MECHANICAL SIGNALS PROMOTING HEALTHY VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE CELL FUNCTION IN AGED ARTERIES

A Dissertation

by

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ABSTRACT

Peripheral arterial function is impaired with age, increasing the risk of cardiovascular disease. One therapeutic modality for improving arterial function in aged arteries is aerobic exercise; however, the signals associated with exercise that lead to improved arterial function are not fully understood. The aim of the research in this dissertation is to determine the importance of hemodynamic changes associated with aerobic exercise (increased intraluminal pressure and shear stress) which may serve as mechanical signals to promote healthy vascular endothelial and smooth muscle function in aged arteries. We hypothesized that a short-duration increase in intraluminal pressure would: 1) improve nitric oxide (NO)-mediated endothelium-dependent dilation in aged soleus muscle feed arteries (SFA); 2) improve vasoconstrictor responses in aged SFA via the Rho pathway; 3) improve vascular smooth muscle contractility in aged denuded (endothelium removed) SFA through the Rho pathway; and, 4) in combination with increased shear stress, induce greater improvements of endothelium-dependent dilation than a short-duration increase in pressure alone. SFA from young (4 mo) and old (24 mo) Fischer 344 rats were isolated and cannulated. Intact SFA or denuded (endothelium removed) SFA were exposed to increased intraluminal pressure for 1 h before assessment of vasodilator or vasoconstrictor function. The results from these experiments demonstrated that exposure to a short-duration increase in pressure, within a range believed to be present in SFA during exercise 1) improved NO-mediated, endothelium-dependent vasodilator responses in aged SFA; 2) improved vasoconstrictor responses in aged intact SFA via the Rho pathway; 3) impaired vasoconstrictor responses in aged denuded SFA; and, 4) did not interact with increased shear stress to produce greater improvements in endothelium-dependent dilation than pressure alone. Collectively, these data indicate that a short-duration increase in intraluminal pressure improves age-impaired

arterial function and suggests that increased intraluminal pressure is one mechanical signal associated with aerobic exercise which promotes healthy endothelial and smooth muscle function in aged arteries.

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"It's just endurance."

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CHAPTER I

INTRODUCTION

1.1 Introduction

Cardiovascular Disease

One of the most devastating health related phenomena of the past century is the rampant emergence of cardiovascular disease as a primary cause of death[1]. Cardiovascular disease, manifested by an impairment of the function of the heart and/or blood vessels, occurs due to a variety of causes. Notably, the bursting, occlusion, or dysfunction of blood vessels may cause a reduction or elimination of blood flow to target tissues. Without the ability to adequately deliver O_2 and nutrients, and to remove waste products, the target tissue will become hypoxic and ultimately necrotic.

Cardiovascular disease is a debilitating ailment that reduces the patient's quality of life, and is often fatal. One third of American deaths are the result of cardiovascular disease[2], and Americans spend $315 billion annually in direct and indirect costs associated with the diagnosis and treatment of cardiovascular diseases, more than any other disease[2]. Though the death rate from cardiovascular disease has fallen in recent years, its incidence rate remains relatively unchanged[3]. To this end, it is important to note that the suffering from cardiovascular diseases reaches beyond the individual patient.

When a patient suffers from cardiovascular disease, it affects the patient's entire family. A patient's family will suffer from stress, anxiety, and worry. Additionally, family members often serve as primary caregivers, a professional service worth an estimated $200 billion annually[4]. Caregiving adds an additional layer of stress to the family. Indeed, caregiving itself

serves as a research model for chronic stress[5] and can lead to impaired psychological and physical health of the caregiver[6]. Therefore, identifying ways to prevent and treat cardiovascular disease is a research priority.

Vascular Anatomy

Arteries are composed of three distinct layers of cells. The fatty/connective tissue layer, the tunica adventitia, is the outer most layer. The tunica adventitia anchors the vessel to its surrounding tissue. The vascular smooth muscle layer, the tunica media, consists of layers of concentric vascular smooth muscle cells that wrap around the vessel and regulate the diameter of the blood vessel. The third layer is referred to as the tunica intima and is comprised of a single layer of endothelial cells. The endothelium lines the inner most portion of the artery, separating the vessel lumen from the surrounding vascular smooth muscle. Originally perceived solely as a permeability barrier, the works of Furchgott, Ignarro, and Murad demonstrated that the endothelium plays an integral role in regulating vascular smooth muscle cell (VSMC) relaxation via the signaling molecule endothelium-derived relaxing factor (EDRF), now known to be nitric oxide (NO)[7-9]. To date, there are three known cell signaling pathways that promote endothelium-dependent dilation: NO, endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin (PGI_2).

Aging

An impairment of an artery's endothelial layer to signal vascular smooth muscle relaxation, or endothelial dysfunction, occurs with age and is an independent risk factor for cardiovascular disease. Though relatively well preserved the first 40-50 years of life, endothelial function, as measured by flow-mediated dilation, declines rapidly beginning in the early 40s (men) and early 50s (women)[10]. While aging does not result in reduced skeletal muscle blood

flow at rest, the ability to increase muscle blood flow during exercise is impaired with age, which is believed to contribute to exercise intolerance in the elderly [11,12].

In addition, the ability to direct blood flow to actively contracting muscle fibers is impaired with age. Specifically, Musch et al. documented an age-associated redistribution of blood flow away from highly oxidative muscle fibers and toward the more fatigable, less oxidative muscle fibers during exercise[13]. Importantly, Muller-Delp et al. reported that endothelium-dependent dilation to acetylcholine (ACh) was impaired in first-order arterioles of the oxidative soleus muscle but not in the first-order arterioles of the gastrocnemius muscle[14]. Selective impairment of endothelial function in highly oxidative muscles was also reported by Woodman et al, who documented attenuated endothelium-dependent vasodilator responses in the soleus muscle feed artery, but not in the gastrocnemius muscle feed artery[15]. Thus, in addition to being a risk factor for cardiovascular disease, the decrement in endothelial function is believed to contribute to an impaired muscle blood flow to actively contracting skeletal muscle and decreased exercise tolerance in the elderly[16].

The mechanisms accounting for the age-induced decline in endothelial function are not fully understood but are believed to be due, in large part, to a decrease in NO bioavailability [17], through an impairment in PI3K/AKT signaling[18]. NO bioavailability is determined by the balance between NO production and NO degradation. The primary mechanism of NO degradation is through its interaction with superoxide (O_2^-). NO reacts readily with O_2^- to produce peroxynitrite, thereby degrading NO and decreasing NO bioavailability. O_2^- is produced as a by-product of cellular metabolism through proteins like NAD(P)H oxidase and xanthine oxidase. Trott et al. showed previously that O_2^- produced by NAD(P)H oxidase contributes to impaired NO bioavailability in aged rats[19]. Elevated peroxynitrite formation is a by-product of

O_2^- and NO reactions and has been documented in the aorta of aged rats. Perhaps as a compensatory mechanism to increased O_2^- production, in an attempt to increase NO bioavailability, the aorta of aged rats have shown a sevenfold higher eNOS activity[20].

Importantly, the NO producing protein, eNOS, may become uncoupled and produce O_2^-, contributing to the elevated O_2^- and subsequent decreased NO bioavailability. To produce NO, eNOS requires the co-factor tetrahydrobiopterin (BH_4). In its absence, the eNOS protein becomes uncoupled and produces O_2^-, exacerbating the degradation of NO and reducing the NO available to promote VSMC relaxation. Sindler et al. reported that BH_4 concentrations decrease with age and are restored following exercise training[21]. These data indicate that the age-related decline in NO bioavailibity is associated with curtailed production and enhanced degradation of NO.

Exercise

Physical inactivity is an independent risk factor for cardiovascular disease[22], and is believed to be responsible for 12%[23] of cardiovascular diseases. Furthermore, physical activity, like endothelial function, declines with age [24]. Importantly, participation in regular physical activity can improve or even reverse age-induced endothelial dysfunction[25,26]. DeSouza and Seals measured forearm blood flow (FBF) responses to ACh and SNP in young and old subjects. They noted a 25% reduction in the increase of FBF in response to ACh in aged, sedentary men compared to young, sedentary men, indicating impaired endothelium-dependent dilation. Furthermore, they showed no between group differences in the FBF response to the endothelium-independent dilator SNP, an indicator of vascular smooth muscle function. The age-related decrement in endothelial function was not present in chronically, endurance trained older men[26]. Additionally, age-impaired endothelial function was ameliorated following

completion of an aerobic exercise training program. Thus, De Souza et al. concluded that endothelial function is maintained in age-matched competitive runners and that a short-duration aerobic exercise program can improve endothelial function in aged individuals[26], reducing an important risk factor for cardiovascular disease[27].

Though the signals associated with exercise that lead to improved endothelial function are unclear, DeSouza and others have postulated that the endothelial improvements may be due, at least in part, to mechanical forces experienced by the endothelial layer during individual bouts of exercise. Specifically, short-duration increases in intraluminal pressure and/or shear stress associated with exercise may be responsible for the promotion of a healthy endothelial phenotype[26,28-31].

The mechanism accounting for the beneficial effect of exercise on endothelial function is not fully understood but it is believed to be due, in large part, to enhanced NO signaling [25], with improvements in endothelial function observable even after a single bout of aerobic exercise [32]. Using an isolated cannulated artery preparation, Trott et al. showed that a moderate-aerobic exercise training program restored endothelium-dependent, ACh-induced dilation of aged rodent SFAs to the levels of young rodent SFAs, and that this benefit was mediated by improvements in NO bioavailibity[25]. The signals associated with exercise that lead to an improvement in NO-mediated endothelial function have not yet been fully defined; however, increases in intraluminal pressure and intraluminal shear stress, changes in the hemodynamic forces experienced by the vasculature during exercise, are potential signals responsible for improvement in endothelial function [31].

1.2 Endothelial Function

Mechanisms of Endothelium-Dependent Vasodilation

The vascular endothelium elicits relaxation of surrounding smooth muscle via three different cell signaling pathways: PGI_2, EDHF, and NO. The production of PGI_2 in the endothelial cell begins with the phospholipids of the plasmalemma[33]. These phospholipids are first converted to arachadonic acid (AA) by the enzyme phospholipase A_2. Subsequent modification by the enzyme cyclooxygenase (COX) converts AA into prostaglandin H_2 (PGH_2). Lastly, PGH_2 is converted into PGI_2 by prostacyclin synthase. PGI_2 is then free to diffuse across the plasma membrane of the endothelial cell and stimulate a Gs protein receptor on the vascular smooth muscle cell[33].

EDHF is a term that refers to several vasoactive molecules that can hyperpolarize vascular smooth muscle cells and promote vasorelaxation. Although much less is known about EDHFs, they are thought to be derived, in part, from arachidonic acid in a reaction catalyzed by Cytochrome P450[34]. The epoxide, 11,12-epoxyeicosatrienoic acid (11,12-EET) has been suggested to be a Cytochrome P450-derived EDHF metabolite[34,35]. Fisslthaler demonstrated EDHF-mediated dilation following enhanced formation of 11,12-EET in coronary arteries[34]. Alternatively, Sindler et al. showed that H_2O_2, the byproduct of O_2^- scavenging by SOD, is an important vasodilator in skeletal muscle arterioles[21], and has been demonstrated to be an important contributor to flow-induced dilation in coronary arterioles[36]. Though the mechanism of action has not been fully elucidated, it is believed that EDHFs promote vasorelaxation via the hyperpolarization of smooth muscle cells through the reduction of intracellular Ca^{2+} and the opening of VSMC K^+ channels[37,38].

Nitric oxide is produced by a reaction catalyzed by the eNOS protein in the endothelial cell. eNOS is regulated by a Ca^{2+}/calmodulin-dependent cycle with caveolin[39], in which, eNOS remains inactive while attached to the golgi apparatus, along with caveolin-1 and a polmytoyl group. Once eNOS is polmytoylated, myristoylated, and transferred to a caveolus with caveolin-1, eNOS and caveolin-1 become incorporated into the plasmalemma. Phosphorylation of eNOS by Akt releases eNOS from the plasmalemma into the cytosol. Concomitant increases in Ca^{2+} are promoted by IP_3 stimulation of calcium channels on the sarcoplasmic reticulum. Ca^{2+} associates with calmodulin, and further interacts with eNOS, thus activating the protein[33,39]. eNOS produces NO as long as it has the substrate, L-arginine, and the co-factor, BH_4. eNOS is deactivated by dephosphorylation and the removal of intracellular calcium. eNOS becomes re-polmytoylated and reattached to the golgi apparatus, completing the cycle. Once NO is produced, it is free to diffuse across the plasma membrane into the VSMC and stimulate vasodilation[33].

Endothelium-dependent dilation can be stimulated by chemical ligands or by shear stress. Shear stress is the mechanical force experienced by endothelial cells as blood flows across the surface of the endothelium. The plasmalemma of the endothelium is disturbed by shear stress which prompts the influx of Ca^{2+} into the endothelial cell, where it binds to calmodulin and associates with eNOS. The association of calmodulin with eNOS is enhanced by heat shock protein 90 (Hsp90), which facilitates the production of NO by eNOS[40-42]. Additionally, the vascular endothelial growth factor (VEGF) receptor is sensitive to shear stress and can activate PI3K and subsequently Akt. Akt phosphorylates and activates eNOS along with Ca^{2+}/calmodulin to produce NO[33].

One ligand that promotes endothelium-dependent vasodilation is ACh. It is thought that ACh released at the myoneural junction can "spillover" and stimulate the M_2 receptor on the endothelium. The M_2 receptor is associated with a g-protein-coupled receptor and activates the PI3K/Akt pathway to promote NO formation and vascular smooth muscle relaxation[33].

Any impairment in endothelium-dependent dilation, regardless of the mechanism of impairment, is classified as endothelial dysfunction. Endothelial dysfunction is an independent risk factor for cardiovascular disease and is therefore an important research target for the treatment and prevention of cardiovascular disease[43-48].

Exercise and NO-Dependent Endothelial Function

In young, healthy individuals, aerobic exercise training increases maximal cardiac output, which is directed primarily to the actively contracting skeletal muscle during a bout of exercise[49]. Exercise also has a beneficial effect on the NO pathway, which may contribute to enhanced muscle blood flow and may act to preserve healthy endothelial function with age. Laughlin et al., using miniature swine, showed that eNOS protein content in the arterial network is increased differentially throughout the vascular tree following completion of 4-5 months of combined sprinting and endurance exercise training[50]. Interestingly, Laughlin reported variable increases in eNOS protein content in the coronary resistance arteries and arterioles, but no changes in eNOS protein content of the coronary conduit arteries in fully trained animals. Laughlin suggested that the differences in local shear stress and intraluminal pressures experienced by the different arteries and arterioles may be responsible for the differential changes in eNOS protein content[50]. The increase in eNOS protein content is consistent with the demonstrated increase in eNOS mRNA[51] and improved NO-mediated endothelium-dependent dilation documented by exercise training studies[52,53]. Along with increases in eNOS mRNA and

protein content, the O_2^- scavenger, Cu/Zn SOD is increased following exercise training, thus promoting a decrease in oxidative stress and an increase in NO bioavailibity[54]. Furthermore, Haram and colleagues demonstrated that a single bout of exercise improves NO-dependent vasodilation for up to 24 h, and that chronic exercise training extends these NO-dependent benefits[32]. These adaptations in NO-dependent vasodilation following exercise training likely contribute to the functional improvements in aged endothelium.

Exercise and Age-Induced Endothelial Dysfunction

Aerobic exercise has been shown to improve or restore endothelial function in aged humans and animals. DeSouza et al. measured ACh-induced FBF responses in young, aged, and chronically trained aged individuals and showed that age-related decrements in endothelium-dependent FBF responses of the aged group were absent when compared to chronically trained, aged individuals[26]. Other work by Taddei et al. attributed the beneficial effect of exercise on endothelial function to improvements in NO bioavailability[55]. Additionally, Spier et al. showed increased eNOS mRNA and protein content in aged, rodent skeletal muscle arterioles following an exercise training program[56] and further showed that age-induced impairments of flow-induced dilation are mitigated with exercise, primarily through the NO pathway[57]. Furthermore, exercise preserves mechanisms responsible for preventing degradation of NO by oxidative stress in aged subjects[58,59]. Trott et al. extended these findings by showing that a 12 week moderate, aerobic exercise program improved NO-mediated, endothelium-dependent vasodilation and increased the extracellular superoxide dismutase (SOD) protein content in the SFA of aged rats[25].

Regulation of Endothelial Cell Phenotype by Shear Stress

The endothelium is a dynamic layer of cells, which responds to its local environment. Blood flow is pulsatile, increasing during systole and decreasing during diastole. The velocity of

blood flow is a vector quantity defined by the speed and direction of blood flow. Shear stress experienced by the vascular endothelium is determined by the viscosity, volumetric flow rate, and vessel radius relationship ($\tau = 4\eta Q/\pi r^3$): where η refers to the viscosity, Q to the volumetric flow rate, and r to the vessel radius[29]. Blood is a non-Newtonian fluid composed primarily of a mixture of erythrocytes, lipoproteins, proteins, and water. The viscosity of blood is not constant, but is often cited as 4 times that of water, or 4 centipoise, and can change with low flow rates and in smaller branch order arterioles and capillaries[60]. The volumetric flow rate is a measure of the volume of blood flowing through an area and is affected by the contractile state of the heart and by its location in the circulatory system[60]. The volume of blood that the vessel can accept at any given time is determined by the radius of the blood vessel.

Blood flowing through a vessel lumen and under its Reynolds number (Re) will adopt a laminar flow pattern or layered, cylindrical sheets, of linear, non-mixing blood flow. The Reynolds number is an indication of the blood's inertial force to viscous force ratio. As the velocity of blood flow increases, so do the inertial and viscous forces. The inertial forces increase faster than the viscous force. When the inertial forces surpass the viscous force, laminar flow will become turbulent[61]. Laminar flow is the condition where the blood nearest the endothelial layer of the vessel experiences frictional forces, or drag. This drag is greatest along the tunica intima and weakest at the radial center, creating a force characteristic that increases in magnitude from the radial center of the vessel lumen. Turbulent flow is a departure from the layered, non-mixing flow patterns and characterized as swirling and chaotic, with vortices or eddies that can be stationary or propagated through the blood vessel[62]. Endothelial cells modify their morphology and orientation to align with the direction of blood flow, in part through modification of their cytoskeletal organization and in the absence of endothelial cell division, indicating the synthesis of proteins like f-actin and stress fiber proteins[63-65]. Along with

cytoskeletal modifications, shear stress has also been documented to regulate the endothelial genes for COX-2, MnSOD, and eNOS[66].

The force of blood moving along the tunica intima creates shear stress on the endothelial cells. Rodbard was among the first to suggest endothelial vasomotor regulation by shear stress[67,68]. Subsequent studies using endothelial cell cultures demonstrated that eNOS, COX-2, MnSOD , and Cu/Zn SOD mRNA are up-regulated following exposure to laminar shear stress, while the mRNA for the vasoconstrictor gene, endothelin, is down-regulated and the constitutively expressed COX-1 protein remains unchanged[66,69-73]. These data suggest the potential for improved endothelium-dependent vasodilation following exposure to laminar shear stress. Further cell culture work by Uematsu and Harrison demonstrated that increased shear stress of up to 15 dyn/cm^2 for as little as 3 h increased eNOS mRNA expression in a concentration-dependent manner[74]. The initial NO release in response to shear stress, in cultured cells, has been shown to be Ca^{2+}-calmodulin-dependent[75], while the NO released during sustained exposures to shear stress appears to occur via Ca^{2+}-independent mechanisms, possibly through serine/threonine and tyrosine kinases[75-80].

Modulation of endothelial phenotype by shear stress was first documented at the tissue level by Fry in 1968[62]. Fry reported that exposure of dog thoracic aorta to high levels of shear stress, for as little as 1 h, created endothelial cytoplasmic swelling, deformation, and disintegration of the endothelium. However, it is important to note that the increased blood flow produced by the intra-aortic device used by Fry exposed the thoracic aorta to turbulent flow patterns[62]. Subsequent experiments revealed that laminar shear stress exerted positive modifications of the vasculature through increases in NO production, vascular remodeling, and by promoting the formation of new blood vessels[81]. In isolated coronary arterioles exposed to a

high shear stress with laminar flow, Woodman et al. showed improved NO-dependent vasodilation, an increased eNOS mRNA content and an increased Cu/Zn SOD mRNA content[29,82]. Maximal flow-mediated dilation is achieved at around 6 dyn/cm^2 in rat soleus feed arteries[83]. The vasodilation to shear stress in isolated vessels is NO-dependent, and mediated only in part by changes in intracellular Ca^{2+} [84-86] and is largely attributed to Ca^{2+}-independent mechanisms[86].

While exposure to shear stress can positively benefit vascular function, too much or too little shear stress can be detrimental. Too little shear stress can negatively modulate endothelial cell phenotype, as in the case of atherosclerosis, where atherosclerotic lesions occur preferentially in areas of low shear-stress, non-laminar flow regions near branch points and curves in the arteries[87,88]. Low shear stress and non-laminar flow generate a greater time in contact of plaque forming particles with the vessel wall, while high shear stress and laminar flow promotes the rapid removal of those particles from an individual blood vessel area[87,89]. Furthermore, exposure to low shear stress (1.8 dyn/cm^2) or a short-duration exposure to 10 dyn/cm^2 promotes the release of the vasoconstrictor endothelin-1[90]. Although low shear stress has been associated with disease states like atherosclerosis, in the right magnitude and duration it remains an important mechanical signal for healthy vascular function and is likely a key component to the exercise-induced improvements in the vascular function of the elderly.

Regulation of Endothelial Cell Phenotype by Intraluminal Pressure

Pressure within the vasculature fluctuates cyclically and is dependent on the contractile status of the heart. Blood pressure increases during systole and falls continuously through diastole. Blood pressure may be increased chronically in disease states, like hypertension, or

may be acutely increased during physical activity to aid in the perfusion of exercising skeletal muscle[91-95].

Cell culture studies have revealed that endothelial cells are sensitive to changes in pressure. When exposed to cyclic stretching, endothelial cells orient themselves to a position of least mechanical deformation and undergo a reorganization of actin filaments to produce parallel arrays of actin filaments[96]. Mechanical stress not only affects cellular structure, but has been shown to increase the release of the vasoconstrictor endothelin in cultured endothelial cells[97,98]. Furthermore, cell culture studies by Hishikawa et al. indicate that NAD(P)H-derived O_2^- production is increased following exposure to pulsatile stretch. Following 24 h of stretch, total cytosolic eNOS RNA and protein content are increased[99] and the increase in O_2^- is coupled with an increase in BH_4-mediated NO synthesis, perhaps as a compensatory mechanism by which NO scavenges the O_2^- free radical.[100]

At the artery level, chronic exposure to elevated intraluminal pressure, as in hypertension, is associated with an increased peripheral vascular resistance[101] and associated with a dysfunctional endothelial phenotype[102,103]. This hypertension-associated endothelial dysfunction is largely attributed to decreased NO bioavailability[102,104-107]. Similarly to Hishikawa's cell culture studies, Gan et al. suggest that the mRNA of the vasoconstrictor endothelin is increased after 6 h of exposure to an increase intraluminal pressure; however, unlike Hishikawa's cell culture studies, the mRNA of the vasodilator promoting protein eNOS, is increased after only 3 h of an increase in intraluminal pressure[108]. Gan et al. further showed by immunohistochemical localization, a greater immunoreactivity of eNOS in umbilical veins perfused under high pressure than those perfused with low pressure[108]. A decrease in NO

bioavailibity, an increase in endothelium-derived O_2^-, and an impaired NO-dependent vasodilation have also been demonstrated in spontaneously hypertensive rats[109,110].

At the arterial level, long-term exposures to elevated intraluminal pressure, as in hypertension, can be detrimental to endothelial function. Taddei et al. characterized hypertension-associated endothelial dysfunction as a form of accelerated NO-mediated, age-induced endothelial dysfunction, or premature aging[111]. Further studies by Taddei et al. concluded that regular physical activity serves to prevent age-induced endothelial dysfunction[55]. DeSouza et al. also showed that regular physical activity can prevent age-induced endothelial dysfunction, and that chronic physical activity can reverse age-induced endothelial dysfunction[26]. DeSouza and others have proposed that exercise-induced improvements in endothelial function are due, at least in part, to mechanical forces experienced during exercise, like increased intraluminal pressure or shear stress[26,28-31]. Additionally, Nelson et al. documented a decrease in the blood pressure of essential hypertensive patients following a regular exercise training program[112]. These data indicate that there are components of physical exercise, i.e. a transient increase in intraluminal pressure, that benefit NO-dependent dilation, while chronic elevations of intraluminal pressure are detrimental to NO-dependent dilation. Indeed, isolated cannulated artery experiments performed by Woodman et al. demonstrated improved endothelium-dependent dilation in aged arteries following exposure to a 4 h increased intraluminal pressure[30]. These data suggest that unlike the chronic elevations of intraluminal pressure in hypertension, a short-duration increase in intraluminal pressure, as experienced during an exercise bout, may be an important mechanical signal for the promotion of a healthy endothelium.

1.3 Vascular Smooth Muscle Cell (VSMC) Function

Mechanisms of Vascular Smooth Muscle Relaxation

Endothelial cells produce vasodilatory factors, but the vascular smooth muscle must respond appropriately to the vasodilator signal. EDHFs have been proposed to work through potassium channels, inhibiting calcium channels, and hyperpolarizing smooth muscle cells. Both PGI_2 and NO have separate protein kinase pathways to induce vasorelaxation of the VSMC. NO diffuses through the smooth muscle plasmalemma and activates the enzyme guanylate cyclase to make cGMP from GTP. cGMP activates protein kinase G (PKG). PGI_2 stimulates a Gs protein that activates adenylate cyclase. Adenylate cyclase produces cAMP from ATP and subsequently activates protein kinase A (PKA). Both PKA and PKG inhibit the phosphorylation of the myosin/actin contractile elements, impair Ca^{2+} entry into the cell, promote Ca^{2+}sequestering by the sarcoplasmic reticulum, and inhibit the Gq/IP_3 pathway to relax the VSMC[33].

Myogenic Response

Vascular smooth muscle cells respond dynamically to changes in intraluminal pressure. The myogenic response is classically defined as the constriction of a blood vessel in response to elevations in intravascular pressure[113]. The myogenic response was first proposed by W.M. Bayliss, in 1902, and later became a key stanchion of the auto-regulation of blood flow concept[113,114]. Though the mechanism has not been fully elucidated, vascular smooth muscle contraction following an increase in intraluminal pressure is believed to play an integral role in maintaining optimal sarcomere length[115,116] and wall tension[117]. It has been proposed that the greater excitation-contraction coupling achieved by the myogenic response is accomplished through membrane depolarization and enhanced Ca^{2+} permeability[118] of stretch activated Ca^{2+} channels[119,120].

Vasoconstrictor Response of Vascular Smooth Muscle

The most prominent vasoconstrictor mechanism in vascular smooth muscle is the stimulation of Gq protein and the formation of IP3. When an agonist such as norepinephrine, phenylephrine, endothelin, or IP3 diffuses into the cytosol, DAG will travel the plasma membrane. Both IP3 via phospholipase C (PLC) and DAG will increase Ca^{2+} influx through stimulation of the sarcoplasmic reticulum to release Ca^{2+}. Once in the cytosol, Ca^{2+} associates with calmodulin and activates myosin light chain kinase, which phosphorylates myosin at the 20 kDa residue and initiates contraction[121-123].

There is some evidence in the literature that suggests that IP3-induced contractions are transient, and that sustained contractions are mediated by DAG[124]. An IP3-independent source of DAG has been documented. Phosphatidylcholine (PC), in the plasma membrane, may be converted into DAG, by an independent PC specific PLC (PC-PLC). Additionally, PC may be converted into phosphatidic acid by phospholipase D, and subsequently converted into DAG[125]. DAG subsequently opens calcium channels and promotes phosphorylation of myosin light chain kinase at residues other than 20 kDa[122].

An additional mechanism of vasoconstriction is through voltage gated calcium channels in smooth muscle. When potassium leaves the cell, as in depolarization, or when the concentration of potassium outside the cell is elevated, the membrane potential is reduced and potassium-dependent and voltage-gated calcium channels open, allowing calcium to flow into the cell[121].

Exercise and Aged-Vascular Smooth Muscle Cell Function

Morphologically, aged vascular smooth muscle cells show an increased thickening and an invasion into the endothelial layer[126]. Functionally, the current dogma suggests that NO-mediated vascular smooth muscle cell relaxation is preserved with age, which is well supported by exogenous NO donor studies[15,18,19,25,30,127] and not altered with exercise training[25]. However, there is some evidence that the hormone stimulated relaxation response of VSMC, specifically β adrenergic relaxation, is decreased with age[128].

Vascular smooth muscle cell constriction is altered with age; the age-effect is dependent on the location of the vessel and vasoconstriction may be enhanced or impaired with age[129-131]. Exercise training improves age-impaired myogenic constriction in soleus first order arterioles[129]. In aged gastrocnemius muscle first order arterioles, Donato et al. demonstrated an increased sensitivity of vascular smooth muscle to the vasoconstrictor ET-1, likely mediated by the ETa receptor. Furthermore, they showed that exercise training did not alter ET-1 vasoconstriction in these aged arterioles[132]. Vascular smooth muscle cell production of the potent vasoconstrictor, Angiotensin II, is increased with age[133]. Griendling et al. reported that Ang II also increases NAD(P)H derived O_2^- in cultured VSMC[134]. An increased sensitivity to, or production of, vasoconstrictors coupled with impaired endothelium-dependent dilation, may lead to an increased peripheral vascular resistance, chronically increased intraluminal pressures, and ultimately to pathological hypertension[135].

In addition to increased production and sensitivity to vasoconstrictors, aging also disrupts proteins responsible for mechano-force transduction, providing a possible mechanism for age-impaired vasoconstriction. RhoA is a protein that helps regulate vascular smooth muscle cell contraction by increasing the phosphorylation of myosin light chain proteins and by

strengthening agonist-induced contraction of the VSMC[136]. RhoA is stimulated by O_2^{-}[137,138] and has been shown to increase with age[136,139].

Guanosine triphosphate bound RhoA (GTP-RhoA) is the active isoform of RhoA[140]. RhoA is activated from its inactive, cytosolic state upon fatty acyl-mediated assimilation into the plasma membrane[141]. By overexpressing RhoA in cultured NIH 3T3 cells, Kimura et al. showed increased phosphorylation of myosin light chain and the myosin binding subunit of myosin phosphatase, which promote smooth muscle contraction. These investigations proposed Rho-associated kinase (ROK) as a mediator for RhoA-induced, Ca^{2+}-dependent smooth muscle contraction[140].

RhoA also plays a critical role in enhancing the formation of actin and focal adhesions[142]. Focal adhesions are complex groups of proteins that serve as important regulators of the contractile actin fibers, and also transfer force from the cytoskeleton to the extracellular matrix[143]. RhoA is a potential target for improving age-impaired VSMC function. Aerobic exercise training has been shown to be successful in reducing RhoA gene expression [144], and has been suggested to inhibit the RhoA signaling pathway[145]. Taken together, these data indicate that there is a component of exercise that is responsible for maintaining healthy vascular smooth muscle function.

Regulation of Vascular Smooth Muscle Cell Phenotype by Intraluminal Pressure

One exercise-associated hemodynamic force experienced by vascular smooth muscle is an increase in intraluminal pressure. Using cell culture techniques, the morphological deformation caused by increased intraluminal pressure, is simulated by stretching cultured VSMC. Cyclic stretching of cultured VSMC reorganizes the cytoskeletal F-actin[146], increases the number of focal adhesions[146], and increases the vascular endothelial growth factor (VEGF)

mitogen[147]. Chronic exposure to elevated intraluminal pressure (i.e. hypertension) is detrimental and associated with elevated RhoA, increased RhoA activation[148], VSMC hypertrophy, and increased vascular tone in resistance vessels[149]. However, previous studies suggest that exposure to baseline pressure and mechanical stimulation is important for inhibiting cell proliferation[150,151], preserving sensitivity to vasoconstrictors and contractile function[152], and maintaining cell morphology[153,154]. Therefore, exposure to short-duration increases in intraluminal pressure during individual bouts of exercise may be an important mechanical signal for the maintenance of a healthy vascular smooth muscle function in aged arteries.

