted in an upward manner, relative to the Below position, with greater changes within the values of C rather than R (Figure 15a). In contrast, NE infusion induced a shift towards greater R and lower C in the Control trial (Figure 15b).

15. a



15. b

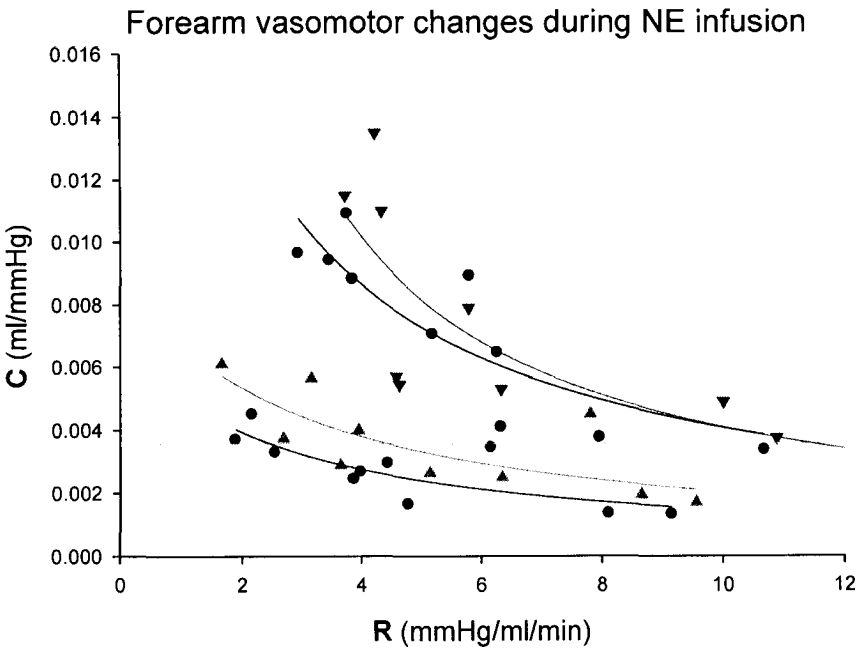


Figure 15 a. & b. – Resistance vs. Compliance. Forearm Vasomotor Changes during Baseline (a) & during norepinephrine (NE; b) infusion. Measures of vascular resistance (R; mmHg/ml/min) and vascular compliance (C; ml/mmHg) (n=10) during a period of rest for four conditions: arm above (Above), Above with phentolamine (Above PH) arm below (Below) and Below with PH (Below PH).

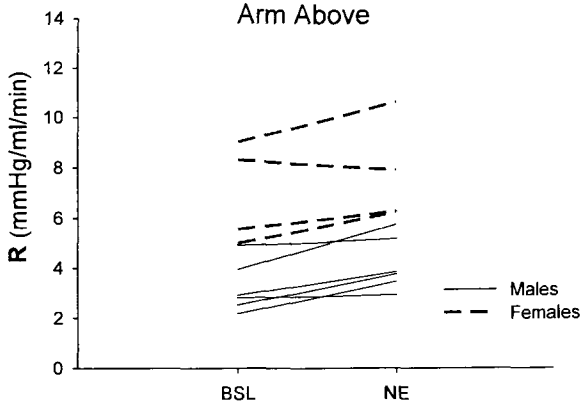
Horizontal line (--) represents mean of compliance.

