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Vascular function, physical performance and aging

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VASCULAR FUNCTION, PHYSICAL PERFORMANCE
AND AGING

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
requirement for the degree of
Doctor of Philosophy

In

The Department of Kinesiology

By

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Abstract

This is a series of three experiments with brachial artery flow-mediated dilation (BAFMD) as the major outcome variable. The first study examined the temporal response of the brachial artery diameter following forearm occlusion in sixteen young (28 ± 8 years) and fifteen older (85 ± 8 years) men. Following release of the pressure cuff there is a significant reduction in brachial diameter compared to baseline, followed by a rapid increase to a PEAK. When comparing the magnitude of the decrease in diameter and the BAFMD between Young and Old, older subjects demonstrated a blunted response. A significant relation was noted between the magnitude of decrease and BAFMD ($r = -0.44, p = 0.04$). Specific features of the biphasic pattern are blunted in older adults compared with younger subjects. The magnitude of the drop in diameter following forearm occlusion correlates with the magnitude of the BAFMD.

The second study examined the relation between BAFMD and the total score from the Continuous Scale Physical Function Performance Test (PFP-10). Sixty-four men (84 ± 11 years) were studied. BAFMD was associated with the total PFP-10 score ($r = 0.45, p = 0.0001$) and age ($r = -0.36, p = 0.003$). When individuals were categorized based on their PFP-10 score, those in the highest functional class, exhibited the highest BAFMD, compared to those in the middle class, who had greater vasoreactivity than those in the lowest functional class.

The final study examined the effects of four weeks of unilateral handgrip exercise training on BAFMD and the features of the vasoreactivity curve. Twelve men (81 ± 5 years) were studied before, during and after four weeks of handgrip training of the non-dominant arm. Following training, a 40% increase in BAFMD was observed in the trained arm only. Significant improvements in BAFMD were

observed after the second week of training ($p=0.024$). Also, BAFMD was significantly related to the estimated shear rate ($r=0.341$, $p<0.001$). A biphasic pattern was not observed in the present study. A significant improvement in BAFMD was observed following unilateral handgrip training. These improvements take place very rapidly and may be partly mediated through increases in shear stress resulting from changes in resistance vessel function.

Chapter 1

Introduction

The purpose of this introductory chapter is to provide a foundation upon which the subsequent doctoral work is based. Chapter 2 is an extensive review of the scientific literature pertinent to the author's area of interest. Chapters 3-5 are original investigations conducted by the author. Chapter 6 is a conclusion chapter designed to give the reader a summary and connect the findings of the projects outlined.

According to the most recent projections from the U.S. Census Bureau the number of individuals over the age of 65 years is expected to increase from approximately 35 million in 2000 to an estimated 71 million in 2030 [1]. Furthermore, the fastest growing portion of the U.S. population is the age group 85 years and older. This growing population of older adults will place a high demand on an already strained public health system and on our medical services. The cost of treating older adults is exacerbated by the disproportionate increase in chronic diseases among this group. However, a recent article published in the *New England Journal of Medicine* suggested that better health, characterized partly by functional independence, results in a longer life but not necessarily in higher health care costs [2]. Accordingly, expanding the current understanding of "healthy" physiological aging and its determinants is critical to improving functional status of the older population and for keeping health care costs in check.

That said, the author's interest lies in examining the effects of aging on the circulatory system. This system (composed of the heart and blood vessels) requires the complex interaction of central, local and reflex controllers in order to ensure proper regulation of blood pressure and optimal perfusion of body tissues during times of stress. Thus, it is nearly impossible to discuss the system in its entirety.

Furthermore, extensive reviews discussing the impact of aging on heart function can be found elsewhere [3-6]. Consequently, the focus of this work is to examine the effects of aging on the peripheral vascular system, its impact on physiological performance and the effects of exercise training.

Non-invasive evaluation of brachial artery flow-mediated dilation (BAFMD), first described by Celermajer and colleagues [7], is a technique currently used in the Department of Kinesiology at Louisiana State University to study vascular function. This technique utilizes high-resolution ultrasonography to measure brachial artery diameter at rest and throughout a period of reactive hyperemia, induced by forearm cuff occlusion. The dilatory response associated with increased flow is thought to be endothelium-dependent, and is used as a marker of global vascular health because of its close association with coronary endothelial function [8]. Reduced BAFMD has been found in the presence of numerous CVD risk factors [9-15] and holds predictive value for cardiovascular events [16].

Previous work at Louisiana State University focused on establishing the stability and reproducibility of BAFMD under controlled conditions [17]. Continuous image acquisition of the brachial artery throughout hyperemic period has allowed the author to refine the BAFMD protocol in an attempt to enhance its clinical application and resulted in the discovery of a distinct biphasic pattern whereby the diameter of the vessel decreases before gradually increasing to its peak diameter. This observation has not been reported in the literature and it was hypothesized that such a finding may allow for a better understanding of the physiological processes dictating vascular function. Consequently the goal of the first dissertation study was to examine the features of this biphasic pattern in a group of older adults and compare these features to a group of young individuals.

A second step in the research process was to examine the relation between vascular function (using the BAFMD protocol) and measures of physical function in older adults. The Disablement Pathway, described by Verbrugge and Jette [18], serves as the basis for this research. This model suggests that disablement begins with some pathology/injury that result in impairment at the tissue, organ and/or system level. Furthermore, it has been suggested that the decline in peak aerobic capacity seen in advancing age [5, 19] may be partly attributable to a diminished limb blood flow and poor vascular conductance [20] resulting from an age related decline in vasodilatory function of resistance vessels [21] and larger conduits [22].

Finally, the third objective in the research process was to examine whether vascular function is modifiable with exercise training in a group of elderly adults. Others [21, 23] have observed a significant improvement in vascular function of older adults engaged in long term exercise training. Despite these findings the time course of changes in vasoreactivity with exercise training in aging has not been established. Previous evidence from Louisiana State University [24] indicates that modifications in vascular responses of younger men occur rapidly after only four days of localized handgrip exercise. Also, it is unknown how exercise training may influence the features of the vasoreactivity curve. Last, recent findings [25] suggest that BAFMD is partially influenced by the strength of the shear stimulus. It is not entirely clear whether exercise training will result in alterations of the shear rate stimulus and whether these changes partly modify the vascular response in the elderly.

Chapter 2

Review of Literature

2.1 Structural Anatomy

Any practical discussion of a physiological system requires a prerequisite understanding of its parts. The purpose of this section of the literature review to identify the various compartments which make up the cardiovascular system and describe their function. Prior to doing so we will examine the structural components of the blood vessels themselves.

The walls of arteries and veins generally consist of three layers, or tunics. The tunica adventitia, the outermost layer consists of dense fibroelastic tissue, which provides stability to the vessel and anchors it to the surrounding tissues. Also contained within this layer is the vaso vasorum, which supply blood to the vessel, and nerves that are partly responsible for vascular control (see next section) The middle layer, or media, consists of smooth muscle cells arranged within elastic fibers that are embedded in a matrix of elastin and collagen. Finally, the innermost layer or tunica intima is composed of endothelial cells. Capillaries consist of endothelial cells only, while in larger vessels these cells are connected to a thin basal lamina which in turn is connected to a sub-endothelial layer. This layer is comprised of collagenous fibers, elastin, smooth muscle cells and fibroblasts [26]

2.1.1 Arteries

Large arteries, such as the aorta, have large amounts of elastin in their walls. Thus the walls expand when the pressure of the blood rises as a result of systole (up to approximately 120 mmHg) and recoils when the blood pressure falls during diastole (up to 80 mmHg). The elastic recoil helps to reduce these fluctuations in pressure and ultimately produce a smoother flow of blood through smaller arteries and arterioles.

Consequently, large arteries are referred to as “pressure storers” of the circulation [27].

Small arteries, i.e., brachial and femoral arteries, and arterioles are less elastic than larger arteries and have a thicker layer of smooth layer in proportion to their diameters, which range from 10 mm to 3 mm. Because of the relatively large number of smooth muscle fibers, these vessels have contractile capabilities and can therefore constrict their lumen to increase pressure within the system. For this reason these vessels are referred to as “resistance vessels” with pressures ranging from 60 to 90 mm Hg in small arteries to approximately 40 to 60 mm Hg in the arterioles. The thickness of the vessel wall decreases as these vessels become arterioles [28].

The arterioles are the last branches of the arterial system, with diameters between 0.3 mm to 10um. These vessels are control conduits through which blood is released into the capillaries. Arterioles have a muscular wall that is under the influence of nervous innervation, local metabolites or chemicals contained within the blood. These vessels have the capability to greatly alter blood flow to the capillaries in response to the needs of the tissue. Most of the vascular resistance of the organs and tissues are arranged in parallel. This enables greater flows to be achieved with small changes of pressure and allows flow through one circuit to be increased or decreased without necessarily affecting flow through other circuits. On the contrary, in certain organs of the body, i.e., kidney and liver, the blood traverses two capillary beds in tandem so that their resistances are in series. Consequently, the second capillary bed has a lower pressure and lesser ability to increase its flow when such increases are needed [26].

2.1.2 Capillaries

The smallest vessels are the capillaries with diameters between 8 and 10 μm . The function of the capillaries is to exchange fluid, nutrients, hormones and other substances between the blood and interstitial fluid. Hence, the capillary walls are very thin and have numerous capillary pores permeable to water and other small molecular substances.

Two types of capillaries exist in the body; 1) metarterioles – these vessels act as a direct route between arteries and veins and 2) true capillaries – surround the metarterioles to form a capillary bed to supply adjacent tissues with appropriate needs. True capillaries contain sphincters, which regulate the flow of blood to meet the local demands of the tissue. There are two general methods by which substances pass through capillary walls. Lipid soluble materials such as oxygen and carbon dioxide diffuse rapidly through the lipoprotein membrane of the endothelial cell wall. Water soluble substances are filtered through intercellular slit-pores of the cell wall. The process of filtration- absorption, described by Starling, states that hydrostatic and osmotic pressures on each side of the membrane regulate exchange of fluid across the capillary wall. At the arterial end, there is an excess of hydrostatic over osmotic pressure thus promoting movement into tissues and at the venous end an excess of osmotic over hydrostatic pressure promotes reabsorption [27]

2.1.3 Veins

The venules collect blood from the capillaries and gradually coalesce into progressively larger veins. The pressure within the venous system ranges from about 10 mmHg at the venular ends of the capillary to about 0 mmHg at the entrance of the vena cavae to the heart. Veins are about eight times as distensible as the arteries. Distensibility refers to the relative change in volume per unit of pressure ($[\Delta V/V]/\Delta P$).

Therefore, a given increase in pressure causes about eight times as much extra blood in a vein as in an artery of comparable size. For this reason, veins are known as “volume storers”. Indeed, approximately 64% of the blood volume is stored within the venous system.

Because the pressure in the venous system is low the venous walls are thin. In fact, the lumens of veins are larger than those of the corresponding arteries but the walls are thinner and contain less elastin. One way “cusp like” valves, formed from the intima layer, help to prevent back flow of blood at such low pressures. Although the walls of veins are thin, they are muscular enough to contract and expand to help facilitate blood flow back to the heart. In addition, the compression of the venous walls by contracting skeletal muscle aids in propelling blood back to the heart, a mechanism referred to as the muscle pump [29].

In summary, the structure of the arteries allows them to transport blood from the heart to the capillaries, which in turn, permit the exchange of oxygen and nutrients to the surrounding tissues. Finally, the structure of the veins allows transport of blood back to the heart. However, keeping the blood circulating through the system and the needs of the cells met during times of acute stress requires the influence of vascular control mechanisms. In the next chapter we will discuss these vascular controllers.

2.2 Vascular Control

In the previous section the functional anatomy of the vascular compartments was discussed. The following section will review the physiological principles which govern vascular function. In addition, the mechanisms which underlie these principles will be reviewed. While proceeding through this section the reader is reminded of the main principles which underlie circulatory function; 1) The blood flow to each tissue of the body is controlled in relation to the tissue needs, 2) Cardiac output is dependent

upon the sum of local blood flows returning to the heart and 3) Blood pressure is maintained independent of cardiac output and local blood flow.

2.2.1 Determinants of Vascular Control

Vascular control is governed by two important factors. Poiseuille's Law states

...

$$Q = \frac{\pi \Delta P r^4}{8 \eta l}$$

... whereby, Q is blood flow, ΔP is the pressure difference over the vessel length, r is the radius of the vessel, l is the vessel length, and η is the viscosity of the blood. It is apparent that radius has the greatest effect upon blood flow considering the length of the vasculature and blood viscosity does not change transiently. Specifically, a 4-fold increase in vessel radius causes a 256-fold increase in blood flow [28].

The second factor is based on the derivative of Ohm's Law which states ...

$$\Delta P = Q * R \dots$$

... whereby, ΔP = the change in pressure across a system or vessel, Q = cardiac output or flow, and R = resistance to flow. Therefore, the most efficient way to increase pressure in a system or vessel is to increase flow by increasing cardiac output, increase resistance to flow through peripheral vasoconstriction, or both [28].

The following sections outline the major vascular control mechanisms underlying Poiseuille's Law and central blood flow/pressure regulation. These include neurohumoral control, metabolic and intrinsic regulation and muscle afferents. Extrinsic neural control is directed toward maintaining blood pressure whereas local regulation of blood flow is directed toward meeting tissue requirements.

2.2.2 Neurohumoral Control

2.2.2.1 Baroreceptor Reflex

The best known of the nervous mechanisms responsible for arterial pressure control is the baroreflex. Baroreceptors are nerve endings which lie in the walls of internal carotid artery in an area known as the carotid sinus, and in the wall of the aortic arch. These receptors respond to increases in pressure between 60 and 180 mmHg for the carotids and between 90 and 210 in the aorta. An increase in pressure causes nerve transmission to the tractus solitarius in the medulla, which leads to an inhibition of the vasoconstrictor center of the medulla and excitation of the vagal parasympathetic center. The subsequent peripheral vasodilation and decreased heart rate and strength of contraction cause blood pressure to decrease. Conversely, low pressure causes the pressure to rise back toward normal [28].

The importance of sympathetically mediated vasoconstrictor response is most evident when moving from a supine to an upright posture [28]. In this particular circumstance blood volume in the thoracic region is translocated to the lower body thus reducing venous return (cardiac filling pressure). Subsequently, stroke volume and cardiac output decline considering the heart can only pump what passively fills it during diastole [30]. Hence, during orthostasis the reflex autonomic-circulatory adjustments described above must occur in order to maintain blood pressure [31].

2.2.2.2 Medium/Long Term Blood Pressure Control

2.2.2.2.1 Vasopressin

The maintenance of medium-term (hours) blood pressure is predominantly controlled by hormonal regulation of free water excretion by the kidney and vasoconstriction of the vasculature. Vasopressin, in particular, is a hormone released into the blood stream by the pituitary gland in response to increased electrolytes in

blood plasma (rise in hemoconcentration) and hypovolemia. This hormone travels to the kidney, where it increases the permeability of the collecting ducts and tubules to water, thereby allowing the water to be reabsorbed as the tubular fluid passes through these ducts [28]. Consequently, water is conserved and blood pressure is maintained.

2.2.2.2 Renin-Angiotensin-Aldosterone Axis

Long-term (days to weeks) blood pressure control is primarily regulated by the renin-angiotensin-aldosterone axis. Renin is a protein released by the juxtaglomerular cells of the kidney in response to fall in arterial pressure and decreased flow to the kidney. Most of the renin enters the renal blood and passes out of the kidney to circulate throughout the entire body where it acts enzymatically on angiotensinogen to release the amino acid peptide, angiotensin I. Within a few seconds of formation of angiotensin I, an angiotensin converting enzyme (ACE) bound to the endothelial cells in the vascular system catalyzes the formation of yet another amino acid peptide, angiotensin II. Angiotensin II elevates blood pressure by three means; 1) it constricts arterioles thereby increasing total peripheral resistance, 2) it acts directly on the kidneys to cause salt and water retention, and 3) it acts on the adrenal glands to secrete aldosterone, which subsequently increases salt and water reabsorption by the kidney tubules [28].

2.2.2.3 Nervous Control

Nervous control of circulation occurs almost entirely through the autonomic nervous system, with the sympathetic nervous system being the most important branch. Sympathetic vasomotor nerve fibers leave the spinal cord through all the thoracic and the first two lumbar spinal nerves and these fibers innervate the entire vascular tree except the capillaries and most of the metarterioles. The postganglionic fibers are usually nonmyelinated and form two plexuses, both of which insert into the

adventitia of the vessel. Transmitter release leads to changes in vascular smooth muscle tone. The sympathetic neurons release norepinephrine and are therefore referred to as adrenergic. This neurotransmitter binds to α receptors on the cell membrane, where a vasoconstrictor response is initiated.

Most of the norepinephrine release is reincorporated into the releasing nerve terminal while a small amount spills over into the circulation. Sympathetic impulses are also transmitted to the adrenal medulla which causes the release of norepinephrine and epinephrine directly into the blood. These catecholamines contribute significantly to basal vascular tone. While epinephrine affects both α and β receptors, norepinephrine has a much greater affinity for α receptors.