1.4 Hypothesis and Specific Aims

Hypothesis

The general aim of the research in this dissertation is to determine the importance of intraluminal pressure and shear stress in regulating vascular endothelial and smooth muscle function in aged arteries. We *hypothesized* that short-duration increases in intraluminal pressure and shear stress promote healthy endothelial and smooth muscle cell function in aged arteries. Our hypothesis was tested by the following specific aims:

Specific Aim 1 (SA1)

Determined whether a short-duration increase in intraluminal pressure improved endothelium-dependent vasodilator responses in aged SFA. We utilized an isolated artery preparation and immunoblot analysis to test the hypothesis that a short-duration (1 h) increase in intraluminal pressure, to mimic a time duration representative of an exercise bout, would attenuate age-induced impairments in endothelium-dependent dilation in SFA via the NO pathway.

Specific Aim 2 (SA2)

Determined whether a short-duration increase in intraluminal pressure improved vascular smooth muscle cell constrictor responses in aged, intact SFA. We utilized an isolated artery preparation to test the hypothesis that a short-duration (1 h) increase in intraluminal pressure would attenuate age-induced impairments of vasoconstrictor responses in soleus muscle feed arteries via the Rho pathway.

Specific Aim 3 (SA3)

Determined whether a short-duration increase in intraluminal pressure would improve vascular smooth muscle contractility in aged, endothelium-denuded SFA. We utilized an isolated artery preparation to test the hypothesis that a short-duration (1 h) increase in intraluminal pressure would attenuate age-induced impairments of vascular smooth muscle contraction in denuded (endothelium removed) soleus muscle feed arteries via the Rho pathway.

Specific Aim 4 (SA4)

Determined whether intraluminal pressure and shear stress interact to produce greater improvements in endothelium-dependent vasodilator responses in aged SFA than increased intraluminal pressure alone. We utilized an isolated artery preparation and immunoblot analysis to test the hypothesis that increased intraluminal pressure and shear stress interact to produce greater improvements in endothelium-dependent dilation than pressure alone.

CHAPTER II

ACUTE INCREASES IN INTRALUMINAL PRESSURE IMPROVE VASODILATOR RESPONSES IN AGED SOLEUS MUSCLE FEED ARTERIES*

2.1 Introduction

Endothelial function declines with age in humans and animals [10,15,18,56,155,156]. The decrement in endothelial function is characterized, in part, by impaired nitric oxide (NO)-mediated, endothelium-dependent dilation in conduit and resistance arteries [15,55,56,155,156]. Endothelial dysfunction in conduit arteries is a risk factor for cardiovascular disease in older adults [157,158], whereas impaired endothelium-dependent dilation in resistance arteries may result in impaired exercise hyperemia and reduced exercise tolerance in the elderly [11,13,157,159].

Aerobic exercise training improves endothelium-dependent vasodilator responses in aged arteries [25,26,56,57]. Although the mechanism accounting for the beneficial effect of exercise training has not been fully elucidated, training-induced enhancement of NO bioavailability is believed to play an integral role [21,25] .

Haram et al. [32] reported previously that NO-mediated endothelium-dependent vasodilation in rat aorta was improved following a single bout of exercise, and that enhanced endothelial function persisted for up to 48 h following the exercise bout. The signal(s) associated with exercise that leads to training-induced improvements in endothelial function is not known; however, it has been proposed that short-duration increases in arterial pressure, which occur

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during individual bouts of exercise, may provide a mechanical signal leading to improved endothelial function [26,30]. Specifically, short-duration mechanical stimulation of vascular endothelium during exercise, as a result of increased arterial pressure, may induce long-term changes in the capacity of endothelial cells to produce NO. This speculation is supported by previous studies indicating that treatment of isolated arteries with increased intraluminal pressure for 4 h, restored endothelium-dependent dilation in aged arteries to levels exhibited in young arteries [30].

While the study by Woodman et al. revealed that exposure to increased intraluminal pressure improved endothelial function in aged arteries, the 4 h pressure stimulus used in their study was substantially longer than a typical bout of exercise [30]. Therefore, it is not known whether exposure to an acute increase in intraluminal pressure, within a time frame representative of an exercise bout, improves endothelium-dependent dilation in aged arteries. In addition, no experiments were performed to determine how long the adaptation persisted after return to normal pressure. Therefore, the purpose of this study was to test the hypothesis that exposure to an acute increase in intraluminal pressure, for a time duration representative of an exercise bout (60 min), improves NO-mediated endothelium-dependent dilation in aged soleus muscle feed arteries (SFA). In addition, we tested the hypothesis that improved endothelial function in aged arteries would persist after a 2 h recovery period at normal pressure. SFA were studied, because they provide an active site of regulation of resistance to blood flow to the soleus muscle at rest and during exercise [160] and because an age-related decline in endothelium-dependent dilation is well documented in these arteries[15,29,156].

2.2 Methods

Animals

This study was approved by the Institutional Animal Care and Use Committee at Texas A&M University and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Fischer 344 rats (4 and 24 month) were obtained from the National Institute on Aging (NIA) and housed at the College of Veterinary Medicine's Comparative Medicine Program Facility. Animals were housed under a 12:12-h light-dark cycle, and food and water were provided ad libitum. The rats were examined daily by the Comparative Medicine Program's veterinarians. Fischer 344 rats were studied, in part, because of the absence of atherosclerosis and hypertension in this animal model [161].

Isolation of Feed Arteries

Soleus muscle feed arteries were isolated as described previously[15,19,25,30,156]. In brief, rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg body wt. ip). The soleus/gastrocnemius/plantaris muscle complex from each hind limb was dissected out and placed in cold (4 ºC) MOPS-buffered physiological saline solution (PSS), composed of (in mM) 145.0 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA and 25.0 MOPS (pH 7.4). SFA were then transferred to a Lucite chamber containing MOPS-PSS for cannulation.

Cannulation of Feed Arteries

SFA were cannulated on both ends with resistance-matched glass micropipettes and secured with a single strand of surgical thread. Micropipettes were attached to pressure reservoirs filled with MOPS-PSS containing albumin (1g/100ml). All SFA were initially

pressurized to 60 cmH_2O (1 mmHg = 1.36 cmH_2O) and checked for leaks. When a SFA was determined to be leak free, intraluminal pressure was increased to 90 cmH_2O or 130 cmH_2O for 60 min at 37 ºC. These pressures were selected to correspond to mean arterial pressures believed to be present in these arteries at rest and during exercise, respectively[160]. At the end of the 60 min pressure treatment, intraluminal pressure in the P130 SFA was lowered to 90 cm H_2O, and SFA were allowed to develop spontaneous, stable tone for 10 minutes before assessment of vasodilator responses.

Assessment of Vasodilation

Procedures used to assess vasodilator responses in SFA have been published previously[15,30,162]. Briefly, endothelium-dependent, flow-induced dilation was assessed by establishing intraluminal flow in the SFA by raising and lowering the heights of the pressure reservoirs in equal but opposite directions while maintaining constant pressure at the midpoint of the artery[84]. Flow-induced dilation was assessed at pressure gradients of 0, 2, 4, 6, 8, 10, 15, 20, 30 and 40 cmH_2O, corresponding to flow rates of 0-62 µl/min[83]. Endothelium-dependent, acetylcholine (ACh)-induced dilation was assessed in SFA by adding increasing concentrations of ACh over the range of 10^{-9}-10^{-4} M in whole log increments. Endothelium-independent dilation was assessed by adding increasing concentrations of sodium nitroprusside (SNP) over the range of 10^{-9}-10^{-4} M in whole log increments. The use of SNP to assess vascular smooth muscle responses to NO has been validated previously by Hirai et al[163]. After the SNP concentration response curve, SFA were incubated in Ca^{2+} free PSS for 60 min to assess maximal passive diameter.

Experimental Protocol 1: Effect of Acute High Pressure Treatment on Vasodilator Responses

To test the hypothesis that treatment of aged SFA with an acute increase in intraluminal pressure attenuates or reverses age-induced impairments of endothelium-dependent dilation, SFA were pressurized for 60 min at 90 or 130 cmH_2O to approximate mean arterial pressures believed to be present in these arteries at rest and during exercise, respectively[160]. The same pressures were used for young and old SFA since Musch et al reported previously that mean arterial pressure during submaximal exercise was similar in young and old rats[13]. At the end of the 60 min treatment period, intraluminal pressure in the P130 SFA was lowered to 90 cm H_2O, and SFA were allowed to develop stable spontaneous tone for 10 minutes. Endothelium-dependent vasodilator function was assessed by measuring vasodilator responses to intraluminal flow or ACh. Endothelium-independent vasodilator function was assessed by measuring vasodilator responses to SNP.

Experimental Protocol 2: Role of Nitric Oxide

On the basis of the results indicating that treatment of old SFA with high pressure improved endothelium-dependent vasodilator responses in the aged arteries, we completed an additional experiment to determine whether the beneficial effect of the high pressure treatment was mediated by NO. Specifically, old SFA were pressurized for 60 min at 90 or 130 cmH_2O as described in protocol 1, and endothelium-dependent vasodilator responses to flow and ACh were assessed in the absence or presence of N^{ω}-nitro-L-arginine (L-NNA; 300 μM) to inhibit nitric oxide synthase (NOS). L-NNA was added to the vessel bath 20 min before the assessment of vasodilator responses and remained in the bath for the duration of the experiment.

Experimental Protocol 3: Recovery from Acute High Pressure Treatment

To determine whether the beneficial effect of the acute high pressure treatment in the old SFA persisted following a recovery period at normal pressure, old SFA were pressurized for 60 min at 90 or 130 cmH_2O as described in experimental protocol 1. At the end of the 60 min pressure treatment, intraluminal pressure in the P130 SFA was lowered to 90 cmH_2O for 2 h before assessment of vasodilator responses.

Quantification of p-eNOS^{ser1177} and eNOS Protein Content

Relative differences in p-eNOS^{ser1177} and total eNOS protein contents were assessed in SFA using immunoblot analysis as described previously in detail[50,164]. p-eNOS^{ser1177} was assessed using a monoclonal antibody (1:250, BD Biosciences catalog no. 612393). Total eNOS was assessed using a monoclonal antibody (1:1,250, BD Biosciences catalog no. 610297) after the membrane was stripped using Restore Western Blot Stripping Buffer (Thermo). Immunoblots were evaluated using enhanced chemiluminescence (ECL, Amersham) and densitometry using a LAS-4000 Luminescent Image Analyzer and Multi-Gauge Image Analysis Software (FUJIFILM Medical Systems). Protein data were expressed as a ratio of p-eNOS^{ser1177}-to-total eNOS.

Statistical Analysis

All data are presented as means ± SE. Between-group differences in body mass, maximal diameters, total eNOS, p-eNOS^{ser1177} and p-eNOS^{ser1177}/eNOS protein ratios were assessed using Student's *t*-test or one-way ANOVA where appropriate. Vasodilator response data were assessed as percent possible dilation calculated as $[(D_{dose} - D_B)/(D_P - D_B)] \times 100$ where D_{dose} is the measured diameter for a given dose/flow rate, D_B is the baseline diameter before the dose response curve and D_P is maximal passive diameter. Two-way ANOVA with repeated

measures on one factor (flow rate or dose) was used to determine differences in vasodilator responses. Statistical significance was defined as $P \leq 0.05$.

2.3 Results

Characteristics of Rats and SFA

Body weights were significantly greater in old (430 ± 7 g) compared to young rats (353 ± 6 g). Maximal passive diameter was similar in SFA from old (179 ± 4 µm) and young (173 ± 5 µm) rats.

ACh-Induced Dilation

ACh-induced vasodilator responses were significantly impaired in old P90 SFA relative to young P90 SFA (Fig. 2.1). Treatment of SFA with increased intraluminal pressure (130 cmH_20) for 60 min improved ACh-induced vasodilation in old (not young) SFA (Fig. 2.1).

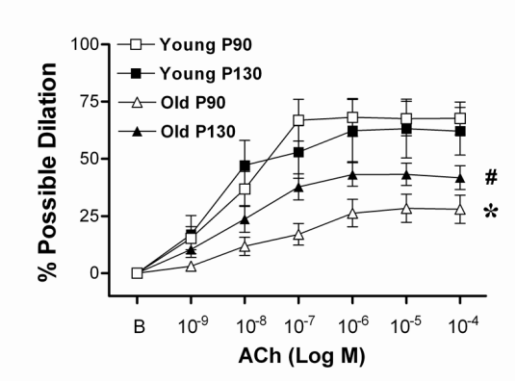

Figure 2.1. Effect of an acute increase in intraluminal pressure on acetylcholine (ACh)-induced vasodilation in young and old soleus muscle feed arteries (SFA). SFA were pressurized to 90 (P90) or 130 (P130) cmH_2O for 60 min. At the end of the 60 min treatment period, intraluminal pressure in P130 SFA was lowered to 90 cmH_2O and allowed to develop spontaneous stable tone for 10 minutes. ACh-induced dilation was then assessed in all SFA at an intraluminal pressure of 90 cmH_2O. B is baseline diameter before the first dose of ACh. Values are mean ± SE; *n* = 10-20 rats/group. *Dose-response curve significantly different from all other curves, $p \leq 0.05$. #Dose-response curve significantly different from young P90, $p \leq 0.05$

In the presence of L-NNA, to inhibit NOS, ACh-induced vasodilator responses in Old P130 SFA were abolished (Fig. 2.2). In addition, the beneficial effect of pressure treatment on old P130 SFA was not present following the 2 h recovery period at normal pressure (Fig. 2.3).

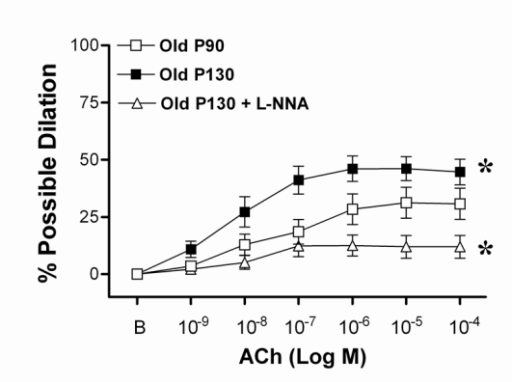

Figure 2.2. Role of nitric oxide (NO) in ACh-induced vasodilation in old SFA treated with an acute increase in intraluminal pressure. Old SFA were pressurized to 90 (P90) or 130 (P130) cmH_2O for 60 min. At the end of the 60 min treatment period, intraluminal pressure in P130 SFA was lowered to 90 cmH_2O and allowed to develop spontaneous stable tone. Vasodilator responses were assessed in the absence or presence of N^{ω}-nitro-L-arginine (L-NNA; 300 μM), to inhibit NOS. B, baseline diameter before the first dose of ACh. Values are mean ± SE; *n* = 7-19 rats/group. *Dose-response curve significantly different from all other curves, $p \leq 0.05$

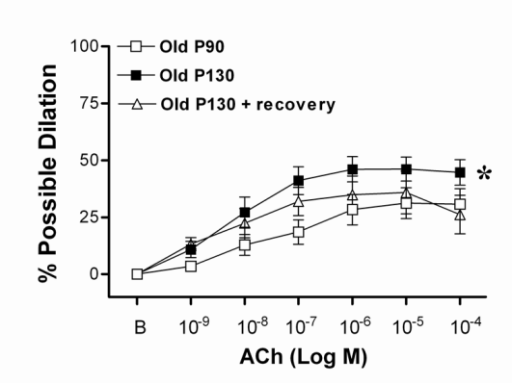

Figure 2.3. Effect of an acute increase in intraluminal pressure on acetylcholine (ACh)-induced vasodilation in old soleus muscle feed arteries following a 2 h recovery period at normal pressure. Old SFA were pressurized at 90 (P90) or 130 (P130) cmH_2O for 60 min. At the end of the 60 min treatment period, intraluminal pressure in the p130 SFA was lowered to 90 cmH_2O for 2 h before ACh-induced vasodilator responses were assessed. B, baseline diameter before the first dose of ACh. Values are mean ± SE; *n* = 7-19 rats/group. *Dose-response curve significantly different from Old p90, $p \leq 0.05$

Flow-Induced Dilation

Flow-induced vasodilation was significantly impaired in old P90 SFA relative to young P90 SFA (Fig. 2.4). Treatment of young SFA with elevated pressure for 60 min did not alter flow-induced dilation (Fig. 2.4). Treatment of old SFA with increased pressure significantly improved flow-induced dilation (Fig. 2.4) such that flow-induced vasodilator responses in the old P130 arteries were significantly greater than old P90 arteries and were not different from young P90 SFA (Fig. 2.4).

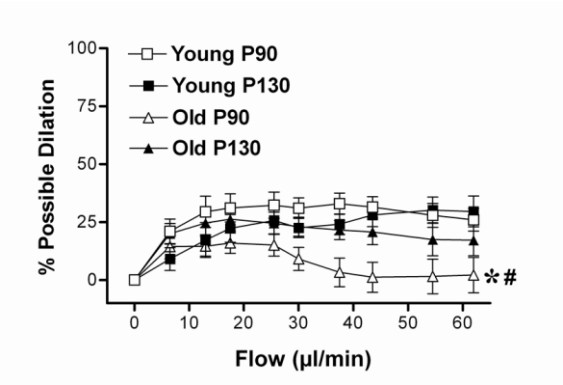

Figure 2.4. Effect of an acute increase in intraluminal pressure on flow-induced vasodilation in young and old soleus muscle feed arteries. SFA were pressurized to 90 (P90) or 130 (P130) cmH_2O for 60 min. At the end of the 60 min treatment period, intraluminal pressure in P130 SFA was lowered to 90 cmH_2O and allowed to develop spontaneous stable tone for 10 minutes. Flow-induced dilation was then assessed in all SFA at an intraluminal pressure of 90 cmH_2O. Values are mean ± SE; *n* = 10-20 rats/group. *Dose-response curve significantly different from old p130, $p \leq 0.05$. #Dose-response curve significantly different from young p90, $p \leq 0.05$

In the presence of L-NNA, flow-induced vasodilator responses in Old P130 SFA were abolished (Fig. 2.5). Furthermore, the beneficial effect of pressure treatment on flow-induced dilation in old P130 SFA was not present following a 2 h recovery period at normal pressure (Fig. 2.6).

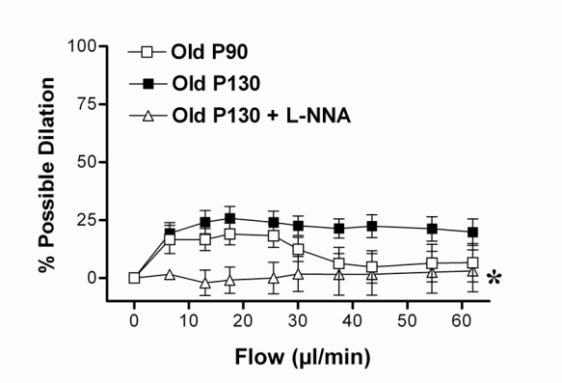

Figure 2.5. Role of nitric oxide (NO) in flow-induced vasodilation in old SFA treated with an acute increase in intraluminal pressure. Old SFA were pressurized to 90 (P90) or 130 (P130) cmH_2O for 60 min. At the end of the 60 min treatment period, intraluminal pressure in p130 SFA was lowered to 90 cmH_2O. Vasodilator responses were assessed in the absence or presence of L-NNA (300 µM), to inhibit NOS. Values are mean ± SE; *n* = 7-19 rats/group. *Dose-response curve significantly different from old p130, $p \leq 0.05$

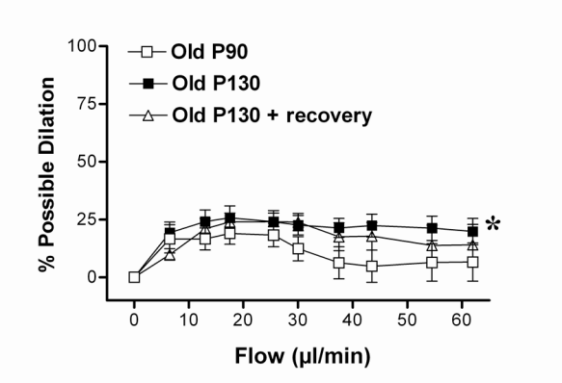

Figure 2.6. Effect of an acute increase in intraluminal pressure on flow-induced vasodilation in old soleus muscle feed arteries following a 2 h recovery period at normal pressure. Old SFA were pressurized at 90 (P90) or 130 (P130) cmH_2O for 60 min. At the end of the 60 min treatment period, intraluminal pressure was lowered to 90 cmH_2O for 2 h before assessment. Values are mean ± SE; *n* = 7-19 rats/group. *Dose-response curve significantly different from old p90, $p \leq 0.05$

SNP-Induced Dilation

SNP-induced vasodilation was significantly impaired in old P90 SFA compared to young, P90 SFA (Fig. 2.7). Treatment of young SFA with elevated pressure for 60 min improved maximal SNP-induced dilation (Fig 2.7). Treatment of old SFA with increased pressure significantly improved SNP-induced dilation such that SNP-induced vasodilator responses in the old P130 arteries were significantly greater than old P90 arteries and were not different from young P90 SFA (Fig. 2.7). The beneficial effect of pressure treatment on SNP-induced dilation was not present after a 2 h recovery (Fig. 2.8).

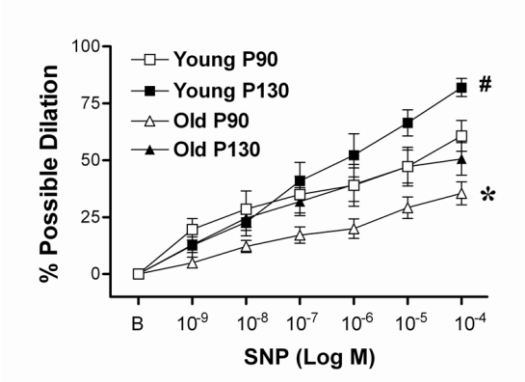

Figure 2.7. Effect of an acute increase in intraluminal pressure on SNP-induced vasodilation in young and old soleus muscle feed arteries. SFA were pressurized to 90 (P90) or 130 (P130) cmH_2O for 60 min. At the end of the 60 min treatment period, intraluminal pressure in P130 SFA was lowered to 90 cmH_2O and allowed to develop spontaneous stable tone for 10 minutes. SNP-induced dilation was then assessed in all SFA at an intraluminal pressure of 90 cmH_2O. Values are mean ± SE; *n* = 10-20 rats/group. *Dose-response curve significantly different from all other curves, $p \leq 0.05$. #Maximal dilation different from all other groups, $p \leq 0.05$

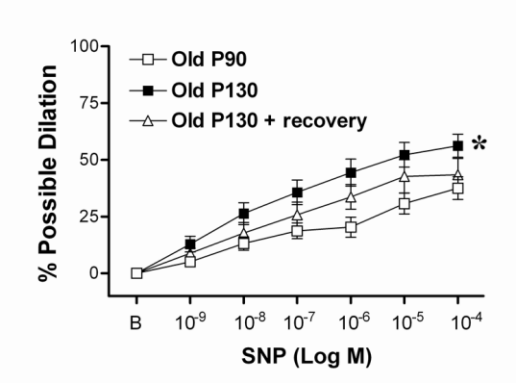

Figure 2.8. Effect of an acute increase in intraluminal pressure on SNP-induced vasodilation in old soleus muscle feed arteries following a 2 h recovery period at normal pressure. Old SFA were pressurized at 90 (P90) or 130 (P130) cmH_2O for 60 min. At the end of the 60 min treatment period, intraluminal pressure in the p130 SFA was lowered to 90 cmH_2O for 2 h before assessment of vasodilator responses. Values are mean ± SE; *n* = 7-19 rats/group. *Dose-response curve significantly different from old p90, $p \leq 0.05$

p-eNOS/eNOS Protein Content

Immunoblot analysis revealed that eNOS, p-eNOS^{ser1177} and the p-eNOS^{ser1177}/eNOS ratio were not altered by age or pressure treatment (Data not shown).

2.4 Discussion

The purpose of this study was to test the hypothesis that exposure to an acute increase in intraluminal pressure, to mimic mean arterial pressure associated with a bout of exercise, improves NO-mediated endothelium-dependent dilation in aged soleus muscle feed arteries (SFA). In addition, we tested the hypothesis that improved endothelial function in aged arteries would persist after a 2 h recovery period at normal pressure. The primary new findings of this study were as follows: (1) Treatment with increased pressure for 60 min improved ACh- and flow-induced dilations in old (not young) SFA. (2) The beneficial effect of pressure treatment on ACh- and flow-induced dilation in old SFA was abolished in the presence of L-NNA and was not present after a 2 h recovery period. (3) Treatment with increased pressure for 60 min improved SNP-induced dilation in old and young SFA. (4) The beneficial effect of pressure treatment on SNP-induced dilation was not present following a 2 h recovery period at normal pressure. Collectively, these results indicate that exposure to an acute increase in intraluminal pressure improves NO-mediated endothelium-dependent vasodilator function in old SFA. Importantly, the beneficial effect of the pressure treatment occurs within a time frame representative of a bout of exercise (i.e. 60 min). Contrary to our hypothesis, the beneficial effect of pressure does not persist after a 2 h recovery period.

SFA were used in the present study because these arteries provide an active site of regulation of resistance to blood flow to the soleus muscle at rest and during exercise [160] and because NO-mediated, endothelium-dependent dilation declines with age in these arteries[15,18,19,30,156]. In addition, endurance exercise training reverses age-induced endothelial dysfunction in SFA[25]. Thus these arteries are well suited for experiments to determine the signals

associated with exercise resulting in improved endothelium-dependent vasodilator responses in aged arteries.

Woodman and colleagues demonstrated previously that treatment of aged SFA with increased intraluminal pressure for 4 h restored endothelial function in old SFA by enhancing NO bioavailability[30]. While the study by Woodman et al. revealed that exposure to a short-duration increase in intraluminal pressure improved endothelial function in aged arteries, it is important to note that the 4 h treatment period used was substantially longer than a typical bout of exercise[30]. Therefore, in the present study we wished to extend these findings to determine whether exposure to an acute increase in intraluminal pressure, within a time frame representative of an actual exercise bout (i.e. 60 min), would improve or restore endothelium-dependent dilation in aged SFA.

Results indicating that flow- and ACh-induced dilations were significantly blunted in old P90 SFA relative to young P90 SFA (Figs. 2.1, 2.4) are consistent with previous studies and confirmed that endothelial function was impaired in the old SFA[15,18,19,25,156]. Treatment of SFA with an acute (60 min) increase in intraluminal pressure, within a range of pressure believed to be present in these arteries during exercise, significantly improved ACh-induced dilation in old SFA (Fig. 2.1). In addition, pressure treatment completely restored flow-induced vasodilator responses in old SFA such that the response was not different from young SFA (Fig. 2.4). While previous studies revealed improved endothelium-dependent dilation following 4 h exposure to elevated intraluminal pressure[30], these data are the first to demonstrate a beneficial effect of an acute increase in pressure on endothelial function within a time frame (60 min) representative of an actual bout of exercise.

Previous studies indicate that aerobic exercise training improves endothelium-dependent vasodilator responses in aged arteries by enhancing NO bioavailability[21,25]. In addition, we demonstrated previously that treatment of aged SFA with increased arterial pressure for 4 h restored NO-mediated endothelium-dependent vasodilation in old SFA[30]. To determine whether the acute pressure stimulus (i.e. 60 min) used in the present study also improved endothelium-dependent dilation in old SFA by enhancing NO-mediated dilation, ACh- and flow-induced vasodilator responses were assessed in the presence of a nitric oxide synthase inhibitor (L-NNA). Addition of L-NNA abolished endothelium-dependent vasodilator responses to ACh and flow in old SFA treated with a short-duration increase in pressure. These results indicate that the beneficial effect of the acute pressure treatment in old SFA was mediated by NO. Equally important, these results demonstrate that the beneficial effect of pressure on NO-mediated vasodilator function occurred within a time frame representative of a bout of exercise (i.e. 60 min).

Trott et al. [25] reported previously that endurance exercise training improved NO-mediated endothelium-dependent vasodilator responses in old SFA. These findings, taken together with results of the present study, suggest that an acute increase in intraluminal pressure that occurs during individual bouts of exercise may provide an important mechanical signal by which exercise improves or restores NO-mediated endothelium-dependent vasodilation in arteries that perfuse skeletal muscle that is recruited during exercise.

Importantly, McCullough et al. [165] reported that exercise training also reverses age-induced endothelial dysfunction in arteries perfusing skeletal muscle that is not recruited during exercise. Improved endothelial function in vascular beds that do not exhibit a hyperemic response to exercise cannot be attributed to increased blood flow/shear stress. Therefore, these

investigators proposed that increased blood pressure during exercise may provide a mechanism for enhanced endothelium-dependent vasodilation in aged arteries that would be independent of changes in shear stress. Results presented in the current study, indicating that acute increases in pressure improved or restored endothelium-dependent dilation in aged SFA, are consistent with the interpretation that increased arterial pressure associated with individual bouts of exercise contributes to the beneficial effect of exercise on endothelial function in aged arteries.

Based on our results indicating that exposure to an acute increase in pressure improved endothelium-dependent dilation in old SFA, we completed an additional experiment to determine whether the beneficial effect of the high pressure treatment persisted following a recovery period at normal pressure. Contrary to our hypothesis, the beneficial effect of pressure treatment on ACh- and flow-induced dilation in old SFA was not present following a 2 h recovery period at normal pressure (Figs. 2.3, 2.6).

Interestingly, Haram et al. [32] reported previously that endothelium-dependent dilation was improved for up to 48 hours following a single bout of treadmill exercise. The mechanism accounting for the persistent improvement in NO-mediated endothelium-dependent dilation following a bout of exercise is not known; however, arteries in an exercising animal would be exposed to multiple mechanical signals, not just an increase in intraluminal pressure. Woodman et al. [29] reported previously that exposure to a short-duration increase in intraluminal shear stress also improves NO-mediated endothelium-dependent dilation in aged SFA. Thus, during exercise it is conceivable that exercise-induced increases in intraluminal pressure and shear stress interact to produce longer lasting improvements in endothelial function than pressure alone. Further studies are needed to determine whether pressure and shear stress work synergistically to promote a healthy endothelium in aged arteries. In addition, studies are needed to determine

whether pulsatile increases in pressure augment the beneficial effects of pressure on vasodilator function in aged arteries.

In the present study, treatment of young SFA with increased pressure for 60 min did not improve endothelium-dependent dilation (Figs. 2.1, 2.4). These results are in agreement with findings indicating that treatment of young SFA with increased intraluminal pressure for 4 h did not improve endothelium-dependent dilation in young SFA[30]. In addition, these results are in accord with the findings by Trott et al.[25] indicating that endurance exercise training did not enhance endothelium-dependent dilation in young SFA.

Based on results indicating that exposure to increased intraluminal pressure for 60 min improved NO-mediated endothelium-dependent dilation in old SFA, we wished to determine whether the acute pressure stimulus resulted in enhanced phoshphorylation of eNOS. In vascular endothelial cells, eNOS is the enzyme primarily responsible for the synthesis on NO and phosphorylation of eNOS on serine residue 1177 plays an integral role in its activation[41,166]. Thus, enhanced phosphorylation of eNOS on ser^{1177} could increase eNOS activity and contribute to enhanced NO-mediated vasodilator responses observed in the old SFA exposed to the high pressure stimulus. Results indicated that the 60 min pressure treatment did not alter the phosphorylation state of eNOS on ser^{1177} indicating that the beneficial effect of pressure treatment on NO-mediated dilation was not mediated by enhanced phosphorylation of eNOS on ser^{1177}.

In the present study, acute pressure treatment improved vasodilator responses to SNP (a NO donor) in old SFA. Based on these findings, it could be argued that the improvement in ACh- and flow-induced dilation observed in the old SFA was due to enhanced responsiveness of vascular smooth muscle to NO rather than an enhanced capacity of vascular endothelium to

produce NO. It is important to note however, that acute pressure treatment also improved SNP-induced vasodilation in young SFA, but did not enhance endothelium-dependent vasodilator responses to ACh or flow in the young arteries. Thus, it is likely that improved vasodilator responses in old SFA treated with high pressure were due to enhanced endothelium-dependent and endothelium-independent vasodilator responses. The mechanism by which pressure treatment improved vascular smooth muscle responses to NO in old SFA is not known; however, it is conceivable that they are a result of alterations in the cyclic GMP signaling pathway[167].

In summary, the results of this study indicate that exposure of old SFA to an acute increase in intraluminal pressure, to mimic mean arterial pressure associated with a bout of exercise, improves vasodilator responses. The beneficial effect of pressure was mediated by enhanced NO-mediated endothelium-dependent dilation and occurred within a time frame (60 min) representative of a bout of exercise. Contrary to our hypothesis, the beneficial effect of the pressure treatment was not present following a 2 h recovery period at normal pressure. These results suggest that increased intraluminal pressure is one signal by which NO-mediated, endothelium-dependent vasodilator function is improved by exercise in aged arteries. Further study is needed to determine whether repeated exposures to increased pressure, as well as pulsatile increases in pressure, augment the effects of pressure on vasodilator function in aged arteries.