4.3.2 Sub-Group Analysis – Source of Variability

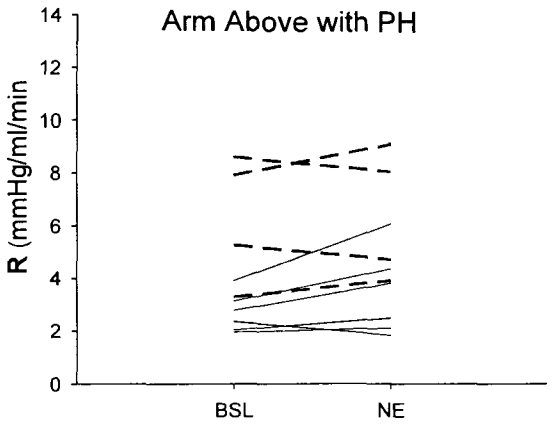
4.3.2.1 Effect of Sex

To address the large variability in the baseline data, the effect of sex was examined by comparing individual data points during baseline and during NE infusion to examine whether there were distinct group difference with forearm vascular R (Figures 16a-d) and C (Figures 17a-d). Overall, during the Control trial, R values were consistently greater, and C consistently lower, in females versus males ($P<0.05$). This effect of one's sex was less apparent during the PH infusion for R. However, the sex dependent levels of C were minimally affected by PH.

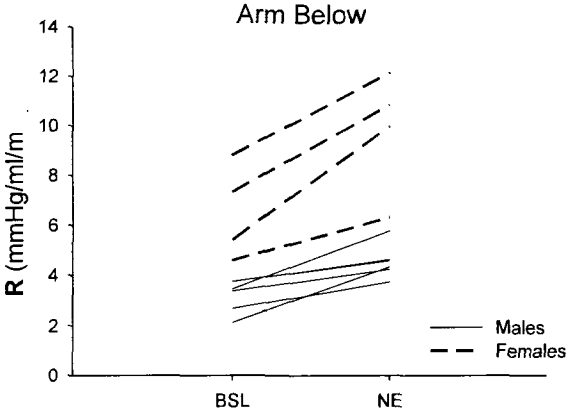
16. a



16. b



16. c



16. d

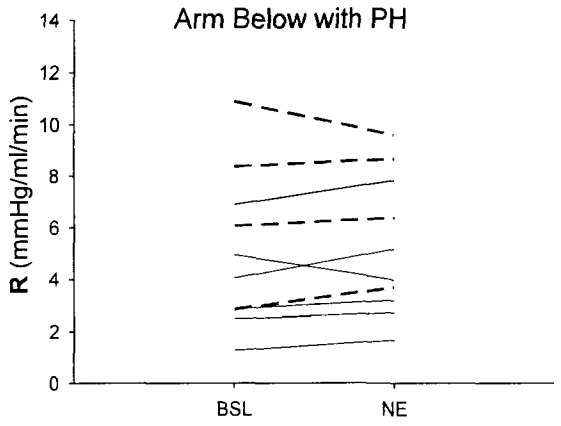


Figure 16 a-d- Individual changes in forearm vascular resistance. Changes in forearm vascular resistance (R; mmHg/ml/min) across all individuals during baseline (BSL) and during norepinephrine (NE) infusion of 100ng/kg/min for the arm Above Control (a), arm Above with phentolamine(PH; b), arm Below Control (c) and arm Below with PH (d).