The parasympathetic system releases acetylcholine via the vagus nerve. Parasympathetic discharge at the heart causes a decrease in heart rate and force of myocardial contraction. The parasympathetic system has little if any effect on the peripheral vascular resistance [28].

2.2.2.4 Local Control

2.2.2.4.1 Myogenic Reflex

The myogenic reflex has been defined as the contraction of a blood vessel that takes place when intravascular pressure is elevated thus allowing for flow through the vessel to remain constant despite increases in arterial pressure. This behavior of the vessel is inherent to the smooth muscle, is independent of neural, metabolic and humoral influences and is most pronounced in the arterioles as this may act as a protective mechanism to avoid over-perfusion of capillary beds.

The stimulus for the myogenic response is currently not known but it may be a response to tension on the vessel wall and/or to stretch on the myofibrils. The wall tension theory is supported by La Place's law . . .

$$T = P_t * r$$

... whereby wall tension (T) is a function of transmural pressure across the vessel wall (Pt) and radius (r). Therefore, if wall tension increases due to a rise in pressure inside the vessel, a reduction in radius can counteract this increased wall tension and create a homeostatic feedback loop. The structural component of the vessel is most likely the smooth muscle. The increase in vessel wall tension and activation of stretch-operated ion channels leads to depolarization. Sufficient entry of Ca^{2+} through stretch-operated channels could directly stimulate contraction and-or trigger Ca^{2+} release from intracellular stores. A cascade of second messenger pathways would then result in smooth muscle contraction. This pathway and alternate pathways are illustrated elsewhere [32].

2.2.2.4.2 Endothelial Control

Furchgott and Zawadzki were the first to recognize the importance of the endothelium in modulating vascular smooth muscle contractile activity when they discovered that an intact endothelium is necessary for normal vasodilation of an arterial ring [33]. The endothelium responds to physical and chemical stimuli by synthesis or release of vasoactive and thromboregulatory factors which include but is not limited to nitric oxide [33], endothelial derived hyperpolarizing factor [34], bradykinin, thromboxane, endothelin[35] and tissue-type plasminogen activator [36].

Two classes of stimuli elicit an endothelial-dependent response- (1) Pharmacological stimuli such as norepinephrine, acetylcholine and bradykinin stimulate α_2 , muscarinic and β_2 receptors on the endothelial surface, and (2) Flow induced shear stress increases calcium concentration via stretch activated potassium channels and/or some form of endothelial cell cytoskeleton which transduces the signal into the cell. Intracellular calcium concentration is believed to

be the key mediator for vasodilation by activation of three mechanisms: (i) phospholipase A2 and the production of prostacyclin [prostaglandin (PGI₂)], and/or (ii) NO synthase (NOS) and the production of nitric oxide (NO). Endothelium-derived hyperpolarizing factor (EDHF) is also produced but the mechanism has not been firmly established [37]. These substances, in turn, cause relaxation of the smooth muscle and subsequently regulate vascular tone. Endothelium dependent flow induced vasodilation is an important mechanism in the regulation of radius in large and small skeletal muscle resistance arteries.

2.2.2.4.3 Metabolic Control

The importance of metabolic control is most apparent in the small resistance arteries [38]. Metabolic regulation allows active tissues (like skeletal muscle during exercise) to vasodilate while less active tissues are vasoconstricted [28]. Vasodilator substances (e.g. adenosine, adenine nucleotides, carbon dioxide, potassium, phosphate ions, lactate and Krebs cycle intermediates), which are released from active muscle fibers, diffuse through the interstitial space to act on the smooth muscle cells of arterioles and increase blood flow [39].

No consensus has been reached as to which vasodilating substance is most important in regulating blood flow and it is now generally agreed that a number of the substances work together to produce vasodilation [38]. For example, Ishibashi et al. [40] measured coronary blood flow in dogs during acute exercise and observed that when they blocked adenosine receptors there was no change in the relationship between coronary blood flow and exercise intensity. They observed similar results when they blocked ATP-sensitive K⁺ channels. However, when both receptors were blocked coronary blood flow no longer increased with exercise, suggesting that

redundancy of control mechanisms exist for metabolic control of vascular smooth muscle [40].

It has also been suggested that low levels of oxygen in the tissue cause vasodilation. This “oxygen lack” theory reasons that since oxygen is required for vascular smooth muscle contraction, a lack of O₂ supply would cause the vessel to relax and naturally dilate [28].

In summary, several mechanisms contribute to the maintenance of adequate blood flow and blood pressure throughout the vascular tree while the relative importance of each mechanism is dependent upon the specific vascular compartment. For example, the small resistance arteries are more responsive to the myogenic reflex whereas larger conduit vessels respond more to flow and sympathetic stimulation. This can be best explained by referring to Jones et al. [41]. They used the coronary circulation to propose a theory of interconnected vascular “microdomains”. Each domain, or vascular compartment, is under the control of a specific, dominant regulatory mechanism. The microdomains are then proposed to be integrated by interactions of the controlling mechanism. Accordingly, if one mechanism is diminished others can be increased to maintain optimal cardiovascular function.

2.3 Assessing Vascular Physiology

Now that the vascular anatomy and the controllers of circulation have been clearly established it is necessary to introduce the reader to the various assessment tools and discuss their application in vascular research. Although a number of techniques are currently being used in cardiovascular laboratories across the world only those tools relevant to this review will be discussed. As such, this section is limited to mostly non-invasive tools used in human subjects research. The reader is encouraged to use this chapter for the purpose of clarification when experimental

designs and results of scientific investigations are presented in the subsequent chapters.

Clearly, the various compartments of the circulatory system share the same anatomical features and therefore are influenced by the same structural components and vascular controllers, although to different degrees. However, it must also be pointed out that assessment of vascular physiology is made within the context of the individual physiological roles of each vascular compartment. For example, as noted previously, large arteries are responsible for converting pulsatile output of the heart into a more continuous pattern of flow in the vessels of the periphery. Subsequently, the emphasis here is determining the role of the “structural” components, which influence pressure and vascular elasticity. This is often evaluated noninvasively with applanation tonometry. On the other hand, the peripheral circulation is greatly influenced by local vascular control mechanisms which regulate blood flow and vascular resistance. Doppler ultrasonography (which can also assess vessel structure), plethysmography and dilator methods are used for examining these mechanisms. Particular emphasis will be placed on Doppler ultrasonography as this is most pertinent to the author’s field of study. Finally, the venous system is a capacitance system and is the major determinant of cardiac preload. Consequently, it is evaluated, in part, based on the structural factors that influence the venous system [42]. Venous plethysmography can be applied for this purpose. This discussion begins with the assessment of the central elastic arteries using applanation tonometry.

2.3.1. Applanation Tonometry

2.3.1.1 Augmentation Index

The contour of the pressure pulse in an artery provides a measure of systemic arterial stiffness [43]. This can be acquired non-invasively using a pencil-shaped

probe held on the skin over a maximal arterial pulsation. Contained within the tip of this probe is a high fidelity micromanometer. Use of the probe is based on the principle of applanation tonometry, which states that if one can flatten (applanate) the curved surface of a pressure-containing structure, then the circumferential stresses in the wall of the structure are balanced and the pressure registered by the sensor is the true intra-arterial pressure [44]. This technique is commonly used on the radial, common carotid and femoral arteries. Kelly et al. [45] showed a strong correlation between applanation tonometry recordings of carotid artery pulse pressure and the more invasive cardiac catheterization procedures. Waveforms are typically recorded and further analyzed to measure the shoulder of the pressure wave, defined as the first concavity on the upstroke of the wave. This separates the initial pressure rise from the peak systolic wave. The augmentation index is defined as the ratio of height of the peak above the shoulder of the wave to the pulse pressure [44] and indicates the pressure rise resulting from peak flow input into the vasculature before the effects of wave reflection.

An increase in the pulse amplitude as well as changes in the pulse pressure contour reflects changes in the vasculature that increase cardiac afterload. One such change is an increased aortic stiffness. An increase in the late systolic pressure peak is thought to occur from early reflected pressure waves secondary to an increase in pulse wave velocity[46].

2.3.1.2 Pulse Wave Velocity

An alternative noninvasive method used to evaluate the status of the central arteries is pulse wave velocity analysis. This is based on the physiological principle that the pressure pulse generated by ventricular ejection is propagated throughout the arterial tree at a certain speed. This speed at which the pressure pulse travels is

determined mainly by the elastic properties of the arterial wall. This propagation velocity constitutes an index of arterial distensibility and stiffness. Higher velocities indicate higher rigidity of the vascular wall and the lower distensibility [47].

Arterial flow waves are recorded simultaneously at two different sites along the vascular tree using transcutaneous Doppler flow probes. Pulse wave velocity can be derived from these waveforms by measure in the time delay (Dt) or foot-to-foot transit time and the distance (D) between these two waves [47].

$$PWV = D/Dt$$

Most pulse pressure recordings are taken at over the aortic arch (mid point of the manubrium sterni) and femoral sites because it is believed that the aorta is the major component of arterial elasticity and regional stiffness. The distance between these two sites is measured using a tape measure. This may present a limitation because the vessels follow a more circuitous path and therefore superficial measurements underestimate path lengths. Alternatively, the right common carotid artery flow wave may be used in place of the aortic arch. In this case, the distance between the midpoint of the manubrium sterni and the carotid sampling site is measured and subtracted from the manubrium-to-femoral artery distance [46].

2.3.2 Plethysmography

Venous occlusion plethysmography remains a popular tool to study limb blood flow [48] and limb venous compliance (see Application of Plethysmography for Assessing Venous Compliance)[49]. In regard to limb blood flow, the general idea behind this technique is that a “collecting” cuff is inflated around the upper limb (either arm or thigh) to a pressure less than diastolic in order to obstruct venous outflow without affecting arterial inflow. Thus, the limb swells and the volume of the

limb increases at a rate proportional to the rate of arterial inflow [48]. The limb under investigation should be at or above heart level to ensure venous drainage.

The subsequent change in volume is most commonly recorded with the use of mercury-in-silastic strain gauges. Briefly, a thin, mercury filled Silastic tube is wrapped around the experimental limb. A small electric current is passed through the mercury. The Silastic is stretched when the veins are occluded and the limb expands, thus reducing the diameter of the tubing and increasing the electrical resistance. Therefore, a properly calibrated strain gauge will provide an estimate of volume and flow because the change in electrical resistance has a linear relationship with change in limb circumference [48]. Strain gauge plethysmography appears to be highly reproducible for serial measurements following reactive hyperemia [50] and during 5 minutes of forearm occlusion with exercise [51].

2.3.3 Doppler Ultrasound

The underlying principle behind this technique is that the frequency of reflected ultrasound waves is dependent upon the speed of the blood from which the waves have been reflected [52]. The addition of the pulsed Doppler allows for “ultrasonic arteriography. A short-burst of ultrasound is emitted and the crystal used to detect the returning ‘echo’ is only activated for a brief moment, referred to as a time gate. The depth of the structure from which the returning signals enter the gate can be estimated by measuring the length of time between signal emission and receiver activation. This allows for the construction of a three dimensional map of the vessel [52].

The Doppler’s capabilities have expanded to include Duplex scanning which combines real time ultrasonic imaging with Doppler flow measurements [53] and color duplex scanning in which flow is represented on a real time image by a color

scale, subsequently allowing investigators to determine velocity and direction. Most contemporary studies now utilize waveform pattern recognition and analysis [54].

Perhaps the most widely used application of Doppler ultrasound has been to examine conduit arteries of the peripheral vasculature and their responses to various physical and physiological stimuli. Many of these studies utilized Doppler ultrasonography to obtain an image of the brachial artery at rest and following a period of forearm occlusion. The percent change in vessel diameter between the two is used as a marker for vascular function and is thought to be a potential marker for coronary artery disease [55]. This protocol was developed by Celermajer and colleagues in 1992 [7] because brachial artery could be easily imaged by conventional ultrasound and the vessel itself is of similar size to the major coronary arteries. Therefore, they reasoned it may give useful insight in atherosclerosis [56]. Of course, this model is of limited consequence to systemic cardiovascular control due to its small muscle mass and blood flow requirements. Hence, recent studies have used Doppler ultrasonography to study the leg vasculature [57].

Still today, the brachial artery model sees widespread use. This led to the publication of “The Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery” in 2002 [58]. The purpose of these guidelines was in part to discuss the technical and interpretive limitations of the technique and provide a universal standardized approach. Despite this attempt to minimize measurement error the problem of biological and environmental variability is readily apparent. For example, several factors have been known to influence vasoreactivity, including time of day of testing [59], relationship to meals [60] and more recently magnitude of the shear stress imposed on the vessel wall [61]. To date only three accuracy/reproducibility studies pertaining to the

brachial artery vasoreactivity assessment have been published in the literature [17, 62, 63].

The most recent was conducted in the vascular physiology laboratory at Louisiana State University. Welsch and colleagues [17] collected data on a group of men (mean age 41 years) free from overt signs of disease, acute medical conditions or pharmacotherapy with known vascular effects. Subjects were instructed to fast and refrain from exercise 12 to 48 hours. Ultrasound imaging was conducted at the same time of day, following 15 minutes of supine rest in a quiet environment. The study design was such that intraclass correlation coefficients and covariance parameter estimates could be made for between and within days, ultrasound technicians, image readers and subjects. Average mean percent artery dilation differences for days and testers were 1.91% and 1.40%, respectively. The mean absolute difference in artery dilation for readers was 0.21 mm. When components were combined days, testers, readers and subjects accounted for 14%, 19%, 0% and 43%, respectively, indicating adequate accuracy and reproducibility of this technique under controlled conditions [17].

2.3.4 Indicator Methods

Perhaps the most successful approach to studying muscle blood flow during exercise is constant infusion of dye or ice-cold saline through a catheter designed to provide adequate mixing [64].

Dye dilution is based upon the principle that the concentration of an indicator is dependent upon the volume of blood flow through the artery. It is performed by infusion of a known concentration of dye at a specific rate followed by determination of the dye concentration (using a photo-densitometer) in blood sample withdrawn downstream. It is assumed there is no recirculation of the mixture or samples are also

drawn from a bi-lateral limb (working at the same level of activity) and the sample values adjusted accordingly. This technique is limited to steady state exercise but can be used during incremental exercise up to maximal intensities. The major disadvantage with this technique include, the invasive nature, the involvement of blood handling, and the possible need to replace fluids lost due to repeated sampling.

Thermodilution techniques are based upon a thermodynamic heat balance relationship, and knowing the specific heats of the blood, the infusate as well as the masses of the blood and the infusate. Blood flow is determined from the temperature change in the investigation vessel during continuous infusion of cold saline. The temperature deflection is proportional to the temperature, rate of infusion, and rate of blood flow [65]. Measurements are taken from a thermostat inserted proximally in the artery from a catheter. Bolus and continuous infusion techniques can be used at rest but the changes in intra muscular pressures during steady state exercise make only continuous infusions useful. The advantage of this technique is that recirculation effects can be ignored and measures can be made more frequently than with dye dilution techniques.

2.3.5 Application of Plethysmography for Assessing Venous Compliance

Using the principles of plethysmography described above, Halliwill and colleagues [49] demonstrated a simple technique to assess limb venous compliance, a major factor associated with venous pooling. The authors inflated a collecting cuff to 60 mmHg for four minutes then decreased cuff pressure at 1 mmHg/s (over a one minute period) to 0 mmHg, during which time limb volume was measured with mercury-in-Silastic strain gauges. This pressure-volume relationship was used to generate pressure-volume curves as depicted in Figure 2.5. Notably, the volume-pressure relationship is nonlinear. As such, compliance is variable and depends on

distending pressure. Compliance is greatest at low pressures and decreases as pressure increases. Subsequent comparison of pressure volume curves between individuals was made using the following quadratic equation:

$$(\Delta \text{ limb volume}) = \beta_0 + \beta_1 * (\text{cuff pressure}) + \beta_2 * (\text{cuff pressure})^2$$

The curvilinear relationship between pressure and volume necessitates that the parameters β_1 and β_2 be used together to provide an estimate of compliance. Hence, compliance is defined as the derivative of the pressure-volume curve [49].

In most established methods it is assumed that resting venous pressure in the limb equals zero and compliance is subsequently characterized by a change in limb volume in the transition from no cuff inflation to cuff inflation at a set pressure (40 mmHg). However, in the present investigation direct intravenous pressure was approximately 6 mmHg while venous collecting cuff pressure was 0. Therefore, it would be an error to assume the difference between 0 mmHg and 40 mmHg represents a 40mmHg rise. Accordingly, the authors considered the overall slope of the curve independent of resting venous pressure [49]

In summary, the complexity of the cardiovascular system renders it impossible to study function using one particular tool. Rather, various tools are required to assess its individual parts. More information regarding these assessment techniques can be found elsewhere [66].