CHAPTER III

A SHORT-DURATION INCREASE IN INTRALUMINAL PRESSURE PROMOTES HEALTHY VASOCONSTRICTOR RESPONSES IN AGED SKELETAL MUSCLE FEED ARTERIES

3.1 Introduction

Aging is associated with a decline in vascular smooth muscle constrictor responses in central[168] and peripheral arteries[169]. The age-related decrement in vascular function is characterized by impaired constrictor responses to KCl, NE, and vasopressin[168]. The reduced ability of the vasculature to promote constriction may contribute to orthostatic intolerance, impaired ability to properly distribute skeletal muscle blood flow, and reduced exercise capacity in aged individuals[13].

The ability of the vascular system to respond to molecular or mechanical signals declines with age [10,14,15,18,19,21,56,155,168,170]. Aging alters the normal, healthy function of vessels by increasing or decreasing their vasoconstrictor responses. This aging response appears to be dependent on the location and function of the vessel. Age-related alterations in vasoconstrictor function have been reported in gastrocnemius and soleus first-order arterioles to both receptor-dependent and receptor-independent stimuli. Receptor-dependent Ang II- and endothelin-1-induced vasoconstriction has been reported to be increased in some vessels, while receptor-dependent NE and vasopressin and receptor-independent KCL-induced vasoconstriction is reduced with age[132,168,170].

The mechanism(s) through which age-impaired vasoconstriction occurs has yet to be fully elucidated. There is some evidence indicating that aging disrupts proteins responsible for

mechano-force transduction and may provide a mechanistic insight and a target for preventing or improving vascular function with age. The vascular smooth muscle small g-protein, RhoA, and its downstream effector Rho Kinase (ROCK) play an important role in the vasoconstrictor response. ROCK and RhoA are essential for Ca^{2+}-dependent smooth muscle contraction and are responsible for mechano-force transduction, in part, by actin organization and increasing myosin light chain phosphorylation[136]. Furthermore, it is reported that defects in the Rho pathway can lead to cardiovascular diseases like hypertension and atherosclerosis[171]. Because the Rho pathway's association with cardiovascular diseases and it's integral role in the vasoconstrictor response, the Rho pathway may play a role in age-impaired vasoconstriction.

Exercise training has been shown to be effective in attenuating or reversing the detrimental effects of aging on vasoconstrictor responses. In soleus and gastrocnemius muscle first-order arterioles, Park et al. demonstrated that aerobic exercise training reduced age-increased Ang II-induced constriction in these arterioles, thereby mitigating age-induced vasoconstrictor dysfunction[170]. Donato et al. have shown that aging increases α-adrenergic vasoconstriction in aged soleus first order arterioles and that this vasoconstrictor dysfunction is mitigated following exercise training[172]. Moreover, aerobic exercise training improves impaired myogenic constrictor responses in aged arterioles[129]. These data indicate that aerobic exercise may be an important modality for maintaining or improving a healthy vasoconstrictor function in some arteries.

Additionally, aerobic exercise has been shown to decrease RhoA gene expression in an endothelial cell[145], while long-term increases in intraluminal pressure have been shown to increase RhoA in vascular smooth muscle cells[148], suggesting the Rho pathway is sensitive to changes in pressure. Although the mechanism(s) through which aerobic exercise improves age-

impaired vasoconstriction have yet to be fully elucidated, it is possible that short-duration increases in intraluminal pressure during a bout of exercise are important for maintaining healthy vascular smooth muscle function via the Rho pathway.

The purpose of the present study was to test the hypothesis that exposure to a short-duration (1 h) increase in intraluminal pressure, to mimic the pressure believed to be present in the soleus muscle feed artery during a bout of exercise, would attenuate age-induced impairments of vascular smooth muscle constrictor responses in the soleus muscle feed arteries via the Rho pathway.

3.2 Methods

Animals

This study followed the Guide for the Care and Use of Laboratory Animals provided by National Institutes of Health and was approved by the Institutional Animal Care and Use Committee at Texas A&M University. Male Fischer 344 rats aged 4 and 24 mo were received from the National Institute on Aging (NIA) and housed at the Laboratory Animal Resources and Research facility at Texas A&M University. Care was provided by the Comparative Medicine Program through the College of Veterinary Medicine. Rats were maintained on a 12:12-h light:dark cycle and were provided food and water ad libitum.

Isolation of Feed Arteries

SFA were isolated as previously described[15,25,30,173]. Briefly, rats were anesthetized with an intraperitoneal injection with Ketamine (80 mg/kg body weight) and Xylazine (5 mg/kg body weight). Once anesthetized, the soleus-plantaris-gastrocnemius muscle complex was removed from each hindlimb and placed in a cold (4 °C) MOPS-buffered physiological saline solution

(PSS) - (in mM) 145.0 NaCl. 4.7 KCL, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 25.0 MOPS (pH of 7.4). Rats were euthanized by excising the heart. SFA were isolated, excised, and placed in a Lucite dissection chamber containing MOPS-PSS (pH 7.4; 37 °C).

Cannulation of Feed Arteries

SFA were cannulated on each end with a glass micropipette and secured with surgical thread. Each micropipette was attached to a pressure reservoir filled with MOPS-PSS supplemented with albumin (1g/ml). SFA were pressurized at 60 cmH_2O (1 mmHg = 1.36 cmH_2O) and checked for leaks. Once determined to be leak free, SFA were pressurized to 90 cmH_2O or 130 cmH_2O for 1 h. These pressures were selected to correspond to pressure believed to be present in rat SFA at rest and during exercise, respectively[160]. MOPS-PSS in the vessel chamber was maintained at 37 °C and changed at 20 min intervals during the pressure treatment. Immediately following the 1 h pressure treatment period, intraluminal pressures of all SFA were set to 90 cmH_2O for the assessment of vasoconstrictor responses. Lastly, to determine the maximal passive diameter, SFA were incubated in Ca^{2+}-free PSS for 30 min.

Assessment of Vasoconstrictor Responses

Vasoconstrictor responses were assessed in SFA by measuring the change in diameter in response to cumulative, increasing, whole log additions of norepinephrine (NE), angiotensin II (Ang II) and phenylephrine (PE). NE was assessed at concentrations ranging from 10^{-9} to 10^{-4} M and was selected for use because it stimulates both the α1 and α2 adrenergic receptors. Ang II was assessed at concentrations ranging from 10^{-11} to 10^{-4} M. Ang II stimulates the AT1 and AT2 receptors. PE was assessed at concentrations ranging from 10^{-9} to 10^{-4} M and is selective for the α1 adrenergic receptor.

To evaluate the contribution of ROCK, vasoconstrictor responses to NE, Ang II , and PE were assessed in the absence or presence of a 20 min incubation with Y27632 (10^{-6} M), a RhoA-kinase selective inhibitor. Additionally, vasoconstrictor responses were assessed in the absence or presence of a 20 min incubation with lipophosphatidic acid (LPA) (10^{-5} M), a short-duration activator of RhoA-kinase.

NE was obtained from Sigma-Aldrich (Cat. A9512). Ang II was obtained from Bachem through VWR (Cat. H-1705) and PE was obtained from Sigma-Aldrich (Cat. P6126). Y27632 was obtained from Sigma Aldrich (Cat. Y0503). LPA was obtained from Sigma Aldrich (Cat. L7260).

Statistical Analysis

All values are means ± SE. Between group differences in body mass and maximal passive diameter were assessed using One-Way ANOVA. Two-Way ANOVA with repeated measures on one factor (concentration) was used to determine whether constrictor responses to NE, Ang II, or PE differed by group. Concentration-response data were expressed as percent constriction and were calculated as $[(D_b-D_c)/D_b] \times 100$, where D_c is the measured diameter for a given concentration. D_b is the baseline diameter measured before each concentration response curve[170]. When a significant p-value was obtained, post hoc analyses were performed with Duncan's Multiple-Range Test. Statistical significance was set at the $p \leq 0.05$ probability level.

3.3 Results

Characteristics of Rats and SFA

Old rats (416 ± 6 g) weighed significantly more than young rats (346 ± 6 g). SFA from old rats (145 ± 8 μm) had significantly smaller maximal passive diameter than SFA from young rats (200 ± 13 μm).

Vasoconstrictor Responses

Vasoconstrictor responses to NE, Ang II, and PE were significantly blunted in Old P90 SFA relative to Young P90 SFA (Fig. 3.1).

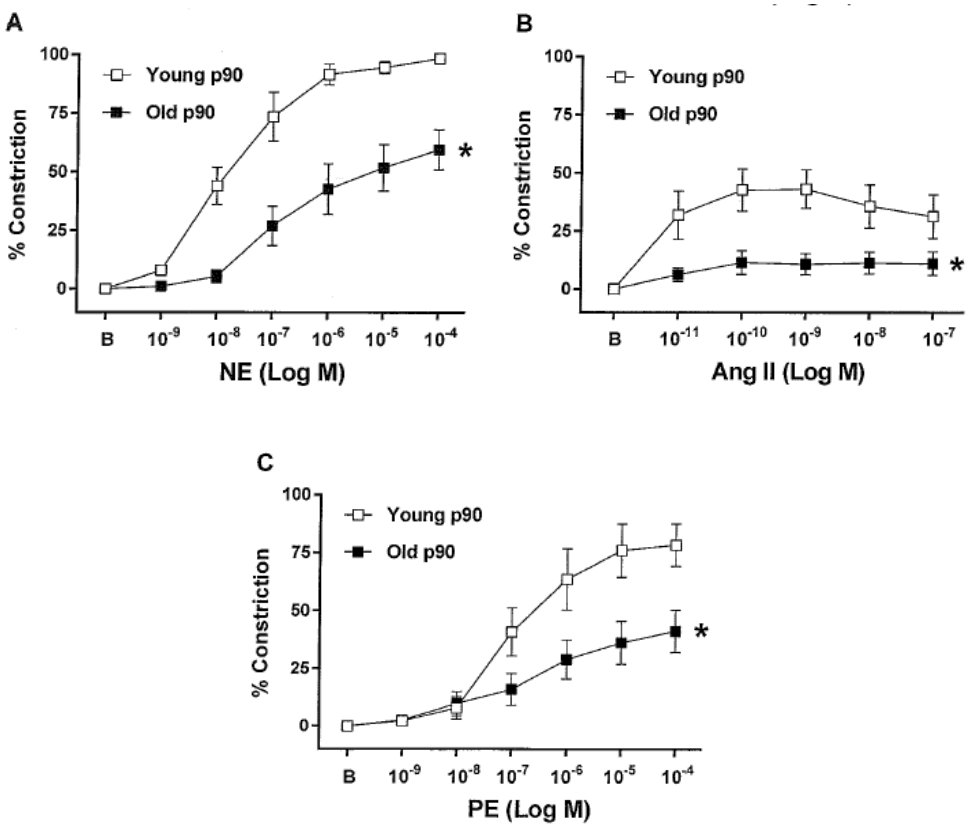

Figure 3.1. Effect of aging on vasoconstrictor responses of SFA to NE (A), Ang II (B), and PE (C). All SFA were pressurized to 90 cmH_2O (P90) for 1 h before assessment of vasoconstrictor function. B, baseline diameter before the first dose of NE, Ang II, or PE. Values are means ± SE; n = 6-8 rats per group. * Significantly different from Young P90; $P \leq 0.05$.

Pressure Treatment

Pretreatment of SFA with 1 h increased intraluminal pressure (130 cmH_2O) , to mimic the pressure believed to be present during exercise, improved, but did not restore vasoconstrictor responses to NE in old SFA (Fig. 3.2A). Pretreatment with increased intraluminal pressure restored Ang II-induced vasoconstrictor responses in aged SFA such that they were no longer different from the Young P90 SFA vasoconstrictor responses (Fig 3.2B). Pressure pretreatment in old SFA did not significantly improve vasoconstrictor responses to PE (Fig. 3.2C).

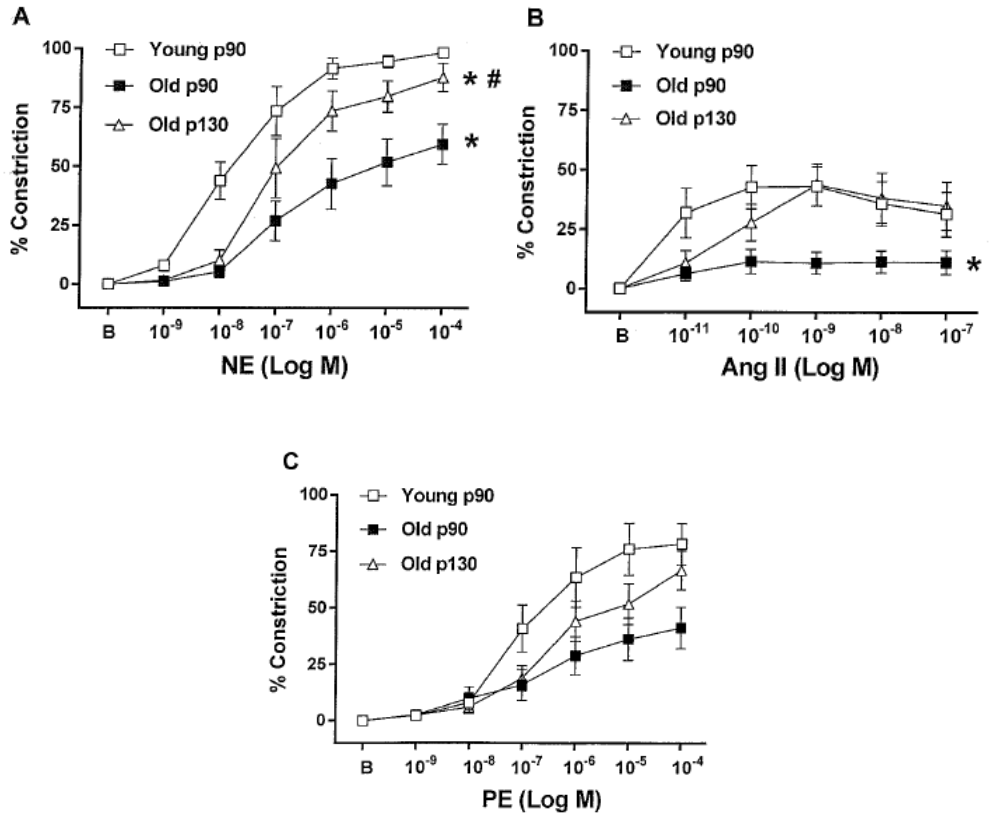

Figure 3.2. Effect of a short-duration increase in intraluminal pressure on vasoconstrictor responses to NE (A), Ang II (B), and PE (C). SFA were pressurized to 90 cmH_2O (P90) or 130 cmH_2O (P130) for 1 h. At the end of the 1 h treatment period, intraluminal pressure was set to 90 cmH_2O in all SFA for assessment of vasoconstrictor function. B, baseline diameter before the first dose of NE, Ang II, or PE. Values are means ± SE; n = 6-8 rats per group. * Significantly different from Young P90; # Significantly different from Old P90; $P \leq 0.05$.

Role of Rho Kinase

To assess the role of the Rho pathway, vasoconstrictor responses were assessed in the absence or presence of a Rho Kinase inhibitor (Y27632). In the presence of Y27632, NE- and PE-induced vasoconstriction was reduced in Old P130 SFA (Fig. 3.3A and Fig. 3.3C). ROCK inhibition eliminated Ang II-induced constriction in Old P130 SFA (Fig. 3.3B).

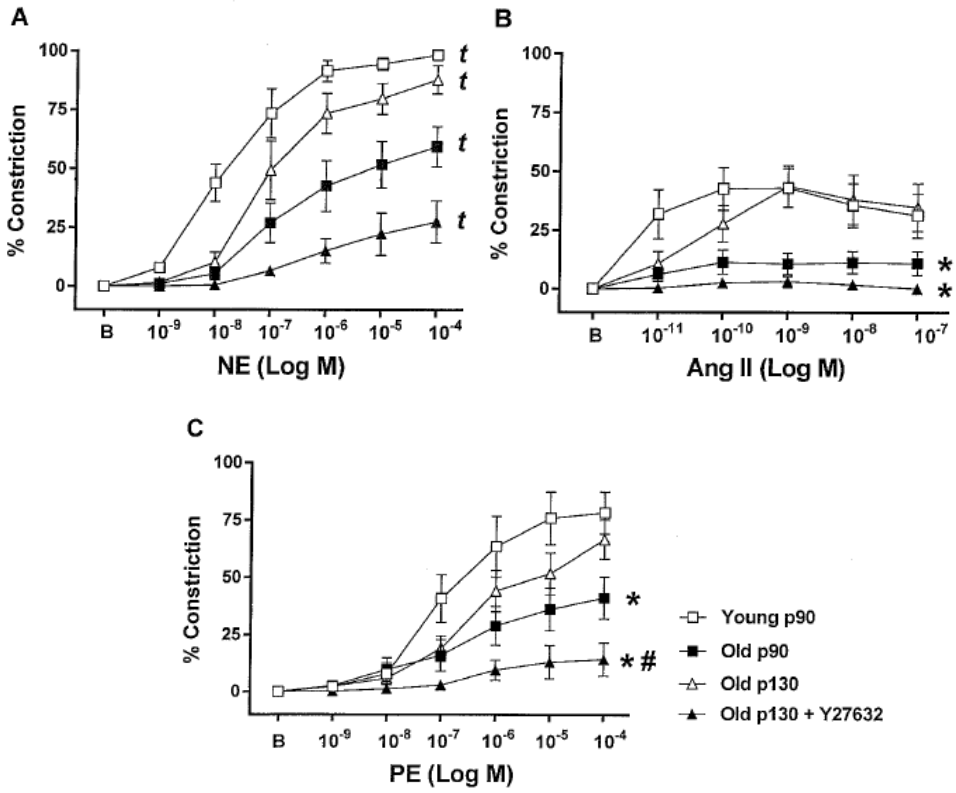

Figure 3.3. Effect of Rho Kinase inhibition with a short-duration increase in intraluminal pressure on vasoconstrictor responses to NE (A), Ang II (B), and PE (C). Vasoconstrictor responses were assessed in the absence or presence of Y27632, a selective inhibitor of Rho Kinase. B, baseline diameter before the first dose of NE, Ang II, or PE. Values are means ± SE; n = 5-8 rats per group. t Significantly different from all other curves; * Significantly different from Old P130; # Significantly different from Old P90; $P \leq 0.05$.

To further assess the importance of the Rho signaling pathway, vasoconstrictor responses were assessed in the absence or presence of a Rho Kinase activator (LPA). Constrictor responses to NE were not improved by LPA (Fig. 3.4A). However, LPA significantly improved Ang II-induced vasoconstrictor responses in Old P90 SFA, such that the vasoconstrictor responses were no longer different than Young P90 SFA (Fig. 3.4B). Statistical analysis did not reveal any significant differences in PE-induced vasoconstriction following incubation with LPA (Fig 3.4C).

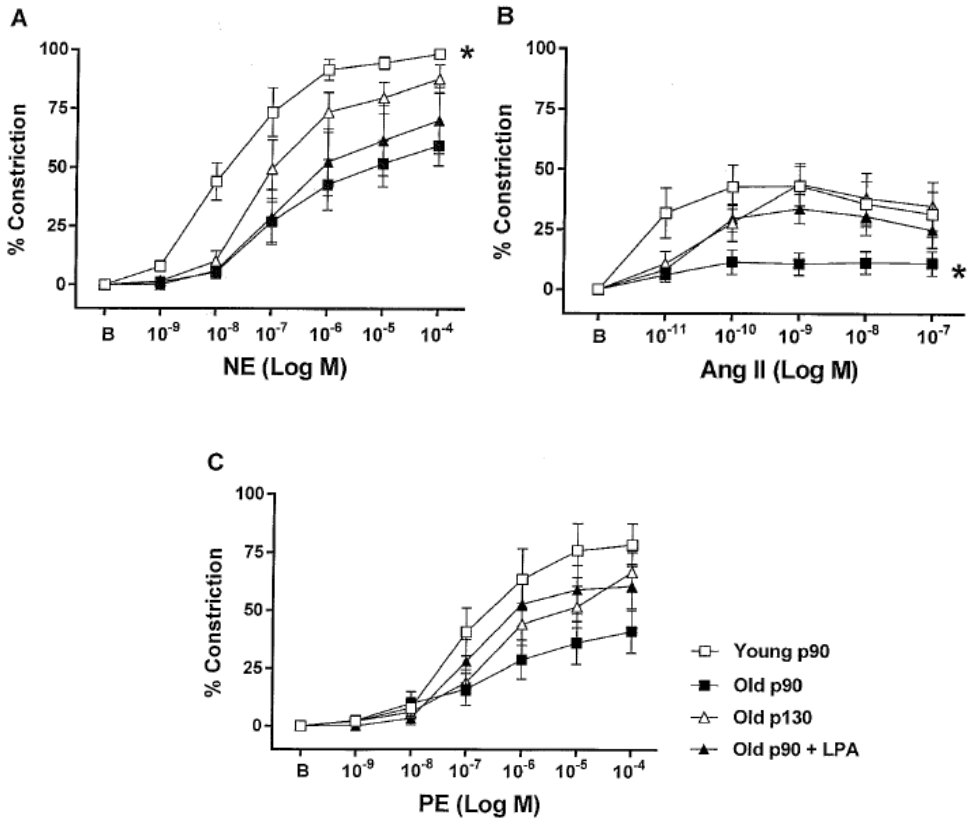

Figure 3.4. Effect of Rho Kinase activation on vasoconstrictor responses to NE (A), Ang II (B), and PE (C). Vasoconstrictor responses were assessed in the absence or presence of LPA, a selective activator of Rho Kinase. B, baseline diameter before the first dose of NE, Ang II, or PE. Values are means ± SE; n = 5-8 rats per group. * Significantly different from Old P130; # Significantly different from Old P90; $P \leq 0.05$.

3.4 Discussion

The purpose of the present study was to test the hypothesis that exposure to a short-duration (1 h) increase in intraluminal pressure, to mimic the pressure believed to be present during a bout of exercise, would attenuate age-induced impairments of vascular smooth muscle constrictor responses in SFA via the Rho pathway. SFA were studied because they play an integral role in regulating blood flow to the soleus muscle at rest and during exercise and are susceptible to decreased vascular function with age, which can be improved with exercise[15,25,174]. The novel findings of this study were as follows: 1) Constrictor responses to NE, Ang II, and PE were impaired in old P90 SFA. 2) A short-duration exposure of aged SFA to increased intraluminal pressure (P130 cmH_2O), to mimic the pressure believed to be present during a bout of exercise, improved NE- and PE-induced vasoconstrictor responses and restored Ang II-induced vasoconstrictor responses such that the responses in Old P130 SFA were not different from Young P90 vasoconstrictor responses. 3) The beneficial effect of increased intraluminal pressure on vasoconstrictor responses in aged SFA were eliminated in the presence of a ROCK inhibitor (Y27632). 4) Activation of ROCK with LPA restored Old P90 Ang II vasoconstrictor responses so that they were no longer different than Young P90 vasoconstrictor responses.

Previous studies indicate that vasoconstrictor responses are impaired with age[130,169,175]. To our knowledge, this study is the first to document an age-related decline in vasoconstrictor responses in rat SFA (Fig. 3.1). Ishihata and Delp both have shown an age-impaired vasoconstrictor response to endothelin-1 and NE, respectively, in aorta from aged rats[168,176]. Park et al. and Donato previously showed enhanced vasoconstrictor responses to Ang II and endothelin-1 in rat soleus 1A arterioles[132,170]. These studies indicate that the location of the vessel

within the vascular tree, the oxidative/glycolytic state of the vessel's tissue, and function of the vessel in the vascular system plays an important role in determining the response to aging.

Previous studies have reported that exercise can reduce age-induced augmented vasoconstriction and improve age-impaired vasoconstriction[129,170]. Our current study demonstrates that a short-duration increase in intraluminal pressure, within a range believed to be present during a bout of exercise, improves age-impaired vasoconstrictor responses in aged SFA (Fig. 3.2). Together these data are consistent with the interpretation that short-duration increases in intraluminal pressure that occur during exercise may promote healthy vascular function by improving vasoconstrictor function.

Our data showed that exposure to a short-duration increase in pressure improved NE-induced constriction, while having a minimal effect on PE-induced constrictor responses (Fig. 3.2, 3.3). Since NE binds to both α1 and α2 receptors, whereas phenylephrine selectively binds to the α1 receptor, these results suggest a preferential effect of a pressure treatment on the α2 receptor, or that there is an additive vasoconstrictor effect by stimulating both α1 and α2 receptor stimulation on vasoconstrictor response following a short-duration increase in intraluminal pressure.

To directly test the hypothesis that increased intraluminal pressure improves constrictor responses in aged arteries via the Rho pathway, vasoconstrictor responses were assessed in the absence or presence of a Rho Kinase inhibitor. The finding that the beneficial effect of pressure treatment was eliminated in the presence of Y27632 indicates that the beneficial effect of pressure was Rho-dependent (Fig. 3.3, 3.4). ROCK signaling is an important mechanism of vascular smooth muscle cell constriction by decreasing myosin light chain phosphatase activity and increasing the formation of actin fibers[140,142,177]. In cultured vascular smooth muscle cells,

Lim et al. reported an increase in myosin light chain phosphorylation following treatment with LPA and a decrease in myosin light chain phosphorylation following treatment with Y27632[178]. In the current study, Ang II-induced constriction, following treatment with a short-duration increase in intraluminal pressure, was nearly abolished following inhibition with the ROCK inhibitor Y27632 (Fig. 3.3B). These results indicate that Ang II-induced constriction operates almost exclusively through the ROCK pathway. Furthermore, Ang II-induced constriction in aged SFA was fully restored following incubation with LPA, a ROCK activator, compared to young SFA (Fig. 3.4B). These data further suggest that the impairment of aged Ang II-induced vasoconstriction lies within the ROCK pathway.

Lim et al. have previously reported that the application of tensile stress to live, cultured vascular smooth muscle cells produced vascular smooth muscle cell remodeling that was RhoA dependent[178]. Additional studies by Lim et al. revealed that the RhoA-mediated response to mechanical stimuli was likely modulated through the extracellular matrix[179]. Collectively, these data suggest that the improvement in ROCK-mediated vasoconstriction in intact SFA is largely due to vascular smooth muscle modifications.

In summary, the results of this study indicate that aging impairs vasoconstrictor responses in rat SFA and that pretreatment with 1 h increased intraluminal pressure can improve or restore vasoconstrictor responses in aged arteries. Furthermore, improved vasoconstrictor responses following increased intraluminal pressure pre-treatment are mediated by Rho Kinase. Collectively, these data suggest that a short-duration increase in intraluminal pressure is one mechanical signal associated with exercise that is important for promoting healthy vascular function in aged arteries.

CHAPTER IV

A SHORT-DURATION INCREASE IN INTRALUMINAL PRESSURE IMPAIRS VASCULAR SMOOTH MUSCLE CONTRACTILITY IN AGED, DENUDED SKELETAL MUSCLE FEED ARTERIES

4.1 Introduction

Aging impairs vasoconstrictor responses in central and peripheral arteries[130,168,169]. In the previous chapter, impaired vasoconstrictor responses were observed in soleus muscle feed arteries. An impaired ability to properly regulate blood flow through appropriate vasoconstrictor responses may lead to orthostatic intolerance, reduced blood flow to skeletal muscle, and reduced exercise capacity[13].

The mechanism(s) by which vasoconstrictor responses are impaired with age has not yet been fully elucidated, but may include impaired ability of the vascular smooth muscle to develop contractile tension. In the previous chapter, vasoconstriction of aged soleus muscle feed arteries to Ang II was decreased. However, in the first-order arterioles studied by Park et al., Ang II vasoconstriction was increased with age[170]. Angiotensin II (Ang II) is a potent vasoconstrictor through its stimulation of vascular smooth muscle receptors (AT_1R and AT_2R) but also can induce nitric oxide-dependent vasodilation by stimulating endothelial receptors (AT_1R)[180-186]. Ang II sensitivity is increased in aged, cultured vascular smooth muscle cells[133]. Park et al. showed, in aged first-order arterioles, increased vasoconstriction to Ang II; however, removing the endothelium eliminated the age effect, suggesting that increased vasoconstriction in aged first-order arterioles is due, at least in part, to the biphasic response to Ang II and is both nitric oxide synthase-dependent and endothelium mediated[170]. Therefore, it is conceivable, that the

decreased vasoconstriction to Ang II in aged SFA observed in the previous chapter may be due to an impairment of vascular smooth muscle contractility.

One component of vascular smooth muscle constriction that may be responsible for age-impaired vasoconstriction lies within the Rho-Rho kinase pathway. Kimura et al. overexpressed RhoA in cultured NIH 3T3 fibroblast cells and showed that RhoA contributes to phosphorylation of myosin light chain in these cells, thus setting a precedent and suggesting that RhoA's role in vascular smooth muscle contraction may be through the inhibition of myosin phosphatase by Rho kinase[140]. Furthermore, RhoA also contributes to increasing the formation of actin and focal adhesions, proteins important for the mechano-transduction of contractile force from the cytoskeleton to the extracellular matrix[142,143].

Aerobic exercise training has been associated with the restoration of a young vascular vasoconstrictor phenotype to aged arterioles, through an endothelium-mediated mechanism(s)[170,172]. In the previous chapter, our lab showed that age-impaired vasoconstrictor function is improved with age following exposure to a short-duration increase in intraluminal pressure, one hemodynamic force experienced by the vascular smooth muscle during a bout of exercise. However, the contribution of vascular smooth muscle to the improved response remains unclear. Exposure to elevated intraluminal pressure is associated with elevated RhoA, increased RhoA activation, and increased vascular tone[148,149]. Furthermore, mechanical stimulation is important for preserving sensitivity to vasoconstrictors and contractile function[152]. These data suggest that exposure to short-duration increases in intraluminal pressure, as believed to be present during a bout of exercise, may serve to improve or maintain healthy vascular smooth muscle constrictor function through improvements in the Rho-Rho kinase pathway.

Therefore, the purpose of the present study was to test the hypothesis that a short-duration (1 h) increase in intraluminal pressure, to mimic the duration and magnitude of pressure believed to be present during a typical exercise bout, would improve vascular smooth muscle constriction through the Rho-Rho kinase pathway. All studies were performed in denuded (endothelium removed) SFA to isolate the effects of age and pressure on vascular smooth muscle.

4.2 Methods

Animals

This study was approved by the Institutional Animal Care and Use Committee of Texas A&M University and followed the National Institutes of Health Guide for the Care and use of Laboratory Animals. Young (4 mo) and Old (23-24 Mo), male Fischer 344 rats were obtained from the National Institute on Aging (NIA) and were housed and cared for at the Texas A&M College of Veterinary Medicine's facility for the Comparative Medicine program. Animals were examined daily by the Comparative Medicine Veterinary staff and by study investigators. Rats were housed on a 12:12 hour light-dark cycle and provided food and water *ad libitum*.

Isolation of Feed Arteries

Soleus muscle feed arteries were isolated as previously described[25,30,173]. In brief, rats were anesthetized using an intraperitoneal injection of a Ketamine:Xylazine (80 mg/kg body weight Ketamine: 5 mg/kg body weight Xylazine). After rats were fully anesthetized, the soleus-gastrocnemius-plantaris muscle complex was removed from each hindlimb and placed in a 4 °C MOPS-buffered physiological saline solution (PSS) composed of (in mM) 145.0 NaCl. 4.7 KCL, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 25.0

MOPS (pH of 7.4). Rats were euthanized by excising the heart. SFA were isolated, excised, and placed in a Lucite dissection chamber containing MOPS-PSS for cannulation.

Cannulation of Feed Arteries

One end of the SFA was cannulated with a glass micropipette and secured with surgical thread. The endothelium was removed from SFA by passing 5 ml of air through the vessel lumen and the remaining end of the SFA was the cannulated and secured with surgical thread. Once both ends of the SFA were cannulated and secured, each glass micropipette was connected to a pressure reservoir containing MOPS-PSS with Albumin (1 g/mL). SFA were pressurized at 60 cmH_2O and were checked for leaks. SFA determined to be leak free were pressurized at 90 cmH_2O (P90) or 130 cmH_2O (P130) for 1 h, at 37 ºC. Intraluminal pressures of 90 cmH_2O and 130 cmH_2O were selected based on previous studies[30,173] and are representative of the intraluminal pressure thought to be present in a rat SFA at rest and during exercise, respectively[174]. After 1 h, intraluminal pressure was set at 90 cmH_2O for all vessels and vasoconstrictor responses were assessed. Endothelial removal was confirmed at the completion of the experiment by the lack of vasodilation (< 5%) following addition of ACh (3x10-4 M). Lastly, to determine the maximal passive diameter, SFA were incubated in Ca^{2+}-free PSS for 30 min.