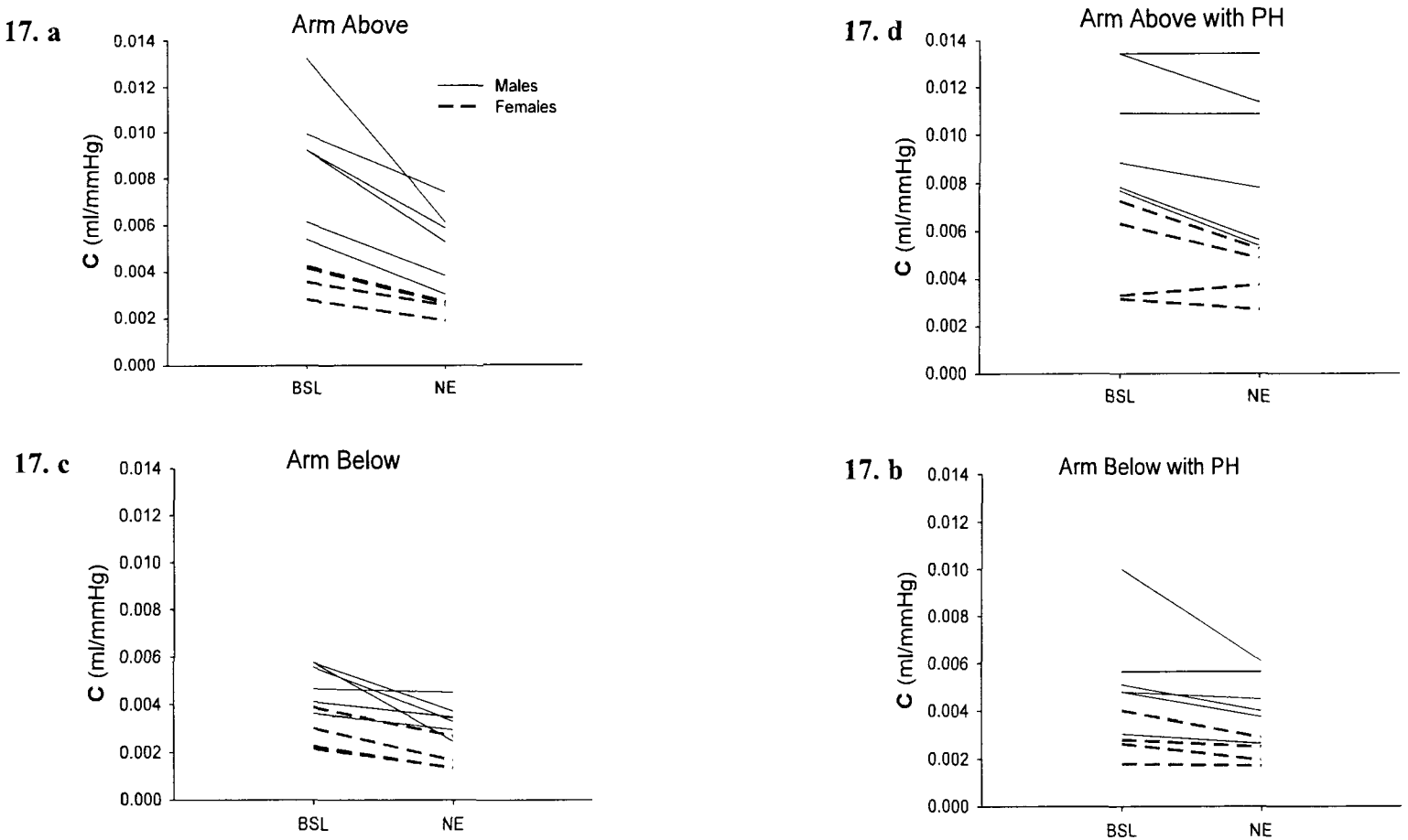


Figure 17a-d- Individual changes in forearm vascular compliance. Changes in forearm vascular compliance (C; ml/mmHg) across all individuals during baseline (BSL) and during norepinephrine (NE) infusion of 100ng/kg/min for the arm Above Control (a), arm Above with phentolamine(PH; b), arm Below Control (c) and arm Below with PH (d).

Forearm vascular C was further examined to determine whether the magnitude of change of C during NE infusion was dependent on the starting value of C (Figure 18). Data were plotted for all conditions during both the Control and PH trials and linear regression analysis was applied. While variable and modest, both males and females showed a greater degree of change in C when starting with greater forearm vascular C (r^2=0.75 and r^2=0.61 for males and females respectively; P<0.01).