2.4 Effects of Aging on Vascular Structure

In the previous sections we learned of the structural components of the vascular system, the mechanisms of vascular control and the various techniques used to assess vascular physiology. At this point in the review we shift our focus to the discussion of the physiology of vascular aging and the potential impact on physiological functioning. First, we explore the profound effect aging has on vascular

structure and control. The organization of this section and Section 2.5 is consistent with Sections 2.1 and 2.2. Thus, structural changes will be discussed in the context of each individual vascular compartment, i.e., large elastic arteries, peripheral conduits, resistance vessels, capillaries and systemic veins. Similarly, the discussion on vascular control moves from the *neurohumoral control* mechanisms, i.e., those which control blood pressure to *local control* mechanisms, i.e., those which regulate blood flow to the tissues. Separate sections within these chapters will provide mechanistic insight to these changes. Although the author makes a concerted effort to separate structural and vascular control changes they do interact in an attempt to satisfy the three principles of circulatory function (See Section 2.2). Thus, most changes represent adaptations consistent with the laws that govern vascular control. This will, in turn, set the stage to discuss the consequences of such changes on physiological function with a particular emphasis on exercise performance. The reader should have an understanding of effects of aging on cardiovascular reflexes, the ability to regulate blood pressure and cardiac output and blood flow distribution during exercise. Finally, this document will conclude with a discussion of the effects of age on physiological functional capacity and the role of exercise training in improving this capacity and its vascular determinants.

Our discussion of the aging effects on vascular physiology begins with a look at structural changes. As stated above, we start at the point where blood leaves the heart, the large elastic arteries and then move to the blood flow distribution vessels which deliver the oxygenated blood to the tissues. We finish with a discussion of the veins, which are ultimately responsible for returning the blood from the tissue to the heart.

2.4.1 Central Arteries

The central elastic arteries have received a great deal of attention considering the ease with which investigators can assess structural changes with aging by noninvasive means. It has generally been found that central artery stiffness increases with age.

Vaitkevicius et al. [46] measured pulse wave velocity to define age-associated changes in arterial stiffness in healthy men and women from the Baltimore Longitudinal Study of Aging. Individuals with occult cardiovascular disease, hypertension and diabetes were excluded from analysis. The authors found a two-fold increase in aortic PWV between the ages of 20 and 91 years.

Similarly, Tanaka et al, [67] compared arterial compliance (the inverse of stiffness) of the common carotid artery between groups of young (aged 18-37 years), middle-aged (aged 38-57 years), and older (aged 58-77 years) men. All participants were normotensive (<140/90 mmHg) and free from overt chronic diseases. Central artery compliance was lower ($P<0.05$) in the middle-aged and older men compared with the young group. No differences were observed between the middle aged and older groups. The authors concluded that aging has an intrinsic physiological effect on central arterial compliance.

Others have observed a decrease in aortic compliance with age in women [68, 69] [46]. Further, van der Heijden-Spek and colleagues [68] found that after adjustment for confounding factors such as blood pressure, body mass index and pulse rate, PWV across the life-span was similar between men and women.

Changes in central arterial stiffness with age are accompanied by an increase in arterial diameter and wall thickness. For instance, Nichols and colleagues [70] measured the mean systolic internal radius of the ascending aorta of individuals free

from cardiovascular disease. The authors reported a 9% increase in vessel radius per decade of life. Aortic sections collected in postmortem studies indicate intimal thickening contributes mostly to the increase in wall thickness seen with age [71]. Increase in the common carotid intima-media thickness (IMT) has been typically observed in those with established cardiovascular disease or with the presence of traditional risk factors [72, 73].

Nagai and colleagues [74] performed carotid ultrasound on apparently healthy volunteers, those with possible CAD and those with definite CAD as diagnosed by a medical history and resting electrocardiogram. Indeed, the investigators found a graded increase in IMT from CAD free subjects to those with possible CAD to those with confirmed CAD. However, there was a progressive increase in IMT with age in CAD free group indicating that IMT thickening is a consequence of physiological aging.

2.4.2 Peripheral Conduits

It is clear that with advancing age large elastic arteries become stiffer, dilate and show an increase in wall thickening. The reason for extensive work in this vessel is due to the accessibility of the animal aorta. No consensus has been reached concerning changes in vascular parameters of the medium sized muscular arteries. Often, the changes in the central vessels are inferred in the more peripheral conduit vessels. The structural properties do, however, differ between these different vascular compartments.

Recently, Van der Heijden-Spek and colleagues [68] compared vessel wall properties of the aorta to those of the brachial artery in a group of men and women ranging in age from 20 to 79 years. Arterial compliance of the aorta and brachial artery were measured with the use of pulse-wave velocity and a vessel wall movement

detector system. After adjustment for confounders, PWV of the aorta increased with age (decrease in compliance) to the same extent in both men and women whereas brachial artery compliance increased in women and remained unchanged in men with increasing age. A progressive increase in brachial artery diameter was observed in both sexes but to a greater extent in women than in men. The reason for a gender difference could be due to differences in smooth muscle tone and the elastin/collagen ratio difference between men and women. The increase in brachial artery compliance with age is caused by a larger diameter, without a significant change in distensibility, i.e., relative change in volume per unit pressure. While it has been suggested that the larger diameter with age is due to loss of elastic fibers in the arterial wall this is clearly not the case in the brachial artery because elasticity, reflected by distensibility, appears to be not significantly changed with age. Instead, the authors propose that the brachial artery may undergo adaptive remodeling, and this may be more pronounced in women than men [68].

Conversely, Urbina et al [75] found an inverse relation between brachial artery distensibility and age in a group of younger adults. The reason for the conflicting findings is unclear but differences in the results may be partly attributed to measurement tools and subsequently the manner in which distensibility is estimated between studies.

Alternatively, whereas the above investigators measured distensibility in the distal portion of the brachial artery Bjarnegard et al [76] assessed proximal brachial artery distensibility in a slightly younger population at the level of the major pectoral muscle. The authors noted a decrease in distensibility and diameter with age emphasizing the need for investigators to define the exact location of measurement when performing these studies. The composition of elastin and collagen differs in

the arterial tree. Whereas elastin is the dominant component in the central arteries, collagen is the dominant component in more peripheral arteries. The transition from elastic to more muscular artery may occur along the brachial artery and as such the effects of aging are different depending on territory.

2.4.3 Resistance Vessels

The dilatation of the central elastic arteries and conduits results in the structural narrowing of resistance vessels. This appears to be an adaptive mechanism designed to maintain or augment mean arterial pressure. This adaptation of the radius of resistance vessels occurs in accordance with Poiseuille's Law, whereby an elevation of perfusion pressure would be balanced by narrowing of the resistance vessels, since flow changes with the fourth power of radius[3]

Lawrenson measured quadriceps muscle blood flow (QMBF) and leg vascular resistance during incremental knee extensor exercise in a group of sedentary young and sedentary older men[77]. The investigators observed a consistently attenuated blood flow response and elevated leg vascular resistance (indicative of structural narrowing) in the old compared to the young across all work rates. These findings indicate that during small muscle mass exercise peripheral vascular limitations (vascular resistance and reduced blood flow) persist in the old.

The resistance vessels also appear to undergo wall thickening like that seen in larger arteries. Auerbach et al. [78] observed fibrous thickening in the walls of the trachea, esophagus, the stomach wall, the pancreas and the adrenal glands. This increased wall thickening of resistance vessels offsets any decline in smooth muscle strength with age by keeping wall stress constant, thereby normalizing the strain on individual contractile elements (see Laplace's Law). Consequently, wall compliance

is reduced leading to decreased flow capacity. Gothberg and colleagues observed reduced stiffness of the resistance vessels with increasing age [79]

2.4.4 Capillaries

The effects of aging on the capillary exchange vessels in the various systemic circuits are not well known. However, histological studies of the microvasculature in skeletal muscle of aging athletes show that these vessels beds remain largely intact [3] at least when related to maximal flow capacity. In fact, in the study by Lawrenson et al. discussed above they found avO_2 diff was maintained in older subjects during incremental bouts [77](See later). Filtration rates in the kidneys may decline with age but these high pressure type vessels may be exposed to wear and tear than most systemic capillaries [3]

While lower limb edema is common in older adults other factors (not capillary function) become less effective with aging. These factors may include structural changes, like failing valvular incompetence in dependent veins or functional changes, such as reduced efficiency of myogenic autoregulatory function in the smallest precapillary resistance vessels or declining reflex constriction of both resistance vessels and capacitance vessels [3]

2.4.5 Systemic Veins

The venous capacitance vessels are exposed to considerably increased transmural pressures since in the upright posture most of this volume is below heart level. Peripheral pooling of blood and subsequent reduction in venous return is a problem that may be exacerbated with advancing age. In general the affects of aging on venous structure remain largely underexplored. However, like the arteries discussed previously the venous side gradually shows a reduction of wall compliance, Monahan and colleagues [80] measured venous calf compliance using

plethysmography in a group of young and older sedentary, and young and older endurance trained men. The resulting pressure-dependent compliance lines are presented in Figure 2.9. These data indicate that pressure volume curves in the older men were less steep than observed in younger men, indicating lower venous compliance and providing evidence for an independent negative influence of age on venous compliance.

In summary, increased stiffness and wall thickening is a consistent pattern across most of the vascular network. The increased thickness per se appears to be an adaptive remodeling process in accordance with LaPlace's Law. In other words, wall thickening takes place in order to keep tension constant, thereby normalizing the strain on individual smooth muscle contractile elements produced from an increase in pressure and/or radius or age related decrease in smooth muscle strength. The following paragraphs will offer the reader insight into the potential mechanisms underlying these structural changes.

2.4.6 Mechanisms of Structural Change

2.4.6.1 Increased Vascular Stiffness

2.4.6.1.1 Decreased Elasticity

The smooth muscle cells of the tunica media are surrounded by non-living macromolecules collectively referred to as the extracellular matrix (EC). The EC is made up of long carbohydrate chains linked to proteins which make up collagen and elastin. Collagen is the main structural component of the vessel and elastin is responsible for the elasticity of the vessel. Surrounding the elastin is a sheath of microfibrils, which maintain its integrity. With aging the microfibrils disappear [81]. There is also evidence in aging rat models that the elastin becomes frayed [82] and as a consequence, elastin loses much of its elastic properties.

2.4.6.1.2 Elastin Calcification

Smooth muscle cells (SMC) produce proteases and elastases, e.g. matrix metalloproteinase-2 (MMP-2). MMP-2 degrades collagen and elastin and is involved in vascular remodeling associated with vascular injury to promote protein degradation and smooth muscle cell migration. The activation of this enzyme is stimulated by cytokines. Bailey and colleagues [83] recently found that in the rat model increased calcification of elastin was associated with and up regulation of MMP mRNA expression and increased activity of MMP-2 in the early stages of calcification.

2.4.6.1.3. Augmented Vascular Smooth Muscle Tone

Smooth muscle can alter the distribution of forces between the collagen and elastin fibers, thus altering vascular stiffness. Vascular smooth muscle tone is controlled in part by neurohumoral factors [84]. Carroll and colleagues [85] studies a group of individuals diagnosed with dilated cardiomyopathy ranging in age from 16 to 71 years old. These individuals were categorized into three groups (young, middle-aged and older). As expected, aortic stiffness increased with age. Infusion of nitroprusside reduced aortic stiffness in all groups but the reduction was greatest in the older group, suggesting that augmented vascular smooth muscle tone may be partly responsible for increased stiffening with age.

2.4.6.1.4 Increase Intima-Media Thickness

- Matrix metalloproteinase-2 (MMP-2)

As described above this enzyme is involved in the digestion of the basement membrane surrounding smooth muscle cells (SMC). These proteins also facilitate the migration of SMC and their subsequent invasion of the extracellular matrix of the vessel wall. Total MMP-2 protein of young rats was increased 8 fold over old rats and activity was regionally localized to the intima. This appears to partly account for

age associated breaks of the internal elastic lamina in the aorta of the older rats. Thus, it is hypothesized that increased elastase activity with aging, as observed in humans, along with age associated increases in Ca^{2+} contribute to elastin fragmentation or reduction in its content with aging. In addition, enhanced MMP-2 levels were observed in old versus young SMC after stimulation by cytokines, potentially reflecting enhanced level of cytokine stimuli in vivo [86]

- Transforming growth factor- β (TGF- β)

The thickness of the intima in old rats was 5 times greater than the intima in young rats and was characterized by a significant increase in TGF- β . TGF- β helps to synthesize extracellular matrix proteins and its expression can lead to excessive fibrosis. The accumulation of TGF- β may account for the concurrent increase in fibronectin and collagen, which can alter SMC properties. Given that fibronectin levels increase with age it is apparent that the increased levels of TGF- β are associated with increase in TGF- β activation [86]

- Intracellular adhesive molecule-1 (ICAM-1)

An increase in ICAM-1, similar to that of TGF- β is also observed in the aorta of aged rats. The concomitant increase in both factors may be related since TGF- β is known to induce the synthesis of cell adhesion receptors. This may lead to increased adhesion and interaction of cells with the surrounding extracellular matrix [86].

- Angiotensin converting enzyme (ACE)

It is known that age-associated increase in aortic angiotensin converting enzyme (ACE) activity occurs in rats [87]. Interestingly, treatment with ACE inhibitors significantly postpone the increase in medial and intimal thickening, suggesting age associated changes in local vascular angiotensin system plays a central role in age-associated vascular remodeling [88]

- Increased sympathetic vascular smooth muscle tone

The peripheral conduits contain a greater degree of sympathetic innervation compared to larger elastic vessels. Dinneno and colleagues [89] tested muscle sympathetic nerve activity and measured femoral artery intima media thickness in a group of younger and older men. Neural activity was 70% higher and IMT thickness 70% higher in the older compared to the younger men. Further these two outcomes were strongly related, indicating that sympathetic nervous activity could be an important mechanism contributing to arterial hypertrophy and consequently arterial stiffness in conduit vessels [89].

It is clear, based on the data discussed above, that a number of mechanisms could be potentially responsible for the structural changes associated with aging in the human. Our discussion now moves to the vascular control changes observed in advancing age.

2.5 Effect of Aging on Vascular Function

Our discussion on age-related changes in vascular control begins with the vascular smooth muscle cell, since this “effector cell” is ultimately responsible for altering blood vessel diameter. Next, this chapter discusses the baroreceptor reflex and factor associated with altered sympathetic control. Finally, we end this chapter by discussing endothelium-mediated control, which is primarily responsible for regulating tissue perfusion. The endothelium has received a great deal of attention in recent years because of its apparent links to atherosclerosis [90]. However, as the reader shall see, poor endothelial function has been observed as a result of “normal” physiological aging.

2.5.1 Vascular Smooth Muscle

Little is known about the functional changes of the vascular smooth muscle with aging. This is complicated by the fact that the smooth muscle is affected by a number of control mechanisms. For example, the pronounced basal tone generally displayed in resistance vessels is mainly due to myogenic activity [91] whereas smooth muscle activity in the venous capacitance vessels seems to be mainly a consequence of sympathetic fiber discharge [92]. Most studies utilize the vascular smooth muscle derived from the aorta to study how aging affects smooth muscle tone, but it would be inappropriate to generalize aging affects to all compartments of the vascular system.

Early work indicates that vascular smooth muscle taken from aorta showed modest reduction in maximal contractile strength with age to sympathetic stimuli, suggesting that basic excitation-contraction links and/or the contractile machinery have become altered with age [3]. Other evidence suggests that structural adaptive increases in the wall-to-lumen ratio may offset a decline in vascular smooth muscle strength (See Chapter 5) [78]. Additionally, Carvajal and colleagues [93] observed increased contractile response of aged rat aorta to noradrenaline and potassium. No such changes were observed in a mesenteric artery indicating the age related changes in vascular smooth muscle function may be different for different blood vessels.

2.5.2 Neurohumoral Control Mechanisms

2.5.2.1 Baroreceptor Reflex

It is known that short term reflex cardiovascular control is compromised in advanced age [3]. Studies indicate that this effect may be the result of reduced sensitivity of baroreceptors located in the aorta and carotid arteries [94]. There is evidence to suggest that decreased compliance of the large elastic arteries impairs

responsiveness of the cardiovagal baroreflex [95]. Specifically, stiffening of the ascending aorta and carotid sinus with increasing age causes less deformation of the arterial wall in response to changes in pressure, and therefore, reduced ability of the baroreceptors to transmit vasoregulatory signals [96, 97].

Also, α_2 mediated responses seem to be attenuated with age, and since the venous side contains a high degree of α_2 receptors, effector responses to central or reflex activations would also be diminished[3]. These effects are exacerbated by decline in strength and speed of contraction in venous smooth muscle cells and by increasing valvular incompetence [3].

Finally, Halliwill and colleagues [98] demonstrated that by preventing blood pooling with antishock medical trousers attenuated the reflex tachycardia and vasoconstriction to lower body negative pressure. This is an experimental approach used to pool blood in the capacitance vessels in the lower part of the body to create hypovolemia in order to examine autonomic-circulatory mechanisms [99]. Their findings indicate that the compliance of the veins of the legs is an important factor in determining the cardiovascular response to orthostasis. It has already been established in Chapter 5 that venous compliance decreases with increasing age, but no general consensus has been reached regarding whether this actually contributes orthostatic intolerance in the old.