Assessment of Vasoconstriction

Vasoconstrictor responses to adrenergic receptor stimulation were assessed by the addition of increasing whole log doses of norepinephrine (NE; 10^{-9} – 10^{-4} M: Sigma Aldrich, A9512) and phenylephrine (PE; 10^{-9} – 10^{-4} M: Sigma Aldrich P6126). NE stimulates α1 and α2 adrenergic receptors. PE is selective for the α1 adrenergic receptor. To determine the vasoconstrictor responses to the stimulation of the AT1 and AT2 receptors, vasoconstriction was

assessed by the addition of increasing whole log doses of angiotensin II (Ang II; 10-11 – 10-6 M: Bachem via VWR, H-1705).

To evaluate the role of Rho Kinase, vasoconstrictor responses in aged, denuded SFA not receiving the high pressure treatment were assessed in the absence or presence of a Rho Kinase inhibitor, Y27632. A 20 minute incubation with (10^{-6} M) Y27632 was used to inhibit Rho Kinase prior to the assessment of vasoconstrictor responses.

Statistical Analysis

All values are means ± SE. Between group differences in body mass and maximal passive diameter were assessed using One-Way ANOVA. Two-Way ANOVA with repeated measures on one factor (concentration) was used to determine whether constrictor responses to NE, ANG II, or PE differed by group. Concentration-response data were expressed as percent constriction and were calculated as $[(D_b-D_c)/D_b] \times 100$, where D_c is the measured diameter for a given concentration. D_b is the baseline diameter measured before each concentration response curve[170]. When a statistical difference was observed, Duncan's Multiple Range Test was used for post-hoc analysis. Statistical significance was set at the $p \leq 0.05$ level.

4.3 Results

Animal and SFA Characteristics

Young rats (340 ± 22 g) weighed significantly less than old rats (424 ± 36 g). The maximal passive diameters of SFAs were not different across groups: Young P90 (153 ± 41 g), Old P90 (163 ± 47 g), OldP130 (132 ± 24 g), OldP90+Y27632 (115 ± 18 g).

Vasoconstrictor Responses

Vasoconstrictor responses to NE, Ang II, and PE were not significantly impaired with age (Fig. 4.1). Exposure to a 1 h increase in intraluminal pressure, to mimic the pressure believed to be present during exercise, significantly impaired vasoconstrictor responses to Ang II and PE in aged, denuded SFA (Fig. 4.2B, 4.2C). The aged, denuded Ang II vasoconstrictor response curve (Old P130) was abolished after exposure to a 1 h increase in intraluminal pressure (Fig. 4.2B).

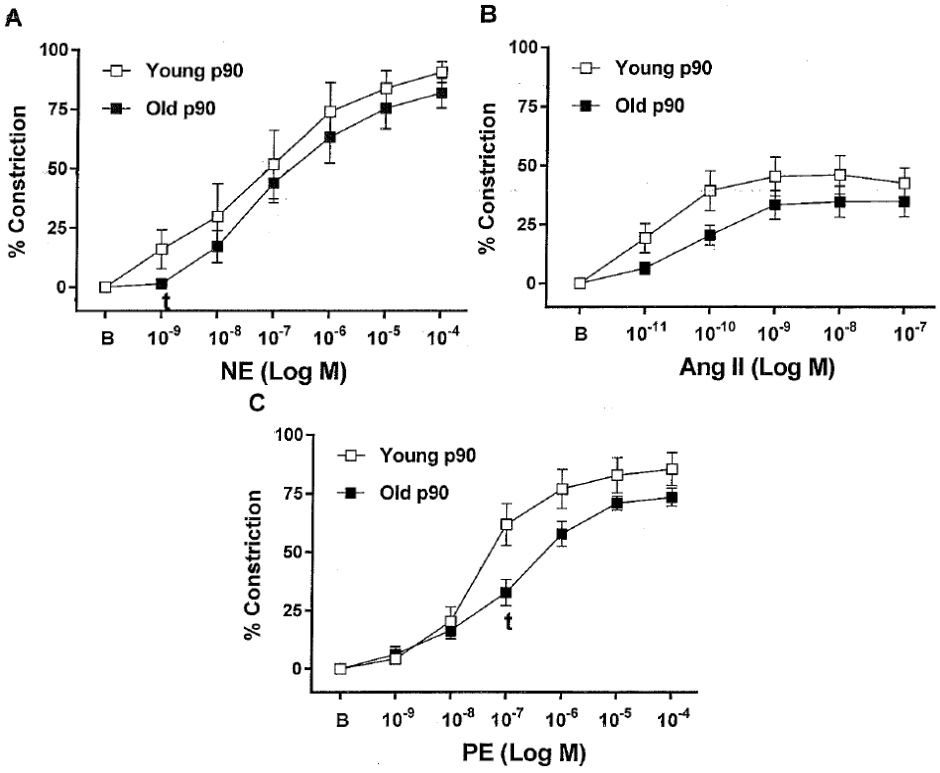

Figure 4.1. Effect of aging on vasoconstrictor responses to NE (A), Ang II (B), and PE (C). All SFA were denuded and pressurized to 90 cm H_2O for 1 h before assessment of vasoconstrictor responses. B, baseline diameter before the first dose of NE, Ang II, or PE. Values are means ± SE. n = 6-12 rats per group. Statistical analysis revealed no significant between group differences.

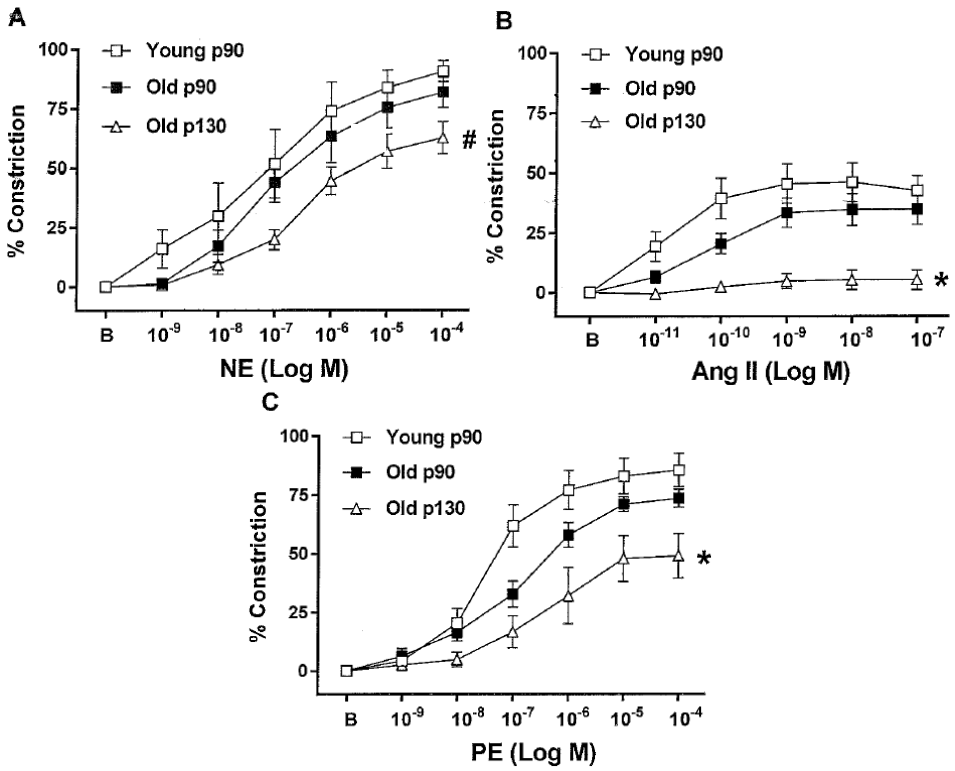

Figure 4.2. Effect of a short-duration increase in intraluminal pressure on constrictor responses to NE (A), Ang II (B), and PE (C). SFA were denuded and pressurized to 90 cmH_2O (P90) or 130 cmH_2O (P130) for 1 h. At the end of the 1 h treatment period, all SFA were pressurized to 90 cmH_2O (P90) and vasoconstrictor responses were assessed. B, baseline diameter before the first dose of NE, Ang II, or PE. Values are means ± SE, n = 5-12 rats per group. * Significantly different than all other curves. $P \leq 0.05$.

To determine the contribution of Rho kinase to vascular smooth muscle constrictor responses, Rho Kinase was inhibited with Y27632 before assessment of vasoconstrictor responses. In the presence of Y27632, vasoconstriction was significantly blunted in aged, denuded SFA (Fig. 4.3). Following incubation with Y27632, NE-induced vasoconstriction was significantly impaired in Old P90 denuded SFA (Fig. 4.3A). In aged, denuded SFA, Ang II-induced vasoconstriction, following incubation with Y27632, was significantly impaired

compared to Young P90 and Old P90 denuded SFA, and was not different from the Ang-II induced vasoconstrictor response of Old denuded SFA pretreated with 1 h of increased intraluminal pressure (Fig. 4.3B). Incubation with Y27632 abolished the vasoconstrictor response of Old, denuded SFA to PE (Fig. 4.3C).

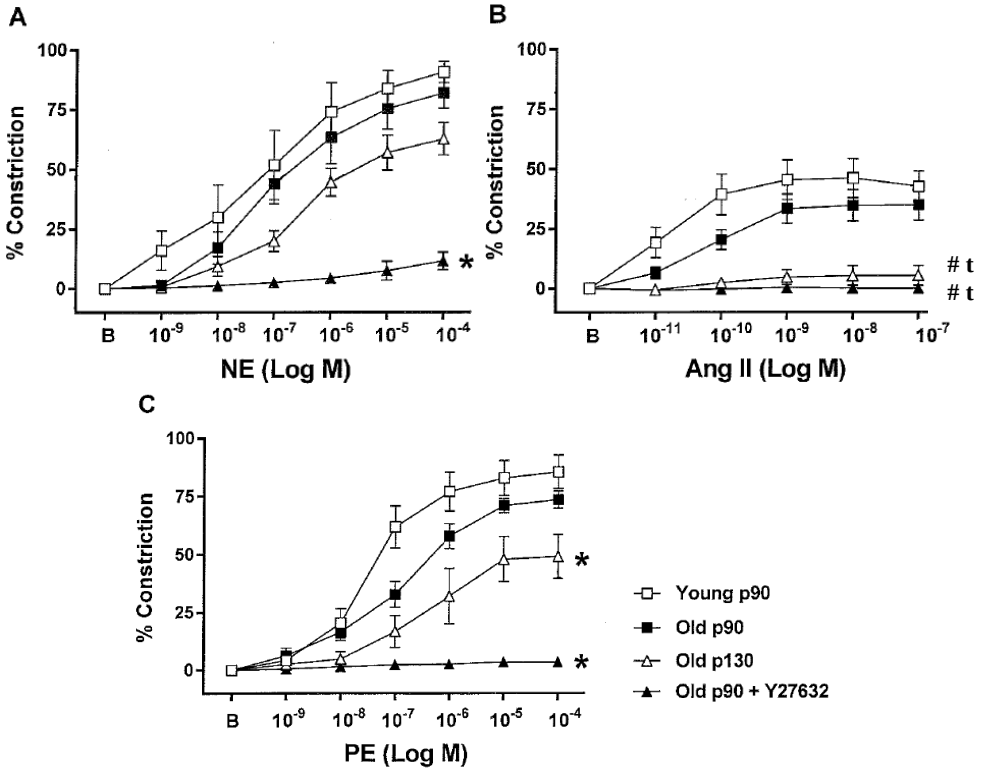

Figure 4.3. Effect of Rho kinase inhibition and a short-duration increase in intraluminal pressure on vasoconstrictor responses to NE (A), Ang II (B), and PE (C). SFA were denuded and pressurized to 90 cmH_2O (P90) or 130 cmH_2O (P130) for 1 h. At the end of the 1 h treatment period, all SFA were pressurized to 90 cmH_2O (P90) and vasoconstrictor responses were assessed. Vasoconstrictor responses were assessed in the absence or presence of Y27632, a selective inhibitor of Rho kinase. B, baseline diameter before the first dose of NE, Ang II, or PE. Values are means ± SE. n = 5 – 11 rats per group.* Significantly different than all other curves. # Significantly different than Young P90. t Significantly different than Old P90. $P \leq 0.05$.

4.4 Discussion

The purpose of this study was to test the hypothesis that a short-duration (1 h) increase in intraluminal pressure, within a range of pressures believed to be present during exercise, would improve vascular smooth muscle constriction in aged SFA. Denuded SFA were studied to isolate the effects of age and pressure on vascular smooth muscle function. This study revealed the following primary findings: 1) Vascular smooth muscle constriction was not different between young and old denuded P90 SFA. 2) Exposure to 1 h increased intraluminal pressure impaired Ang II and PE vasoconstrictor function in old denuded P130 SFA. 3). Incubation with a Rho Kinase inhibitor (Y27632) impaired NE- and abolished PE- induced vasoconstriction in old denuded P90 SFA, and also eliminated Ang II-induced vasoconstriction so that it was no longer different than Ang II-induced constriction in old denuded P130 SFA.

In the present study, vasoconstrictor responses were not significantly impaired in denuded SFA (Fig. 4.1). Vasoconstrictor responses are impaired with age in intact vessels[130,169,175]. These results are consistent with data presented by Park et al., who showed impaired Ang II- induced vasoconstriction in aged, intact soleus and gastrocnemius first-order arterioles, but not in denuded soleus or gastrocnemius first-order arterioles[170]. The present study extends the findings of Park et al. to the soleus feed artery and also indicate that aged associated impairments of $\alpha 1$ and $\alpha 2$ adrenergic receptor-dependent vasoconstriction are also likely mediated by the endothelium.

Previously, Park et al. reported that in first-order arterioles, aged endothelium-dependent Ang II-impaired vasoconstrictor responses were likely due to the biphasic response of Ang II, the stimulation of the endothelium at the AT_1R and AT_2R to produce the vasodilator NO, and its stimulation of the vascular smooth muscle at AT1R receptor to induce

vasoconstriction[170,180,181,183-186]. In a similar manner to Ang II receptors, α adrenergic receptors are present on both endothelium and vascular smooth muscle. The α2 adrenergic receptor on the vascular smooth muscle stimulates the vascular smooth muscle to constrict; however, α2 adrenergic receptors are also present on the vascular endothelial cell and stimulate the production of an endothelial-derived relaxing factor[187]. The endothelial-derived relaxing factor, NO, not only serves as a vasodilator stimulus in competition with the vasoconstrictor stimulus, but also inhibits α2 adrenergic receptor-induced constriction[187]. In an effort to observe the vascular smooth muscle response alone, the endothelium from the SFA of the present study was removed, thus competition between endothelium derived- vasodilator and vasoconstrictor responses was removed. These data suggest that age-impaired vasoconstriction is endothelium-dependent and likely dependent on vasodilator and vasoconstrictor competition.

In the current study, denuded SFA were exposed to 1 h increased intraluminal pressure, to mimic the intraluminal pressure believed to be present during a bout of exercise. Contrary to our hypothoses, Ang II- and PE- induced vasoconstriction following the increased intraluminal pressure treatment was impaired, not improved, in old SFA (Fig. 4.2). The data from the current study demonstrates a similar response in SFA to existing data from first-order arterioles. In first-order arterioles, both age-increased Ang II- and α –adrenergic – induced vasoconstriction were reduced following aerobic exercise training[170,172]. NE stimulates both the α1 and α2 adrenergic receptor. PE stimulates the α1 adrenergic receptor. Following exposure to increased intraluminal pressure in old SFA, vasoconstriction to PE was impaired while NE-induced vasoconstriction remained similar to old P90 SFA (Fig 4.2). This may indicate a redundancy in NE-induced vasoconstriction that is not present in PE or ANG-II induced vasoconstriction which functions to preserve the vasoconstrictor response to NE following increased intraluminal pressure. This

study demonstrates that a short-duration exposure to an increased intraluminal pressure reduces vasoconstrictor function in aged SFA.

In order to identify a mechanism of reduced vascular smooth muscle constriction following exposure to an exercise-like increase in intraluminal pressure in aged arteries, vasoconstrictor function was assessed following incubation with a Rho inhibitor (Y27632) in aged, denuded SFA. In the presence of the Rho inhibitor, NE- and PE- induced vasoconstriction was reduced, and Ang II-induced vasoconstriction was abolished and not different than Ang II-induced constriction in old denuded P130 SFA (Fig. 4.3). These data indicate that the reduced vasoconstrictor function following exposure to 1 h increased intraluminal pressure is mediated by the Rho pathway. Aerobic exercise has been shown to reduce the expression of the RhoA gene and has also been suggested to inhibit the Rho signaling pathway[144,145]. The Rho pathway plays an integral role the mechano-transduction of force from the cytoskeleton to the extracellular matrix via actin fiber and focal adhesion formation and contributes to the phosphorylation of myosin light chains[140,142,143]. The data in the present study suggest that the reduction of vasoconstrictor responses following a short-duration increase in intraluminal pressure in denuded SFA is mediated by the Rho pathway.

In conclusion, results of this study indicate that vascular smooth muscle-dependent vasoconstriction is not impaired in aged SFA. Furthermore, pretreatment with 1 h intraluminal pressure, to mimic the pressure believed to be present during a bout of exercise, impairs vascular smooth muscle vasoconstrictor responses in the absence of the endothelium, and that this detrimental response is mediated, at least in part, through the Rho pathway. Collectively, these data suggest that the endothelium is an important target for the age-mitigating effects of a short-duration increase in intraluminal pressure as experienced during exercise.

CHAPTER V

REGULATION OF ENDOTHELIAL PHENOTYPE BY PRESSURE AND SHEAR STRESS IN AGED SKELETAL MUSCLE FEED ARTERIES

5.1 Introduction

Endothelial function declines with age[10,15,18,56,155,156]. The age-related decrements in endothelial function are characterized in part by impaired endothelium-dependent vasodilator responses. Celermajer et al. demonstrated that endothelium-dependent, flow-induced dilation is preserved throughout middle age but begins to decline in the early 40s for men and early 50s for women[188]. The age-related decline in endothelial function is an independent risk factor for the development of cardiovascular disease[157,158,189].

It has been proposed that the age-related decrement in endothelial function can result in reduced exercise hyperemia and a decrease in exercise tolerance[13,157]. Moderate, aerobic exercise can attenuate or reverse age-induced endothelial dysfunction in some arteries[25,26,56]. Hambrecht et al. documented improved, nitric oxide (NO) – dependent endothelial function in patients with cardiovascular disease following the completion of a regular physical exercise training program[159]. In addition, Haram et al. showed that endothelial function is improved for up to two days following just a single bout of exercise. The mechanism(s) responsible for improved endothelial function following physical exercise has yet to be fully elucidated; however, it has been proposed that short-duration increases in intraluminal pressure and shear stress associated with individual bouts of exercise may provide important mechanical signals that promote healthy endothelial function[26,29,30].

Woodman et al. reported previously that exposure of skeletal muscle feed arteries to increased intraluminal pressure or shear stress, for a duration of 4 hours, improved endothelium-dependent dilation in aged arteries[29,30]. Results from these studies are consistent with the interpretation that short-term increases in intraluminal pressure and shear stress promote healthy endothelial function. Seawright et al. previously demonstrated improved endothelium-dependent vasodilator responses in aged skeletal muscle feed arteries following a 1 h increase in intraluminal pressure[173], suggesting that the beneficial effect of pressure on endothelial function occurs within a duration of a typical exercise bout. While these two mechanical signals have been investigated separately, the question remains if pressure and shear stress interact to produce greater benefits than pressure or shear stress alone.

Therefore, the purpose of the present study was to test the hypothesis that exposure to a short-duration (1 h) increase in intraluminal pressure combined with increased shear stress (flow), to mimic two mechanical signals associated with a bout of exercise, would induce greater improvements of endothelium-dependent dilation in aged arteries than a short-duration increase in intraluminal pressure alone.

5.2 Methods

Animals

Approval for this study was provided by the Institutional Animal Care and Use Committee at Texas A&M University and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Fischer 344 rats (4 and 24 mo) were provided by the National Institute of Aging (NIA) and housed by the College of Veterinary Medicine through the Comparative Medicine Program at the facility for Laboratory Animal Resources and Research. Animals were provided food and water ad libitum and were maintained in a 12:12-h

light-dark cycle. Fischer 344 rats were selected for use in these studies because they remain free of atherosclerosis and hypertension[161].

Isolation of Feed Arteries

Soleus muscle feed arteries (SFA) were isolated as previously described [25,29,30,173]. Briefly, rats were anesthetized with an intraperitoneal injection of Ketamine (80 mg/kg body weight) and Xylazine (5 mg/kg body weight). After anesthetization, the soleus-gastrocnemius-plantaris muscle complex was dissected free from each hindlimb and placed in a cold (4 °C) MOPS-buffered physiological saline solution (PSS). The PSS (pH 7.4) was comprised of (in mM) 145.0 NaCl. 4.7 KCL, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 25.0 MOPS. Rats were euthanized by excising the heart. The SFA were isolated, excised, and transferred to a Lucite dissection chamber containing MOPS-PSS for cannulation.

Cannulation of Feed Arteries

SFA were cannulated on both ends with resistance-matched glass micropipettes and secured with surgical thread. The micropipettes were then attached to pressure reservoirs which contained MOPS-PSS supplemented with albumin (1g/mL). SFA were pressurized to 60 cmH_2O (1 mmHg = 1.36 cmH_2O) and checked for leaks. SFA with leaks were re-secured to eliminate the leak. Once a SFA was determined to be leak-free, the pressure reservoirs were raised to 90 cmH_2O or 130 cmH_2O for 1 h and the vessel chamber warmed to 37 ºC. Additional SFA were exposed to 130 cmH_2O pressure and simultaneously exposed to a flow gradient of 40 cmH_2O (65 µl/min). This flow gradient was achieved by raising one pressure reservoir by 20 cmH_2O and concurrently lowering the other pressure reservoir by an equal 20 cmH_2O. Intraluminal pressures of 90 cmH_2O and 130 cmH_2O correspond to the pressures believed to be present in the rat SFA

at rest and during exercise, respectively[160] . The 40 cmH_2O flow gradient was chosen based on previous publication by Woodman et al, describing improved endothelial function following 4 h exposure to a 40 cmH_2O flow gradient[29]. SFA baths were changed at 20 minute intervals during the 1 h treatment period and after the completion of each concentration response curve. Immediately following the 1 h treatment period, pressure in all SFA was set to at 90 cmH_2O with no flow (0 µl/min). SFA were allowed to develop 10 min of spontaneous, stable tone prior to the assessment of vasodilator responses.

Assessment of Vasodilator Responses

Vasodilator responses were assessed as previously described[29,30,173]. Briefly, endothelium-dependent, flow-induced dilation was assessed by raising and lowering the pressure reservoirs in equal and opposite directions. Flow-induced dilation was assessed at flow gradients of 0, 2, 4, 6, 8, 10, 15, 20 and 40 cm H_2O, which correspond to flow rates of 0-62 µl/min[164]. Endothelium-dependent dilation was assessed by adding increasing whole log increments of acetylcholine (ACh) from 10^{-9} – 10^{-4} M. Endothelium-independent dilation was assessed by adding increasing whole log increments from 10^{-9} – 10^{-4} M sodium nitroprusside (SNP), an exogenous NO donor. Lastly, to determine the maximal passive diameter, SFA were incubated in Ca^{2+}-free PSS for 60 min.

To determine the role of NO, a separate set of SFA was exposed to high pressure (130 cmH_2O) and high shear stress (40 cmH_2O flow gradient) for 1 h and vasodilator responses were assessed in the presence of N^{ω} –nitro-$_L$-arginine (L-NNA; 300 uM), a nitric oxide synthase inhibitor. SFA were incubated in L-NNA for 20 minutes prior to the assessment of vasodilator responses.

Immunoblot Analysis

p-eNOS^{ser1177} and total eNOS protein content were assessed in SFA as previously described[50,164,173]. p-eNOS^{ser1177} was assessed using a monoclonal antibody (1:250, BD Biosciences - 612393). The membrane was subsequently stripped with Thermo Restore Western Stripping Buffer and total eNOS was assessed with a monoclonoal antibody (1:1,250, BD Biosciences – 610297). All immunoblot data were expressed relative to GAPDH, which served as a loading control, and normalized to the Young P90 mean. GAPDH was assessed with a monoclonal antibody (1:10,000, Millipore - MAB374). Immunoblots were evaluated with enhanced chemiluminescence (ECL plus, Amersham) and assessed by densitometry using a LAS-4000 Luminescent Image Analyzer and Multi-Gauge Image Analysis Software (FUJIFILM Medical Systems). The ratio of p-eNOS^{ser1177} to total eNOS was used to quantify ser^{1177} phosphorylated eNOS protein content.

Statistical Analysis

Values are presented as means ± SE. Body weights and maximal passive diameters were analyzed by One-Way ANOVA. To determine whether dilator responses to flow, ACh, and SNP differed by group, statistical analysis was performed with Two-way ANOVA with repeated measures on one factor (concentration). Percent dilation was calculated as: $[(D_{conc} – D_B)/(D_P – D_B)] \times 100$. D_{conc} is the measured diameter for a given concentration or flow rate. D_B is the baseline diameter measured before the concentration response curve. D_P is the maximal passive diameter observed[25,29,30,173]. Total eNOS/GAPDH, p-eNOS^{ser1177}/GAPDH, and p-eNOS^{ser1177}/total eNOS ratios were determined using One-Way ANOVA. Statistical significance was set at $p \leq 0.05$.

5.3 Results

Animal Characteristics

Body weights were significantly lower in Young rats (352 ± 31 g) than Old rats (429 ± 26 g). Maximum diameters were not different between Young P90 (179 ± 40 μm), Old P90 (190 ± 28 μm), Old P130 (183 ± 29 μm) and Old P130 + HSS (195 ± 36 μm) vessels. The maximum diameters of the Old P130 + HSS + L-NNA group (112 ± 45 μm) were significantly smaller than all other vessel groups (Table 5.1).

Animal Characteristics		
Group	**Body Weight (g)**	**Maximal Passive Diameter (μm)**
Young P90	352 ± 31*	179 ± 40
Old P90	431 ± 38	190 ± 28
Old P130	440 ± 24	183 ± 29
Old P130 + HSS	427 ± 36	195 ± 36
Old P130 + HSS + L-NNA	419 ± 7	112 ± 45*

Table 5.1. Animal Characteristics. * Different from all other groups. n= 5-10; P≤ 0.05.

ACh-Induced Dilation

ACh-induced dilation was significantly lower in Old P90 SFA compared to Young P90 SFA (Fig.5.1).

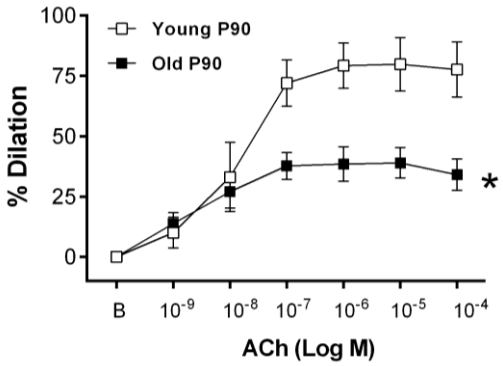

Figure 5.1. Effect of age on ACh- induced dilation in SFA. ACh-induced dilation was assessed in young and old SFA. ACh-induced dilation is impaired in Old SFA. B, baseline diameter before first dose of ACh. * Significantly different from Young P90. n= 6-10; $P \leq 0.05$.

Pre-treatment with increased intraluminal pressure (P130) or increased intraluminal pressure and shear stress (P130 + HSS) SFA for 1 h significantly improved but did not restore the maximal dilatory response to ACh in old SFA. Increased intraluminal pressure alone (P130) did not improve ACh-induced dilation in Old SFA (Fig. 5.2). Incubation with a NO inhibitor (L-NNA) impaired ACh-induced dilation in Old P130 + HSS SFA (Fig. 5.3).

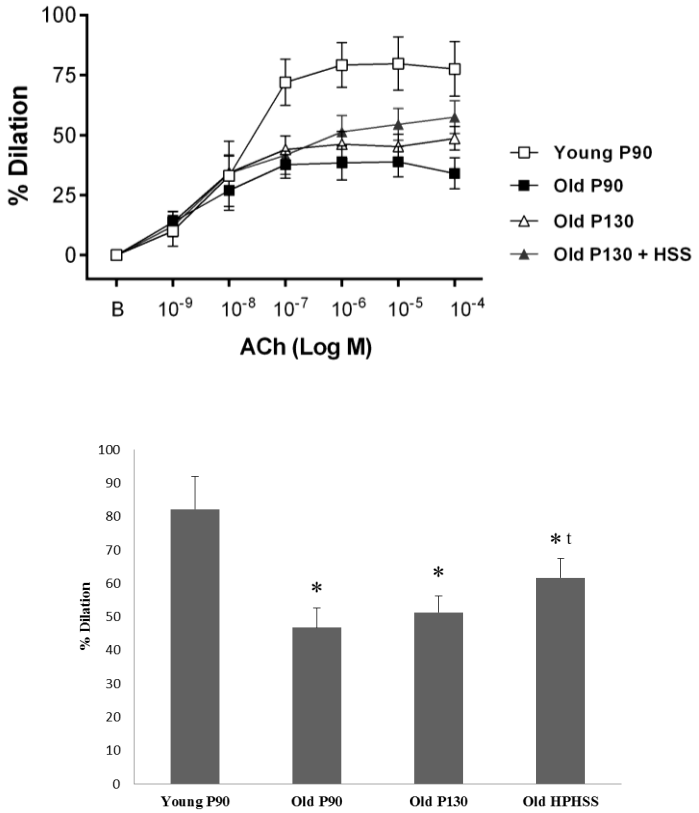

Figure 5.2. Effect of increased pressure, alone or in combination with increased shear stress, on ACh- induced dilation in SFA. ACh-induced dilaton was assessed following pre-treatment with 1 h of increased intraluminal pressure (P130) or increased intraluminal pressure and shear stress (P130 + HSS). Maximal dilation to ACh was improved, but not restored in Old SFA in the P130 + HSS group. B, baseline diameter before first dose of ACh. * Significantly different from Young P90, ^{t} Significantly different from Old P90. n= 6-10; $P \leq 0.05$.

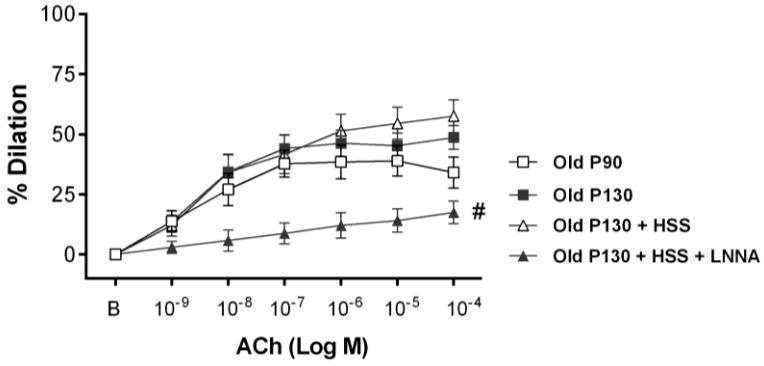

Figure 5.3. Effect of NOS inhibition on ACh-induced dilation in SFA. To determine the contribution of NO, ACh-induced dilation was assessed in the absence or presence of the nitric oxide synthase inhibitor, L-NNA (300 μM). The beneficial effect of the P130 + HSS pre-treatment was eliminated in the pressence of L-NNA. B, baseline diameter before the first dose of ACh. # Different from all other curves. n= 5-10; P≤ 0.05.

Flow-Induced Dilation

Flow-induced dilation was significantly impaired in Old P90 SFA relative to Young P90 SFA (Fig. 5.4). Pre-treatment with increased intraluminal pressure (P130) or increased intraluminal pressure and shear stress (P130 + HSS) restored flow-induced dilation such that it was no longer different than Young P90 flow-induced dilation (Fig. 5.5). Incubation with L-NNA eliminated flow-induced dilation in Old P130 + HSS SFA (Fig. 5.6).

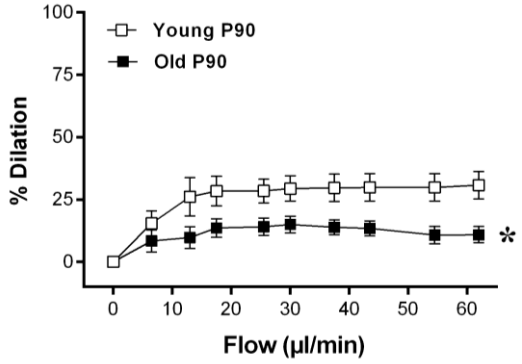

Figure 5.4. Effect of age on flow- induced dilation in SFA. Flow-induced dilation was assessed in young and old SFA. flow-induced dilation was impaired in Old SFA. * Significantly different from Young P90. n= 6-10; P≤ 0.05.