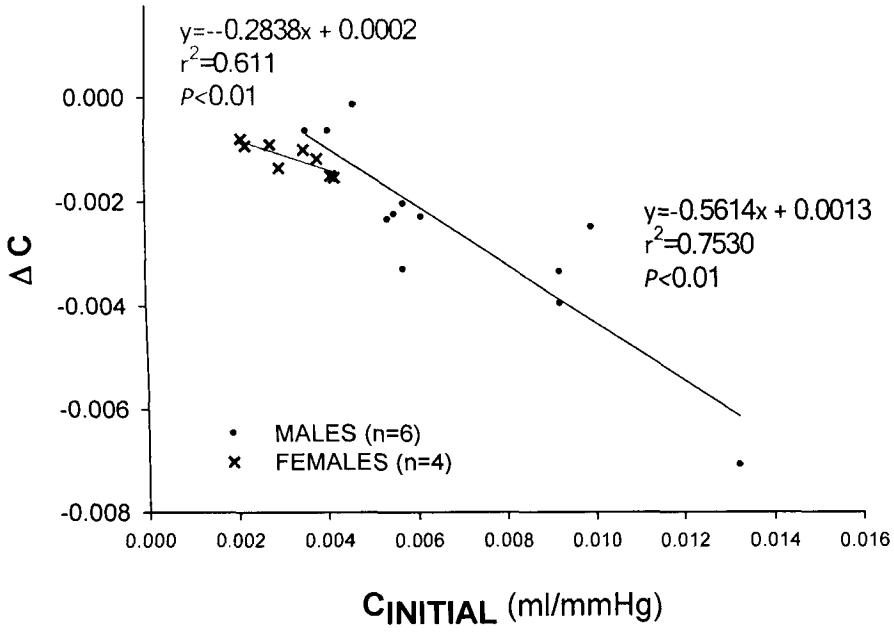


Figure 18- Magnitude of Change in Forearm Compliance between Males and Females. Relationship of changes in forearm vascular compliance (ΔC) during the 4th minute of norepinephrine (NE) infusion at 100ng/kg·min to baseline C ($C_{INITIAL}$; ml/mmHg) values during the Control trial.

The amount of change of forearm C from the arm below heart level to the arm above heart level was then compared for both the control and the PH trials to assess further any sex differences (Figure 19). Males were consistently able to elicit greater changes in C compared to females ($P<0.05$).

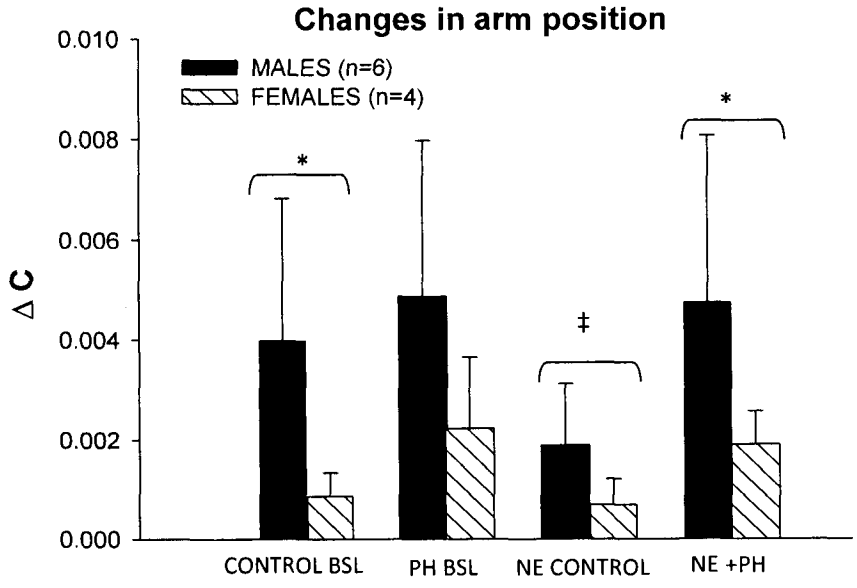


Figure 19 - Changes in forearm vascular compliance (ΔC; ml/mmHg) across females (patterned), and males (solid) during movement in arm position from below to above heart level (Below to Above) during a period of baseline for the Control and the phentolamine (PH) trials, and during the 4th minute of norepinephrine (NE) infusion at 100ng/kg/min for the Control and the PH trials.

* Denotes $P<0.05$ vs. females; ‡$P=0.07$ vs. females.