2.5.2.2 Adrenergic and Cholinergic Receptors

The prominent role of the β receptors is to mediate the important sympathetic excitatory effect on the heart and the renin release in the kidneys, while they play less of a role in mediating the dilator effects on the vasculature. However, Hyland and colleagues [100] reported a progressive loss with increasing age of the β - adrenoceptor-mediated relaxation which was markedly reduced by 6 months and

abolished in 24 months old rats. They concluded that, in the rat aorta, there is a loss of β -adrenoceptor-mediated responses in advancing age. It appears that the decrease in responsiveness is the consequence of a deficiency with aging of the intracellular transduction mechanism considering there is no age related reduction of either the number of β receptors or the agonist binding to these receptors [3]. In other words, the formation of cAMP (the second messenger) becomes impaired with age. The above change results in declining sympathetic positive chronotropic and inotropic effects on the heart with age, a reduction in the release of renin release to sympathetic activation, and an attenuation of vasodilator responses to epinephrine [3].

It has also been established that among normotensives, plasma norepinephrine levels increase significantly with age [101]. Most of the plasma norepinephrine in humans comes from neuronal “spillover” from skeletal muscle vascular beds as well as from the lungs, heart, and kidneys. Normally, approximately 80% of the released adrenergic transmitter is taken up by the nerve fibers while the remaining 20% reaches the bloodstream and shows up as plasma norepinephrine. It has been hypothesized that the elevated plasma norepinephrine concentration in the elderly reflect a 1) concomitant increase of tonic sympathetic nerve discharge 2) an increased release of the neurotransmitter per neural impulse and/or 3) a reduced reuptake at the nerve junctions [102].

While an increased rate of sympathetic discharge has been shown in skeletal muscle vascular beds of resting elderly subjects such findings do not necessarily indicate a general elevation in sympathetic nerve discharge [103]. For example, there is little or no increase in norepinephrine spillover into the urine with age, as would be expected if resting sympathetic activity to the renal vascular bed had been raised [3]. On the other hand, several studies have shown that the rate of NE release is

augmented in the elderly, but production is similar to that of younger individuals, thus favoring the 3rd alternative, i.e. reduced reuptake at the nerve junctions [104-106]. Regardless of the reason for the enhanced effect of adrenergic nervous influences on the vasculature, this effect most likely compensates for the decline in vascular smooth muscle function with age [3].

2.5.2.3 Sympathetic Activity During Exercise

While age related changes in basal sympathetic outflow to skeletal muscles has been fairly well investigated, less is known about the possible sympathoadrenal adjustments during dynamic exercise. In one particular study, norepinephrine spillover was examined at rest and at 50% of peak cycle ergometry work capacity for 20 minutes in a group of young and old men. It was found that whole body norepinephrine spillover did not differ between groups under resting conditions. In response to 20 minutes of sub maximal exercise, whole body spillover rates increased significantly in both groups but did not differ between the groups. On the contrary, hepatomesenteric spillover rates were higher at rest and during exercise suggesting age-dependent regional differences in sympathetic nerve activity [107]. Of course it is not possible to determine whether norepinephrine overflow is due to elevated rates of sympathetic nerve firing or is a result of impaired neuronal reuptake of norepinephrine. Both mechanisms may be present, however, microneurography shows increased firing rates of postganglionic sympathetic efferent nerve fibers to resting skeletal muscle in advancing age [108, 109]

Proctor et al. [110] observed increased norepinephrine spillover at 210 W but not at lower relative sub maximal intensities in endurance trained men. In moderately active men leg NE spillover rates during exercise at 60% VO₂ peak was higher in older men compared to their younger controls [111]. It is likely, based on these

findings, that norepinephrine spillover would be elevated in older adults during exercise at the same absolute workload.

In contrast a more recent investigation by Koch and colleagues [112] suggest that vasoconstrictor responsiveness to acute sympathetic stimulation may be augmented in the exercising legs of moderately active older men. These authors recruited a group of younger and older men to perform cycle ergometer exercise at 60% of peak oxygen consumption during which time leg blood flow, mean arterial pressure and plasma noradrenaline concentrations were measured. In addition, after reaching a steady state these participants placed their hand in ice water in order to achieve an acute sympathetic stimulation. The cold pressor stimulation evoked a significant increase in mean arterial pressure in both groups of men. The increase in sympathetic outflow to the exercising legs was similar between groups, yet the older men demonstrated a larger percentage reduction in leg vascular conductance as a result of local cold stimulation. The authors suggest such an augmented response is necessary in the older adults to maintain arterial blood pressure during dynamic exercise in light of the fact that cardiac output measured at 60% of peak oxygen consumption was approximately $3.7 \text{ l} \cdot \text{min}^{-1}$ lower in the older adults in comparison to the young group [112].

Alternatively, it may reflect age related attenuation in functional sympatholysis. During exercise, vasoconstriction is produced by the sympathetic nervous system to direct cardiac output toward exercising skeletal muscle and to contribute to the maintenance of arterial pressure. This functional sympatholysis phenomenon refers to the decreased sensitivity to sympathetic stimulation or adrenergic agonists in the skeletal muscle during exercise [113] thereby ensuring adequate blood flow and oxygen delivery to the skeletal muscle [114]. In this context,

Dinenno and others [115] measured forearm hemodynamics (forearm blood flow and forearm vascular conductance) in response to α -adrenergic receptor stimulation during rhythmic handgrip exercise and during a control non-exercise vasodilator condition (intra-arterial infusion of adenosine) in young and older men. The forearm blood flow and MAP during the last 30s of α -agonist infusion was used to calculate the vasoconstrictor effect during the exercise and control condition. In young men, vasoconstrictor responses to tyramine (evokes endogenous noradrenaline release), phenylephrine (α 1 agonist) and clonidine (α 2 agonist) were blunted during exercise compared to the control condition. In older men, exercise did not significantly blunt the responses to tyramine and phenylephrine. The vasoconstrictor response to clonidine was blunted, albeit to a lesser degree than that observed in the young. That said, nitric oxide has been a mechanism implicated in functional sympatholysis [116]. Since endothelium-derived bioavailability of NO (a local vasodilator) is reduced with aging [117] it is suspected age-related impairment in endothelial function, could lead to impaired ability to blunt sympathetic vasoconstriction during exercise.

2.5.3 Local Control

2.5.3.1 Basal Endothelial Control

It is well recognized that endothelium derived vasodilating factors (NO in particular) contribute importantly to resting vascular tone and that responsiveness to acetylcholine is blunted with age [15, 21, 118-121] In what is now consider a classic study, Celermajer et al. [22] assessed endothelial function in the brachial artery by measuring flow mediated dilation (endothelium dependent) and endothelium independent dilation in a large sample of men and women without known risk factors for atherosclerosis. Reduced flow-mediated dilation, indicative of compromised function, was inversely related to age while no significant change in the response to

glyceryl trinitrate (endothelium independent mechanism) was observed. The authors concluded that a progressive endothelial dysfunction occurs with normal physiological aging. Other investigations have confirmed age-related impaired function in the coronary arteries [15] the basilar artery [122] and also in resistance vessels [123, 124].

2.5.3.2 Endothelial Control During Exercise

Whether the endothelium derived vasodilator responses play a significant role during exercise is currently under debate. Presently, there are no studies looking at this in aging model. However, a number of investigations have been undertaken clarify the role of endothelium during exercise. Gilligan et al. [125] found that the increased blood flow responses observed during incremental handgrip isometric exercise was reduced by 7% following infusion of L-NMMA, an inhibitor of nitric oxide synthesis. The effect was greater at higher doses of L-NMMA. In contrast, L-NMMA did not affect the forearm vasorelaxation to the selective endothelium-independent vasodilator sodium nitroprusside suggesting nitric oxide production plays a significant role in exercise vasodilation and subsequently vascular resistance. In a similar experiment [126] forearm blood flow (FBF) was measured in young healthy men at rest and after three minutes of static handgrip exercise before and after intra-arterial infusion of L-NMMA. L-NMMA decreased resting blood flow and attenuated the increase in peak FBF immediately after exercise as well as during the middle and late stages of recovery. However, when the authors normalized exercise FBF by resting FBF the percent increase in exercise induced blood flow were similar before and after administration of L-NMMA, suggesting NO plays a minimal role in metabolic arteriolar vasodilation induced by exercise. Both experiments administered NO synthase inhibitors before muscle contractions. Arguably, the

distribution of blood flow in contracting muscles is different from that of resting muscles. Therefore, NO synthase blockers may not reach the vessels involved in hyperemia. Accordingly, Dyke et al. [127] infused L-NMMA *after* the onset of handgrip contractions of mild intensity and observed a significant reduction in blood flow responses compared to what was observed with hand gripping alone.

Subsequent administration of acetylcholine during the exercise caused an attenuated rise in blood flow, suggesting that NO does indeed play a role in exercise hyperemia. Based on these observations the authors concluded that during mild exercise NO may be released from the increase in shear stress on the endothelial cells [128] or via cholinergic nerve stimulation of the endothelium [129], whereas during heavy exercise of prolonged duration muscle metabolites may play a larger role in exercise hyperemia [130].

Shoemaker et al. [131] conducted a study in which a group of younger adults performed rhythmic handgrip contractions during infusions of saline, atropine (to block acetylcholine binding to muscarinic receptors), or atropine + L-NMMA (to further inhibit NO synthase). At rest, FBF, as assessed by Doppler ultrasonography was progressively reduced from control by the infusions atropine and then atropine + L-NMMA. Yet neither drug treatment altered the rate or magnitude of the increase in FBF upon going from rest to mild exercise. Thus, this study failed to support the findings of Dyke and colleagues [127]. The authors speculate that the different methodologies may partly explain these contrasting findings. Whereas Doppler ultrasound allows continuous monitoring of FBF venous occlusion plethysmography requires interruption of the exercise and therefore reflect a combination of exercise and post exercise blood flows. Indeed, the post exercise hyperemic response was

reduced from control in the atropine + L-NMMA treatment indicating a role of both muscarinic and nonmuscarinic receptor-mediated NO release during recovery.

Forearm blood flow measurements are somewhat limiting in that they involve only small muscle mass at low exercise intensities and may not be reflective of the locomotory muscle of the leg. Radegran and colleagues [132] measured the effect of NOS inhibition per se on blood flow (via Doppler ultrasound) by infusing L-NMMA a rest, during sub maximal one-legged knee extension exercise and following recovery. While L-NMMA caused a significant and persistent reduction in FABF during rest and post exercise recovery, no effect was observed during dynamic exercise. Bradley et al. [133] measured leg blood flow using thermodilution technique during supine cycling, a modality more typical of that recommended for cardiovascular health. Similar to the findings of others [132] infusion of L-NMMA had no effect on exercise blood flow. While these findings do not exclude the role of NO, it demonstrates that the role of NO is not essential for the exercise response [132]. It is possible however that in order to compensate for diminished NO levels other vasodilators formed in the skeletal muscle such as adenosine [134], prostacyclin [135] or potassium [136] are produced or released to a greater extent[137]. However, Frandsen and colleagues [137] did not find increased concentrations of interstitial adenosine, PGI₂ and potassium concentrations in contracting skeletal muscle upon inhibition of NOS by infusion of L-NAME suggesting that the lack of response of NOS inhibition on the rate of blood flow in contracting muscle is not due to compensatory formation or release of these substances.

This issue of redundant mechanisms was looked into further by others [138]. EDHF is another factor released by the vascular endothelium which has been shown to illicit vasodilation, independent of NO and prostanoids [139]. Hillig et al. [138]

examined the role of CYP 2C, an EDHF, in regulation of skeletal muscle blood flow at rest and during exercise. This was done by infusing sulfaphenazole (and EDHF inhibitor) alone and in combination with L-NMMA. The authors found that in comparison with a control there was no difference in blood flow at any time with sulfaphenazole alone. However when infused in combination with the NO inhibitor, blood flow was 16% lower than in the control condition. The lack of an effect with sulfaphenazole suggests a redundancy between EDHF and NO, and EDHF can serve as a vasodilating factor during exercise when NO is diminished. Finally, Schrage [140] sought to determine whether local inhibition of NO and prostaglandin synthesis during rhythmic handgrip exercise would reduce exercise hyperemia. Participants performed handgrip exercise for 20 minutes. During the fifth minute L-NAME (a NO inhibitor) was infused, followed by ketorolac infusion (a COX inhibitor). Blood flow responses were measured using Doppler ultrasound. The authors found that after reaching steady state exercise, infusion of L-NAME reduced FBF to 80% of control levels. Subsequent infusion of ketorolac further decreased the blood flow by approximately 10%. At the end of ketorolac infusion, however, the blood flow had returned to L-NAME levels indicating that the vasodilating factor which restored hyperemic response was not NO [140]. In light of the results of studies reviewed above it is possible the EDHF may serve in that capacity since blocking COX may result in increased synthesis of EDHF [140]. Further studies in this area are necessary to elucidate these complex interactions.

We have now established the major age-related alterations in vascular control. It is evident that these changes may result from and/or cause the structural “adaptations”. Together they significantly alter cardiovascular function. Prior to

discussing the consequences we first take a look at some major theories purported to explain endothelial aging.

2.5.4 Mechanisms of endothelial aging

2.5.4.1 Increased Production of $\bullet\text{O}_2^-$ Free Radical

NO is generated by the metabolic conversion of L-arginine into L-citruline by the activity of NO-synthesizing enzyme [NOS]. The eNOS isoform is expressed under basal conditions and the NO generated from this reaction stimulates guanylate cyclase, which subsequently provokes vasodilation of the vascular smooth muscle cells.

Van der loo [141] and colleagues found that eNOS mRNA expression and activity in rat aorta increased in an aged dependent manner while NO-mediated vascular endothelial function declined with age. Interestingly, levels of endogenous NO were lower in old and middle aged animals compared with those from young rats, indicating that decreased NO production in the aged animals was due to neither a reduced expression of NO producing enzymes nor a reduced activity in the L-arginine/NO pathway. Instead, the authors attributed this reduced NO availability to a threefold higher activity in endothelial $\bullet\text{O}_2^-$. This free radical, which is formed by autoxidation of components of the respiratory chain, inactivates NO by forming peroxynitrite. Peroxynitrite (ONOO^-) in turn, inactivates the enzyme manganese SOD (MnSOD), which normally detoxifies $\bullet\text{O}_2^-$ to form H_2O_2 [141]. ONOO^- can also switch the NO synthase from a NO- to an O_2^- - generating enzyme. These reactions increase the concentration of O_2^- further promoting ONOO^- formation and causing DNA damage. It is believed that mitochondrial dysfunction with age is responsible for increased production of O_2^- . NADPH oxidase may also play a similar role given that inhibition of this enzyme reduces O_2^- generation in the aorta of rats [142].

Meanwhile, increased eNOS expression and activity observed by van der pool and colleagues [141] may in part act as a protective mechanism against the age-dependent oxidative stress or be the result of increased shear against the vessel wall due to structural modifications with aging [143, 144]

2.5.4.2 Senescence of Endothelial Cells

Endothelial cells are known to undergo senescence which has been defined as an irreversible state of growth arrest [145]. Telomere length is often used as an index of senescence. Telomeres are base sequences that protect the ends of chromosomes from damage and prevent them from binding to other DNA strands. Approximately 50-200 base pairs of telomeric DNA fail to replicate during each duplication and senescence is reached when the telomeres are shortened below a critical length [146]. Aviv et al evaluated telomere length on the chromosomes of non cultured endothelial cells of the abdominal aorta and found there to be an inverse relationship with age [147]. True stem cells express telomerase reverse transcriptase (TERT), which counteract telomere shortening. Indeed, Murasawa et al. [148] performed gene transfer to achieve constitutive expression of TERT, into human endothelial progenitor cells. This led to a conservation in telomerase activity and delay in senescence[146].

2.5.4.3 Decrease Vascular Endothelial Growth Factor (VEGF)

Aging is associated with increased exposure to various stimuli (i.e., inflammatory cytokines, oxidized lipids, turbulent blood flow) which eventually lead to cell injury or death (apoptosis). Apoptosis leaves gaps in the endothelial monolayer which must be filled by hyperplasia of existing cells, spreading of adjacent cells or engraftment of circulating endothelial progenitor cells [146]. However, the proliferative and regenerative capacity of the endothelium is attenuated with age[149,

150]. Rivard and colleagues measured collateral vessel development following forty days after resection of a femoral artery in young and older rabbits and rats. They found reduced capillary density, indicative of impaired angiogenesis in the older group of both animal models. The authors attributed this impaired angiogenesis to a lower expression of vascular endothelial growth factor (VEGF) because recombinant VEGF protein administration to both the younger and older groups of animals resulted in significantly improved capillary development [150].

In summary, a number of theories have been proposed to explain poor endothelial function with age. These may extend to other cells and tissues to help explain the aging process in general. Continued efforts aimed at understanding these mechanisms will assist in developing effective therapies designed to maintain or improve cardiovascular function throughout the lifespan.