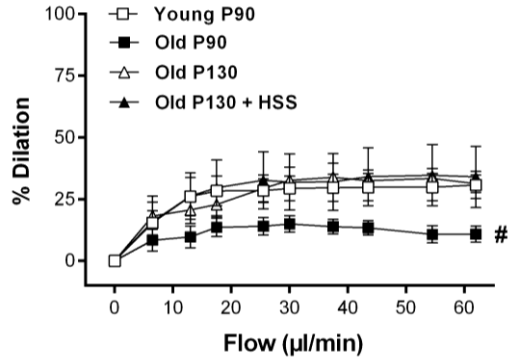

Figure 5.5. Effect of increased pressure, alone or in combination with increased shear stress, on flow- induced dilation in SFA. Flow-induced dilaton was assessed following pre-treatment with 1 h of increased intraluminal pressure (P130) or increased intraluminal pressure + shear stress (P130 + HSS). Flow-induced dilation was restored in both Old P130 SFA and Old P130 + HSS groups. # Significantly different from all other groups. n = 6-10; P≤ 0.05.

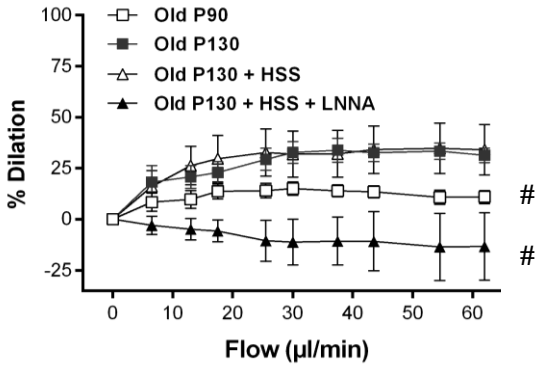

Figure 5.6. Effect of NOS inhibition on flow-induced dilation in SFA. To determine the contribution of NO to flow-induced dilation, flow-induced dilation was assessed in the absence or presence of the nitric oxide synthase inhibitor, L-NNA (300 µM). The beneficial effect of the P130 + HSS pre-treatment was blocked by L-NNA. # Different from all other curves. n= 5-10; P≤ 0.05.

SNP-Induced Dilation

SNP-induced dilation was similar in young an old SFA (Fig. 5.7). Pre-treatment with increased intraluminal pressure alone (P130) or increased intraluminal pressure and shear stress (P130 + HSS) for 1 h did not improve SNP-induced dilation in Old SFA (Fig. 5.8).

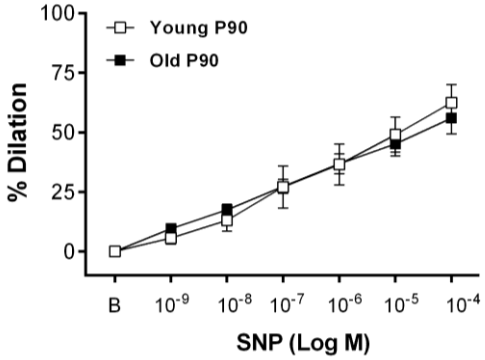

Figure 5.7. Effect of age on SNP- induced dilation in SFA. SNP-induced dilation was assessed in young and old SFA. B, baseline diameter before the first dose of SNP. n= 6-10. Statistical analysis revealed no significant differences between groups. n= 6-10; P≤ 0.05.

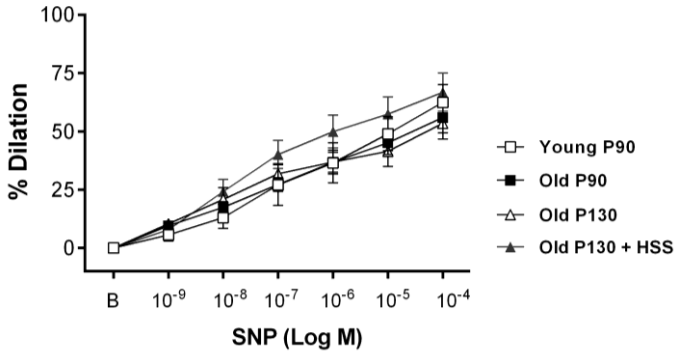

Figure 5.8. Effect of increased pressure, alone or in combination with increased shear stress, on SNP-induced dilation in SFA. SNP-induced dilaton was assessed following pre-treatment with 1 h of increased intraluminal pressure (P130) or increased intraluminal pressure and shear stress (P130 + HSS). B, baseline diameter before the first dose of SNP. Statistical analysis revealed no significant differences in SNP-induced dilation between young and old SFA. n= 6-10; $P \leq 0.05$.

p-eNOS/eNOS Protein Content

Immunoblot analysis revealed that total eNOS/GAPDH, p-eNOS^{ser1177}/GAPDH, nor p-eNOS^{ser1177}/eNOS ratio were altered by age or pressure treatment (Fig. 5.9).

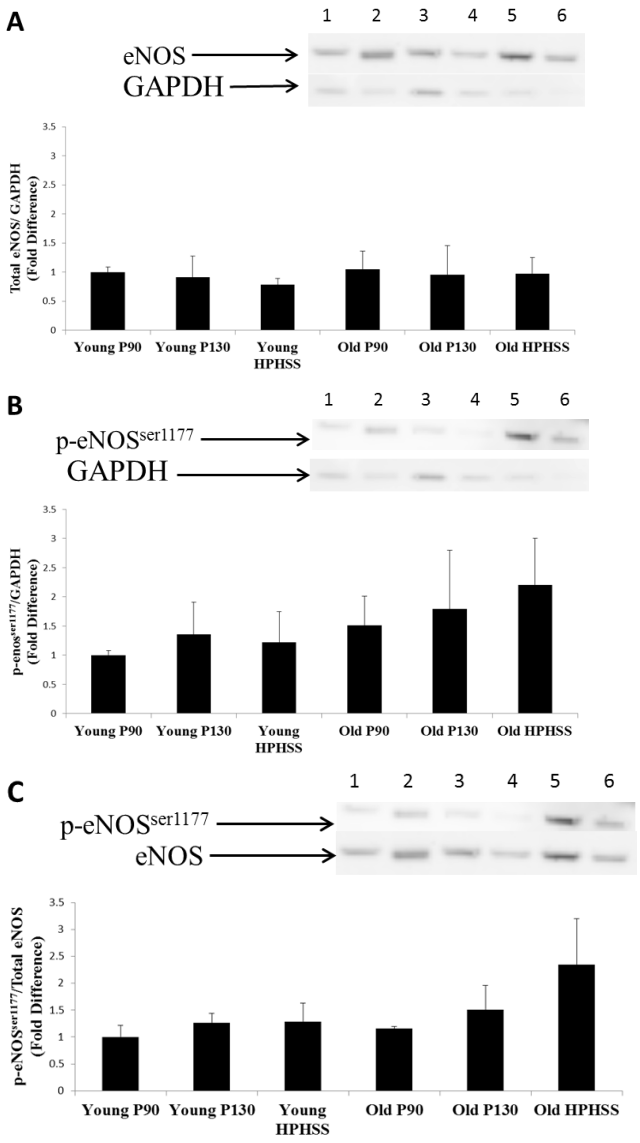

Figure 5.9. Immunoblot analysis for p-eNOS^{ser1177}, eNOS, GAPDH protein content in SFA. Statistical analysis revealed no significant differences in the ratio of eNOS/GAPDH (A), p-eNOS^{ser1177}/GAPDH (B), and p-eNOSser1177/total eNOS (C). 1, Young P90; 2, Young P130; 3, Young HPHSS; 4, Old P90; 5, Old P130; 6, Old HPHSS. Values are means ± SE normalized to Young P90 mean. n= 2-3; P≤ 0.05.

5.4 Discussion

The purpose of this study was to test the hypothesis that exposure to a short-duration (1 h) increase in intraluminal pressure combined with increased shear stress (flow rate), to mimic two mechanical signals associated with a 1 h bout of exercise, would induce greater improvements of endothelium-dependent dilation than a short-duration increase in intraluminal pressure alone. The primary findings of this study are as follows: 1) Endothelium-dependent dilation to flow and ACh was impaired with age. 2) Exposure to a short-duration (1 h) increase in intraluminal pressure alone or in combination with increased shear stress improved endothelium-dependent dilation to flow. Exposure to a combination of increased intraluminal pressure and shear stress for 1 h improved maximal dilation to ACh. Contrary to our hypothesis, the combination of increased intraluminal pressure and shear stress did not produce greater benefits than increased intraluminal pressure alone. 3) The improvements in age-impaired endothelial function following a short-duration (1 h) increase in intraluminal pressure and shear stress were reduced in the presence of the nitric oxide synthase inhibitor, L-NNA, which indicates the beneficial response was mediated, at least in part, by NO.

In the present study endothelium-dependent dilation was impaired in aged SFA (Fig. 5.1, 5.4), which is in agreement with previous published studies[15,18,156,173]. Consequently, the aged arteries were suitable for experiments to determine whether treatment with short-duration increases in intraluminal pressure and shear stress improve or restore healthy endothelial phenotype. One novel finding of this study was that exposure to a short-duration increase in intraluminal pressure restored flow-induced dilation in old SFA such that the flow response was no longer different than Young P90 (Fig. 5.5), but did not improve ACh-induced dilation in old SFA (Fig. 5.2).

The combined increased intraluminal pressure and increased shear stress for 1 h improved the maximal dilation to ACh compared to Old P90, was not different than Old P130 (Fig. 5.2), and restored flow-induced dilation so that it was no longer different than Young P90 (Fig. 5.5). The improvement of ACh-induced dilation following the combination of increased intraluminal pressure and shear stress indicates that pressure and shear stress did not interact to produce greater benefits than increased intraluminal pressure alone on receptor-mediated dilation. Previously, Woodman et al. demonstrated that exposure to increased shear stress for 4 h restored ACh-induced dilation in Old SFA so that it is comparable to Young SFA[29]. Collectively, these data suggest that maintenance of healthy ACh-dependent dilation responses may be more dependent on exposure to increased shear stress than to increased intraluminal pressure.

Treatment of aged SFA with increased intraluminal pressure, alone or in combination with increased shear stress, fully restored flow-induced dilation in aged, SFA so that it was no longer different than Young P90 SFA (Fig. 5.5). However, there was no additional benefit to flow-induced dilation in the P130 + HSS group compared to the P130 group alone (Fig. 5.5). Previously, exposure to increased intraluminal pressure for durations of 1 and 4 h restored flow-induced dilation in aged SFA[30,173]. The present data suggest that exposure to increased intraluminal pressure alone is sufficient to provide that maximum benefit to flow-induced dilation.

NO is a critical endothelial-derived signaling molecule contributing to healthy endothelial-dependent dilation[8]. NO-mediated dilation is impaired with age and has been shown to be improved with exercise, after exposure to 1 h and 4 h increased intraluminal pressure, and after 4 h exposure to increased shear stress[18,25,29,30,173]. In an effort to understand the role of NO in improved endothelial function following exposure to a combination of increased intraluminal

pressure and shear stress for the duration of 1 h, vasodilator responses were assessed in the presence of the nitric oxide synthase inhibitor L-NNA. The improvements in both ACh- and flow-induced dilation following exposure to P130 + HSS were abolished following incubation with the nitric oxide synthase inhibitor L-NNA (Fig. 5.3, 5.6), indicating that the improved endothelial-function is mediated by NO.

In summary, results of this study show that the maximal ACh-induced dilation was improved in aged SFA following exposure to a 1 h increase in both intraluminal pressure and shear stress; and that age-impaired, flow-induced dilation is restored following exposure to a 1 h increase in intraluminal pressure alone. Collectively, these data indicate that increased intraluminal pressure alone or in combination with increased shear stress improves endothelium-dependent dilation in aged SFA. However, contrary to our hypothesis, increases in pressure and shear stress did not interact to produce greater benefits that increased intraluminal pressure alone. The improvements in aged endothelial phenotype appear to be mediated by NO.

CHAPTER VI

SUMMARY AND CONCLUSIONS

6.1 Summary and Conclusions

The purpose of this dissertation was to determine the importance of intraluminal pressure and shear stress in the regulation of vascular endothelial and vascular smooth muscle cell phenotype in aged arteries.

The purpose of the first study was to test the hypothesis that exposure to a short-duration increase in intraluminal pressure, to mimic mean arterial pressure believed to be present in soleus muscle feed arteries during a bout of exercise, improves NO-mediated endothelium-dependent dilation. The novel findings of this study were: 1) Treatment with increased pressure for 1 h improved ACh-and flow-induced dilation in old SFA. 2) The beneficial effects of pressure treatment on ACh- and flow-induced dilation in old SFA were abolished in the presence of L-NNA and were not present after a 2 h recovery period. 3) Treatment with increased pressure for 1 h improved SNP-induced dilation in old and young SFA. 4) The beneficial effect of pressure treatment on SNP-induced dilation was not present following a 2 h recovery period at normal pressure. These results indicate that exposure to 1 h increased intraluminal pressure, as believed to be present during a bout of exercise, improves NO-mediated dilation and promotes a healthy endothelial phenotype in aged arteries. Furthermore, these results suggest that increased intraluminal pressure is one mechanical signal by which aerobic exercise improves age-impaired endothelial function.

The purpose of the second study was to test the hypothesis that exposure to a short-duration (1 h) increase in intraluminal pressure (130 cmH_2O), to mimic the pressure believed to be present in the soleus muscle feed artery during a bout of exercise, would attenuate age-induced impairments of vasoconstrictor responses in the soleus muscle feed arteries via the Rho pathway.

The findings of this study were as follows: 1) Constrictor responses to NE, Ang II, and PE were impaired in old P90 SFA. 2) Short-duration exposure of aged SFA to increased intraluminal pressure, to mimic the pressure believed to be present during a bout of exercise, improved NE- and PE-induced vasoconstrictor responses, and restored Ang II-induced vasoconstrictor responses such that the responses in Old P130 SFA were not different from Young P90 vasoconstrictor responses. 3) The beneficial effect of increased intraluminal pressure on vasoconstrictor responses in aged arteries were eliminated in the presence of a ROCK inhibitor (Y27632). 4) Activation of ROCK with LPA restored Old P90 Ang II vasoconstrictor responses so that they were no longer different than Young P90 vasoconstrictor responses. Collectively, these data indicate that a short-duration increase in intraluminal pressure, as believed to occur during a bout of exercise, is an important mechanical signal for exercise-induced, improvements in vasoconstrictor function in aged SFA via the Rho pathway.

The purpose of the third study was determine whether the beneficial effect of exposure to an increased intraluminal pressure on aged vasoconstrictor responses was mediated by enhanced vascular smooth muscle function. We tested the hypothesis that a short-duration (1 h) increase in intraluminal pressure, to mimic the pressure believed to be present during a typical exercise bout, would improve vascular smooth muscle constriction in aged, denuded SFA, and

that the potential benefit of increased intraluminal pressure on vascular smooth muscle constriction would be mediated by the Rho pathway.

The novel findings of the third study were as follows: 1) Vascular smooth muscle constrictor responses were not different between young and old denuded P90 SFA. 2) Exposure to 1 h increased intraluminal pressure impaired Ang II and PE vasoconstrictor function in old denuded P130 SFA. 3). In denuded old P90 SFA, incubation with a Rho inhibitor (Y27632) impaired NE- and abolished PE- induced vasoconstriction. Incubation with a Rho inhibitor eliminated Ang II-induced vasoconstriction in old P90 SFA so that it was no longer different than Ang II-induced constriction in old denuded P130 SFA. Collectively, these data indicate that a short-duration increase in intraluminal pressure, as believed to be present during exercise, does not improve vascular smooth muscle cell constrictor responses in aged arteries, and that the improved vasoconstrictor responses following exposure to increased intraluminal pressure in intact arteries is endothelium-dependent.

The lack of an age difference in SFA constrictor responses in denuded SFA is a novel finding and underscores the importance of the endothelium. A decline in endothelial function is associated with an increased risk for cardiovascular disease and a decrease in exercise tolerance[43,45,190]. These data indicate that the impairment of vasoconstrictor responses, which may lead to orthostatic intolerance[191], are not a consequence of impaired smooth muscle function, but are a result of an impairment of the endothelium to provide the appropriate vasoconstrictor signal. Taken together with results from study 2, these data indicate that the beneficial effect of pressure on constrictor responses in aged arteries is endothelium-dependent, and that the beneficial effect of aerobic exercise on the endothelium promotes both a healthy vasodilator and vasoconstrictor function.

In the final study of this dissertation we tested the hypothesis that exposure to a short-duration (1 h) increase in intraluminal pressure combined with increased shear stress (flow rate), to mimic two mechanical signals associated with a 1 h bout of exercise, would induce greater improvements of endothelium-dependent dilation than a short-duration increase in intraluminal pressure alone. The major findings of this study were as follows: 1) Endothelium-dependent dilation was impaired with age. 2) Age-impaired endothelial function was improved following exposure to a short-duration (1 h) increase in intraluminal pressure alone or in combination with increased shear stress. 3) The beneficial improvements in age-impaired endothelial function following a short-duration (1 h) increase in intraluminal pressure and shear stress were mediated, in part, by NO. Collectively, these data suggest that the intraluminal pressure increase is sufficient to improve the function of the aged endothelium. While shear-stress has been shown to improve endothelial function[29], the lack of an additional benefit of shear-stress in conjunction with increased intraluminal pressure to improve endothelial function may suggest a redundancy in the mechanism(s) of improved endothelial function in aged arteries following exercise such that increased intraluminal pressure or increased shear stress, both of which occur during exercise, is sufficient to improve aged endothelial function and ensure a vascular benefit to exercise.

Collectively, the data presented in this dissertation are consistent with the interpretation that short-duration increases in intraluminal pressure that occur during exercise promote healthy vascular function in aged arteries through enhanced vasodilator and vasoconstrictor function[26,30,173]. The data in the studies 1 and 4 illustrated a NO-dependent mechanism for enhanced endothelium-dependent dilation following exposure to a short-duration increase in intraluminal pressure. Studies 2 and 3 documented an endothelium-dependent mechanism for enhanced Rho-mediated vasoconstrictor function following exposure to a short-duration increase

in intraluminal pressure. The mechanism by which the vasoconstrictor signal is transmitted from the endothelium to the vascular smooth muscle remains unknown.

There is some indication that catecholamines, like NE, may play a role in vasoconstriction by endothelin-1. NE-induced contraction is potentiated by endothelin-1[192,193]. The potentiation effect of endothelin-1 on NE-induced contraction has been observed in old, not young, rat mesenteric arteries[194]; indicating that some component of endothelin-1 pathway is susceptible to alteration with age. Additionally, Ang II has been shown to be a stimulant of superoxide (O_2^-) production through NAD(P)H in both vessel and cell culture studies and also through eNOS uncoupling[19,134,195,196]. O_2^- increases Ca^{2+} sensitization through RhoA and may serve as a pathway through which Ang II-mediated constriction is impaired in aged arteries following exposure to increased intraluminal pressure [197].

One proposed mechanism of age-impaired vascular function centers on the role reactive oxygen species (O_2^-) and NO bioavailability[17]. NO is a major endothelium-derived signaling molecule for inducing vasodilation and is degraded by O_2^-, which is produced in part by uncoupled eNOS. Sindler et al. demonstrated that aerobic exercise training reduced eNOS uncoupling and improved endothelial dependent dilation in arterioles[21]. Interestingly, Sindler et al. also showed that O_2^- is necessary, on some level, for endothelium-dependent dilation[21]. O_2^- increases with age and impairs vasodilator function and promotes vasoconstriction, yet is also a component of vasodilation[21,136,139,198,199]. Therefore, a balance must exist between vasoconstrictor and vasodilator influences that promote healthy vascular function (Fig. 6.1A). Kellogg et al. suggested that during exercise, competition exists between vasoconstrictor and vasodilator pathways[200], making the balance between vasoconstriction:vasodilation important for determining the net vascular response; if the balance swings too far in one direction, there is an

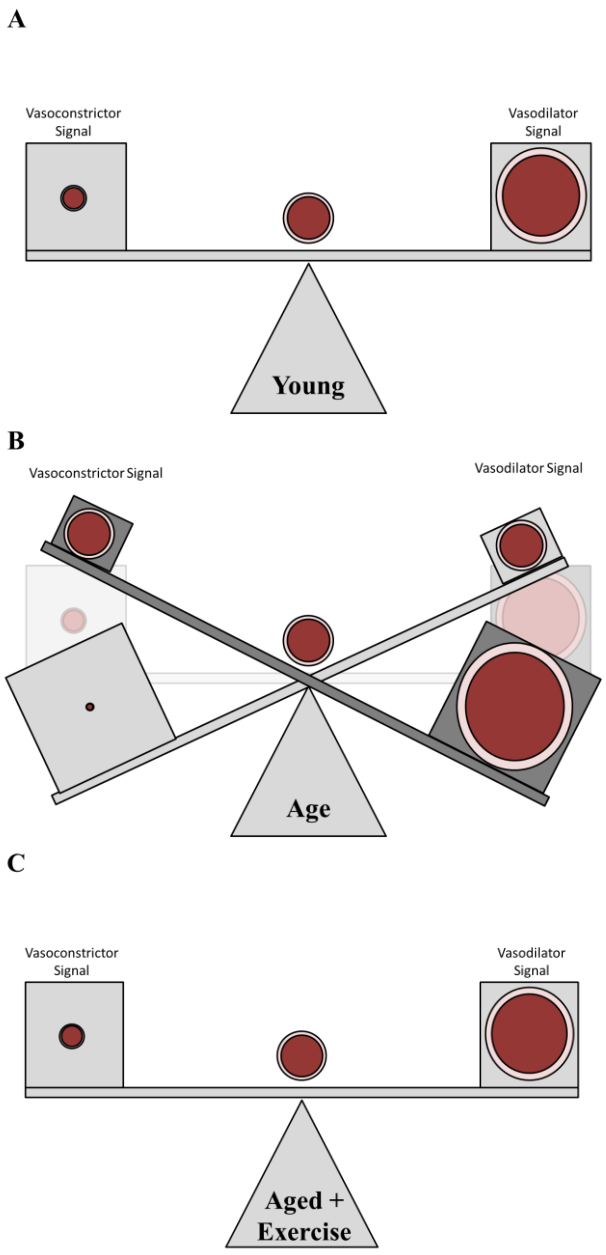

Figure 6.1. Illustration of the vasoconstrictor signal vs. the vasodilator signal balance with age and exercise. Ideal balance between the vasoconstrictor and vasodilator signals in young (A) healthy vessels. Aging (B) disrupts the balance between vasoconstrictor and vasodilator signals. Exercise (C) promotes a healthy vasodilator and vasoconstrictor balance in aged vessels.

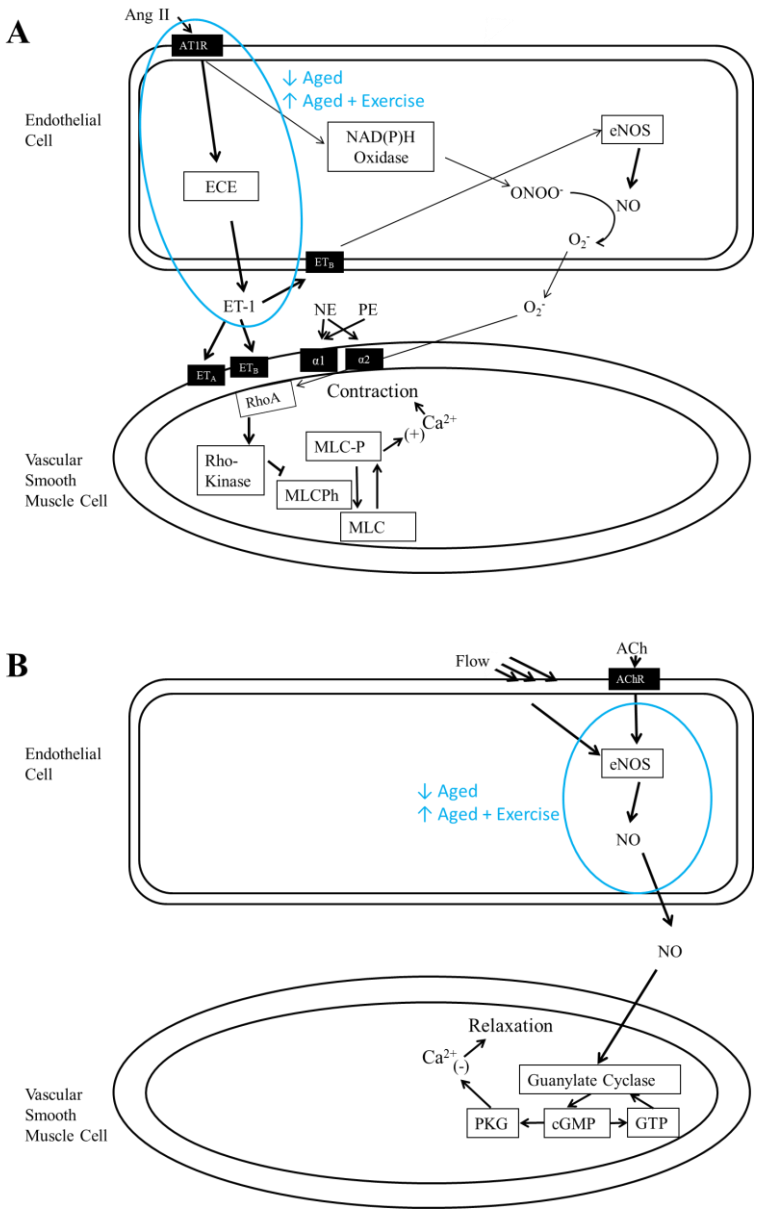

Figure 6.2. Proposed model for the effect of a short-duration increase in intraluminal pressure on vasodilator and vasoconstrictor function.

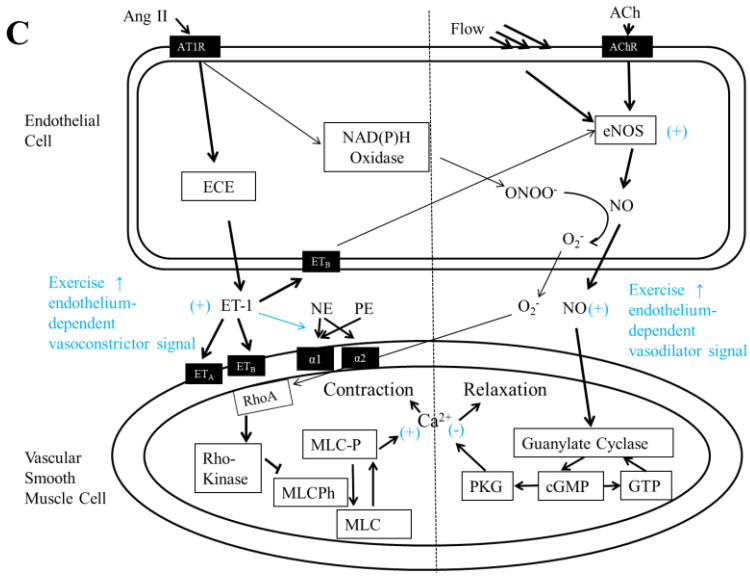

Figure 6.2. (Continued). A short-duration increase in intraluminal pressure improves age-impaired, endothelium-mediated Rho-dependent vasoconstriction, through the endothelin-1 pathway (A). A short-duration increase in intraluminal pressure improves age-impaired endothelium-mediated vasodilation, through the NO pathway (B). A combined model showing the proposed effect of a short-duration increase in intraluminal pressure on vasodilator and vasoconstrictor responses in aged SFA (C). A short-duration increase in intraluminal pressure improved NO-mediated vasodilator function and improved Rho-mediated vasoconstrictor function in aged SFA. A short-duration increase in intraluminal pressure improved aged vasodilator function by increasing NO bioavailability. NO stimulates smooth muscle guanylate cyclase to produce cGMP, which stimulates PKG and decreases Ca^{2+} concentration within the smooth muscle cell to induce relaxation. Aged vasoconstrictor function is improved following a short-duration increase in intraluminal pressure through an endothelium mediator, endothelin-1, that stimulates endothelin recepters A and B and potentiates catecholamine-induced (NE, PE) contraction through a Rho-dependent mechanism. RhoA and its associated Rho Kinase inhibit myosin light chain phosphatase, which increase phospohorylated myosin light chain and increase Ca^{2+} within the smooth muscle cell to induce vasoconstriction. Abbreviations: Acetylcholine Receptor (AChR), α1 Adrenergic receptor (α1), α2 Adrenergic receptor (α2), Angiotensin Receptor 1 (AT1R), Cyclic Guanosine Monophosphate (cGMP), Endothelin Converting Enzyme (ECE), Endothelin-1 (ET-1), Endothelin Receptor A (ET_A), Endothelin Receptor B (ET_B), Endothlial Nitric Oxide Synthase (eNOS), Guanosine Triphosphate (GTP), Myosin Light Chain (MLC), Myosin Light Chain Phosphatase (MLCPh), Phosphorylated Myosin Light Chain (MLC-p), Protein Kinase G (PKG).

unfavorable outcome (Fig 6.1B). It is likely that aerobic exercise is an effective intervention to restore vasoconstrictor:vasodilator balance in the aged population (Fig. 6.1C).

Results from the studies contained within this dissertation illustrate that increased intraluminal pressure is an important mechanical signal associated with exercise that promotes proper vasoconstrictor:vasodilator balance and healthy vascular function (Fig 6.2); thus improving age-induced vascular dysfunction, increasing exercise tolerance in the elderly, and reducing the risk and detrimental effects of cardiovascular disease.

6.2 Limitations

Several limitations must be considered when interpreting these studies. First, the pressure treatment used in these studies was constant (static). *In vivo,* intraluminal pressure is dynamic and oscillates across a range of pressures associated with the systole and diastole of the cardiac cycle. Secondly, the isolated cannulated artery technique used in this dissertation is an *ex vivo* technique, which allowed the determination of the effect of a short-duration increased intraluminal pressure on aged SFA function without systemic influence; however, there may be a component of age-improved vascular function that is dependent on a hormonal or systemic response. Finally, the soleus muscle feed artery was chosen, in part, for its documented history of age-impaired vascular function and improved vascular function following aerobic exercise, which make it an ideal artery for determining the mechanism(s) of exercise improved vascular function. It should be noted, however, that because of the heterogeneous nature between arteries, the mechanical signaling, and the effect of exercise on vascular function, may be different between arteries throughout the vascular tree.

6.3 Clinical Relevance

Cardiovascular disease is the primary cause of death in the United States. Consequently, developing strategies that lead to the prevention and/or treatment of cardiovascular disease are imperative[1]. Vascular dysfunction occurs with age and is an independent risk factor for cardiovascular disease.[43-48,188]. Though the mechanism(s) are yet to be fully elucidated, it has become apparent that aerobic exercise is an effective therapeutic modality for preventing and treating aged vascular dysfunction, and reduces the risk of cardiovascular disease[26,27]. The results from the studies in this dissertation show that a short-duration increase in intraluminal pressure improves age-impaired vasodilator and vasoconstrictor function and suggests that increased intraluminal pressure is one mechanical signal by which aerobic exercise improves age-impaired vascular function. Determining the mechanism(s) by which aerobic exercise improves vascular function will aid in the development of the most effective exercise prescriptions to treat and prevent cardiovascular disease ultimately reducing the incidence of cardiovascular disease, improving the quality of life of those whom suffer from cardiovascular disease, and alleviating devastating effect of cardiovascular disease on the individual and the family.