Chapter 5: Discussion

This study examined the concurrent influence of α-adrenergic and myogenic inputs on local vascular compliance (C) and resistance (R) of the lumped brachial artery vascular bed. The main findings of this study were that 1) forearm vascular C and R responded independently to myogenic inputs, supporting the first hypothesis. Specifically, the myogenic load imposed on the forearm arterial vasculature by changing arm position affected vascular C but not R; moving the arm above heart level produced an increase in C with no change in R and moving the arm below heart level produced a decrease in C with no change in R. 2) Forearm vascular R and C were affected by an α-adrenergic mediated constrictor effect whereby R increased and C decreased with NE infusion and these effects were minimized by PH. 3) Most importantly, an interaction was observed between NE infusion and arm position for both R and C, but differently in each case. Forearm C was reduced more with NE infusion when the arm was positioned above the heart. In contrast, R increased more with NE infusion when the arm was positioned below the heart, with a greater myogenic stimulus. While these latter observations support the general hypothesis of a direct interaction between myogenic and α-adrenergic vasomotor control on vascular R, the response of C was directionally opposite to the proposed response (i.e., greater reduction in C with higher myogenic stimulus). Thus, it was concluded that both R and C are affected by an alpha-adrenergic mechanism, that R and increased myogenic contractile factors interact positively, and that a change in C with adrenergic stimulation is impacted more by the starting point rather than the myogenic load; that is, C must be high in order to observe a NE-induced reduction in C (i.e., a constrictor effect).

Another observation that was not part of the original design, but supports the conclusions, was that of between-subject variability from which certain patterns emerged. For example, individuals with high baseline R displayed lower C and, conversely, those with low R demonstrated high C measures. Also, within the variability observed, females consistently exhibited smaller C and larger R measures, compared to the male counterparts. Thus, the ability to induce a reduction in C depended on the starting value of C and that starting point, also may be affected by inter-individual differences such as one's sex.

5.1 Physiological Data

5.1.1 Myogenic Response

In changing the hydrostatic pressure gradient by raising and lowering the arm with respect to heart level, it was expected that the myogenic tone would be modified without activating neurogenic vascular inputs. As expected, movement of the arm above or below heart level had no effect on systemic hemodynamic variables. Also, the changes in forearm blood pressure were in close agreement with the theoretical linear hydrostatic effect as supported by Netea *et al.* (93) and Suzuki *et al.* (125) and is consistent with previous work (145; 148). Comparable results were reported with similar non-invasive methods by Suzuki *et al.* (125), who observed that the Finapres blood pressure monitor measured changes in pressure that were linearly related to the vertical distance of the limb. These authors offered support that changes in forearm MAP allow for close approximation of the changes in intravascular pressure elicited with a change in arm position (93; 125).

Importantly, moving the arm between the Below and Above positions had no effect on brachial artery diameter or total forearm blood flow. While this was expected, based on some earlier reports (119) others using similar techniques have noted that such arm positions can affect forearm blood flow (137). A reason for the disparate findings may be due to the difference in height of change used between the positions Above to the position Below heart level. For instance, Walker *et al.* (137) had a range of motion between 21 cm above to 21 cm below heart level, compared to this study that used 18 cm Above and 9 cm Below.

Forearm vascular R and C responded independently to myogenic inputs. More specifically, raising the arm above heart level decreased the myogenic load and produced a large increase in C but no change in R. On the other hand, lowering the arm below heart level increased the myogenic load and produced a decrease in C with no change in R. The change in C in the elevated-arm manoeuvre can be attributed to relaxation of vascular smooth muscle following a reduction in transmural pressure. Therefore, the myogenic stimulus affects vascular smooth muscle tone and this appears to affect the C of the bed and not R at least at this magnitude of arm elevation. Furthermore, consistent with previous work (27; 31; 35; 36; 145), this study demonstrated that forearm C can be influenced independently via non-neurogenic mechanisms.

5.1.2 Adrenergic Response

NE infusion resulted in an increase in MAP and TPR as well as a decrease in SVC and SYSC. These constrictor responses were blocked by PH indicating they were induced by an α-adrenergic mechanism. Consistent with previous work, infusion of PH, a systemic non-selective α-adrenergic receptor blocker, did not have an effect on resting

MAP in supine subjects (114; 130). Therefore, PH exerted no independent myogenic stimulus.