2.6 Consequences of Vascular Aging

Having established the structural and vascular control changes taking place in advancing age and discussed their interaction we now examine the consequences of such changes on the physiological functional capacity in the aging adult. This will link the reader to Sections 7 and 8, which focus on the decline in physiological functional capacity seen across the lifespan and the role of exercise training in reversing diminished capacity. A decline in physiological functional capacity is mostly determined by a reduction in maximal oxygen consumption (VO_{2max} or VO_{2peak}). The capacity for VO_{2max} depends on the capacity of the cardiovascular system. The Fick equation serves as a reference point for understanding the components of VO_{2max} .

$$VO_{2max} = \text{Heart Rate}_{max} * \text{Stroke Volume}_{max} * a\bar{v}O_{2diff}$$

Thus, VO_{2max} is determined by the heart rate and the major factors which influence stroke volume. These include intrinsic factors such as cardiac contractility and extrinsic factors such as preload and afterload. Preload is the degree of stretch on the muscle fibers of the ventricular wall prior to systole and is dependent on the ability of the venous system to return blood to the heart. Afterload refers to the pressure the left ventricle must overcome in order for blood to be pumped out of the heart and is determined mostly by compliance of the central elastic vessels. Finally, avO_{2diff} refers to the amount of oxygen extracted by the muscles from the blood. This value is determined by the concentration of hemoglobin in the blood, the oxygen binding capacity, alveolar PO_2 , and alveolar ventilation. However, it also depends on the ability of the peripheral arteries to distribute blood to the skeletal muscle.

2.6.1 Central Arteries

The progressive loss of compliance of the large elastic arteries has several consequences on the function of the aging cardiovascular system. As alluded to above, cardiovascular performance is determined by the interaction of the intrinsic properties of the heart, such as power and stroke capacity and the mechanical load properties, or afterload imposed on the ventricle during ejection [151]. This interaction is referred to as ventricular-vascular coupling. Inappropriate matching of these properties leads to less than optimal maximal cardiac work and cardiac output and poor blood pressure responses [151]. Prior to discussing the consequences of central artery stiffness a brief discussion of ventricular-vascular coupling is needed.

2.6.1.1 The Concept of Ventricular-Vascular Coupling

The cardiac cycle consists of two phases; 1) systole- the period of time during which the muscle cross bridges are uncoupled to the point of maximal mechanical activation, and 2) diastole- period of time during which the muscle relaxes from the

maximally activated state back toward the resting state. Changes in pressure in the ventricular chamber cause blood to move in and out of the ventricle. Therefore, ventricular properties during the cardiac cycle can be characterized by displaying ventricular pressure as a function of ventricular volume on a pressure-volume diagram as depicted in Figure 2.1.

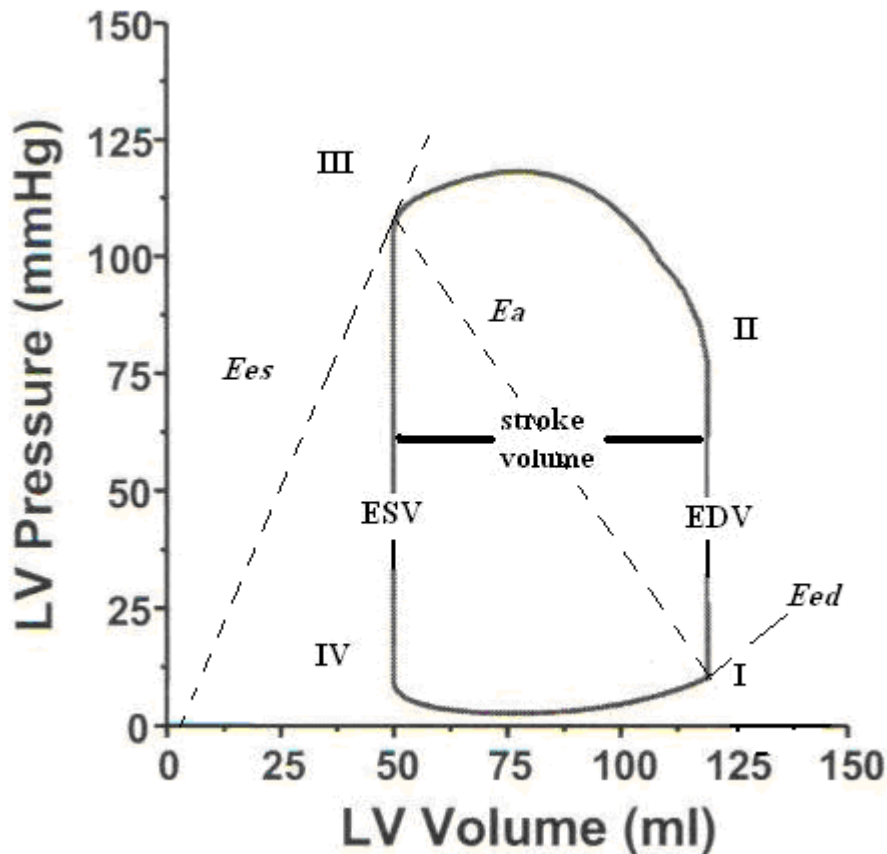


Figure 2.1. Flow volume loop demonstrates the changes in intraventricular volume and pressure during a cardiac cycle. Point I indicates where contraction of the heart begins. From this point until point II both the mitral valve and the aortic valve are closed and therefore no blood leaves the heart. This period is called *isovolumic contraction*. At point II left ventricular pressure exceeds aortic pressure. The aortic valve opens and blood is ejected from the ventricle and the LV volume decreases. This period between point II and III is the ejection phase. As the cardiac muscle reaches its maximal effort, ejection slows down, LV pressure falls below aortic pressure at point III forcing the aortic valve to close. The period between point III and IV is isovolumic relaxation. Both the mitral and aortic valves are closed. As LV pressure falls below left atrium pressure the mitral valve opens (at point IV) and left ventricle filling proceeds.

The plot of pressure versus volume for one cardiac cycle forms a loop, which is called a pressure-volume loop. The maximum volume the heart achieves is the end diastolic volume (EDV). Conversely, the minimum volume the heart attains is the end systolic volume (ESV). The difference between the EDV and the ESV is the stroke volume, which represents the amount of blood ejected during the cardiac cycle. Ventricular diastolic elastance (E_{ed}) defines diastolic stiffness and ventricular systolic elastance (E_{es}) defines stiffness in the ventricular chamber at end-systole. These measures describe the intrinsic properties of the heart and are influenced by preload and afterload, respectively. Preload is the hemodynamic load on the heart muscle wall at the end of diastole before contraction begins and afterload is the hydraulic load imposed on the ventricle by the arterial system during ejection. Accordingly, arterial properties can also be represented on the diagram as effective arterial elastance (E_a), which equals the ratio of P_{es}/SV , where P_{es} is ventricular end-systolic pressure. Arterial elastance is a reflection of the functional properties of the arterial system; notably peripheral vascular resistance, vascular compliance, wave reflections or impedance [152]. By coupling the characteristics of the heart with the arterial loading properties one can determine specific cardiovascular performance variables, such as stroke volume, stroke work, ejection fraction and cardiac efficiency.

Ventricular vascular coupling is indexed by the ratio of E_a/E_{es} . Asanoi and colleagues [153] found that in normal individuals, i.e., those with an ejection fraction of 60% or more, ventricular elastance was nearly twice as large as arterial elastance thus allowing for maximal mechanical efficiency. In contrast, those individuals with severe cardiac dysfunction (ejection fraction less than 40%), ventricular elastance was less than one half of arterial elastance indicating suboptimal ventricular performance and metabolic efficiency.

Altered reserve mechanisms can be easily described using the flow-volume loop diagram. Figure 2.2(A) illustrates the flow volume loops of a hypertensive older adult at rest (solid line) and during exercise (dashed line). During exercise, increased contractility of the heart results in a significant pressure rise (Ees shift to the left) for a given change in stroke volume because the heart is pumping against a high vascular load (notice the steep Ea). In a normal individual depicted in Figure 2.2(B), a similar rise in stroke volume yields less pressure change because of reduced vascular loading (Ea is less steep).

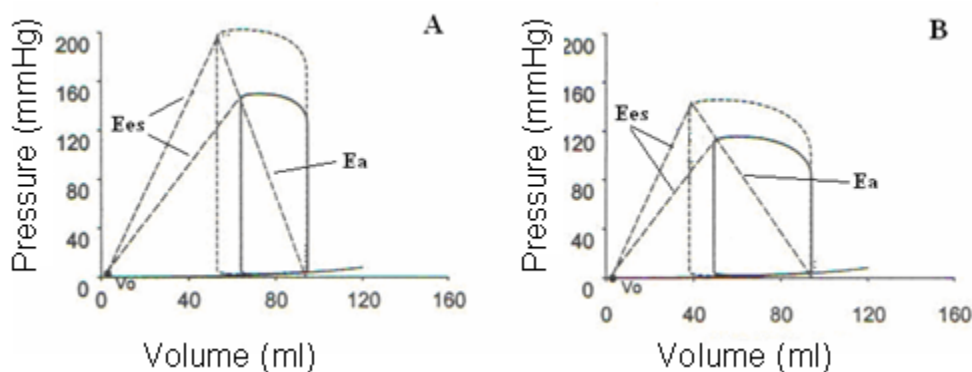


Figure 2.2. Influence of arterial load on the residual effect of higher contractile function on blood pressure and stroke volume. (A) Displays a hypertensive individual with high arterload. With exercise, contractility increases leading to a significant rise in systolic pressure for a given change in stroke volume. (B) Displays the identical contractility change, but a lower afterload. The systolic pressure rise is less relative to a greater stroke volume increase. Thus, the interaction of the heart and arterial system determine the net effect of reserve capacity [151]

2.6.1.2 Effects of Ventricular-Vascular Stiffening on Functional Capacity

As previously discussed in Chapter 5 aging is associated with an increase PWV and augmentation index, both indices of vascular stiffness. Chen et al. [154] recently confirmed the age related increase in vascular stiffness using the Ea parameter. In their investigation of 57 adults aged between 19 and 93 years they found that the age related increase in arterial stiffening (Ea) was matched by a similar increase in ventricular systolic stiffness (Ees), indicating a well-preserved arterial-

heart interaction (E_a/E_{es} ratio) with age. It is believed that increased ventricular wall thickness in the myocardium underlie the changes in E_{es} with age [155]. This appears to an adaptive mechanism designed to overcome the elevated systolic afterload as well as to offset the decline in contractile strength of the myocytes [3, 154]. This ensures that chamber power, stroke work and efficiency are maintained at near maximal levels [151].

Unfortunately, the matched coupling with age can ultimately limit the functional reserve of the cardiovascular system under stress. An increase in cardiac output during exercise is the result of an increased heart rate and systolic contractile function. E_a rises during exercise as a function of increased heart rate and arterial pulsatility, while contractile elevation is reflected by a rise in E_{es} [151]. Since these two parameters may already be elevated in the aged cardiovascular system, see Figure above, then there would be less reserve. Consequently, the capacity of the heart to eject blood is compromised at the cost of increased metabolic demand [151]. Indeed, the increase in ejection fraction during peak exercise becomes blunted with aging [156] while no age-related differences are seen in the resting ejection fraction [157] suggesting that aging is associated with less reserve.

2.6.2 Peripheral Vascular Function

As we move down the arterial tree the focus shift from maintaining blood pressure and cardiac function to supplying local tissues with adequate blood supply. This is the responsibility of the smaller conduits and resistance vessels in the periphery. Active muscles require an adequate local blood supply to meet the metabolic demands of exercise and activities of daily living. Therefore, a discussion of the age associated alterations in blood flow is relevant to aging since attenuated

delivery of flow could contribute to reduced functional capacity attenuated blood flow delivery would potentially have serious functional implications for the older adults.

This discussion is devoted to large muscle dynamic exercise and therefore it is possible that age-related differences in leg blood flow could also be influenced by age-related decline in muscle mass [158]. However in all the studies discussed below younger and older men were matched according to leg muscle mass, therefore essentially eliminating this confounding factor. Also, at no point can limb blood flow exceed cardiac output [64]. Given that reduced cardiac outputs during maximal exercise are often observed in older adults [159, 160] it is to be expected that limb blood flow during peak cycling exercise would also decline with age [161-163]. Yet, differences in blood flow are seen between young and old sedentary individuals persist even during isolated muscle mass exercise, indicating peripheral vascular limitations

To evaluate age related blood flow changes during dynamic exercise Beere et al. [161] studied a group of men aged 61-74 years of age 13 and younger men 21 to 39 years of age. These individuals were tested on a cycle ergometer starting at 25W and advancing 25 W every 3 minutes until maximal exhaustion was reached. The investigators used radionuclide angiography and thermodilution catheter to assess central function and leg blood flow, respectively. The younger group achieved a significantly higher peak workload compared to the older group, 807 kpm vs. 630 kpm respectively. Oxygen consumption and cardiac output were higher in the young both at rest and at peak exercise. Although leg blood flow was similar at rest and sub maximal workloads it was lower in the older men at peak exercise. No differences in avO_2 diff were observed at any point during the exercise but leg oxygen consumption was 33% lower in the older subjects at peak exercise. These results indicate that the

lower peak oxygen consumption in the older group was a result of lower peak cardiac output and central $\text{avO}_{2\text{diff}}$. These findings point to a less efficient redistribution of blood flow considering the femoral venous oxygen content was as low as that seen in the younger subjects, and hemoglobin concentration was not dependent on age.

Similarly, Poole et al. [162] found that limb blood flow at sub maximal workloads of 15 and 30 watts were comparable between young (mean age 20 years) and old (mean age 70 years) sedentary men but was attenuated in the older men at 99 W and maximal work rate. At each absolute work rate (WR) and WRmax the leg $\text{avO}_{2\text{diff}}$ was significantly higher in the older group, apparently in an effort by the older subjects to achieve similar muscle VO_2 at the most taxing sub maximal work level (99 W). Above this intensity however, leg VO_2 was attenuated in the older group. Despite elevated O_2 extraction in the old subjects, the normoxic muscle O_2 transport conductance (DO_2) 100% work rate max was reduced. Muscle O_2 conductance is measured in $\text{ml}/\text{min}/\text{mmHg}$ and reflects the rate at which O_2 can move by diffusion from Hb in the red cell to the muscle mitochondria [164]. Such an effect would indicate an O_2 transport limitation from blood to muscle cells or possibly a mitochondrial O_2 demand limitation. Therefore, even if limb blood flow were restored to the level of the younger adult any benefit may be offset by the poor diffusive component of O_2 transport [162].

Other investigators [165] found that leg blood flow responses at light cycle ergometry workloads (20-40 W) were similar between younger (20-27) compared to older (60-71) recreationally active women. However, at moderate workloads of 50-60 W leg blood flow response were significantly attenuated in the older group. This trend was apparent at peak exercise, for leg blood flow was 29% lower in the older women [163]. Furthermore, the reduced blood flow at moderate and peak intensities was

associated with an attenuated rise in leg VO_2 . This occurred despite elevated perfusion pressures i.e., direct measurement of mean arterial pressure (MAP) was 20-25 mmHg higher in older women across all work rates, thus indicating a potential peripheral limitation.

In a more recent study by Proctor and colleagues [111] eleven younger and eight older normotensive “moderately active” men performed both graded and constant load bouts of leg cycling at the same absolute and relative exercise intensities. In contrast to the findings in sedentary older men and recreationally active women, leg blood flow responses across absolute work rates between 20 and 100 watts were not reduced, and may have been slightly augmented in older normally active men despite an age-associated reduction in cardiac output.

Similar measurement techniques were used in order to compare leg hemodynamic and metabolic responses during sub maximal cycle ergometry in younger and older men who were chronically endurance trained. Proctor et al. [110] showed an approximate 25% reductions in lower leg blood flow at three sub maximal workloads, which was associated with reduced vascular conductance. This occurred despite similar leg muscle mass, cycling experience, or arterial O_2 carrying capacity. Mean leg VO_2 responses across all work rates were similar between the younger and older men, consistent with findings elsewhere [166].

In summary, it appears that during dynamic exercise blood flow to the exercising limb is reduced with age in endurance trained men [110], sedentary men [161] [162] and women [163, 165]. The importance of sex and level of fitness in modulating these differences will be discussed in subsequent sections.

2.6.3 Venous Compliance

The venous system plays a very important and often unappreciated role in cardiovascular function. Specifically, venous tone is a major determinant of cardiac preload because the heart can only pump out what passively fills it during diastole[30]. This cardiac output/venous return relationship was first described by Guyton and colleagues [167]. They explain that the rate of blood pumped by the heart is set principally by the filling pressure, or right atrial pressure. Venous return is determined by the pressure gradient (ΔP) from the peripheral vascular beds to the right side of the heart and the resistance to venous return (R) ($\text{Flow} = \Delta P/R$). If venous return is higher than cardiac output, blood accumulates in the right atrium, thus increasing right atrial pressure and cardiac output. Because the pressure gradient difference between the peripheral tissue and the heart is reduced venous return decreases. On the contrary, if venous return is lower than cardiac output blood is removed from the right atrium, the pressure drops which results in a reduced cardiac output and increased pressure gradient for venous return [167].

Compliance describes the change in blood volume that occurs for each unit of change in transmural pressure in a segment of the blood vessel ($\Delta V/\Delta P$). The volume-pressure slope is nonlinear and therefore compliance depends on distending pressure. At low pressures, a large change in volume accompanies a small change in pressure so compliance is high and the curve is steep. At higher pressures the slope is less steep and compliance is low [168]. The shape of the venous volume-pressure curve represents a compromise in design to meet two requirements; 1) to redistribute blood to the heart to increase filling and restore cardiac output, and 2) serve as a blood-storage reservoir that allows substantial loss of blood with only small changes in venous and arterial pressure. If veins were as stiff as arteries the problem of

hydrostatic pooling would be eliminated but the system's ability to adjust to a sudden blood loss would be compromised [99].