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APPENDIX A CHAPTER II RAW DATA

Chapter II - Animal and Vessel Characteristics: ACh-Induced Dilation

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J39	Young P90	342.0	172.0	44.2
J41	Young P90	351.0	234.0	27.8
J42	Young P90	346.0	168.0	43.5
J43	Young P90	336.0	150.0	31.3
J44	Young P90	369.0	145.0	44.1
J46	Young P90	347.0	141.0	53.2
J51	Young P90	361.0	134.0	51.5
J52	Young P90	394.0	170.0	50.6
J55	Young P90	346.0	168.0	28.0
J65	Young P90	388.0	198.0	40.9
	Mean	358.0	168.0	41.5
	Standard Error	6.3	9.4	3.0

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J39	Young P130	342.0	185.0	43.8
J41	Young P130	351.0	197.0	35.5
J44	Young P130	369.0	179.0	44.1
J51	Young P130	361.0	182.0	37.4
J52	Young P130	394.0	165.0	46.1
J53	Young P130	339.0	110.0	58.2
J63	Young P130	381.0	170.0	38.8
J65	Young P130	388.0	156.0	35.9
	Mean	365.6	168.0	42.5
	Standard Error	7.4	9.4	2.7

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
J48	Old P90	488	168	35.71428571
J50	Old P90	353	123	25.20325203
J56	Old P90	502	179	46.36871508
J57	Old P90	447	129	34.10852713
J58	Old P90	420	86	33.72093023
J59	Old P90	486	220	33.18181818
J61	Old P90	462	216	43.98148148
J69	Old P90	370	149	25.5033557
J72	Old P90	452	153	30.71895425
J73	Old P90	387	176	36.36363636
J80	Old P90	428	168	33.92857143
J81	Old P90	469	211	41.70616114
J82	Old P90	444	199	46.73366834
J84	Old P90	425	145	42.06896552
J87	Old P90	384	176	65.05681818
J88	Old P90	481	225	49.77777778
J90	Old P90	475	197.5	31.89873418
	Mean	439.5882353	171.7941176	38.59033251
	Standard Error	10.82686569	9.260631691	2.401405229

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J45	Old P130	424.0	146.0	44.5
J47	Old P130	426.0	206.0	30.1
J50	Old P130	353.0	147.0	25.2
J56	Old P130	502.0	179.0	55.9
J57	Old P130	447.0	182.0	29.7
J58	Old P130	420.0	188.0	39.4
J60	Old P130	428.0	105.0	25.7
J61	Old P130	462.0	207.0	36.7
J67	Old P130	332.0	157.0	21.7
J68	Old P130	367.0	155.0	32.3
J80	Old P130	428.0	236.0	55.1
J81	Old P130	469.0	161.0	26.7
J82	Old P130	444.0	164.0	42.7
J83	Old P130	352.0	168.0	50.6
J84	Old P130	425.0	174.0	32.2
J87	Old P130	384.0	180.0	30.0
J88	Old P130	481.0	193.0	52.8
J89	Old P130	433.0	158.5	48.9
J90	Old P130	475.0	226.0	46.9
	Mean	423.8	175.4	38.3
	Standard Error	10.8	7.0	2.6

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J80	Old P130 + L-NNA	428.0	187.0	55.6
J81	Old P130 + L-NNA	469.0	165.0	90.3
J82	Old P130 + L-NNA	444.0	167.0	37.1
J86	Old P130 + L-NNA	412.0	150.0	68.7
J86	Old P130 + L-NNA	412.0	217.0	46.1
J87	Old P130 + L-NNA	384.0	168.0	66.1
	Mean	424.8	175.7	60.6
	Standard Error	12.0	9.6	7.7

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J40	Old P130 + recovery	458.0	189.0	28.0
J48	Old P130 + recovery	488.0	181.0	38.7
J50	Old P130 + recovery	353.0	177.0	25.4
J56	Old P130 + recovery	502.0	190.0	39.5
J57	Old P130 + recovery	447.0	142.0	25.4
J58	Old P130 + recovery	420.0	170.0	44.7
J59	Old P130 + recovery	486.0	234.0	26.1
J61	Old P130 + recovery	462.0	215.0	31.2
	Mean	452.0	187.3	32.4
	Standard Error	16.9	9.9	2.7

Chapter II- Raw Vasodilator Responses: ACh-Induced Dilation

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J39	Young P90	0.0	7.9	19.7	55.3	65.8	60.5	60.5
J41	Young P90	0.0	0.0	13.8	27.7	27.7	32.3	32.3
J42	Young P90	0.0	11.0	42.5	75.3	79.5	75.3	75.3
J43	Young P90	0.0	29.8	31.9	100.0	85.1	85.1	80.9
J44	Young P90	0.0	0.0	32.8	89.1	93.8	93.8	93.8
J46	Young P90	0.0	28.0	64.0	88.0	86.7	77.3	77.3
J51	Young P90	0.0	18.8	50.7	75.4	79.7	82.6	82.6
J52	Young P90	0.0	48.8	84.9	90.7	91.9	91.9	91.9
J55	Young P90	0.0	8.5	19.1	55.3	48.9	48.9	34.0
J65	Young P90	0.0	0.0	8.6	12.3	22.2	28.4	48.1
	Mean	0.0	15.3	36.8	66.9	68.1	67.6	67.7
	Standard Error	0.0	5.1	7.6	9.1	8.3	7.6	7.2

Animal	Group	Start	10-9 M	10-8 M	10-7 M	10-6 M	10-5 M	10-4 M
J39	Young P130	0.0	0.0	32.1	77.8	86.4	86.4	86.4
J41	Young P130	0.0	11.4	62.9	47.1	81.4	81.4	47.1
J44	Young P130	0.0	69.6	69.6	86.1	91.1	91.1	91.1
J51	Young P130	0.0	0.0	-11.8	-11.8	-19.1	-11.8	13.2
J52	Young P130	0.0	27.6	73.7	80.3	84.2	84.2	84.2
J53	Young P130	0.0	20.3	26.6	26.6	31.3	31.3	31.3
J63	Young P130	0.0	6.1	43.9	57.6	57.6	57.6	57.6
J65	Young P130	0.0	0.0	80.4	57.1	85.7	85.7	85.7
	Mean	0.0	16.9	47.2	52.6	62.3	63.2	62.1
	Standard Error	0.0	8.4	10.9	11.5	13.6	12.9	10.4

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J48	Old P90	0.0	5.0	5.0	20.0	20.0	28.3	28.3
J50	Old P90	0.0	0.0	0.0	0.0	25.8	35.5	61.3
J56	Old P90	0.0	4.8	12.0	32.5	41.0	41.0	32.5
J57	Old P90	0.0	0.0	0.0	-11.4	22.7	27.3	15.9
J58	Old P90	0.0	20.7	20.7	20.7	31.0	37.9	37.9
J59	Old P90	0.0	0.0	16.4	23.3	30.1	32.9	37.0
J61	Old P90	0.0	0.0	5.3	10.5	10.5	10.5	10.5
J69	Old P90	0.0	18.4	18.4	26.3	26.3	26.3	26.3
J72	Old P90	0.0	0.0	-17.0	-2.1	-2.1	21.3	21.3
J73	Old P90	0.0	0.0	9.4	39.1	39.1	18.8	18.8
J80	Old P90	0.0	0.0	61.4	61.4	61.4	61.4	49.1
J81	Old P90	0.0	0.0	-3.4	-13.6	-19.3	-18.2	-18.2
J82	Old P90	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J84	Old P90	0.0	0.0	23.0	23.0	85.2	93.4	93.4
J87	Old P90	0.0	0.0	-2.5	-6.2	-6.2	-6.2	-6.9
J88	Old P90	0.0	0.0	44.6	50.9	69.6	73.2	68.8
J90	Old P90	0.0	11.6	25.4	41.0	46.8	46.8	46.8
	Mean	0.0	3.6	12.9	18.6	28.4	31.2	30.8
	Standard Error	0.0	1.6	4.6	5.4	6.7	6.8	6.8

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J45	Old P130	0.0	20.0	46.2	46.2	46.2	41.5	41.5
J47	Old P130	0.0	-8.1	1.6	61.3	72.6	72.6	72.6
J50	Old P130	0.0	0.0	0.0	2.7	18.9	21.6	43.2
J56	Old P130	0.0	7.0	62.0	74.0	74.0	62.0	62.0
J57	Old P130	0.0	0.0	0.0	0.0	18.5	31.5	5.6
J58	Old P130	0.0	12.2	35.1	44.6	51.4	51.4	24.3
J60	Old P130	0.0	25.9	33.3	33.3	33.3	33.3	37.0
J61	Old P130	0.0	9.2	9.2	30.3	44.7	51.3	53.9
J67	Old P130	0.0	0.0	0.0	20.6	20.6	20.6	20.6
J68	Old P130	0.0	10.0	16.0	50.0	50.0	46.0	46.0
J80	Old P130	0.0	13.1	41.5	41.5	56.9	55.4	51.5
J81	Old P130	0.0	0.0	7.0	30.2	30.2	37.2	37.2
J82	Old P130	0.0	0.0	-5.7	68.6	68.6	47.1	47.1
J83	Old P130	0.0	62.5	87.5	87.5	87.5	87.5	87.5
J84	Old P130	0.0	10.7	10.7	10.7	10.7	10.7	10.7
J87	Old P130	0.0	22.2	22.2	22.2	29.6	29.6	29.6
J88	Old P130	0.0	0.5	10.3	12.9	16.0	16.0	16.0
J89	Old P130	0.0	10.1	55.0	58.8	60.6	76.8	77.9
J90	Old P130	0.0	0.0	84.9	84.9	84.9	84.9	84.9
	Mean	0.0	10.9	27.2	41.1	46.1	46.2	44.7
	Standard Error	0.0	3.6	6.6	6.1	5.6	5.2	5.6

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J80	Old P130 + L-NNA	0.0	10.6	16.3	26.0	26.0	26.0	26.0
J81	Old P130 + L-NNA	0.0	0.0	4.8	4.8	4.8	4.8	4.8
J82	Old P130 + L-NNA	0.0	0.0	0.0	20.8	20.8	20.8	20.8
J86	Old P130 + L-NNA	0.0	0.0	8.7	8.7	8.7	9.7	9.7
J86	Old P130 + L-NNA	0.0	0.0	0.0	6.0	8.0	8.0	8.0
J87	Old P130 + L-NNA	0.0	0.0	0.0	2.7	2.7	-0.9	-0.9
	Mean	0.0	1.8	5.0	11.5	11.8	11.4	11.4
	Standard Error	0.0	1.8	2.7	3.9	3.8	4.1	4.1

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J40	Old P130 + recovery	0.0	5.7	5.7	15.1	15.1	15.1	-1.9
J48	Old P130 + recovery	0.0	24.3	31.4	54.3	54.3	51.4	32.9
J50	Old P130 + recovery	0.0	13.3	13.3	13.3	13.3	13.3	15.6
J56	Old P130 + recovery	0.0	8.0	12.0	33.3	33.3	30.7	21.3
J57	Old P130 + recovery	0.0	16.7	16.7	16.7	16.7	16.7	16.7
J58	Old P130 + recovery	0.0	18.4	57.9	57.9	80.3	92.1	75.0
J59	Old P130 + recovery	0.0	19.7	36.1	39.3	41.0	42.6	42.6
J61	Old P130 + recovery	0.0	0.0	6.0	25.4	25.4	25.4	7.5
	Mean	0.0	13.3	22.4	31.9	34.9	35.9	26.2
	Standard Error	0.0	2.9	6.4	6.2	8.2	9.3	8.5

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Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
J39	Young P90	342	172	54.1
J41	Young P90	351	234	35.9
J43	Young P90	336	150	28.0
J44	Young P90	369	145	37.2
J46	Young P90	347	141	33.3
J51	Young P90	361	134	53.0
J52	Young P90	394	170	25.9
J53	Young P90	339	204	34.8
J55	Young P90	346	168	32.7
J70	Young P90	324	192	91.7
	Mean	350.9	171.0	42.7
	Standard Error	6.2	9.9	6.2

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
J39	Young P130	342.0	186.0	53.8
J41	Young P130	351.0	197.0	34.5
J43	Young P130	336.0	155.0	25.2
J44	Young P130	369.0	179.0	54.2
J46	Young P130	347.0	149.0	29.5
J51	Young P130	361.0	182.0	52.2
J52	Young P130	394.0	165.0	26.1
J63	Young P130	381.0	170.0	72.4
	Mean	360.1	172.9	43.5
	Standard Error	7.1	5.7	6.0

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J45	Old P90	424.0	173.0	0.0
J48	Old P90	488.0	168.0	31.0
J56	Old P90	502.0	179.0	21.8
J57	Old P90	447.0	129.0	41.9
J58	Old P90	420.0	86.0	25.6
J61	Old P90	426.0	216.0	57.4
J67	Old P90	332.0	179.0	84.9
J69	Old P90	370.0	149.0	26.2
J72	Old P90	452.0	153.0	58.8
J73	Old P90	387.0	176.0	40.3
J80	Old P90	428.0	168.0	32.1
J82	Old P90	469.0	161.0	16.1
J81	Old P90	444.0	164.0	22.0
J84	Old P90	425.0	145.0	24.8
J87	Old P90	384.0	176.0	37.5
J88	Old P90	481.0	225.0	33.8
J90	Old P90	475.0	189.0	33.3
	Mean	432.6	171.8	36.5
	Standard Error	11.0	8.2	4.4

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J45	Old P130	424.0	146.0	39.7
J47	Old P130	426.0	206.0	48.5
J50	Old P130	353.0	147.0	25.9
J56	Old P130	502.0	179.0	32.4
J57	Old P130	447.0	182.0	25.3
J58	Old P130	420.0	188.0	48.4
J59	Old P130	486.0	204.0	33.3
J60	Old P130	428.0	105.0	32.4
J67	Old P130	332.0	157.0	83.4
J73	Old P130	387.0	169.0	41.4
J79	Old P130	368.0	368.0	66.8
J80	Old P130	428.0	236.0	47.0
J81	Old P130	469.0	161.0	42.2
J82	Old P130	444.0	164.0	40.2
J83	Old P130	352.0	163.0	60.7
J84	Old P130	425.0	174.0	31.0
J87	Old P130	384.0	162.0	57.4
J88	Old P130	481.0	193.0	36.8
J89	Old P130	433.0	158.5	56.8
	Mean	420.5	182.2	44.7
	Standard Error	10.9	12.1	3.4

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J80	Old P130 + L-NNA	428.0	187.0	53.5
J81	Old P130 + L-NNA	469.0	165.0	74.5
J82	Old P130 + L-NNA	444.0	167.0	87.4
J86	Old P130 + L-NNA	412.0	150.0	54.0
J86	Old P130 + L-NNA	412.0	217.0	37.3
J87	Old P130 + L-NNA	384.0	168.0	46.4
	Mean	424.8	175.7	58.9
	Standard Error	12.0	9.6	7.6

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J40	Old P130 + recovery	458.0	216.0	29.6
J50	Old P130 + recovery	353.0	177.0	26.6
J56	Old P130 + recovery	502.0	190.0	38.9
J57	Old P130 + recovery	447.0	142.0	45.1
J58	Old P130 + recovery	420.0	170.0	42.9
J59	Old P130 + recovery	486.0	234.0	35.0
J60	Old P130 + recovery	428.0	159.0	30.2
J61	Old P130 + recovery	462.0	215.0	29.8
	Mean	444.5	187.9	34.8
	Standard Error	16.2	11.2	2.4

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J39	Young P90	0.0	40.9	41.9	39.8	39.8	35.5
J41	Young P90	0.0	25.0	34.5	34.5	34.5	34.5
J43	Young P90	0.0	0.0	0.0	9.5	21.4	26.2
J44	Young P90	0.0	18.5	37.0	51.9	51.9	50.0
J46	Young P90	0.0	40.4	66.0	55.3	55.3	36.2
J51	Young P90	0.0	4.2	7.0	16.9	16.9	16.9
J52	Young P90	0.0	29.5	29.5	15.9	15.9	34.1
J53	Young P90	0.0	40.8	42.3	42.3	42.3	42.3
J55	Young P90	0.0	10.9	36.4	45.5	45.5	34.5
J70	Young P90	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	0.0	21.0	29.5	31.2	32.3	31.0
	Standard Error	0.0	5.3	6.7	6.1	5.7	4.4

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J39	Young P90	39.8	41.9	41.9	43.0
J41	Young P90	41.7	41.7	45.2	42.9
J43	Young P90	26.2	23.8	23.8	23.8
J44	Young P90	50.0	48.1	48.1	48.1
J46	Young P90	40.4	40.4	40.4	27.7
J51	Young P90	19.7	19.7	8.5	8.5
J52	Young P90	38.6	38.6	25.0	27.3
J53	Young P90	42.3	29.6	29.6	22.5
J55	Young P90	30.9	30.9	16.4	16.4
J70	Young P90	0.0	0.0	0.0	0.0
	Mean	33.0	31.5	27.9	26.0
	Standard Error	4.6	4.5	5.1	4.9

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J39	Young P130	0.0	0.0	2.0	7.0	8.0	8.0
J41	Young P130	0.0	0.0	13.2	17.6	27.9	27.9
J43	Young P130	0.0	0.0	0.0	0.0	0.0	2.6
J44	Young P130	0.0	13.4	20.6	29.9	29.9	20.6
J46	Young P130	0.0	40.4	66.0	55.3	55.3	36.2
J51	Young P130	0.0	11.6	22.1	22.1	24.2	24.2
J52	Young P130	0.0	0.0	7.0	16.3	30.2	30.2
J63	Young P130	0.0	7.3	7.3	30.1	30.1	30.1
	Mean	0.0	9.3	18.7	21.2	25.1	21.4
	Standard Error	0.0	5.3	8.0	6.3	6.3	4.3

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J39	Young P130	11.0	11.0	11.0	5.0
J41	Young P130	33.8	33.8	33.8	33.8
J43	Young P130	2.6	2.6	2.6	2.6
J44	Young P130	20.6	42.3	48.5	59.8
J46	Young P130	40.4	40.4	40.4	27.7
J51	Young P130	24.2	28.4	28.4	28.4
J52	Young P130	30.2	30.2	39.5	41.9
J63	Young P130	30.1	35.8	37.4	37.4
	Mean	23.3	27.0	29.2	28.4
	Standard Error	4.7	5.3	5.9	7.1

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J45	Old P90	0.0	6.6	9.8	13.1	19.7	29.5
J48	Old P90	0.0	7.7	7.7	7.7	5.8	5.8
J56	Old P90	0.0	0.0	0.0	15.4	15.4	-17.9
J57	Old P90	0.0	7.4	9.3	14.8	14.8	14.8
J58	Old P90	0.0	-4.5	-4.5	13.6	13.6	13.6
J61	Old P90	0.0	71.0	56.5	56.5	41.9	29.0
J67	Old P90	0.0	0.0	5.9	5.9	5.9	5.9
J69	Old P90	0.0	35.9	35.9	35.9	35.9	35.9
J72	Old P90	0.0	0.0	0.0	0.0	0.0	0.0
J73	Old P90	0.0	71.8	49.3	21.1	32.4	32.4
J80	Old P90	0.0	9.3	9.3	14.8	14.8	14.8
J82	Old P90	0.0	0.0	0.0	0.0	0.0	-7.9
J81	Old P90	0.0	8.5	8.5	8.5	8.5	2.8
J84	Old P90	0.0	0.0	19.4	33.3	33.3	33.3
J87	Old P90	0.0	2.5	2.5	-13.9	-31.9	-36.0
J88	Old P90	0.0	51.3	51.3	51.3	51.3	5.3
J90	Old P90	0.0	16.1	21.9	45.2	49.8	49.8
	Mean	0.0	16.7	16.6	19.0	18.3	12.4
	Standard Error	0.0	6.1	4.8	4.7	5.1	5.2

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J45	Old P90	29.5	29.5	26.2	19.7
J48	Old P90	1.9	-11.5	-11.5	-15.4
J56	Old P90	-53.8	-41.0	-41.0	-41.0
J57	Old P90	1.9	1.9	1.9	44.4
J58	Old P90	-40.9	-40.9	-63.6	-50.0
J61	Old P90	21.0	18.5	18.5	19.4
J67	Old P90	5.9	5.9	28.9	28.9
J69	Old P90	35.9	35.9	35.9	35.9
J72	Old P90	0.0	0.0	35.6	35.6
J73	Old P90	32.4	32.4	32.4	25.4
J80	Old P90	14.8	14.8	14.8	1.9
J82	Old P90	-7.9	-7.9	-7.9	-7.9
J81	Old P90	2.8	-7.0	-7.0	-7.0
J84	Old P90	33.3	33.3	33.3	33.3
J87	Old P90	-35.1	-47.4	-52.3	-68.8
J88	Old P90	14.5	14.5	14.5	14.5
J90	Old P90	51.0	51.0	51.0	44.5
	Mean	6.3	4.8	6.4	6.7
	Standard Error	6.9	7.0	8.0	8.3

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J45	Old P130	0.0	15.5	17.2	31.0	31.0	34.5
J47	Old P130	0.0	33.0	71.0	71.0	72.0	72.0
J50	Old P130	0.0	15.8	36.8	23.7	23.7	23.7
J56	Old P130	0.0	24.1	24.1	24.1	24.1	24.1
J57	Old P130	0.0	13.0	15.2	15.2	15.2	8.7
J58	Old P130	0.0	11.0	13.2	26.4	27.5	27.5
J59	Old P130	0.0	16.2	29.4	33.8	33.8	33.8
J60	Old P130	0.0	0.0	2.9	0.0	0.0	17.6
J67	Old P130	0.0	0.0	0.0	0.0	0.0	4.6
J73	Old P130	0.0	64.3	72.9	78.6	67.1	27.1
J79	Old P130	0.0	4.1	4.1	4.1	4.1	4.1
J80	Old P130	0.0	0.0	19.8	28.8	33.3	33.3
J81	Old P130	0.0	53.0	30.3	30.3	22.7	22.7
J82	Old P130	0.0	48.5	48.5	48.5	22.1	22.1
J83	Old P130	0.0	12.1	12.1	15.2	15.2	15.2
J84	Old P130	0.0	44.4	44.4	44.4	50.0	50.0
J87	Old P130	0.0	6.3	6.3	1.8	-0.9	-13.5
J88	Old P130	0.0	5.1	5.1	5.1	5.1	10.3
J89	Old P130	0.0	4.9	4.9	8.1	11.3	11.3
	Mean	0.0	19.6	24.1	25.8	24.1	22.6
	Standard Error	0.0	4.5	5.0	5.2	4.8	4.2

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J45	Old P130	34.5	43.1	43.1	31.0
J47	Old P130	62.0	59.0	49.0	40.0
J50	Old P130	34.2	34.2	18.4	18.4
J56	Old P130	24.1	44.8	55.2	62.1
J57	Old P130	8.7	-19.6	-19.6	-30.4
J58	Old P130	22.0	22.0	22.0	22.0
J59	Old P130	29.4	29.4	23.5	16.2
J60	Old P130	17.6	26.5	32.4	32.4
J67	Old P130	4.6	4.6	4.6	4.6
J73	Old P130	27.1	27.1	27.1	27.1
J79	Old P130	4.1	4.1	4.1	4.1
J80	Old P130	25.2	25.2	25.2	18.9
J81	Old P130	22.7	22.7	22.7	15.2
J82	Old P130	25.0	25.0	25.0	47.1
J83	Old P130	15.2	15.2	21.2	27.3
J84	Old P130	50.0	57.4	57.4	57.4
J87	Old P130	-22.5	-22.5	-35.1	-35.1
J88	Old P130	10.3	10.3	10.3	-1.5
J89	Old P130	12.3	16.7	17.8	19.8
	Mean	21.4	22.4	21.3	19.8
	Standard Error	4.1	5.0	5.3	5.7

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J80	Old P130 + L-NNA	0.0	8.0	17.0	17.0	23.0	26.0
J81	Old P130 + L-NNA	0.0	0.0	-5.7	3.3	3.3	8.9
J82	Old P130 + L-NNA	0.0	0.0	0.0	0.0	0.0	0.0
J86	Old P130 + L-NNA	0.0	0.0	0.0	-6.2	-6.2	-6.2
J86	Old P130 + L-NNA	0.0	0.0	-9.9	-9.9	-9.9	-9.9
J87	Old P130 + L-NNA	0.0	0.0	-16.7	-16.7	-17.9	-17.9
	Mean	0.0	1.3	-2.5	-2.1	-1.3	0.2
	Standard Error	0.0	1.3	4.7	4.8	5.7	6.4

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J80	Old P130 + L-NNA	29.0	29.0	29.0	29.0
J81	Old P130 + L-NNA	11.4	11.4	11.4	11.4
J82	Old P130 + L-NNA	0.0	0.0	4.8	7.5
J86	Old P130 + L-NNA	-6.2	-6.2	-6.2	-6.2
J86	Old P130 + L-NNA	-9.9	-9.9	-9.9	-9.9
J87	Old P130 + L-NNA	-24.4	-24.4	-24.4	-24.4
	Mean	0.0	0.0	0.8	1.3
	Standard Error	7.5	7.5	7.6	7.6

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J40	Old P130 + recovery	0.0	7.8	17.2	28.1	32.8	32.8
J50	Old P130 + recovery	0.0	14.9	27.7	27.7	27.7	23.4
J56	Old P130 + recovery	0.0	0.0	0.0	5.4	10.8	10.8
J57	Old P130 + recovery	0.0	14.1	35.9	35.9	32.8	32.8
J58	Old P130 + recovery	0.0	13.7	37.0	37.0	37.0	37.0
J59	Old P130 + recovery	0.0	18.3	24.4	25.6	25.6	28.0
J60	Old P130 + recovery	0.0	10.4	10.4	16.7	16.7	16.7
J61	Old P130 + recovery	0.0	0.0	15.6	15.6	9.4	9.4
	Mean	0.0	9.9	21.0	24.0	24.1	23.9
	Standard Error	0.0	2.4	4.5	3.8	3.7	3.7

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J40	Old P130 + recovery	15.6	10.9	10.9	10.9
J50	Old P130 + recovery	23.4	23.4	23.4	23.4
J56	Old P130 + recovery	10.8	18.9	18.9	18.9
J57	Old P130 + recovery	32.8	26.6	32.8	35.9
J58	Old P130 + recovery	37.0	37.0	37.0	35.6
J59	Old P130 + recovery	28.0	32.9	35.4	35.4
J60	Old P130 + recovery	-16.7	-16.7	-22.9	-22.9
J61	Old P130 + recovery	9.4	9.4	-25.0	-25.0
	Mean	17.5	17.8	13.8	14.0
	Standard Error	6.0	6.0	8.8	8.9

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J39	Young P90	342.0	172.0	32.6
J41	Young P90	351.0	234.0	30.3
J42	Young P90	346.0	178.0	39.3
J44	Young P90	369.0	145.0	42.1
J46	Young P90	347.0	141.0	60.3
J51	Young P90	361.0	134.0	41.0
J52	Young P90	394.0	170.0	50.6
J53	Young P90	339.0	204.0	47.5
J55	Young P90	346.0	168.0	33.9
J65	Young P90	388.0	198.0	27.3
	Mean	358.3	174.4	40.5
	Standard Error	6.1	9.8	3.2

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J39	Young P130	342.0	186.0	31.7
J41	Young P130	351.0	197.0	31.0
J43	Young P130	336.0	155.0	39.4
J44	Young P130	369.0	179.0	42.5
J46	Young P130	347.0	149.0	24.8
J52	Young P130	394.0	165.0	40.6
J53	Young P130	339.0	110.0	39.1
J65	Young P130	388.0	156.0	25.6
	Mean	358.3	162.1	34.3
	Standard Error	8.0	9.5	2.5

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J48	Old P90	488.0	168.0	35.7
J50	Old P90	353.0	123.0	26.0
J56	Old P90	502.0	179.0	33.5
J57	Old P90	447.0	129.0	38.0
J58	Old P90	420.0	86.0	27.9
J59	Old P90	486.0	220.0	43.2
J61	Old P90	426.0	216.0	41.2
J69	Old P90	370.0	149.0	25.5
J72	Old P90	452.0	153.0	27.5
J73	Old P90	387.0	176.0	29.5
J80	Old P90	428.0	168.0	29.8
J81	Old P90	469.0	211.0	51.2
J82	Old P90	444.0	199.0	42.2
J84	Old P90	412.0	145.0	24.8
J87	Old P90	384.0	176.0	69.0
J88	Old P90	481.0	225.0	64.4
J90	Old P90	475.0	189.0	49.2
	Mean	436.7	171.3	38.7
	Standard Error	10.8	9.2	3.2

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J45	Old P130	424.0	146.0	32.2
J50	Old P130	353.0	147.0	25.9
J56	Old P130	502.0	179.0	49.7
J57	Old P130	447.0	182.0	37.9
J58	Old P130	420.0	188.0	36.2
J59	Old P130	486.0	204.0	25.5
J61	Old P130	462.0	207.0	28.0
J67	Old P130	332.0	157.0	22.9
J68	Old P130	367.0	155.0	25.2
J73	Old P130	387.0	169.0	30.8
J80	Old P130	428.0	236.0	47.5
J81	Old P130	469.0	161.0	26.7
J82	Old P130	444.0	164.0	39.6
J83	Old P130	352.0	163.0	30.1
J84	Old P130	425.0	174.0	28.2
J87	Old P130	384.0	180.0	25.0
J88	Old P130	481.0	193.0	49.0
J89	Old P130	433.0	158.5	62.1
J90	Old P130	475.0	226.0	25.7
	Mean	424.8	178.4	34.1
	Standard Error	11.5	5.8	2.5

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J40	Old P130 + recovery	458.0	216.0	53.2
J47	Old P130 + recovery	426.0	217.0	27.2
J50	Old P130 + recovery	353.0	177.0	25.4
J56	Old P130 + recovery	502.0	190.0	33.2
J57	Old P130 + recovery	447.0	142.0	29.6
J58	Old P130 + recovery	420.0	170.0	35.9
J59	Old P130 + recovery	486.0	234.0	25.6
J61	Old P130 + recovery	462.0	215.0	32.6
	Mean	444.3	195.1	32.8
	Standard Error	16.3	10.9	3.2

Chapter II - Raw Vasodilator Responses: SNP-Induced Dilation

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J39	Young P90	0.0	17.9	32.1	32.1	58.9	58.9	58.9
J41	Young P90	0.0	16.9	12.7	12.7	12.7	12.7	28.2
J42	Young P90	0.0	0.0	-5.7	-5.7	-2.9	1.4	51.4
J44	Young P90	0.0	1.6	1.6	24.6	27.9	50.8	67.2
J46	Young P90	0.0	28.2	43.5	52.9	71.8	71.8	91.8
J51	Young P90	0.0	29.1	29.1	38.2	12.7	29.1	30.9
J52	Young P90	0.0	48.8	84.9	90.7	91.9	91.9	91.9
J53	Young P90	0.0	10.3	18.6	24.7	38.1	47.4	62.9
J55	Young P90	0.0	12.3	36.8	42.1	42.1	54.4	70.2
J65	Young P90	0.0	31.5	31.5	37.0	37.0	53.7	53.7
	Mean	0.0	19.7	28.5	34.9	39.0	47.2	60.7
	Standard Error	0.0	4.7	8.0	8.1	9.2	8.5	6.8

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J39	Young P130	0.0	0.0	0.0	47.5	91.5	91.5	91.5
J41	Young P130	0.0	3.3	18.0	55.7	55.7	68.9	96.7
J43	Young P130	0.0	3.3	23.0	23.0	36.1	49.2	90.2
J44	Young P130	0.0	42.1	38.2	56.6	61.8	67.1	80.3
J46	Young P130	0.0	0.0	0.0	0.0	0.0	48.6	70.3
J52	Young P130	0.0	25.4	25.4	25.4	49.3	53.7	74.6
J53	Young P130	0.0	11.6	30.2	46.5	51.2	65.1	65.1
J65	Young P130	0.0	15.0	47.5	72.5	72.5	87.5	87.5
	Mean	0.0	12.6	22.8	40.9	52.3	66.5	82.0
	Standard Error	0.0	5.2	5.9	8.2	9.5	5.8	4.0

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J48	Old P90	0.0	5.0	5.0	11.7	20.0	40.0	43.3
J50	Old P90	0.0	21.9	21.9	25.0	25.0	25.0	25.0
J56	Old P90	0.0	5.0	13.3	13.3	13.3	26.7	56.7
J57	Old P90	0.0	0.0	4.1	4.1	18.4	24.5	34.7
J58	Old P90	0.0	4.2	4.2	0.0	0.0	12.5	16.7
J59	Old P90	0.0	0.0	10.5	23.2	23.2	36.8	56.8
J61	Old P90	0.0	5.6	21.3	30.3	37.1	37.1	42.7
J69	Old P90	0.0	31.6	31.6	31.6	31.6	31.6	31.6
J72	Old P90	0.0	2.4	21.4	31.0	42.9	42.9	42.9
J73	Old P90	0.0	7.7	7.7	19.2	21.2	32.7	48.1
J80	Old P90	0.0	0.0	20.0	28.0	40.0	66.0	66.0
J81	Old P90	0.0	0.0	5.6	5.6	5.6	5.6	13.0
J82	Old P90	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J84	Old P90	0.0	0.0	44.4	55.6	55.6	66.7	66.7
J87	Old P90	0.0	0.0	2.7	4.8	5.5	5.5	5.5
J88	Old P90	0.0	0.0	0.0	16.6	25.5	25.5	30.3
J90	Old P90	0.0	3.1	9.7	19.1	-18.1	43.9	58.7
	Mean	0.0	5.1	13.1	18.8	20.4	30.8	37.6
	Standard Error	0.0	2.1	3.0	3.5	4.5	4.6	5.0