While it was expected that NE would constrict the arteriolar-level vessels (30; 73; 84; 105; 106), NE infusion did not affect baseline brachial artery diameter in the current study. This stands in contrast to earlier studies, whereby brachial artery constriction was observed when NE was infused (6; 61). However, in those earlier studies the constriction was observed at the site of infusion, whereas, in the current study, diameter measures were taken from the contralateral arm. Thus, our results are consistent with previous work by Salzer *et al.* (113) and Dyson *et al.* (26) who observed unchanged diastolic and systolic brachial diameter measures during sympathoexcitatory sessions of lower body negative pressure and cold pressor tests.

NE infusion induced an α-adrenoceptor mediated increase in R and a decrease in C within the forearm vasculature. Overall, the data indicated the presence of an α-adrenergic effect over the control of these vascular parameters. However, because there was a rise in systemic MAP with the NE infusion, it is difficult to separate the direct effects of NE from the indirect effects due to changes in pressure, as discussed above. Nonetheless, we believe it was the α-adrenergic effect that induced the major changes in C and R with NE infusion because of our earlier results where reductions in forearm C were consistently reduced during sympathoexcitatory reflexes with (cold pressor test) or without (LBNP) changes in blood pressure (113; 145)

5.2 Interaction of myogenic and adrenergic Inputs

The interaction of myogenic and adrenergic inputs on blood flow regulation has been examined previously (30; 45; 73; 84; 105). However, to our knowledge, this is the first study to directly assess the question in humans, particularly as it pertains to vascular C. As aforementioned, the direction of the interaction between arm position and NE-induced "constriction" through changes in C were not in the hypothesized direction. Previous work, focusing on isolated muscle tissue, indicated that the myogenic constriction is enhanced with NE infusion (84; 105). However, upon closer inspection, the data from these studies indicated that there is an optimal range of tone within the arterioles used in these studies, where the myogenic response is most reactive (84). Similar results were observed by Ping & Johnson (105) who measured the interaction between the myogenic and adrenergic inputs on isolated tissues. They examined the autoregulatory capacity of arterioles during periods of decreased transmural pressure in the presence and absence of NE (105). They also observed an increased myogenic regulation with NE infusion (and decreased transmural pressure) and suggested that the starting point of the arterial mechanical properties, specifically C, may be important to achieve an increased level of response.

An explanation for these alternate findings can be considered from studies on the length-tension relationship of vessels in intact vascular beds. Suzuki *et al.* (125) proposed the idea that a change in intravascular pressure as a result of changes in gravitational potential energy may affect the length-tension relationship within the arterial walls and thereby affect its response to NE infusion. This idea was presented by Gore (45) who proposed that the magnitude of constriction upon NE infusion depended on the initial state within the vasculature whereby maximum constriction occurred only when the

initial tangential stress was at an optimum range. The degree of constriction was less than maximum when tangential stress was greater or less than the optimum range. From this perspective, raising and lowering the arm, would have produced different length and tension conformations within the arterial vasculature. More specifically, when intravascular pressure was decreased by raising the arm, the optimum point (the point where arterioles show the greatest response to NE) shifted downstream towards the smaller vasculature and therefore, more distal arterioles could be dilated (45). Similar findings were reached by Bank *et al.* (6) who observed that the magnitude of decrease in C of the brachial artery with NE was most prevalent at low transmural pressures compared to higher transmural pressures. They observed that compliance with smooth muscle relaxation varied from 0.004 $mm^2 \cdot mmHg^{-1}$ at 100 mmHg transmural pressure to 0.140 $mm^2 \cdot mmHg^{-1}$ at 0 mmHg transmural pressure. While these studies examined vascular C in local arterial vasculature, they are congruent with the results of the present study that examined the lumped vasculature of the forearm.