Unfortunately, the impact on changes in venous compliance in the legs on cardiovascular function with aging has been limited to its role in altering the cardiovagal reflex, rather than the impact on $\text{VO}_{2\text{max}}$, per se. Venous return and the subsequent effect on raising end-diastolic volume and stroke volume during exercise is mostly likely influenced by the muscle pump [64], and active venoconstriction, although the role of the second mechanism in humans is currently under debate [169]. However, several investigators argue the passive elastic recoil of the venous bed related to the reduction in cardiac output is more important than active venoconstriction [64, 169]. Furthermore, a positive relation has been found between maximal oxygen consumption and calf venous compliance [80].

It has been suggested by others [170] that the increased venous compliance seen with endurance exercise training may actually be one mechanism which contributes to orthostatic intolerance. Indeed, Pawelczyk [171] observed decrease in systolic blood pressure and greater amount of fluid pooling in the leg (indicating greater compliance) during an orthostatic challenge following seven weeks of exercise training compared to baseline in a group of 18-25 year olds. In this context, endurance training might be contraindicated in older adults at a high risk for falling [172].

Some investigators have reported no relationship between tolerance to lower body negative pressure (LBNP) and compliance [172] while others have attributed a reduced leg compliance to increased tolerance to orthostatic challenge [173]. Differences in study protocol may account for conflicting findings regarding these relationships.

In summary, it appears that consequences of structural and vascular controllers are an inefficient ventricular-vascular coupling relationship, poor blood flow delivery to the active muscles and potentially less than optimal venous return. Collectively, the potential to alter physiological functional capacity is tremendous. Next, we look at the decrease in VO₂max across the lifespan.

2.7 Effects of Aging on Functional Capacity

One can not fully appreciate the potential impact these vascular changes have without actually examining the declines in functional capacity seen with advancing age. In the following section we look at the most recent data looking at the VO₂max / Aging relationship. As you will see studying the master athlete represents a good model because it eliminates potential confounders discussed in the introduction. Yet, reference to the general population, specifically data from the Baltimore Longitudinal Aging Study, will be discussed because it is more applicable to the aging population as a whole.

2.7.1 Insight from the Master Athlete

A popular experimental model used to study the effects of primary aging on physical functional capacity is the master athlete. It is argued that by doing so one can nearly eliminate the confounding effects of reductions in physical activity, changes in body composition and development of clinical diseases across the lifespan [174]. Recent investigations have established that both endurance running performance [174] and swimming performance [175] declines with age in a curvilinear fashion for men and women. For running, performance is maintained until the age of 35 years. Modest decreases are seen until the 5th or 6th decade of life followed by steeper decay thereafter. The declines in swimming performance with advancing age is 30% smaller than observed in running performance and

progressively steeper declines in performance occur nearly a decade later (approximately 70 years) indicating physical functional capacity may be limited by the nature of the task involved [174]. For example, orthopedic injuries are more prevalent among runners and this may in turn limit training volume and intensity and thus account for the earlier decay.

In a subsequent longitudinal trial, swimming performance times of nearly 700 men and women who participated in the US Masters Swimming Championships were followed over a 12 year period. The authors found that the differences in performance between men and women was greatest in the shorter events and became progressively smaller with increasing distance. Thus, age associated declines in physiological determinants of sprint and endurance events may occur at different rates.

The reason for such an accelerated decline in exercise performance in between the ages of 60 and 70 years is not entirely clear but such findings are consistent with investigations examining the affect of age on physical function. For example, Himann et al. examined the effects of age on walking speed in men and women aged 19 to 102 years. They found that before 62 yr, there was a 1 to 2% per decade decline in normal walking speed. After 63 yr, females showed a 12.4% per decade decrease and males showed a 16.1% per decade decrease [176]. Joyner [177] suggested that such a significant impairment in physical function between the ages of 60 and 70 years could occur due to fundamental changes in biological aging. On the other hand, it may reflect a decline in the motivation to train at intensities needed to maintain physical functional performance since greater declines in maximal aerobic capacity in a sample of endurance trained women was due in part to reductions in training volume [178]. Similar findings were observed in men [179]. It is well regarded that the

decline in maximal aerobic capacity is the principal physiological mechanism underlying the age-related reduction in physical functional capacity [180].

2.7.2 Decline in VO_{2max} with Age

This concept that decreases in VO_{2max} contribute to the reductions endurance performance is based upon earlier work by Costill and colleagues who showed a strong inverse relationship between VO_{2max} and time in a 10-mile run in individuals with a range of VO_{2max} between 54.8 ml/kg/min and 81.6 ml/kg/min [180]. This inverse relationship has since been confirmed by more recent investigations involving highly competitive male and female athletes varying in age [177, 179, 181, 182]. Furthermore, it appears that maximal aerobic capacity declines at a greater rate with age in those who are highly trained compared to those who are sedentary [179, 183].

Several reasons have been postulated which may help to explain this steeper decline in aerobically trained individuals. First, this may merely represent a baseline effect, whereby those with the highest levels of aerobic capacity may demonstrate the greatest decline. This theory is supported by that fact that if changes in VO_{2max} are expressed as relative changes the rate of decline in VO_{2max} is similar between fit and sedentary groups [179, 183]. Second, the training volume and intensity may drop off significantly in the later years of life. Therefore, the magnitude of decline in physical activity would be much greater compared to those who do not perform physical activity. In support of this claim, Ezkurza and colleagues [178] followed a group of sedentary and endurance trained women over a seven year follow-up period. The rate of decline in VO_{2max} in the endurance trained women was two fold greater than the sedentary women and this decline was positively related to reductions in training volume. The rates of decline in VO_{2max} for endurance trained women who maintained their training volume was similar to the

sedentary women. Third, a steeper decline in $\text{VO}_{2\text{max}}$ in endurance trained athletes may be the consequence alterations in the physiological determinants of $\text{VO}_{2\text{max}}$; maximal heart rate, maximal stroke volume and maximal $\text{avO}_{2\text{diff}}$. Previous work has failed to observe any significant relation between maximal heart rate and physical activity status [178, 179, 183] indicating that stroke volume and peripheral factors have a greater affect on the decline.

In order to investigate the mechanisms by which aging and physical activity influenced $\text{VO}_{2\text{max}}$ Ogawa and colleagues [184] quantified $\text{VO}_{2\text{max}}$, maximal heart rate, stroke volume and $\text{avO}_{2\text{diff}}$ in a group of younger and older sedentary and endurance trained men and women. As expected, the $\text{VO}_{2\text{max}}$ was significantly lower in older than in younger subjects. For both sedentary and trained individuals a smaller maximal cardiac output explained 72% and approximately 85% of the age related decline in $\text{VO}_{2\text{max}}$, respectively. The remainder was explained by reduced oxygen extraction. Although a slower maximal heart rate accounted for a small portion of this effect on cardiac output, a small stroke volume had a greater impact [184].

2.7.3 Longitudinal Evidence of a Decline in $\text{VO}_{2\text{max}}$ in the General Population

Of the data discussed thus far, much has been cross sectional or has dealt with elite athletic population and therefore not representative of the general population. Cross sectional studies have reported a decline in $\text{VO}_{2\text{max}}$ of approximately 5-15% per decade [185, 186]. Problems with cross sectional investigations include; 1) selection bias- healthy elderly who volunteer for exercise studies may be inherently healthier compared to their younger counterparts due to lifestyle or genetic variability [187], 2) The age related decline in function of physiological systems is progressive but does

not occur at the same rate [188]. In order to fully appreciate the effects of aging on VO_{2max} a longitudinal study is needed.

Perhaps the most comprehensive examination of changes in aerobic fitness across the life span comes from the Baltimore Longitudinal Study of Aging. Fleg and colleagues [19] recently published data on eight hundred volunteers who submitted themselves to medical, physiological and psychological testing every two years starting in 1976. Similar to cross sectional investigations recently reported data from the BLSA indicate that starting at age 30 years longitudinal declines in peak VO_2 average 5% per decade but decline at an accelerated rate in the old. Specifically, peak VO_2 declined approximately 23% in a 10 year period for men over the age of 70 years (see figure below). This age related accelerated decline, which persisted in both sexes was mirrored by a corresponding change in O_2 pulse (product of stroke volume and $avO_{2\ diff}$). Similarly, investigators at the Canadian Centre for Activity and Aging found an age related decline in VO_{2max} of 14% in men and 7 % in women over the age of 60 years [189].

After reviewing this data it is apparent that declines in VO_{2peak} are not linear but rather accelerate with successive age decades in both men and women. In addition, while greater physical activity does not prevent the decline, maintaining a physically active lifestyle may ward off disability which accompanies poor physiological capacity. However, as we shall see in the next section physical training in the old is accompanied by improvements in VO_{2max} .

2.8 Effects of Exercise Training on Vascular Physiology

It is now well recognized that vigorous exercise training can enhance VO_{2max} in older men and women [190-193]. Specifically, Kohrt and colleagues [193] found an approximate 24% increase in VO_{2max} in a group of 60-71 year olds who exercise

between 9-12 months. This improvement was independent of sex, or initial fitness level. Hagberg et al. [191] observed a 22% increase in VO_{2max} in a group of 70-79 year old men and women after 26 weeks of aerobic exercise training.

Until recently, little attention has been given to the “oldest old”, or those over the age of 80 years. This is particularly relevant given that this portion of the population is the fastest growing portion of the population and is more likely to exhibit comorbidities, thus placing them at risk for losing their independence. Binder and colleagues [194] recruited over one hundred sedentary men and women 80 years and older with mild to moderate physical frailty to participate in a nine month exercise intervention which consisted of three months of physical therapy, three months of resistance training followed by three months of endurance exercise. The exercise intervention resulted in a 2 ml/kg/min improvement (approximately 14%) in VO_{2peak} , a significant but modest improvement as compared to that seen in those individuals between the ages of 60-80 [191, 193]. In the paragraphs that follow we discuss the impact of exercise training on some of the proposed vascular determinants of physiological functional capacity.

2.8.1 Central Arteries

Vaitkevicius et al. [46] hypothesized that physical conditioning in older individuals might attenuate the age associated increase in arterial stiffness. Indeed, in those individuals over the age of 70 years, there was a significant decrease in aortic pulse wave velocity with increasing VO_{2max} . Furthermore, the VO_{2max} in senior athletes was significantly higher and APWV and augmentation index significantly lower compared to their sedentary peers[46].

More recent cross sectional analysis provides additional support for the role of exercise in attenuating the age associated decline in large artery compliance. Tanaka

et al. [195] found aortic PWV were 30% lower in post menopausal women who were actively engaged in aerobic exercise vs. those who were sedentary.

To gain better insight into the dose response relation between regular physical activity and age-related decreases in central arterial compliance the same authors studied a group of young, middle aged and older men who were either sedentary, recreationally active or endurance trained. Importantly, central arterial compliance in the endurance trained middle-aged and older men was 20% to 30% higher than in their sedentary and recreationally active peers indicating that vigorous aerobic endurance exercise appears to modulate age-related decreases in central arterial compliance [67].

Evidence now exists that training can restore the age-associated decline in central arterial compliance. In one particular study, previously sedentary middle aged and older men engaged in three months of aerobic exercise training, which resulted in a 25% improvement in central arterial compliance and was similar to levels observed in cross sectional data [67]. Furthermore, it is well recognized that endurance training performed by older men results in improved left ventricular systolic function [192]. The extent to which a reduction in central artery compliance, *pre se*, accounts for this improvement is not yet know. However, evidence from pharmacological studies indicated that both vascular and ventricular properties may need to be optimized in order for there to be an effect on exercise tolerance. For example, Chen et al. [196] pretreated a group of older volunteers with verapamil prior to undergoing a symptom limited exercise test. The verapamil lowered E_a and arterial stiffness as measured by PWV and resulted in a improved exercise capacity as compared to control.

2.8.1.1 Cardiovagal Baroreceptor Sensitivity

Age and habitual exercise-associated differences in cardiovagal BRS are associated with corresponding differences in the compliance of the carotid artery among healthy men. Monahan and others [197] recruited young and old sedentary and endurance trained men and assessed cardiovagal BRS and compliance of the carotid artery. Cardiovagal BRS was greater in endurance-trained than sedentary older men but did not differ with exercise status in young men. Increases in carotid lumen diameter per unit increase in SBP (deformation) were greater in young than older sedentary and endurance-trained men and in the endurance trained compared with the sedentary older men. When these diameter changes were controlled for all group differences were no longer significant.

2.8.2 Peripheral Blood Flow

2.8.2.1 Differences in Blood Flow Between Men of Different Fitness Status

The reason for such a discrepancy in blood flow response between older men who vary in physical fitness status is not immediately clear. However, it has been suggested that attenuated blood flows observed in trained older men indicate an adaptive response given that leg $\text{avO}_{2\text{diff}}$ during exercise were significantly higher in the trained men [110]. Indeed it has been shown that older endurance trained men have a more extensive capillary supply network and higher oxidative enzyme activities than their sedentary counterparts [198].

On the other hand, reduced leg blood flow during sub maximal exercise in sedentary older adults may represent a maladaptive response considering reduced leg blood flow responses at higher sub maximal intensities seen in this group of individuals is associated with reduced leg VO_2 [161, 162, 199] thereby suggesting that

attenuated blood flows are too great to be fully compensated for by increasing leg O₂ extraction.

2.8.2.2 Difference Between Recreationally Active Men and Women

The disparate findings between older men and women of the same fitness category, i.e., recreationally or moderately active, in comparison to their younger counterparts is suggestive of a gender difference in the effect of age on leg blood flow during sub maximal exercise. Alternatively, the attenuated blood flows observed in sedentary men performing sub maximal exercise [162] were similar to those observed in recreationally active women [165], e.g., approximately 2000 ml/min in one leg at 60 watts. These groups had similar pulmonary VO_{2max} (21 ml·kg·min⁻¹ in men vs. 24 ml·kg·min⁻¹ in women), thus suggesting that fitness level could modulate the leg blood flow responses to exercise [20].

Based on the evidence presented above, it is clear that an alteration in active leg blood flow occurs with increasing age and thus may be one factor responsible for the age-related decline in functional capacity. Little information exists regarding potential role of exercise to reverse these alterations. Martin et al. [200] measured lower leg blood flow and conductance by plethysmography at rest and during maximal hyperemia in a group of older men and women (mean age 64 years) before and after 7 months of a walking/jogging exercise program. The exercise program led to a 23% improvement in maximal oxygen uptake and improvements in maximal calf blood flow and conductance of approximately 36% and 35%, respectively.

Only one study has assessed sub maximal and peak leg blood flow in healthy older men before and after exercise training. Following three months of training with cycle ergometry both sub maximal and peak leg blood flow increased and peak leg oxygen consumption increased by 42%. Furthermore, peak oxygen consumption

increased significantly. This training adaptation was most likely due to greater distribution of blood to the exercising limbs considering systemic oxygen difference increased without a change in peripheral oxygen extraction. Although the above study was not designed to elucidate the mechanisms responsible for such a change it may result from alteration in relationship between vasodilator and vasoconstrictor factors [161].

2.8.3 Venous Function

Hernandez et al. [172] described the effect of exercise-associated changes in leg venous compliance on responses to orthostatic stress in older adults. The authors studied 40 men and women who were subsequently divided into four groups based on age and fitness level; young and fit, young and unfit or old and fit or old and unfit. Venous compliance was measured in a manner consistent with what had previously been reported by Monahan. In addition, the participants underwent LBNP testing. Tolerance to LBNP was defined as the point at which a participant could no longer maintain mean arterial pressure and experienced presyncopal symptoms, such as dizziness, nausea or a rapid change in blood pressure defined as either a decrease in SBP by 25 mmHg or a decrease in DBP by 15 mmHg within 1 min. Similar to previous reports [80] the fit participants displayed greater venous compliance compared to their unfit counterparts while the older fit group had greater compliance than did the young unfit and older unfit groups. Despite these differences in compliance with age and fitness, there were no differences in tolerance to simulated maximal orthostatic stress. In a related trial by the same authors [201], young fit individuals had an earlier increase in heart rate and decline in stroke volume compared to young unfit, older unfit and older fit individuals, while tolerance to maximal LBNP did not differ among the groups.

Similar to the above findings, Tsutsui and colleagues [173] found reduced leg compliance in physically active older subjects compared to physically active younger group. Yet, these authors also found that the older participants also had higher orthostatic tolerance to LBNP, which they attributed to reduced leg compliance since central blood return reduction due to LBNP was attenuated in the older group. Several key differences could have explained the disparate findings. Unfortunately, neither fitness nor exercise habits were described by the above authors. Furthermore, individuals who experienced presyncope episodes were excluded from statistical analysis. Moreover, leg compliance was assessed while the participants were undergoing LBNP, whereas Hernandez assessed at rest. Thus, increases in leg muscle sympathetic nerve activity and subsequent alterations in leg blood flow due to LBNP could have confounded the findings.

In summary, it appears that alterations in compliance with age and fitness level do may affect the cardiovascular responses to and orthostatic stress yet these alterations do not affect orthostatic tolerance. Differences in study protocol may account for conflicting findings regarding these relationships.