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J45	Old P130	0.0	0.0	0.0	19.1	29.8	42.6	48.9
J50	Old P130	0.0	18.4	21.1	31.6	36.8	36.8	36.8
J56	Old P130	0.0	19.1	31.5	31.5	39.3	46.1	69.7
J57	Old P130	0.0	0.0	10.1	11.6	18.8	33.3	37.7
J58	Old P130	0.0	58.8	67.6	82.4	75.0	76.5	76.5
J59	Old P130	0.0	9.6	26.9	26.9	26.9	40.4	42.3
J61	Old P130	0.0	29.3	50.0	56.9	62.1	69.0	69.0
J67	Old P130	0.0	16.7	16.7	33.3	33.3	33.3	33.3
J68	Old P130	0.0	15.4	28.2	53.8	100.0	100.0	100.0
J73	Old P130	0.0	0.0	5.8	13.5	19.2	28.8	42.3
J80	Old P130	0.0	10.7	10.7	17.9	26.8	40.2	42.9
J81	Old P130	0.0	30.2	60.5	86.0	86.0	90.7	93.0
J82	Old P130	0.0	9.2	44.6	30.8	67.7	67.7	70.8
J83	Old P130	0.0	0.0	8.2	26.5	26.5	63.3	53.1
J84	Old P130	0.0	0.0	55.1	55.1	71.4	85.7	85.7
J87	Old P130	0.0	20.0	37.8	37.8	37.8	37.8	37.8
J88	Old P130	0.0	0.0	1.3	-6.7	4.1	15.5	21.8
J89	Old P130	0.0	7.3	13.6	18.5	26.6	29.5	40.5
J90	Old P130	0.0	0.0	10.3	51.7	55.2	55.2	67.2
	Mean	0.0	12.9	26.3	35.7	44.4	52.2	56.3
	Standard Error	0.0	3.4	4.8	5.4	5.9	5.4	5.1

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J40	Old P130 + recovery	0.0	0.0	0.0	19.1	19.1	19.1	19.1
J47	Old P130 + recovery	0.0	10.2	11.9	55.9	61.0	61.0	61.0
J50	Old P130 + recovery	0.0	13.3	13.3	13.3	13.3	13.3	15.6
J56	Old P130 + recovery	0.0	6.3	30.2	30.2	47.6	47.6	50.8
J57	Old P130 + recovery	0.0	0.0	2.4	2.4	33.3	33.3	33.3
J58	Old P130 + recovery	0.0	16.4	31.1	32.8	32.8	67.2	67.2
J59	Old P130 + recovery	0.0	13.3	33.3	28.3	38.3	65.0	65.0
J61	Old P130 + recovery	0.0	11.4	20.0	24.3	24.3	35.7	35.7
	Mean	0.0	8.9	17.8	25.8	33.7	42.8	43.5
	Standard Error	0.0	2.2	4.6	5.6	5.5	7.3	7.2

Chapter II - Raw Immunoblot Data

Vessel Number	Group	p-eNOS (AU)	eNOS (AU)	p-eNOS/eNOS (AU)
360	P90	3670619	3383517	1.084853128
354	P90	3690609	5767243	0.639926044
361	P130	3773712	3140305	1.201702382
355	P130	5621689	6153275	0.913609257
345	P130	4569595	5995384	0.762185541
369	P130	2439618	8896825	0.274212205
364	P130	2828118	6835032	0.41376807

p-eNOS / total eNOS	Mean Intensity (AU)	Standard Error
P130	0.713095491	0.167864192
P90	0.862389586	0.222463542

(p-eNOS / total eNOS) / P90mean	Mean Intensity (AU)	Standard Error
P130	0.826883235	0.194650069
P90	1	0.257961767

APPENDIX B CHAPTER III RAW DATA

Chapter III - Raw Animal and Vessel Characteristics: NE-Induced Constriction

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J229	Young P90	361.0	183.0	65.6
J230	Young P90	316.0	228.0	53.9
J231	Young P90	349.0	176.0	50.6
J232	Young P90	350.0	253.0	47.0
J233	Young P90	353.0	142.0	69.0
J234	Young P90	351.0	195.0	40.0
	Mean	346.7	196.2	54.4
	Standard Error	6.4	16.1	4.5

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J219	Old P90	411.0	135.0	51.9
J220	Old P90	426.0	90.0	2.2
J221	Old P90	417.0	221.0	0.0
J223	Old P90	423.0	56.0	3.6
J224	Old P90	444.0	157.0	63.7
J225	Old P90	431.0	147.0	1.4
J226	Old P90	385.0	104.0	1.9
J228	Old P90	414.0	139.0	0.0
	Mean	418.9	131.1	15.6
	Standard Error	6.1	17.5	9.3

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J219	Old P130	411.0	122.0	58.2
J220	Old P130	426.0	119.0	52.9
J222	Old P130	402.0	235.0	8.9
J224	Old P130	444.0	141.0	42.6
J225	Old P130	432.0	158.0	2.5
J226	Old P130	385.0	90.0	2.2
	Mean	416.7	144.2	27.9
	Standard Error	8.8	20.4	10.7

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J223	Old P130 + Y27632	423.0	173.0	1.7
J225	Old P130 + Y27632	432.0	156.0	3.2
J226	Old P130 + Y27632	385.0	162.0	14.8
J227	Old P130 + Y27632	410.0	170.0	0.6
J228	Old P130 + Y27632	414.0	231.0	2.6
	Mean	412.8	178.4	4.6
	Standard Error	7.9	13.5	2.6

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J223	Old P90 + LPA	423.0	122.0	1.6
J224	Old P90 + LPA	444.0	147.0	59.9
J226	Old P90 + LPA	385.0	168.0	38.1
J227	Old P90 + LPA	410.0	159.0	49.7
J228	Old P90 + LPA	414.0	122.0	38.5
	Mean	415.2	143.6	37.6
	Standard Error	9.6	9.4	9.8

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J229	Young P90	0.0	14.3	41.3	79.4	81.0	88.9	90.5
J230	Young P90	0.0	3.8	36.2	87.6	100.0	100.0	100.0
J231	Young P90	0.0	10.3	58.6	82.8	93.1	93.1	100.0
J232	Young P90	0.0	9.7	11.2	21.6	75.4	85.1	100.0
J233	Young P90	0.0	6.8	65.9	84.1	100.0	100.0	100.0
J234	Young P90	0.0	2.6	50.4	85.5	100.0	100.0	100.0
	Mean	0.0	7.9	43.9	73.5	91.6	94.5	98.4
	Standard Error	0.0	1.8	7.9	10.4	4.4	2.7	1.6

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J219	Old P90	0.0	1.5	9.2	38.5	63.1	64.6	67.7
J220	Old P90	0.0	3.4	3.4	6.8	13.6	12.5	26.1
J221	Old P90	0.0	0.0	1.4	46.2	68.8	79.2	80.5
J223	Old P90	0.0	1.9	3.7	18.5	29.6	50.0	59.3
J224	Old P90	0.0	0.0	22.8	71.9	94.7	94.7	100.0
J225	Old P90	0.0	0.0	0.0	10.3	29.7	44.1	55.2
J226	Old P90	0.0	1.0	1.0	21.6	37.3	50.0	56.9
J228	Old P90	0.0	1.4	1.4	1.4	4.3	18.7	30.2
	Mean	0.0	1.2	5.4	26.9	42.6	51.7	59.5
	Standard Error	0.0	0.4	2.7	8.4	10.8	9.9	8.6

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J219	Old P130	0.0	9.8	29.4	86.3	92.2	90.2	90.2
J220	Old P130	0.0	0.0	5.4	75.0	78.6	83.9	100.0
J222	Old P130	0.0	0.0	4.2	38.3	81.3	93.5	95.8
J224	Old P130	0.0	0.0	16.0	65.4	91.4	92.6	93.8
J225	Old P130	0.0	0.0	5.8	17.5	59.1	60.4	88.3
J226	Old P130	0.0	0.0	0.0	13.6	38.6	58.0	59.1
	Mean	0.0	1.6	10.1	49.4	73.5	79.8	87.9
	Standard Error	0.0	1.6	4.4	12.5	8.5	6.7	6.0

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J223	Old P130 + Y27632	0.0	0.0	0.0	4.7	7.1	7.6	7.6
J225	Old P130 + Y27632	0.0	0.0	0.0	2.6	4.0	4.0	6.6
J226	Old P130 + Y27632	0.0	0.0	0.0	8.0	26.8	49.3	52.2
J227	Old P130 + Y27632	0.0	0.6	1.8	7.1	9.5	12.4	31.4
J228	Old P130 + Y27632	0.0	0.0	0.9	10.2	28.4	37.8	39.6
	Mean	0.0	0.1	0.5	6.5	15.2	22.2	27.5
	Standard Error	0.0	0.1	0.4	1.3	5.2	9.0	8.9

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J223	Old P90 + LPA	0.0	0.8	0.8	7.5	38.3	59.2	67.5
J224	Old P90 + LPA	0.0	0.0	0.0	-1.7	3.4	5.1	16.9
J226	Old P90 + LPA	0.0	0.0	9.6	30.8	67.3	73.1	90.4
J227	Old P90 + LPA	0.0	0.0	5.0	60.0	72.5	80.0	81.3
J228	Old P90 + LPA	0.0	0.0	14.7	48.0	80.0	90.7	94.7
	Mean	0.0	0.2	6.0	28.9	52.3	61.6	70.2
	Standard Error	0.0	0.2	2.8	11.7	14.1	15.0	14.1

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J229	Young P90	361.0	183.0	62.8
J230	Young P90	316.0	228.0	0.0
J231	Young P90	349.0	176.0	50.0
J232	Young P90	350.0	253.0	58.5
J233	Young P90	353.0	142.0	69.7
J234	Young P90	351.0	195.0	55.4
	Mean	346.7	196.2	49.4
	Standard Error	6.4	16.1	10.3

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J219	Old P90	411.0	135.0	45.9
J220	Old P90	426.0	90.0	1.1
J221	Old P90	417.0	221.0	45.7
J223	Old P90	423.0	56.0	17.9
J224	Old P90	444.0	157.0	49.0
J225	Old P90	431.0	147.0	15.0
J226	Old P90	385.0	104.0	31.7
J228	Old P90	414.0	139.0	19.4
	Mean	418.9	131.1	28.2
	Standard Error	6.1	17.5	6.2

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J219	Old P130	411.0	122.0	59.8
J220	Old P130	426.0	119.0	-1.7
J222	Old P130	402.0	235.0	54.0
J224	Old P130	444.0	141.0	0.0
J225	Old P130	432.0	158.0	39.9
J226	Old P130	385.0	90.0	16.7
	Mean	416.7	144.2	28.1
	Standard Error	8.8	20.4	11.0

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J223	Old P130 + Y27632	423.0	173.0	2.3
J225	Old P130 + Y27632	432.0	156.0	3.8
J226	Old P130 + Y27632	385.0	162.0	8.6
J227	Old P130 + Y27632	410.0	170.0	5.3
J228	Old P130 + Y27632	414.0	231.0	1.7
	Mean	412.8	178.4	4.4
	Standard Error	7.9	13.5	1.2

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J223	Old P90 + LPA	423.0	122.0	42.6
J224	Old P90 + LPA	444.0	147.0	51.0
J226	Old P90 + LPA	385.0	168.0	58.3
J227	Old P90 + LPA	410.0	159.0	64.8
J228	Old P90 + LPA	414.0	122.0	50.0
	Mean	415.2	143.6	53.4
	Standard Error	9.6	9.4	3.8

Chapter III - Raw Vasoconstrictor Responses: Ang II-Induced Constriction

Animal	Group	Start	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
J229	Young P90	0.0	17.6	17.6	25.0	1.5	1.5
J230	Young P90	0.0	58.3	71.9	69.7	60.1	69.7
J231	Young P90	0.0	3.4	53.4	53.4	38.6	29.5
J232	Young P90	0.0	11.4	15.2	17.1	13.3	7.6
J233	Young P90	0.0	30.2	39.5	37.2	37.2	25.6
J234	Young P90	0.0	21.8	27.6	27.6	27.6	27.6
	Mean	0.0	31.8	42.6	43.1	35.7	31.4
	Standard Error	0.0	10.4	9.1	8.3	9.3	9.4

Animal	Group	Start	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
J219	Old P90	0.0	5.5	11.0	16.4	16.4	12.3
J220	Old P90	0.0	1.1	0.0	1.1	1.1	-1.1
J221	Old P90	0.0	25.8	43.3	38.3	39.2	41.7
J223	Old P90	0.0	2.2	10.9	4.3	2.2	2.2
J224	Old P90	0.0	8.8	17.5	17.5	20.0	20.0
J225	Old P90	0.0	0.0	-1.6	0.8	2.4	0.8
J226	Old P90	0.0	1.4	4.2	5.6	5.6	2.8
J228	Old P90	0.0	4.5	6.3	1.8	3.6	9.8
	Mean	0.0	6.2	11.4	10.7	11.3	11.1
	Standard Error	0.0	3.0	5.1	4.6	4.7	5.0

Animal	Group	Start	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
J219	Old P130	0.0	32.7	44.9	46.9	28.6	28.6
J220	Old P130	0.0	0.0	0.8	70.2	71.9	66.1
J222	Old P130	0.0	17.6	42.6	48.1	48.1	42.6
J224	Old P130	0.0	1.4	42.6	60.3	58.9	56.0
J225	Old P130	0.0	10.5	26.3	22.1	6.3	2.1
J226	Old P130	0.0	2.7	9.3	14.7	14.7	13.3
	Mean	0.0	10.8	27.8	43.7	38.1	34.8
	Standard Error	0.0	5.1	7.7	8.8	10.5	10.1

Animal	Group	Start	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
J223	Old P130 + Y27632	0.0	0.0	0.0	0.0	0.0	-2.4
J225	Old P130 + Y27632	0.0	0.0	1.3	-0.7	-0.7	-0.7
J226	Old P130 + Y27632	0.0	0.0	7.4	12.2	8.8	2.7
J227	Old P130 + Y27632	0.0	0.6	0.6	0.6	-2.5	-2.5
J228	Old P130 + Y27632	0.0	1.3	3.5	3.5	3.5	4.0
	Mean	0.0	0.4	2.6	3.1	1.8	0.2
	Standard Error	0.0	0.3	1.4	2.4	2.0	1.3

Animal	Group	Start	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
J223	Old P90 + LPA	0.0	10.0	10.0	10.0	2.9	1.4
J224	Old P90 + LPA	0.0	5.6	44.4	44.4	48.6	45.8
J226	Old P90 + LPA	0.0	5.7	32.9	40.0	32.9	22.9
J227	Old P90 + LPA	0.0	5.4	28.6	37.5	28.6	21.4
J228	Old P90 + LPA	0.0	14.8	31.1	36.1	39.3	32.8
	Mean	0.0	8.3	29.4	33.6	30.4	24.9
	Standard Error	0.0	1.8	5.6	6.1	7.7	7.3

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J229	Young P90	361.0	183.0	40.4
J230	Young P90	316.0	228.0	46.5
J231	Young P90	349.0	176.0	52.3
J232	Young P90	350.0	253.0	52.2
J233	Young P90	353.0	142.0	41.5
J234	Young P90	351.0	195.0	53.3
	Mean	346.7	196.2	47.7
	Standard Error	6.4	16.1	2.3

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J219	Old P90	411.0	135.0	37.0
J220	Old P90	426.0	90.0	0.0
J221	Old P90	417.0	221.0	43.9
J223	Old P90	423.0	56.0	17.9
J224	Old P90	444.0	157.0	62.4
J225	Old P90	431.0	147.0	30.6
J222	Old P90	385.0	104.0	15.4
J228	Old P90	414.0	139.0	24.5
	Mean	418.9	131.1	29.0
	Standard Error	6.1	17.5	6.8

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J219	Old P130	411.0	122.0	59.8
J220	Old P130	426.0	119.0	48.7
J222	Old P130	402.0	235.0	53.2
J224	Old P130	444.0	141.0	39.0
J225	Old P130	432.0	158.0	22.8
J226	Old P130	385.0	90.0	21.1
	Mean	416.7	144.2	40.8
	Standard Error	8.8	20.4	6.6

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J223	Old P130 + Y27632	423.0	173.0	-0.6
J225	Old P130 + Y27632	432.0	156.0	3.8
J226	Old P130 + Y27632	385.0	162.0	8.0
J227	Old P130 + Y27632	410.0	170.0	1.2
J228	Old P130 + Y27632	414.0	231.0	4.3
	Mean	412.8	178.4	3.4
	Standard Error	7.9	13.5	1.5

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J223	Old P90 + LPA	423.0	122.0	26.2
J224	Old P90 + LPA	444.0	147.0	71.4
J226	Old P90 + LPA	385.0	168.0	54.8
J227	Old P90 + LPA	410.0	159.0	43.4
J228	Old P90 + LPA	414.0	122.0	50.0
	Mean	415.2	143.6	49.2
	Standard Error	9.6	9.4	7.4

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J229	Young P90	0.0	0.9	0.9	32.1	62.4	62.4	62.4
J230	Young P90	0.0	5.7	30.3	72.1	93.4	100.0	100.0
J231	Young P90	0.0	0.0	0.0	46.4	32.1	88.1	90.5
J232	Young P90	0.0	6.6	9.1	26.4	85.1	83.5	78.5
J233	Young P90	0.0	0.0	0.0	3.6	16.9	25.3	42.2
J234	Young P90	0.0	0.0	6.6	63.7	91.2	96.7	96.7
	Mean	0.0	2.2	7.8	40.7	63.5	76.0	78.4
	Standard Error	0.0	1.3	4.8	10.3	13.3	11.5	9.2

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J219	Old P90	0.0	2.4	3.5	8.2	22.4	32.9	47.1
J220	Old P90	0.0	3.3	3.3	3.3	3.3	3.3	5.6
J221	Old P90	0.0	4.8	8.9	16.9	43.5	66.9	67.7
J223	Old P90	0.0	2.2	2.2	4.3	17.4	19.6	26.1
J224	Old P90	0.0	0.0	44.1	61.0	78.0	81.4	86.4
J225	Old P90	0.0	0.0	-1.0	6.9	15.7	28.4	36.3
J222	Old P90	0.0	3.4	3.4	4.5	11.4	14.8	21.6
J228	Old P90	0.0	4.8	13.3	21.9	39.0	41.9	38.1
	Mean	0.0	2.6	9.7	15.9	28.8	36.2	41.1
	Standard Error	0.0	0.7	5.1	6.9	8.5	9.4	9.2

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J219	Old P130	0.0	4.1	16.3	42.9	71.4	71.4	75.5
J220	Old P130	0.0	0.0	0.0	11.5	21.3	24.6	62.3
J222	Old P130	0.0	0.0	1.8	22.7	58.2	60.0	76.4
J224	Old P130	0.0	5.8	4.7	15.1	55.8	76.7	91.9
J225	Old P130	0.0	4.1	9.8	18.0	42.6	50.8	64.8
J226	Old P130	0.0	0.0	2.8	2.8	15.5	26.8	29.6
	Mean	0.0	2.3	5.9	18.8	44.1	51.7	66.7
	Standard Error	0.0	1.1	2.5	5.5	9.0	9.0	8.6

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J223	Old P130 + Y27632	0.0	0.6	0.6	1.7	3.4	2.9	4.6
J225	Old P130 + Y27632	0.0	0.0	2.0	2.0	4.0	4.0	6.0
J226	Old P130 + Y27632	0.0	0.0	2.0	4.7	26.8	41.6	43.0
J227	Old P130 + Y27632	0.0	0.0	1.8	3.6	6.0	6.5	5.4
J228	Old P130 + Y27632	0.0	0.5	0.5	2.3	7.2	10.4	12.2
	Mean	0.0	0.2	1.4	2.9	9.5	13.1	14.2
	Standard Error	0.0	0.1	0.4	0.6	4.4	7.2	7.3

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J223	Old P90 + LPA	0.0	0.0	1.1	3.3	21.1	22.2	30.0
J224	Old P90 + LPA	0.0	0.0	0.0	42.9	47.6	52.4	52.4
J226	Old P90 + LPA	0.0	1.3	1.3	13.2	52.6	63.2	60.5
J227	Old P90 + LPA	0.0	0.0	0.0	26.7	64.4	75.6	73.3
J228	Old P90 + LPA	0.0	0.0	14.8	54.1	78.7	82.0	86.9
	Mean	0.0	0.3	3.4	28.0	52.9	59.1	60.6
	Standard Error	0.0	0.3	2.8	9.3	9.6	10.5	9.6

APPENDIX C CHAPTER IV RAW DATA

Chapter IV - Raw Animal and Vessel Characteristics: NE-Induced Constriction

Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
Young P90	345.0	181.0	34.8
Young P90	364.0	149.0	77.9
Young P90	367.0	178.0	66.9
Young P90	357.0	93.0	5.4
Young P90	335.0	200.0	0.0
Mean	353.6	160.2	37.0
Standard Error	6.0	18.7	15.7

Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
Old P90	400.0	112.0	47.3
Old P90	436.0	95.0	33.7
Old P90	417.0	95.0	63.2
Old P90	417.0	72.0	0.0
Old P90	417.0	111.0	75.7
Old P90	394.0	118.0	26.3
Old P90	444.0	109.0	39.4
Old P90	444.0	112.0	42.0
Old P90	436.0	115.0	52.2
Mean	422.8	104.3	42.2
Standard Error	6.1	4.9	7.3

Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
Old P130	417.0	113.0	30.1
Old P130	417.0	170.0	25.9
Old P130	444.0	121.0	26.4
Old P130	436.0	140.0	26.4
Old P130	436.0	115.0	26.1
Mean	430.0	131.8	27.0
Standard Error	5.5	10.7	0.8

Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
Old P90 + Y27632	400.0	144.0	3.5
Old P90 + Y27632	417.0	100.0	-4.0
Old P90 + Y27632	436.0	118.0	8.5
Old P90 + Y27632	436.0	99.0	6.1
Mean	422.3	115.3	3.5
Standard Error	8.7	10.5	2.7

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J210	Young P90	0.0	14.4	22.0	28.0	66.9	83.1	92.4
J211	Young P90	0.0	18.2	54.5	87.9	100.0	100.0	100.0
J213	Young P90	0.0	54.2	86.4	100.0	100.0	100.0	100.0
J216	Young P90	0.0	3.4	4.5	15.9	18.2	50.0	70.5
J217	Young P90	0.0	0.0	4.5	55.5	76.5	84.0	90.0
	Mean	0.0	18.0	34.4	57.5	72.3	83.4	90.6
	Standard Error	0.0	9.7	15.9	16.3	15.0	9.1	5.4

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J191	Old P90	0.0	0.0	5.1	30.5	39.0	59.3	54.2
J192	Old P90	0.0	0.0	3.2	20.6	77.8	100.0	100.0
J193	Old P90	0.0	0.0	0.0	11.4	11.4	40.0	60.0
J194	Old P90	0.0	0.0	8.3	15.3	18.1	33.3	61.1
J194	Old P90	0.0	7.4	33.3	55.6	100.0	100.0	100.0
J195	Old P90	0.0	0.0	10.3	54.0	62.1	73.6	77.0
J196	Old P90	0.0	7.6	63.6	80.3	87.9	92.4	93.9
J196	Old P90	0.0	-7.7	6.2	70.8	95.4	100.0	100.0
J198	Old P90	0.0	5.5	23.6	56.4	78.2	80.0	90.9
	Mean	0.0	1.4	17.1	43.9	63.3	75.4	81.9
	Standard Error	0.0	1.6	6.8	8.4	11.0	8.7	6.4

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J193	Old P130	0.0	2.5	24.1	24.1	59.5	64.6	59.5
J194	Old P130	0.0	0.8	5.6	33.3	42.1	46.8	57.1
J196	Old P130	0.0	0.0	2.2	12.4	24.7	38.2	44.9
J198	Old P130	0.0	-1.0	1.9	9.7	44.7	55.3	66.0
J199	Old P130	0.0	1.2	12.9	20.0	51.8	80.0	85.9
	Mean	0.0	0.7	9.3	19.9	44.5	57.0	62.7
	Standard Error	0.0	0.6	4.2	4.2	5.8	7.2	6.7

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J191	Old P90 + Y27632	0.0	1.4	2.9	3.6	2.2	10.1	11.5
J194	Old P90 + Y27632	0.0	0.0	1.9	2.9	7.7	16.3	20.2
J199	Old P90 + Y27632	0.0	0.0	0.0	3.7	6.5	5.6	12.0
J199	Old P90 + Y27632	0.0	0.0	0.0	0.0	1.1	-2.2	2.2
	Mean	0.0	0.4	1.2	2.5	4.4	7.5	11.5
	Standard Error	0.0	0.4	0.7	0.9	1.6	3.9	3.7

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J182	Young P90	364.0	200.0	13.0
J182	Young P90	364.0	123.0	82.1
J183	Young P90	332.0	158.0	33.5
J184	Young P90	354.0	237.0	77.2
J184	Young P90	354.0	133.0	63.9
J185	Young P90	357.0	105.0	29.5
J187	Young P90	311.0	176.0	10.2
J187	Young P90	311.0	126.0	52.4
J188	Young P90	351.0	118.0	33.9
J189	Young P90	312.0	159.0	53.5
J235	Young P90	336.0	155.0	1.3
J236	Young P90	370.0	194.0	28.4
	Mean	343.0	157.0	39.9
	Standard Error	6.3	11.3	7.5

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J175	Old P90	455.0	150.0	32.0
J176	Old P90	407.0	218.0	20.6
J177	Old P90	324.0	200.0	40.5
J177	Old P90	324.0	203.0	38.4
J179	Old P90	486.0	201.0	64.7
J191	Old P90	400.0	112.0	58.9
J194	Old P90	417.0	101.0	60.4
J194	Old P90	417.0	72.0	23.6
J195	Old P90	394.0	118.0	32.2
J196	Old P90	444.0	109.0	57.8
J196	Old P90	444.0	112.0	62.5
	Mean	410.2	145.1	44.7
	Standard Error	15.2	15.5	5.0

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J192	Old P130	436.0	132.0	12.1
J193	Old P130	417.0	113.0	48.7
J194	Old P130	417.0	170.0	14.1
J196	Old P130	444.0	121.0	43.0
J198	Old P130	436.0	140.0	39.3
J199	Old P130	436.0	115.0	24.3
	Mean	431.0	131.8	30.3
	Standard Error	4.6	8.7	6.3

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J191	Old P90 + Y27632	400.0	144.0	3.5
J194	Old P90 + Y27632	417.0	100.0	-8.0
J198	Old P90 + Y27632	436.0	115.0	0.9
J199	Old P90 + Y27632	436.0	118.0	5.9
J199	Old P90 + Y27632	436.0	99.0	9.1
	Mean	425.0	115.2	2.3
	Standard Error	7.3	8.2	2.9

Chapter IV - Raw Vasoconstrictor Responses: Ang II-Induced Constriction

Animal	Group	Start	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
J182	Young P90	0.0	0.0	5.2	7.5	13.2	13.2
J182	Young P90	0.0	13.6	27.3	45.5	54.5	59.1
J183	Young P90	0.0	8.6	89.5	100.0	100.0	59.0
J184	Young P90	0.0	48.1	48.1	48.1	40.7	40.7
J184	Young P90	0.0	62.5	85.4	79.2	83.3	83.3
J185	Young P90	0.0	31.1	67.6	67.6	62.2	50.0
J187	Young P90	0.0	17.1	15.2	25.3	21.5	32.9
J187	Young P90	0.0	20.0	41.7	55.0	55.0	48.3
J188	Young P90	0.0	-7.7	32.1	46.2	48.7	56.4
J189	Young P90	0.0	0.0	5.4	16.2	23.0	23.0
J235	Young P90	0.0	2.6	7.8	7.8	3.9	2.0
J236	Young P90	0.0	34.5	46.0	46.0	46.0	41.7
	Mean	0.0	19.2	39.3	45.4	46.0	42.5
	Standard Error	0.0	6.1	8.6	8.2	8.1	6.4

Animal	Group	Start	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
J175	Old P90	0.0	24.5	24.5	27.5	18.6	18.6
J176	Old P90	0.0	19.7	39.9	46.8	48.6	49.1
J177	Old P90	0.0	5.9	33.6	39.5	41.2	41.2
J177	Old P90	0.0	8.0	39.2	39.2	38.4	37.6
J179	Old P90	0.0	0.0	14.1	39.4	39.4	39.4
J191	Old P90	0.0	4.3	4.3	13.0	19.6	19.6
J194	Old P90	0.0	0.0	17.5	70.0	70.0	70.0
J194	Old P90	0.0	0.0	3.6	3.6	3.6	3.6
J195	Old P90	0.0	0.0	0.0	6.3	6.3	8.8
J196	Old P90	0.0	8.7	21.7	28.3	28.3	28.3
J196	Old P90	0.0	0.0	26.2	52.4	66.7	66.7
	Mean	0.0	6.5	20.4	33.3	34.6	34.8
	Standard Error	0.0	2.6	4.2	6.1	6.6	6.5

Animal	Group	Start	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
J192	Old P130	0.0	0.0	0.0	0.0	-1.7	-1.7
J193	Old P130	0.0	0.0	-1.7	-1.7	-1.7	0.0
J194	Old P130	0.0	3.4	4.1	17.1	24.0	24.7
J196	Old P130	0.0	0.0	0.0	1.4	-1.4	-1.4
J198	Old P130	0.0	0.0	0.0	0.0	4.7	4.7
J199	Old P130	0.0	-6.9	11.5	11.5	8.0	5.7
	Mean	0.0	-0.6	2.3	4.7	5.3	5.3
	Standard Error	0.0	1.4	2.0	3.1	4.1	4.1

Animal	Group	Start	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
J191	Old P90 + Y27632	0.0	0.0	0.0	0.0	0.7	0.7
J194	Old P90 + Y27632	0.0	-1.9	-1.9	-1.9	-0.9	-1.9
J198	Old P90 + Y27632	0.0	0.0	0.0	2.6	2.6	2.6
J199	Old P90 + Y27632	0.0	0.0	0.0	0.9	0.9	0.9
J199	Old P90 + Y27632	0.0	-2.2	0.0	0.0	-3.3	-3.3
	Mean	0.0	-0.8	-0.4	0.3	0.0	-0.2
	Standard Error	0.0	0.5	0.4	0.7	1.0	1.1

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J182	Young P90	364.0	200.0	29.5
J182	Young P90	364.0	123.0	80.5
J183	Young P90	332.0	157.0	51.0
J184	Young P90	354.0	237.0	60.8
J184	Young P90	354.0	133.0	78.9
J185	Young P90	357.0	105.0	61.9
J187	Young P90	311.0	176.0	65.3
J187	Young P90	311.0	126.0	53.2
J188	Young P90	351.0	118.0	64.4
J189	Young P90	312.0	159.0	78.6
	Mean	341.0	153.4	62.4
	Standard Error	7.1	13.1	4.9

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J175	Old P90	455.0	150.0	33.3
J176	Old P90	407.0	218.0	59.2
J177	Old P90	324.0	200.0	57.5
J177	Old P90	324.0	203.0	36.5
J179	Old P90	486.0	201.0	65.2
J191	Old P90	400.0	112.0	52.7
J196	Old P90	444.0	109.0	56.0
J196	Old P90	444.0	112.0	61.6
	Mean	410.5	163.1	52.7
	Standard Error	21.1	16.8	4.1

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J193	Old P130	417.0	113.0	30.1
J194	Old P130	417.0	170.0	51.8
J196	Old P130	444.0	121.0	33.9
J198	Old P130	436.0	140.0	37.1
J199	Old P130	436.0	115.0	30.4
	Mean	430.0	131.8	36.7
	Standard Error	5.5	10.7	4.0

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J194	Old P90 + Y27632	417.0	100.0	31.0
J191	Old P90 + Y27632	400.0	144.0	4.2
J198	Old P90 + Y27632	436.0	115.0	-1.7
J199	Old P90 + Y27632	436.0	118.0	8.5
J199	Old P90 + Y27632	436.0	99.0	4.0
	Mean	425.0	115.2	9.2
	Standard Error	7.3	8.2	5.7