Furthermore, the results for arterial R were also consistent with those of Faber & Meininger (30; 84) as well as Ping & Johnson (105; 106), where greatest increases in vascular R were observed under combined increases in transmural pressure and adrenergic inputs. Thus, a myogenic background affects the α-adrenergic vasoconstrictor effect positively if studying R but negatively if studying C. Mechanisms behind this are not certain but are consistent with new data that R and C are regulated independently (145).

5.3 Variability within Vasomotor Properties

The wide variability in R and C amongst different individuals suggests that the mechanical properties of the human forearm vascular bed are not constant from person to person. These results are consistent with those of Zamir *et al.* (146) who observed that there are no set level values for R and C at a group or population level. Rather, baseline values appear to be specific to each individual. Furthermore, each variable may work either independently or may interact in order to adequately adapt to a given stimulus. Even though individual variability was large, the variability between R and C always appeared as a hyperbolic function regardless of the condition. Individuals with higher C values showed smaller R measures and conversely, those with small C had larger R measures (Figures 15a & b).

The variability in baseline values resulted in variable responses to NE. Variability between individuals has been previously addressed (23). Rowell (110) affirmed that within a population, there is great variance between the absolute changes in vascular sympathetic nerve activity and the corresponding absolute changes in plasma concentrations of NE and vascular R. Thus, while there is a close relationship between adrenergic input and forearm vascular changes, the margin of change within each vascular parameter will not be the same across all individuals. Furthermore, at any given time, various humoral and local factors are acting on the vasculature and these may be different across all individuals.

5.3.1 Sub-group Analysis – Effect of Sex

Because variability was observed within the subject pool, further sub-group analysis was completed to determine if this variability was consistent across all

participants or whether there were distinct differences between sex groups (Figure 16 & 17 a-d). The reason for suspecting such differences comes from a growing body of literature that has exposed sex-dependent differences in cardiovascular control in humans (42; 52; 77; 123; 124; 136). For example, whereas associations exist between cardiac output, peripheral vascular resistance and sympathetic discharge at rest in males, no such associations are observed in females (52). These findings, and other observations of varying reflex responses to stress in males and females (52; 77) lead to the question of whether sex-dependent differences are present in sympathetic and myogenic vasomotor interactions. This is in accordance with Lott *et al.* (77) who observed that when comparing the response in blood flow to increases in transmural pressure in both men and women, women showed attenuated brachial vasoconstrictor responses to arm negative pressure ranging from -25 to -100 mmHg as well as attenuated femoral vasoconstrictor responses at -100 mmHg compared to men.

In view of this question, the current analysis showed differences between males and females with respect to baseline R and C. Compared with females, males exhibited lower R and higher C measures throughout the different conditions. Also, males demonstrated a more responsive vascular system with robust changes in C to arm position and NE infusion.

While the division of groups into male and females leaves two smaller unbalanced groups, statistically significant effects were observed for arm position and sex and also with NE infusion and sex, indicating that a type II error was unlikely. Nonetheless, because the present study was not designed to test sex differences, and the effect of sex was not part of the hypothesis, the comparison of males and females as a

sub-group analysis must be considered cautiously. The present study observed that forearm parameters are less responsive to myogenic and α-adrenergic inputs in females compared to males. Possible mechanisms for the difference in response remain uncertain, although studies (42; 124; 136) suggest that estrogen and its vasodilator effects may be a factor. It has also been proposed, that shear rate, which is affected by both blood velocity as well as arterial diameter, is greater in smaller vessels (women in general have smaller arterial vasculature); thus, greater shear rate may induce greater nitric oxide release, and thus greater opposition to vasoconstriction in females (108).

While the mechanism of sex difference was not determined in the present study, one option is arm volume. The variability observed within individuals and within sex may have been reduced by standardizing the forearm arterial dynamics to actual forearm size. However, it is worth noting that Lott *et al* (77) observed a similar attenuation in the vascular response to both low and high levels of transmural pressure in females even after normalizing for limb size to male counterparts. A future study examining a larger sample of the two groups would be valuable.