2.8.3.1 Effect of Exercise Training on Venous Compliance and LBNP

Due to the cross sectional design, the above studies were unable to determine the extent to which differences in compliance may have affected findings. Furthermore, a longitudinal design could elucidate possible mechanisms and determine the time course of possible changes. In a recent trial a group of older men and women (mean age 73 years) underwent graded LBNP to presyncope or 4 minutes before and after a 6 month endurance-exercise training program consisting primarily of the use of treadmills and upright and recumbent bicycles [202]. In regards to the LBNP trial cardiac output was lower than rest for all groups in the last completed

stage of LBNP, but it was lower than rest at -20 mmHg for the exercise group pre intervention and at -30 mmHg post intervention. A 20-30% increase in calf venous compliance was also noted in the exercise group. No relationship was found either pre or post intervention between LTI and venous compliance. Nor was there a relation between change in LTI versus a change in compliance at 20 mmHg for both groups over the 6 month intervention. Thus, the authors concluded that significant changes in venous compliance can be observed in older adults with endurance exercise training, and these changes will not affect orthostatic tolerance.

In both the above trial [202] and in their previous cross sectional investigation [201] the authors found slight but statistically significant differences in cardiovascular responses to orthostatic stress in this older population following endurance exercise training program. In contrast, Carroll and colleagues [203] and Gabbet et al. [204] did not find differences in cardiovascular responses to head up tilt following a 26 week and 12 week endurance training programs. In another trial, cardiovascular performance was monitored during graded lower body negative pressure in nine highly trained male senior athletes with maximum O_2 uptake 52.4 ml/kg/min and nine age-matched control subjects with maximal O_2 uptake of 31.0 ml/kg/min. During lower body negative pressure (0 to -50 mmHg), left ventricular end-diastolic and end-systolic volume indexes and stroke volume index decreased in both groups while heart rate increased. These changes were less significant in the athletes than the controls suggesting that increased $VO_{2\max}$ among older men is associated with improved orthostatic responses. Hernandez et al. [202] found a 14 % improvement in fitness as assessed by six minute walking test. It is unclear whether greater improvements could have altered the cardiovascular responses to LBNP to a greater degree.

Although chronic endurance training did not appear to alter tolerance to maximal LBNP [202] it was apparent that less tolerant participants became more tolerant after six months of training. Similarly, older individuals who experienced lightheadedness, nausea, sweating or syncope during an orthostatic challenge (i.e., symptomatic individuals) were able to complete 15 minutes of head up tilt following 6 months of endurance exercise training [205]. Together, these findings suggest that endurance training may improve orthostatic tolerance in those with symptomatic orthostatic hypotension and subsequently allow the individuals to continue exercise training with limited risk of falling resulting from hypotension.

Based on the existing evidence, exercise training in older adults appears to be an effective intervention improving VO_{2max} due in part to a reversal of some of the age related vascular changes associated with poor physiological function. The potential mechanisms for such improvements will be discussed in Section 2.9.

2.9 Mechanisms for Improved Physiological Function with Exercise Training

In order to better understand relative contributions of increases in cardiac output and avO_{2diff} at maximal exercise to the training-induced increases in VO_{2max} Spina and colleagues [206] measured VO_{2max} and CO, via acetylene rebreathing technique, in a group of 60-69 year olds before and after 9-12 month endurance exercise training program which consisted of 70-85% heart rate maximum for 45 min/day, 4 days per week. Arteriovenous O_2 content difference was expressed as VO_2/Q . VO_{2max} increased 19% in men and 22% in women. In the men, increase in VO_{2max} was attributed to a 12% increase in Q and a 7% increase in arteriovenous O_2 content. Heart rate maximum decreased significantly after training. In women, $avO_2 diff$ increased significantly a result of the training, which in turn accounted for the entire increase in VO_{2max} .

It is believed the larger stroke volume resulting from exercise training in the older men is a result of left ventricular volume overload, an enhanced contractile response to β -adrenergic stimulation or reduced afterload [192]. Evidence in endurance trained older adults shows significant direct correlations between plasma and total blood volumes and peak exercise left ventricular end diastolic volume [207]. Thus expanded intravascular volumes may play a significant role in drawing out the chronic volume-overload left ventricular hypertrophy that may account for increased stroke volume [207]. Spina and colleagues [206] did not observe any differences in SBP, DBP and TPR following exercise training in their group of 60-69 year old men. Therefore, it is unlikely that the higher stroke volume during maximal exercise was due entirely to alteration in afterload [206].

In contrast to men, the increase in VO_{2max} seen in women was due solely to increase in avO_{2diff} . This may be the result of increased capillary density and increased activities of the mitochondrial enzymes as shown previously[208]. Whether peripheral vascular alterations had an influence on the increased avO_{2diff} at maximal exercise in the women was not examined.

With regard to performance in the oldest old, peak exercise cardiac output increased similarly in response to training but avO_{2diff} did not change. Regression analysis showed that 5% increase in maximal heart rate contributed significantly to the training induced increases in aerobic capacity in these octogenarians. While not statistically significant, the magnitude of increase in stroke volume was 10% in this group and could have accounted for the significant VO_{2max} change. As with the younger groups the larger cardiac output is indicative of enhanced systolic function.

The reason for the attenuated improvement is unclear. However, the fact that these individuals were frail may have precluded them from reaching an appropriate

stimulus. Notably, the absolute exercise intensities may have been too low to elicit positive adaptations. Furthermore, the frequency of training (ave 2.6 days per week) and duration (69 min per session, including warm-up, cool-down and rest periods) was significantly less than that required to improve VO_{2max} by 22% in 60-80 year old groups [191, 193]. Alternatively, the biological capacity to adapt to training may be limited once one reaches their ninth decade of life. This seems to fit with the accelerated decline seen in the longitudinal data [19]. The last argument is supported by the fact that even healthy non-frail octogenarians, who were presumably less affected by the orthopedic and morbidity related limitations, experienced less than a 13% increase as a result of a similar exercise training program [209].

These differences in findings between men and women and different leave it open to many possibilities and worthy of further exploration. Are central factors mainly responsible for changes in men whereas peripheral factors the primary change in women? The potential vascular affects will be dealt with in the next sections.

2.9.1 Changes in Vessel Compliance with Training

Vascular compliance is determined by intrinsic elastic properties of the arteries, which is composed of structural determinants, i.e., composition of elastin and collagen and functional determinant, i.e., smooth muscle cell function. That said, several mechanisms have been proposed that might explain increased arterial compliance with aerobic exercise

Biochemical changes in the elastin-collagen composition of the arterial wall occur over years and therefore short-term training is unlikely to increase compliance by this mechanism. It is possible that arterial compliance increases as a result of the volume loading imposed by aerobic exercise. Mechanical distension during exercise sessions stretches collagen fibers and may modify their cross linking. On the other

hand pressure loading induced by heavy resistance training may result in increased smooth muscle content of the arterial wall and load bearing properties of collagen and elastin [210], an adaptive mechanism intended to maintain normal wall stress. Yet, no significant differences in measures of carotid IMT are observed between sedentary and endurance-trained men at any age [211]. Furthermore, three months of aerobic exercise training failed to alter carotid artery IMT in a group of previously sedentary men [211]. Given that resistance training leads to decline and return to baseline within 2 months following cessation of training it is unlikely any structural remodeling would account for positive effects associated with aerobic training or detrimental effects associated with resistance training.

A more likely scenario is improved compliance with exercise is dependent on functional determinant. Increases in flow may evoke the release of nitric oxide as well as lead to the up-regulation of nitric oxide production and other vasodilating substances. Indeed, Maeda and colleagues [212] determined the association between aortic stiffness and the level of mRNA expression in the aorta of male rats who exercised for three weeks. Exercised trained rats demonstrated a significantly lower aortic PWV compared to sedentary. Twenty nine genes known to be involved in arterial vasoconstriction/vasodilation were altered by the training. Conversely, they did not find altered expression of genes associated with structural proteins (collagen, procollagen) or enzymes that modulate structural proteins and the extracellular matrix (e.g., matrix metalloproteinase). The protein expression of eNOS in the abdominal aorta was significantly higher in the trained group than in the control group. It is likely that structural changes represent more chronic component of stiffness. Exercise increases NO production through up-regulation of eNOS and its signaling mechanisms.

In regard to improvements in venous compliance, as discussed earlier it has been suggested by others that 13 weeks of endurance training improved aortic compliance by reducing sympathetic-adrenergic tone directly or by enhancing endothelial release of nitric oxide [67]. However, Halliwill and colleagues noted that sympathetic stimulation reduced unstressed venous volume without affecting venous compliance [49]. More than likely, training may alter the composition of the vessel wall by increasing the ratio of elastin to collagen. This is a more likely mechanism for improved calf venous compliance considering it took 6 months for significant changes to occur in the studies discussed above.

2.9.2 Mechanisms for Improved Blood Flow Delivery

2.9.2.1 Altered Distribution of Cardiac Output

It is possible that reduced distribution of cardiac output to the exercising limbs may partly account for the attenuated blood flow responses during sub maximal and peak exercise. For instance, Proctor and colleagues [165] observed that non leg VO_2 was significantly higher in the older compared with younger women at 60 W, suggesting greater blood flow or O_2 extraction by stabilizing muscles. Similarly, at peak exercise, older men had lower avO_2 diff than their younger counterparts despite similar femoral venous oxygen content and hemoglobin concentration again suggesting less efficient redistribution of blood flow to the exercising limbs [163]. In this context, improved blood flow to exercising muscle could represent a better blood flow distribution.

2.9.2.2 Altered Balance Between Vasodilation/Vasoconstriction Factors

During sub maximal cycle ergometer exercise of moderate intensity, leg vascular conductance was reduced in sedentary older men [162] and recreationally active women [165] despite increased mean arterial pressure. Similarly, during small

muscle dynamic exercise leg vascular conductance was also reduced relative to younger controls in sedentary older men [77] and recreationally active middle aged men [213]. The decreased vascular conductance seen in these studies implies a change in the balance between vasoconstriction and vasodilation [20]. In order to maintain blood pressure in light of reduced cardiac output vasoconstriction must be enhanced and/or vasodilation must be blunted. Both possibilities are discussed below.

2.9.2.2.1 Vasodilator mechanisms

The effect of exercise training on vasodilator function has been well documented elsewhere [214]. It has been consistently shown that localized or whole body exercise training improves endothelial vasodilator function of major conduits or resistance vessels in those groups of patients in whom it has been initially attenuated, notably; CHF patients [215, 216], CAD patients [217, 218], hypertensives[219], diabetics [220] and those with hypercholesterolemia [221]and the obese [222]. Data is less consistent regarding those with normal endothelial function at baseline.

Whereas some studies show an improvement in endothelial vasodilator function in normal subjects [24, 223] others have reported no change [224, 225]. Together, these findings suggest that individuals with endothelial dysfunction may respond more favorably to an exercise stimulus compared to those with normal function at baseline.

Given the fact that vasodilator function is diminished with increasing age [22, 118] it is reasonable to suggest that exercise training would have favorable effects on endothelial vasodilator capacity. Indeed, DeSouza [21] studied a group of sedentary middle aged and older healthy men who completed 3 months of aerobic exercise. The intervention resulted in a 30% improvement in localized forearm blood flow to acetylcholine administration without a significant improvement in VO_{2peak} . This response was similar to those observed in younger adults and middle aged and older

endurance trained men suggesting regular aerobic exercise can restore the loss in endothelium dependent vasodilation in healthy middle aged and older men.

With regard to the relation between functional capacity and EDD exercise training in this CHF patients improves both stroke volume [226] and functional capacity [216] which are significantly correlated with positive changes in endothelium-dependent vasodilation suggesting that improvements in endothelium-dependent blood flow could be partly responsible for mediating improvements in maximal oxygen capacity by altering peripheral vascular resistance and subsequently redistributing blood flow to the working muscles. Improvements in endothelium dependent control were not however, correlated to positive changes in VO_{2peak} in the Desouza study [21]. It must be pointed out that the mean age of the “older” group in that study was 56 years. Still, in older men aged 66 to 83 years VO_{2max} is significantly related to endothelium dependent dilation. Furthermore, master athletes have significantly higher VO_{2max} and EDD than sedentary controls (see Figure 2.20) [227]. Whether exercise training can modify functional capacity through improvements in EDD in the very old (>70 years) is currently not known.

2.9.2.2.2 Vasoconstrictor Mechanisms

It has already been mentioned above that reduced blood flow to active skeletal muscle in older men may be the result of augmented vasoconstrictor responsiveness to acute sympathetic stimulation [112] or an age related attenuation of functional sympatholysis [115]. Therefore, it is conceivable that improvements in exercise blood flow may be improved by favorable changes in these mechanisms. However, since little attention has been given to sympathetic vasoconstrictor tone in exercising muscle the information addressing changes with exercise training are scant.

Spina and colleagues [228] measured α adrenergic responses in a group of older men and women induced by graded doses of phenylephrine infusion before and after 12 months of exercise training. They found that systolic and diastolic blood pressure increased progressively in response to phenylephrine both before and after training. However the increase in mean blood pressure from the basal level was greater after than before training in response to a given dose of phenylephrine. Further, doses of phenylephrine required to raise either systolic or diastolic blood pressure to comparable level were significantly smaller. Whether this enhanced responsiveness had any affect on blood flow in exercising limbs or limb vascular conductance could not be determined from the study design, but it does provide evidence for enhanced responsiveness to an α -adrenergic agonist.

2.9.2.2.3 Increased Capillary Density?

Evidence exists for increased capillarization with exercise training in older adults. Coggan et al. [208] reported an approximate 20% increase in capillary density of the gastrocnemius in a group of 60-70 year old individuals following 12 month exercise program of high intensity (80% maximal heart rate, 45 min, 4 days per week). Muscle blood flow was not measured. Similarly, Ades et al. [229] studied a group of older (mean age 68 years) coronary patients who underwent one year of intense exercise. They found a 34% increase in capillary density which probably contributed to a 20% improvement in peak aerobic capacity by facilitating diffusion of glucose and oxygen into the cytoplasm and mitochondria of muscle cells. Hyperemic calf blood flow, as assessed by plethysmography was unaffected by the exercise intervention. However, this may be due to the fact that hyperemic blood flow is more dependent on dilation of arteriolar and larger conductance vessels as opposed to capillary capacitance [229]. Thus, while increased capillarization may

explain the improvements in oxygen uptake kinetics seen with training in older adults [230] it is unlikely that this adaptation has any effect on alterations in blood flow to the skeletal muscles.

In summary, maximal oxygen uptake improvements seen in older adults undergoing exercise training have been linked to both central and peripheral vascular changes. The extent to which the vascular changes, i.e., improved central artery stiffness, enhanced blood flow delivery during dynamic exercise and better venous compliance in the exercising limbs contribute to improvements in VO_{2max} is still not clear and may differ between men and women and with each successive age decade. Consequently, continued research efforts in this area are warranted.

2.10 Conclusion

In conclusion, the vascular system is a complex network of arteries, capillaries and veins which share a common purpose, to distribute the blood it receives from the heart to the body tissues and return it back from whence it came. Under basal conditions and during times of stress, several vascular control mechanisms interact with the vascular network in order to maintain homeostasis and subsequently satisfy the three main principles of circulatory function; 1) The blood flow to each tissue of the body is controlled in relation to the tissue needs, 2) Cardiac output is dependent upon the sum of local blood flows returning to the heart and 3) Blood pressure is maintained independent of cardiac output and local blood flow. The relative importance of each mechanism is dependent upon the specific vascular compartment it influences but redundancies exist to allow for optimal cardiovascular function. Assessing vascular structure and control is a formidable endeavor and requires a number of tools in order to fully appreciate the individual roles of each vascular compartment.

The aging process places a high demand on vascular physiology. Most vessels become stiffer and their walls thicken, in part, to make up for a decline in smooth muscle strength. These age related “adaptations” in vascular structure interact with alterations in vascular control mechanisms. Together, these changes represent attempts by the system to maintain blood pressure and ensure optimal perfusion of body tissues. However, during times of acute stress vascular function is compromised, resulting in several negative consequences that may ultimately affect physiological functional capacity. These include an inefficient ventricular-vascular coupling relationship, poor blood flow delivery to the active muscles and potentially less than optimal venous return.

It is now clear that declines in VO_{2peak} are not linear but rather accelerate with successive age decades in both men and women. Fortunately, while greater physical activity does not prevent the decline, maintaining a physically active lifestyle may ward off disability which accompanies poor physiological capacity. Furthermore, exercise training in older adults appears to be an effective intervention for improving VO_{2max} due in part to a reversal of some of the age related vascular changes associated with poor physiological function.

In fact, the improvements in VO_{2max} seen in older adults undergoing exercise training have been linked to both central and peripheral vascular changes. The extent to which improved central artery stiffness, enhanced blood flow delivery during dynamic exercise and better venous compliance in the exercising limbs contribute to improvements in VO_{2max} is still not clear and may differ between men and women and with each successive age decade. Considering the high demand on the health care system associated with a growing population of older adults in the United States it would behoove the scientific community to continue efforts which expand the current

understanding of “healthy” vascular aging. Implementing exercise intervention designed at improving vascular physiology may be critical to improving functional status of the older population.