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Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J182	Young P90	0.0	0.0	0.7	14.2	18.4	24.8	28.4
J182	Young P90	0.0	0.0	29.2	70.8	87.5	100.0	100.0
J183	Young P90	0.0	0.0	13.0	100.0	100.0	100.0	100.0
J184	Young P90	0.0	6.5	57.0	92.5	100.0	100.0	100.0
J184	Young P90	0.0	14.3	28.6	78.6	82.1	85.7	85.7
J185	Young P90	0.0	0.0	7.5	45.0	50.0	65.0	70.0
J187	Young P90	0.0	0.0	6.6	77.0	85.2	85.2	83.6
J187	Young P90	0.0	0.0	3.4	30.5	61.0	72.9	89.8
J188	Young P90	0.0	19.0	7.1	35.7	85.7	95.2	97.6
J189	Young P90	0.0	2.9	50.0	73.5	100.0	100.0	100.0
	Mean	0.0	4.3	20.3	61.8	77.0	82.9	85.5
	Standard Error	0.0	2.2	6.3	9.0	8.4	7.5	7.1

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J175	Old P90	0.0	15.0	28.0	45.0	72.0	78.0	83.0
J176	Old P90	0.0	25.8	30.3	33.7	64.0	73.0	80.9
J177	Old P90	0.0	5.9	17.6	34.1	45.9	68.2	76.5
J177	Old P90	0.0	4.7	10.9	14.7	35.7	64.3	69.0
J179	Old P90	0.0	-1.4	25.7	52.9	67.1	67.1	67.1
J191	Old P90	0.0	0.0	5.7	11.3	39.6	56.6	50.9
J196	Old P90	0.0	0.0	4.2	20.8	66.7	79.2	79.2
J196	Old P90	0.0	0.0	9.3	48.8	72.1	81.4	81.4
	Mean	0.0	6.2	16.5	32.7	57.9	71.0	73.5
	Standard Error	0.0	3.4	3.7	5.6	5.3	3.0	3.8

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J193	Old P130	0.0	12.7	16.5	40.5	78.5	86.1	86.1
J194	Old P130	0.0	0.0	6.1	23.2	31.7	34.1	36.6
J196	Old P130	0.0	0.0	0.0	11.3	20.0	37.5	36.3
J198	Old P130	0.0	0.0	0.0	3.4	15.9	35.2	39.8
J199	Old P130	0.0	0.0	1.3	5.0	13.8	46.3	46.3
	Mean	0.0	2.5	4.8	16.7	32.0	47.8	49.0
	Standard Error	0.0	2.5	3.1	6.9	12.0	9.8	9.4

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J194	Old P90 + Y27632	0.0	0.0	5.8	4.3	4.3	4.3	1.4
J191	Old P90 + Y27632	0.0	0.0	0.0	0.0	0.7	2.2	2.9
J198	Old P90 + Y27632	0.0	1.7	1.7	3.4	4.3	4.3	4.3
J199	Old P90 + Y27632	0.0	0.9	0.0	3.7	2.8	3.7	3.7
J199	Old P90 + Y27632	0.0	0.0	0.0	0.0	1.1	3.2	5.3
	Mean	0.0	0.5	1.5	2.3	2.6	3.5	3.5
	Standard Error	0.0	0.3	1.1	0.9	0.8	0.4	0.6

APPENDIX D CHAPTER V RAW DATA

Chapter V - Raw Animal and Vessel Characteristics: ACh-Induced Dilation

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J114	Young P90	292.0	182.0	55.5
J123	Young P90	367.0	130.0	46.2
J124	Young P90	355.0	158.0	44.3
J125	Young P90	357.0	199.0	79.9
J130	Young P90	355.0	223.0	43.0
J146	Young P90	368.0	147.0	57.8
J147	Young P90	394.0	192.0	69.8
	Mean	355.4	175.9	56.6
	Standard Error	11.8	12.3	5.3

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J67	Old P90	332.0	179.0	64.8
J115	Old P90	432.0	221.0	69.7
J117	Old P90	460.0	161.0	57.8
J118	Old P90	428.0	216.0	66.7
J120	Old P90	428.0	141.0	28.4
J126	Old P90	390.0	210.0	52.9
J127	Old P90	419.0	169.0	49.1
J129	Old P90	413.0	184.0	59.8
J131	Old P90	470.0	226.0	53.1
J141	Old P90	454.0	178.0	53.9
J143	Old P90	446.0	224.0	54.5
J145	Old P90	463.0	192.0	70.8
	Mean	427.9	191.8	56.8
	Standard Error	11.0	8.0	3.3

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J117	Old P130	460.0	204.0	47.5
J118	Old P130	428.0	193.0	79.8
J119	Old P130	424.0	204.0	38.7
J120	Old P130	428.0	182.0	44.5
J127	Old P130	419.0	133.0	39.1
J129	Old P130	413.0	146.0	51.4
J142	Old P130	433.0	227.0	59.5
J143	Old P130	446.0	159.0	54.1
J144	Old P130	469.0	196.0	44.4
J145	Old P130	463.0	186.0	38.7
	Mean	438.3	183.0	49.8
	Standard Error	6.3	9.2	4.0

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J64	Old HPHSS	425.0	230.0	30.9
J67	Old HPHSS	332.0	207.0	29.0
J69	Old HPHSS	370.0	146.0	24.7
J115	Old HPHSS	432.0	247.0	41.7
J117	Old HPHSS	460.0	214.0	56.1
J118	Old HPHSS	428.0	201.0	36.3
J120	Old HPHSS	428.0	182.0	53.3
J127	Old HPHSS	419.0	156.0	28.2
J128	Old HPHSS	443.0	191.0	28.3
J129	Old HPHSS	413.0	201.0	71.6
J131	Old HPHSS	470.0	243.0	30.5
J141	Old HPHSS	454.0	161.0	28.6
J142	Old HPHSS	433.0	130.0	49.2
J143	Old HPHSS	446.0	241.0	38.2
J145	Old HPHSS	463.0	174.0	50.6
	Mean	427.7	194.9	39.8
	Standard Error	9.3	9.5	3.5

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J114	Young P90	0.0	11.9	56.4	82.2	87.1	87.1	67.3
J123	Young P90	0.0	0.0	1.7	15.0	23.3	13.3	13.3
J124	Young P90	0.0	0.0	-24.3	78.6	84.3	90.0	90.0
J125	Young P90	0.0	45.9	68.6	84.3	86.8	88.1	86.8
J130	Young P90	0.0	0.0	49.0	82.3	90.6	92.7	92.7
J146	Young P90	0.0	0.0	7.1	87.1	92.9	94.1	100.0
J147	Young P90	0.0	12.7	73.1	74.6	89.6	93.3	93.3
	Mean	0.0	10.1	33.1	72.0	79.2	79.8	77.6
	Standard Error	0.0	6.4	14.3	9.6	9.4	11.1	11.4

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J67	Old P90	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J115	Old P90	0.0	33.1	62.3	52.6	61.7	58.4	53.2
J117	Old P90	0.0	4.3	46.2	43.0	58.1	51.6	38.7
J118	Old P90	0.0	6.3	-2.8	18.1	17.4	18.1	18.1
J120	Old P90	0.0	15.0	20.0	32.5	37.5	37.5	37.5
J126	Old P90	0.0	3.6	9.0	9.0	9.0	9.0	9.0
J127	Old P90	0.0	44.6	59.0	59.0	60.2	49.4	43.4
J129	Old P90	0.0	14.5	33.6	38.2	38.2	44.5	44.5
J131	Old P90	0.0	5.0	36.7	51.7	51.7	26.7	26.7
J141	Old P90	0.0	35.4	43.8	53.1	59.4	63.5	63.5
J143	Old P90	0.0	4.1	4.1	39.3	39.3	39.3	39.3
J145	Old P90	0.0	0.0	11.8	55.9	67.6	69.1	69.1
	Mean	0.0	13.8	27.0	37.7	38.5	38.9	34.1
	Standard Error	0.0	4.4	6.6	5.6	7.0	6.3	6.4

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J117	Old P130	0.0	6.2	22.7	22.7	29.9	29.9	40.2
J118	Old P130	0.0	18.8	53.9	52.6	52.6	50.6	49.4
J119	Old P130	0.0	17.7	58.2	62.0	62.0	53.2	53.2
J120	Old P130	0.0	3.7	51.9	59.3	63.0	63.0	63.0
J127	Old P130	0.0	46.2	57.7	57.7	51.9	51.9	46.2
J129	Old P130	0.0	5.3	9.3	24.0	30.7	32.0	29.3
J142	Old P130	0.0	15.6	33.3	44.4	59.3	59.3	59.3
J143	Old P130	0.0	23.3	55.8	64.0	72.1	72.1	72.1
J144	Old P130	0.0	0.0	0.0	21.8	24.1	24.1	24.1
J145	Old P130	0.0	0.0	0.0	31.9	16.7	16.7	50.0
	Mean	0.0	13.7	34.3	44.0	46.2	45.3	48.7
	Standard Error	0.0	4.5	7.1	5.6	5.4	5.2	4.9

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J64	Old HPHSS	0.0	23.9	78.9	78.9	87.3	87.3	87.3
J67	Old HPHSS	0.0	0.0	30.0	38.3	38.3	38.3	63.3
J69	Old HPHSS	0.0	0.0	0.0	2.8	8.3	8.3	8.3
J115	Old HPHSS	0.0	46.6	82.5	90.3	90.3	90.3	97.1
J117	Old HPHSS	0.0	0.0	0.0	0.0	33.3	39.2	39.2
J118	Old HPHSS	0.0	0.0	0.0	19.2	19.2	31.5	34.2
J120	Old HPHSS	0.0	6.2	42.3	42.3	38.1	40.2	60.8
J127	Old HPHSS	0.0	50.0	56.8	72.7	75.0	75.0	75.0
J128	Old HPHSS	0.0	0.0	0.0	18.5	40.7	48.1	37.0
J129	Old HPHSS	0.0	23.6	35.4	35.4	42.4	42.4	41.0
J131	Old HPHSS	0.0	0.0	39.2	17.6	64.9	64.9	64.9
J141	Old HPHSS	0.0	6.5	6.5	19.6	34.8	34.8	34.8
J142	Old HPHSS	0.0	0.0	29.7	51.6	53.1	60.9	60.9
J143	Old HPHSS	0.0	8.7	71.7	93.5	96.7	100.0	100.0
J145	Old HPHSS	0.0	14.8	36.4	39.8	43.2	52.3	54.5
	Mean	0.0	12.0	34.0	41.4	51.0	54.2	57.2
	Standard Error	0.0	4.4	7.5	7.8	6.8	6.5	6.6

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J219	Old HPHSS + L-NNA	0.0	10.3	17.2	20.7	27.6	27.6	31.0
J220	Old HPHSS + L-NNA	0.0	0.0	6.3	6.3	6.3	12.7	14.3
J221	Old HPHSS + L-NNA	0.0	0.0	-4.5	0.0	4.5	4.5	9.1
J223	Old HPHSS + L-NNA	0.0	1.6	4.1	8.1	9.8	11.4	15.4
	Mean	0.0	3.0	5.8	8.8	12.1	14.1	17.5
	Standard Error	0.0	2.5	4.5	4.3	5.3	4.9	4.7

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J114	Young P90	292.0	182.0	48.4
J123	Young P90	367.0	130.0	66.2
J124	Young P90	355.0	158.0	40.5
J130	Young P90	355.0	223.0	74.0
J147	Young P90	394.0	192.0	52.1
J148	Young P90	326.0	197.0	66.0
	Mean	348.2	180.3	57.8
	Standard Error	14.4	13.3	5.2

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J115	Old P90	432	221	47.05882353
J117	Old P90	460	161	50.31055901
J118	Old P90	428	216	56.48148148
J120	Old P90	428	141	31.91489362
J126	Old P90	390	210	40.47619048
J129	Old P90	413	184	52.17391304
J131	Old P90	470	226	47.78761062
J141	Old P90	454	178	41.01123596
J143	Old P90	446	224	50
J145	Old P90	463	192	48.95833333
	Mean	438.4	195.3	46.61730411
	Standard Error	7.899648375	9.209958378	2.225128678

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J117	Old P130	460.0	204.0	40.2
J118	Old P130	428.0	193.0	75.6
J119	Old P130	424.0	204.0	29.4
J120	Old P130	428.0	182.0	45.6
J142	Old P130	433.0	227.0	54.2
J143	Old P130	446.0	159.0	56.6
J144	Old P130	469.0	196.0	65.3
J145	Old P130	463.0	186.0	52.2
	Mean	443.9	193.9	52.4
	Standard Error	6.4	7.0	5.1

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J219	Old HPHSS + L-NNA	411.0	54.0	38.9
J220	Old HPHSS + L-NNA	426.0	107.0	80.4
J221	Old HPHSS + L-NNA	417.0	98.0	76.5
J222	Old HPHSS + L-NNA	402.0	124.0	55.6
J223	Old HPHSS + L-NNA	423.0	179.0	50.3
	Mean	415.8	112.4	60.3
	Standard Error	4.3	20.3	7.9

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J114	Young P90	0.0	26.1	37.5	37.5	33.0	33.0
J123	Young P90	0.0	8.1	8.1	12.8	12.8	12.8
J124	Young P90	0.0	0.0	10.9	23.4	35.9	40.6
J130	Young P90	0.0	33.3	58.2	53.3	44.8	44.8
J147	Young P90	0.0	12.0	24.0	24.0	24.0	25.0
J148	Young P90	0.0	13.1	17.7	19.2	20.0	20.0
	Mean	0.0	15.4	26.1	28.4	28.4	29.4
	Standard Error	0.0	5.0	7.7	6.0	4.8	5.0

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J114	Young P90	26.1	25.0	26.1	30.7
J123	Young P90	12.8	12.8	12.8	12.8
J124	Young P90	40.6	40.6	40.6	40.6
J130	Young P90	50.3	50.3	50.3	50.9
J147	Young P90	25.0	25.0	24.0	24.0
J148	Young P90	23.1	25.4	25.4	25.4
	Mean	29.7	29.9	29.9	30.7
	Standard Error	5.5	5.5	5.5	5.5

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J115	Old P90	0.0	4.8	4.8	12.5	12.5	17.3
J117	Old P90	0.0	0.0	3.7	6.2	6.2	8.6
J118	Old P90	0.0	1.6	4.1	9.8	12.3	23.8
J120	Old P90	0.0	0.0	0.0	11.1	11.1	4.4
J126	Old P90	0.0	9.4	16.5	16.5	21.2	21.2
J129	Old P90	0.0	0.0	0.0	0.0	-2.1	-2.1
J131	Old P90	0.0	35.2	35.2	33.3	23.1	23.1
J141	Old P90	0.0	0.0	0.0	13.7	23.3	23.3
J143	Old P90	0.0	0.0	0.0	0.0	0.0	3.6
J145	Old P90	0.0	33.0	33.0	33.0	33.0	26.6
	Mean	0.0	7.6	8.8	12.1	12.5	12.9
	Standard Error	0.0	4.2	4.2	3.8	3.7	3.8

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J115	Old P90	17.3	17.3	17.3	22.1
J117	Old P90	8.6	8.6	8.6	8.6
J118	Old P90	23.8	21.3	10.7	10.7
J120	Old P90	13.3	11.1	17.8	15.6
J126	Old P90	14.1	14.1	4.7	4.7
J129	Old P90	-2.1	-2.1	4.2	9.4
J131	Old P90	9.3	9.3	-10.2	-10.2
J141	Old P90	23.3	23.3	23.3	23.3
J143	Old P90	3.6	3.6	3.6	3.6
J145	Old P90	27.7	27.7	27.7	21.3
	Mean	11.9	11.6	9.2	9.2
	Standard Error	3.5	3.3	3.7	3.6

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J117	Old P130	0.0	12.2	26.8	26.8	25.6	25.6
J118	Old P130	0.0	71.2	44.5	40.4	40.4	39.0
J119	Old P130	0.0	0.0	11.7	20.0	26.7	30.0
J120	Old P130	0.0	12.0	19.3	19.3	25.3	25.3
J142	Old P130	0.0	11.4	15.4	21.1	41.5	56.9
J143	Old P130	0.0	22.2	26.7	30.0	41.1	41.1
J144	Old P130	0.0	16.4	30.5	34.4	38.3	38.3
J145	Old P130	0.0	0.0	-9.3	-9.3	-4.1	5.2
	Mean	0.0	13.2	11.5	11.4	13.7	16.1
	Standard Error	0.0	7.4	7.8	8.6	11.2	11.6

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J117	Old P130	17.1	17.1	20.7	20.7
J118	Old P130	39.0	37.0	37.0	37.0
J119	Old P130	30.0	30.0	33.3	33.3
J120	Old P130	25.3	25.3	25.3	25.3
J142	Old P130	65.0	46.3	46.3	28.5
J143	Old P130	41.1	43.3	43.3	43.3
J144	Old P130	40.6	44.5	44.5	44.5
J145	Old P130	12.4	16.5	16.5	17.5
	Mean	16.6	15.5	12.6	14.1
	Standard Error	12.2	11.7	15.2	12.0

Animal	Group	Start	2cmH_2O	4cmH_2O	6cmH_2O	8cmH_2O	10cmH_2O
J219	Old HPHSS + L-NNA	0.0	-19.0	-23.8	-23.8	-47.6	-52.4
J220	Old HPHSS + L-NNA	0.0	0.0	0.0	1.2	3.5	4.7
J221	Old HPHSS + L-NNA	0.0	8.0	8.0	8.0	10.7	13.3
J222	Old HPHSS + L-NNA	0.0	-2.9	-4.3	-8.7	-11.6	-11.6
J223	Old HPHSS + L-NNA	0.0	-1.1	-4.4	-5.6	-7.8	-10.0
	Mean	0.0	-3.0	-4.9	-5.8	-10.6	-11.2
	Standard Error	0.0	4.4	5.2	5.3	10.1	11.3

Animal	Group	15cmH_2O	20cmH_2O	30cmH_2O	40cmH_2O
J219	Old HPHSS + L-NNA	-52.4	-61.9	-71.4	-71.4
J220	Old HPHSS + L-NNA	7.0	9.3	5.8	5.8
J221	Old HPHSS + L-NNA	16.0	22.7	26.7	28.0
J222	Old HPHSS + L-NNA	-13.0	-8.7	-10.1	-10.1
J223	Old HPHSS + L-NNA	-11.1	-15.6	-18.9	-18.9
	Mean	-10.7	-10.8	-13.6	-13.3
	Standard Error	11.8	14.4	16.4	16.6

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J114	Young P90	292.0	182.0	44.5
J123	Young P90	367.0	130.0	43.8
J124	Young P90	355.0	158.0	33.5
J125	Young P90	357.0	199.0	54.8
J130	Young P90	355.0	223.0	40.4
J146	Young P90	368.0	147.0	44.9
J147	Young P90	394.0	192.0	66.7
J148	Young P90	326.0	197.0	51.3
	Mean	351.8	178.5	47.5
	Standard Error	10.2	10.3	3.4

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J67	Old P90	332	179	64.80446927
J115	Old P90	432.0	221.0	47.1
J117	Old P90	460.0	161.0	47.8
J118	Old P90	428.0	216.0	62.5
J120	Old P90	428.0	141.0	30.5
J127	Old P90	419.0	169.0	52.7
J129	Old P90	413.0	184.0	34.2
J131	Old P90	470.0	226.0	42.0
J141	Old P90	454.0	178.0	35.4
J143	Old P90	446.0	224.0	31.7
J145	Old P90	463.0	192.0	31.8
	Mean	431.4	190.1	43.7
	Standard Error	11.5	8.6	3.7

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J117	Old P130	460.0	204.0	43.1
J118	Old P130	428.0	193.0	93.8
J119	Old P130	424.0	204.0	30.9
J120	Old P130	428.0	182.0	30.8
J127	Old P130	419.0	133.0	44.4
J129	Old P130	413.0	146.0	50.7
J141	Old P130	433.0	227.0	28.6
J143	Old P130	446.0	159.0	31.4
J144	Old P130	469.0	196.0	41.3
J145	Old P130	463.0	186.0	38.7
	Mean	438.3	183.0	43.4
	Standard Error	6.3	9.2	6.1

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J63	Old HPHSS	381.0	218.0	28.0
J70	Old HPHSS	324.0	137.0	71.5
J114	Old HPHSS	292.0	209.0	43.1
J122	Old HPHSS	341.0	127.0	26.8
J123	Old HPHSS	367.0	130.0	12.3
J124	Old HPHSS	355.0	192.0	41.1
J125	Old HPHSS	357.0	143.0	35.0
J146	Old HPHSS	368.0	154.0	46.8
J148	Old HPHSS	326.0	154.0	32.5
	Mean	345.7	162.7	37.4
	Standard Error	9.3	11.5	5.5

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
J219	Old HPHSS + L-NNA	411.0	54.0	33.3
J220	Old HPHSS + L-NNA	426.0	107.0	62.6
J221	Old HPHSS + L-NNA	417.0	98.0	41.8
J223	Old HPHSS + L-NNA	423.0	179.0	69.3
	Mean	419.3	109.5	51.8
	Standard Error	3.3	25.9	8.5

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J114	Young P90	0.0	6.2	9.9	17.3	29.6	48.1	64.2
J123	Young P90	0.0	0.0	5.3	5.3	14.0	21.1	21.1
J124	Young P90	0.0	11.3	17.0	17.0	17.0	47.2	75.5
J125	Young P90	0.0	5.5	9.2	15.6	30.3	43.1	57.8
J130	Young P90	0.0	22.2	44.4	81.1	92.2	92.2	100.0
J146	Young P90	0.0	0.0	0.0	0.0	16.7	31.8	48.5
J147	Young P90	0.0	0.0	12.5	44.5	50.8	67.2	74.2
J148	Young P90	0.0	0.0	5.9	35.6	41.6	41.6	58.4
	Mean	0.0	5.7	13.0	27.1	36.5	49.0	62.5
	Standard Error	0.0	2.6	4.6	8.8	8.6	7.3	7.6

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J67	Old P90	0.0	2.6	6.0	6.0	6.0	6.9	6.9
J115	Old P90	0.0	14.4	29.8	38.5	45.2	69.2	85.6
J117	Old P90	0.0	0.0	15.6	32.5	37.7	53.2	64.9
J118	Old P90	0.0	12.6	20.0	20.0	31.9	37.8	49.6
J120	Old P90	0.0	20.9	27.9	37.2	60.5	60.5	72.1
J127	Old P90	0.0	13.5	24.7	30.3	41.6	49.4	67.4
J129	Old P90	0.0	11.1	11.1	25.4	25.4	30.2	41.3
J131	Old P90	0.0	0.0	7.4	21.1	32.6	56.8	70.5
J141	Old P90	0.0	3.2	17.5	36.5	50.8	50.8	68.3
J143	Old P90	0.0	14.1	14.1	31.0	33.8	39.4	39.4
J145	Old P90	0.0	13.1	18.0	21.3	39.3	42.6	49.2
	Mean	0.0	9.6	17.5	27.3	36.8	45.2	55.9
	Standard Error	0.0	2.1	2.4	2.9	4.2	5.1	6.5

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J117	Old P130	0.0	13.6	18.2	23.9	29.5	29.5	31.8
J118	Old P130	0.0	14.4	9.4	16.0	16.0	16.0	16.0
J119	Old P130	0.0	7.9	17.5	41.3	42.9	54.0	54.0
J120	Old P130	0.0	12.5	19.6	28.6	39.3	46.4	58.9
J127	Old P130	0.0	13.6	54.2	37.3	40.7	40.7	62.7
J129	Old P130	0.0	21.6	37.8	55.4	60.8	78.4	82.4
J141	Old P130	0.0	12.3	30.8	49.2	60.0	60.0	73.8
J143	Old P130	0.0	8.0	14.0	18.0	22.0	22.0	32.0
J144	Old P130	0.0	0.0	7.4	17.3	40.7	50.6	72.8
J145	Old P130	0.0	0.0	0.0	31.9	16.7	16.7	50.0
	Mean	0.0	10.4	20.9	31.9	36.9	41.4	53.5
	Standard Error	0.0	2.1	5.1	4.4	5.1	6.5	6.7

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J63	Old HPHSS	0.0	0.0	0.0	-11.5	-11.5	-11.5	-4.9
J70	Old HPHSS	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J114	Old HPHSS	0.0	15.6	17.8	24.4	24.4	57.8	66.7
J122	Old HPHSS	0.0	0.0	0.0	0.0	2.9	17.6	17.6
J123	Old HPHSS	0.0	37.5	50.0	50.0	62.5	62.5	62.5
J124	Old HPHSS	0.0	7.6	7.6	19.0	24.1	50.6	50.6
J125	Old HPHSS	0.0	16.0	28.0	46.0	46.0	68.0	80.0
J146	Old HPHSS	0.0	4.2	26.4	31.9	31.9	31.9	65.3
J148	Old HPHSS	0.0	0.0	0.0	18.0	34.0	34.0	38.0
	Mean	0.0	9.0	14.4	19.8	23.8	34.6	41.8
	Standard Error	0.0	4.2	5.9	7.0	7.8	9.4	10.3

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
J219	Old HPHSS + L-NNA	0.0	22.2	50.0	72.2	88.9	94.4	100.0
J220	Old HPHSS + L-NNA	0.0	29.9	59.7	74.6	82.1	89.6	94.0
J221	Old HPHSS + L-NNA	0.0	12.2	31.7	53.7	65.9	82.9	90.2
J223	Old HPHSS + L-NNA	0.0	8.9	25.8	30.6	37.9	41.1	67.7
	Mean	0.0	18.3	41.8	57.8	68.7	77.0	88.0
	Standard Error	0.0	4.8	7.9	10.2	11.3	12.2	7.0

Chapter V – Raw Immunoblot Data

Vessel Number	Group	p-eNOS (AU)	GAPDH (AU)	eNOS (AU)	p-eNOS/eNOS (AU)
23	Young P90	7049423	3541279	8612774	0.818484614
28	Young P90	6035031	2582973	4693192	1.285911806
15	Young P90		2578274	6078506	
22	Young P130	10563665	6105513	6982380	1.512903193
27	Young P130	8290191	2005734	7207833	1.150164134
17	Young P130		3774056	4897565	
21	Young HPHSS	10082046	2670927	5877678	1.715311046
24	Young HPHSS	5534233	3686680	5564259	0.994603774
16	Young HPHSS		3068108	4478580	
12	Old P90	14998157	3447798	12720134	1.179087972
13	Old P90	4580879	2094186	3637923	1.259201748
8	Old P90		4226605	6441522	
7	Old P130	4877122	1445052	2166950	2.250685064
14	Old P130	15680721	1996756	8458625	1.85381442
9	Old P130	6662436	16712135	10107164	0.659179568
5	Old HPHSS	6786598	1515446	1683500	4.031243243
6	Old HPHSS	11658465	1480357	4721402	2.469280311
3	Old HPHSS	7242649	3680499	7878838	0.919253448

Vessel Number	Group	eNOS/GAPDH (AU)	p-eNOS/GAPDH (AU)	peNOS/eNOS Normalized to Young P90 (AU)
23	Young P90	2.432108286	1.990643211	0.777880637
28	Young P90	1.816972922	2.336466932	1.222119363
15	Young P90	2.357587285		
22	Young P130	1.14361889	1.730184671	1.437849998
27	Young P130	3.59361361	4.133245485	1.09310596
17	Young P130	1.297692721		
21	Young HPHSS	2.200613495	3.774736636	1.630216655
24	Young HPHSS	1.509287218	1.501142763	0.94526275
16	Young HPHSS	1.459720453		
12	Old P90	3.68935013	4.350068362	1.120594923
13	Old P90	1.73715372	2.187427	1.196734357
8	Old P90	1.524041636		
7	Old P130	1.499565414	3.375049479	2.139031452
14	Old P130	4.23618359	7.853098225	1.761849054
9	Old P130	0.60477994	0.39865858	0.626478511
5	Old HPHSS	1.110894087	4.478284281	3.831258413
6	Old HPHSS	3.189367159	7.875441532	2.346782468
3	Old HPHSS	2.140698313	1.967844306	0.873650458

Vessel Number	Group	eNOS/GAPDH Normalized to Young P90 (AU)	P-eNOS/GAPDH Normalized to Young P90 (AU)
23	Young P90	1.104387917	0.920079751
28	Young P90	0.825063157	1.079920249
15	Young P90	1.070548925	
22	Young P130	0.519302077	0.79969523
27	Young P130	1.631811985	1.910395321
17	Young P130	0.589264948	
21	Young HPHSS	0.999269222	1.744691728
24	Young HPHSS	0.685347185	0.693831547
16	Young HPHSS	0.662839578	
12	Old P90	1.675284661	2.010611341
13	Old P90	0.788818323	1.011033659
8	Old P90	0.692046969	
7	Old P130	0.680932643	1.559955429
14	Old P130	1.923594438	3.629719589
9	Old P130	0.2746225	0.184260888
5	Old HPHSS	0.50444218	2.069873025
6	Old HPHSS	1.448249066	3.640046716
3	Old HPHSS	0.972062537	0.909542046

eNOS/GAPDH Normalized to Young P90	Mean	SE
Young P90	1	0.088012203
Young P130	0.91345967	0.359743537
Young HPHSS	0.782485328	0.10858651
Old P90	1.052049984	0.312866995
Old P130	0.959716527	0.496006537
Old HPHSS	0.974917928	0.272457321

p-eNOS/GAPDH Normalized to Young P90	Mean	SE
Young P90	1	0.079920249
Young P130	1.355045276	0.555350045
Young HPHSS	1.219261638	0.52543009
Old P90	1.5108225	0.499788841
Old P130	1.791311969	1.001322592
Old HPHSS	2.206487262	0.79118298

p-eNOS/eNOS Normalized to Young P90	Mean	SE
Young P90	1	0.222119363
Young P130	1.265477979	0.172372019
Young HPHSS	1.287739703	0.342476952
Old P90	1.15866464	0.038069717
Old P130	1.509119672	0.454554069
Old HPHSS	2.35056378	0.853789968

APPENDIX E CURRICULUM VITA

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Education:

Texas A&M University	Ph. D.	Exercise Physiology	2016
Texas A&M University	B.S.	Exercise Physiology	2008

Professional Experience:

Fall 2013, Spring 2015	Invited Speaker, Saturday Morning Biophysics: Image Life! *NSF funded outreach to introduce and encourage 6-12 grade girls to pursue education and careers in STEM fields.* Health Science Center, Texas A&M University, College Station, TX
August 22, 2013	Teaching Assistant Institute, *Training workshop,* Center for Teaching Excellence, Texas A&M University, College Station, TX
June 3-21, 2013	NASA Space Radiation Summer School, *Training course in Space Radiation Physics/Biology*, Directed by Francis Cucinatta, PhD and Dudley & Linda Goodhead, PhDs; Brookhaven National Laboratory, Upton, NY
August 2012 – May 2013	Fundamentals of Exercise Physiology Teaching Assistant, *Developed and implemented a Learning Community curriculum for freshman Fundaments of Exercise Physiology students.* Department of Health and Kinesiology; Texas A&M University, College Station, TX
June – July 2011	NASA Summer Internship, *Induction of Radio-Adaptive Response in Mouse Cardiomyocytes following Low-Dose Gamma Irradiation*, Supervised by *Chris Westby, PhD and Steve Platts, PhD;* Johnson Space Center, Clear Lake, TX
May 31 - June 3, 2011	National Space Biomedical Research Institute (NSBRI) - Summer Bioastronautics Institute, *Training course in Space Life Sciences, Research Presentation, and Communication.* Directed by Ron McNeel, PhD. Houston, TX

Fall 2009 – Spring 2010	Anatomy & Physiology Graduate Teaching Assistant, Department of Biology; Texas A&M University, College Station, TX 77843
June – August 2009	Exercise Biomechanics Graduate Teaching Assistant, Department of Health and Kinesiology; Texas A&M University, College Station, TX 77843

Peer-Reviewed Publications:

1. Seawright, JW, Luttrell, MJ, Woodman, CR. (2014). Acute increases in intraluminal pressure improve vasodilator responses in aged soleus muscle feed arteries. *Eur J Appl Physiol.* 114 (10):2213-2221.

2. Luttrell M, Seawright J, Woodman CR. (2013) Effect of age and exercise training on protein:protein interactions among eNOS and its regulatory proteins in rat aortas. *Eur J Appl Physiol*. 113(11):2761-2768.

3. Trott D, Luttrell M, Seawright J, Woodman CR. (2013) Aging impairs PI3K/Akt signaling and NO-mediated dilation in soleus muscle feed arteries. *Eur J Appl Physiol.* 113(8):2039-2046.

4. Trott DW, Seawright JW, Luttrell, MJ, and Woodman CR. (2011) NAD(P)H oxidase-derived reactive oxygen species contribute to age-related impairments of endothelium-dependent dilation in rat soleus feed arteries. *J Appl Physiol*. 110(5):1171-1180.

5. Trott DW and Seawright JW. (2010) Rethinking the role of superoxide in the ageing skeletal muscle vasculature. *J Physiol*. 588(3):397-398.