5.4 Limitations

One of the aims of this study was to determine the effects of the sympathetic nervous system on forearm vascular control by testing α-adrenergic inputs through the infusion of NE. However, it is important to note that during heightened levels of sympathetic nerve activity, more than one type of neurotransmitter may be released. For instance, NE has been shown to be co-released with adenosine triphosphate (56); as well, neuropeptide Y has been shown to be released under high frequency stimulation (100).

Jackson *et al.* (60) demonstrated that the manner of increase in baseline hindlimb blood flow and vascular conductance in rats differed after sympathetic α_1 versus Y_1 receptor blockades. Thus, there may be a series of neurotransmitters that are involved in vascular regulation during changes in pressure and sympathetic nerve activation. That being said, Ping & Johnson (105) have demonstrated that both sympathetic nerve activity via stimulation and direct NE infusion elicited equivalent responses, indicating that NE has an important if not dominant role in peripheral vascular auto-regulation.

While the present study used a novel modeling method to examine forearm vascular C and R, the model examines these properties in a lumped method approach. As a result, the difference in control of vascular C and R over the different levels of the arterial tree cannot be determined. For instance, variability in the constrictor response to increases in transmural pressure have been observed between the larger 1A and 2A arterioles, and the 3A and 4A arterioles, where no constrictor response, and a large constrictor response were observed, respectively for the same increase in transmural pressure (85). In addition to this, it has been shown that there is a differential distribution and density of α_1 and α_2 adrenoceptors within different levels of the vascular tree, with a prevalence α_1 adrenoceptors in large arterioles and α_2 adrenoceptors being more dominant within small arterioles (29). Similarly, the mechanisms coupling adrenoceptor activation to contraction have been shown to be different for α_1 and α_2 receptors (30; 33; 89; 101). It has been demonstrated that the degree and type of α-adrenoceptor tone at different microvasculature levels may be important in myogenic autoregulatory blood flow adjustments during changes in perfusion pressure. Whereas, the adrenergic receptor

density and locations cannot be determined in humans, the lumped model approach provides a global and easily-grasped response with minimum invasiveness of measures.

To date, modeling approaches used to examine arterial properties have relied on the assumption that the arterial vasculature is either purely resistant (18; 50) or purely elastic (147; 148). By accommodating both of these components the present study accounts for the steady state and oscillatory nature of blood flow through the forearm. This vascular bed does include both arterial and venous segments. It is uncertain what role the venous segment plays in the R, C, K, and L modeling approach, particularly when arm position affects venous volume as well as pressure in this distensible tissue. While the present study did not quantify the role that the venous side may have played in the arterial vasomotor responses, recent work in our laboratory has shown that cuff occlusion of the forearm during the arm Above condition, affecting the venous pressures, had no effect on arterial hemodynamics compared to arm Above with no venous cuff (May & Goswami, unpublished data). Thus, prominent changes in forearm C appear to reside in the arterial side, under the experimental conditions of the current study.

Chapter 6: Conclusions

In summary, the findings of the study demonstrated that differential control of α-adrenergic and myogenic inputs exist over forearm vascular R and C. It was found that forearm C was highly responsive to a myogenic stimulus. Second, it was observed that both C and R were affected by α-adrenergic receptor activation. Finally, interactions between myogenic and α-adrenergic stimuli were observed but the direction and magnitude of the effect depended on the variable of interest and the starting value of that variable. Specifically, interactions between myogenic and adrenergic inputs affected forearm vascular C, but the adrenergic impact was observed only when the myogenic load was smallest in the elevated arm position. These results provide further evidence that the C of a vascular bed is an important neurogenic target that can be used to exert control over blood pressure and the distribution of blood flow. In addition, the large individual variability within the data demonstrates that there is no set standard within each parameter. Rather, these act as modifiable variables in a dynamic system that can defend the responsiveness of the system to changes in pressure or flow (146). The mechanisms to explain how this can be achieved are not clear and may differ between males and females.

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