Chapter 3

Biphasic Response of the Brachial Artery Diameter Following Forearm Occlusion: A Blunted Response in the Elderly

3.1 Introduction

Non-invasive evaluation of brachial artery flow-mediated dilation (BAFMD) has emerged as a useful tool to study vascular function. Celermajer and colleagues [7] are the first to describe this technique, in which high-resolution ultrasonography is used to measure brachial artery diameter at rest and following reactive hyperemia, induced by forearm cuff occlusion. The dilatory response associated with increased flow is thought to be endothelium-dependent, and is subsequently used as a marker of endothelial function. In fact, reduced BAFMD has been found in the presence of numerous CVD risk factors [9-15] and holds predictive value for cardiovascular events [16].

Currently, use of the BAFMD technique is limited mostly to research, but continued refinement of the methodologies may help to enhance its clinical application. For example, seminal work using this technique report BAFMD as absolute and percentage change in vessel diameter from rest to peak dilation, usually 60 seconds following five minutes of forearm occlusion [7, 24]. However, advanced technology now makes it possible to collect multiple images of the brachial artery by automatically using the ECG signal as a trigger. Furthermore, computer-based edge detection software allow for semi-automated measurements of the arterial diameter [58]. Accordingly, Bressler and colleagues [231] obtained vessel diameter measurements in an adult sample at 20 second intervals following release of cuff pressure. They found that the average time to reach peak dilatory response was 60 seconds, but values ranged between 40 and 140 seconds. Jarvisalo et al. [232] found

similar results in a group of children, indicating that more frequent sampling of the data is needed to identify an individual's true peak response. Assessing variables such as the time taken to reach peak diameter may improve the utility of the BAFMD technique and subsequently allow for better understanding of the physiological processes dictating vascular function.

Hence, the purpose of this study was to examine the temporal pattern of the BAFMD vasoreactivity curve immediately following five minutes of forearm occlusion in a group of younger sedentary men. Also, given that BAFMD declines progressively throughout the lifespan [233], we speculate that age may also influence specific features of the vasoreactivity curve. Therefore, a second objective of this study was to compare these features between a sample of young and older men.

3.2 Methods

3.2.1 Participants and Experimental Protocol

Sixteen healthy, young men, aged 21 to 44 years were recruited to participate in our study. Smokers, those with renal impairment and proteinuria, hepatic impairment, gout, anemia, hypercholesterolemia, hypertension, diabetes, acute medical conditions and active infection were excluded from the study. In addition, brachial artery vascular data from fifteen older men, aged 71 to 100 years, were used to allow for comparison of specific features of the vasoreactivity curves against the younger subjects. The only exclusion criteria for the older adults were individuals in the American Heart Association Class D (i.e., symptoms of cardiovascular and/or metabolic disease at rest). Each participant signed an informed consent approved by the Pennington Biomedical Research Center Institutional Review Board and Louisiana State University.

All brachial artery imaging and analyses were conducted in accordance with the Guidelines set forth by the Brachial Artery Reactivity Task Force [58]. Brachial artery ultrasound measures (Toshiba Power Vision SSA-380A) were obtained with participants in the supine position using a 7.5-MHz linear array transducer prior to, during and following five minutes of forearm occlusion. Prior to scanning, participants were instructed to fast and refrain from exercise and alcohol intake for 12 and 48 hours, respectively. Baseline ultrasound images were obtained after 10 minutes of supine rest. All images were obtained in the longitudinal view, approximately 4 cm proximal to the olecranon process, in the anterior/medial plane. Image depth was initially set at 4 cm gain settings were adjusted to provide an optimal view of the anterior and posterior intimal interfaces of the artery and kept constant throughout. The participant's arm was immobilized and slightly supinated. Forearm occlusion consisted of inflation of a blood pressure cuff, positioned approximately 1 cm distal to the olecranon process, to 240 mm Hg for five minutes. Images for vessel diameter and velocity profiles were obtained at rest, and continuously from the final 30 seconds of occlusion until five minutes following the release of the blood pressure cuff. In addition, blood pressure and heart rate were monitored throughout the imaging process. All ultrasound images were recorded on compact discs for subsequent analysis.

3.2.2 Data and Statistical Analyses

The Brachial Imager software (Medical Imaging Applications, LLC) was used to analyze the images. Arterial diameters were calculated as the mean distance between the anterior and posterior wall at the blood vessel interface, with the image in diastole, defined as the peak of the R wave on the ECG. Base diameter (BASE) was defined by the average of 30 seconds of data obtained after 10 minutes of resting

conditions. Peak dilation (PEAK) was defined (by visual inspection of the arterial diameter curve) as the largest diameter following release of the occluding cuff. Its value was calculated by the average of 10 images (five seconds) surrounding this highest observable peak.

Three other characteristics of the vasoreactivity curve were identified and subsequently analyzed. The pre release diameter (PRE) was defined as the average of 20 seconds of data collected during occlusion. The initial decrease in arterial diameter following cuff release was quantified as the average of 10 images surrounding the smallest diameter (NIL). The magnitude of the initial decrease following occlusion was defined as the absolute (mm) and percent change in vessel diameter from BASE to NIL. Finally, BAFMD was defined as the absolute (mm) and percent change in vessel diameter from BASE to PEAK.

Flow velocity profiles were obtained at rest and immediately following release of the blood pressure cuff. From each velocity profile, the flow velocity integral (FVI)(cm) was manually traced, using Image Pro 4.0 software. The FVI was then divided by the ejection time (s) from that cardiac cycle to subsequently determine the mean velocity ($\text{cm}\cdot\text{s}^{-1}$). At rest, the average of three velocity profiles was used to calculate resting mean velocity (V_{meanrest}). Within 10 seconds of cuff deflation, an average of three velocity profiles was used to calculate mean hyperaemic velocity ($V_{\text{meanhyper}}$). The vessel radius (cm) at rest and immediately post occlusion was used in the equation, $(V_{\text{mean}} \cdot \text{heart rate}) \cdot \pi r^2$ to calculate mean blood flows ($\text{ml}\cdot\text{min}^{-1}$) at rest and hyperemia, respectively. Finally, the mean wall shear rate upon release was calculated according to recently published findings [234].

Independent t-tests were used to compare age groups for descriptive characteristics such as age, weight, height, BMI, resting heart rate, and blood

pressure. To examine the main features of the temporal pattern of the brachial artery diameter before and after occlusion, a repeated measure ANOVA (Feature: BASE, PRE, NIL, PEAK) was used. Post-hoc comparisons were made using t-tests. To examine the difference in the main features of the temporal pattern between young and old, a 2 (Young and Old) * 4 (BASE, PRE, NIL, PEAK) ANOVA was used. To further appreciate differences between the younger and older subjects a multivariate analysis was performed to examine the magnitude of the decrease in diameter and BAFMD. Finally, associations between features of the temporal pattern of the brachial artery were examined using Pearson Product Moment Correlations.

3.3 Results

Participant characteristics: None of the participants had any overt signs of disease. Participant characteristics are shown in Table 3.1. Independent t-tests reveal that the older adults had a higher BMI and systolic blood pressure as compared to their young counterparts (all p-values<0.05).

Temporal pattern of the vasoreactivity curve: The temporal pattern and main features of the brachial artery vasoreactivity curve, for the young and old men, are displayed in Figure 3.1. Following the forearm occlusion a biphasic pattern is evident with an initial significant decrease in the diameter of the vessel (NIL) as compared to the BASE and PRE diameters (p=0.03). No significant difference was observed between BASE and PRE.

After approximately 10 seconds the vessel diameter begins to increase, reaching PEAK at 41 seconds (p=0.001 vs. BASE). Subsequently, the vessel diameter gradually returns toward the baseline value over the next four minutes. Absolute values of BASE, PRE, NIL and PEAK are shown in Table 3.2. In addition, the magnitude of the decrease and BAFMD are also shown in Table 3.2.

Comparison of the main features of the temporal pattern between young and old men: Absolute values of the brachial artery diameter at the BASE, PRE, NIL and PEAK time points for the older adults are also shown in Table 3.2. The 2*4 ANOVA revealed a significant main effect of group such that vessel diameter was greater in the older adults at all points compared to the young ($p=0.0001$). Moreover, blood flow estimates immediately following cuff release appeared greater in the older subjects ($p=0.15$), whereas the mean wall shear rates were higher in the Young ($p=0.06$). Results of the multivariate ANOVA revealed a significant difference between the Young and Old for the magnitude of the decrease in diameter, (2.2% vs. 0.4%, $p=0.015$) and the BAFMD (7.7% vs. 2.6%, $p=0.001$) (see Table 3). The difference in the magnitude of the decrease in diameter was not significant ($p=0.14$) when the BASE and mean wall shear rate were used as covariates in the model. In contrast, the BAFMD difference remained significant ($p=0.004$), using the ANCOVA. Finally, no differences were noted for time to reach NIL and PEAK diameters.

Relation between the magnitude of the NIL and BAFMD: The BASE was inversely associated with BAFMD ($r=-0.48$, $p=0.006$). Moreover, the mean wall shear rate was significantly related to BAFMD ($r=0.62$, $p=0.003$) and the magnitude of the decrease in diameter following occlusion (from BASE to NIL) ($r=-0.51$, $p=0.05$). Finally, there was also a significant relationship between the magnitude of the decrease and BAFMD ($r=-0.44$, $p=0.04$) with approximately 21% of the variability in BAFMD explained by the magnitude of change between BASE and NIL.

3.4 Discussion

This study examined the temporal pattern of brachial artery diameter after five minutes of forearm occlusion. The findings indicate a biphasic pattern whereby immediately following cuff release brachial diameter decreases from baseline. This is

Table 3.1. Participant Characteristics

Variable		Young			Old			<i>p</i> value
		Mean	±	SD	Mean	±	SD	
Age	(years)	28	±	7	85	±	11	0.001
Weight	(kg)	70	±	9.5	71.4	±	11	0.757
Height	(cm)	177	±	7	165	±	11	0.006
BMI	(kg/m ²)	22	±	2	26	±	4	0.003
Resting SBP	(mmHg)	114	±	10	139	±	4	0.004
Resting DBP	(mmHg)	71	±	8	77	±	13	0.373
Resting HR	(bpm)	68	±	9	60	±	6	0.4

Values are means ± SD for 16 young subjects, 15 old subjects BMI, Body Mass Index, SBP, systolic blood pressure, DBP, diastolic blood pressure, HR, heart rate

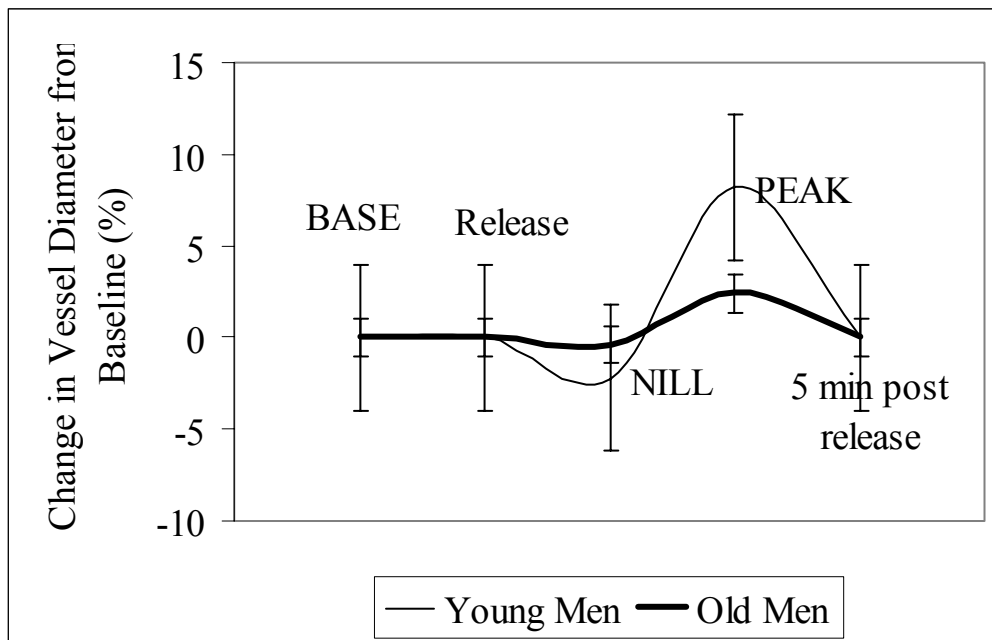


Figure 3.1. Features of the vasoreactivity curve: Young vs. Old; BASE: base diameter (30 sec average); NIL: lowest diameter post cuff release (5 second average); and PEAK: largest diameter post cuff release (5 second average). Data represents percentage change from baseline.

Table 3.2. Between groups comparison of the main features of the vasoreactivity curve

Features	Young	Old	<i>p</i> value
BASE (mm)	3.6 ± 0.5	4.4 ± 0.7	0.002
PRE (mm)	3.5 ± 0.5	4.5 ± 0.6	0.001
NIL (mm)	3.5 ± 0.5	4.4 ± 0.6	0.001
PEAK (mm)	3.9 ± 0.5	4.5 ± 0.7	0.015
Time to NIL (sec)	10 ± 4	14 ± 9	0.126
Time to PEAK (sec)	41 ± 16	59 ± 26	0.297
Magnitude of Decrease (% Δ)	2.2 ± 0.8	0.4 ± 2.1	0.015
BAFMD (% Δ)	7.7 ± 3.5	2.6 ± 1.2	0.001
Resting Blood Flow (ml/min)	131 ± 63	232 ± 131	0.046
Hyperemic Blood Flow (ml/min)	396 ± 95	495 ± 199	0.161
Mean Wall Shear Rate (s ⁻¹)	191 ± 64	135 ± 63	0.064

Values are mean ± SD

followed by a gradual increase in diameter for approximately 41 seconds to a peak and subsequent return toward baseline values. This biphasic pattern is evident in both young and older healthy participants but appears to be significantly blunted in the older group as indicated by differences in the main features of the vasoreactivity curve. Finally, significant relationships were noted between the magnitude of the initial drop and BAFMD, and the shear rate and the magnitude of the initial drop and BAFMD.

Given the hypothesis that endothelial dysfunction is an initiating event in atherosclerosis, a significant amount of research has led to the development of non-invasive tools to assess vasoreactivity in an attempt to identify individuals at risk for vascular disease or to examine the influence of treatment strategies [16]. The underlying assumption of this research is that a sudden increase in shear stress can activate stretch receptors on the endothelial surface, which trigger the release of vasodilatory molecules, and subsequent arterial vasodilation [235].

Probably the most commonly used technique to study this phenomenon is the BAFMD model. Traditionally, BAFMD has been assessed by measuring vessel diameter at baseline and at a specified time point, usually 60 seconds after five minutes of forearm occlusion [58]. The basis for selection of the 60 second time point stems from research that measured the brachial diameter at one-minute intervals following cuff release [58]. The results indicated a peak response at the first minute after release. However, since no measurements were made within the first minute after release, the true peak diameter may not have been observed. Nonetheless, given the evidence that the BAFMD response is related to coronary vasoreactivity [236] and is, generally, reduced in individuals with

greater severity of coronary artery stenosis [237], the technique is increasingly viewed as a surrogate marker of cardiovascular health.

Since Coretti's original work, technological advances have allowed more detailed assessment of arterial behavior that question the suitability of a set arterial dilation assessment point. For example, subjects with documented coronary artery disease have been shown to reach peak dilation at approximately 80 seconds [231]. Arterial diameters in this group were sampled every 20 seconds following cuff release and demonstrated a wide individual variation in time to peak (40 to 140 seconds), suggesting this sampling method may be still too large. In fact, we surmised, knowledge of vessel behavior over the entire period of examination may yield more significant information concerning vascular function/health.

The image acquisition used in this study allowed for discovery of a distinct biphasic pattern following the occlusion period. The arterial diameter initially decreases to a nadir at 10 seconds before gradually increasing in size to a peak at 41 seconds post cuff release. To our knowledge, this biphasic pattern has not been previously reported in the literature. The reason for the initial decrease is unclear but must be the consequence of, (1) mechanical, (2) physiological and/or (3) structural mechanisms. Although a definitive answer to this puzzle is beyond the scope of this study we have taken the liberty to speculate on each of these areas. Upon release of the blood pressure cuff there is an immediate and rapid increase in flow velocity of blood through the vessel, which may contribute to a decrease in the vessel diameter due to flow kinetics or the response to a rapidly emptying artery (and concomitant fall in local blood pressure). In fact, a significant association between the V_{mean} at release and the magnitude of the drop ($r=-$

0.44, $p=0.05$) in the present study may support this speculation. The immediate decrease could also be the results of a myogenic reflex or constriction to counter the sudden stretch induced by a greater volume of blood perfusing through the vessel. This mechanism is commonly seen in smaller arterioles [32]. However, in the present study there was no association between the estimated hyperaemic blood flow and the magnitude of the drop. Finally, the load bearing properties of the collagen and elastin components of the vascular wall, which influence arterial distensibility, may determine how the vessel responds to sudden changes in intravascular pressure [68]. In this context, a stiffer vessel would experience less recoil in response to a change in pressure compared to a more distensible vessel.

Peak dilation (BAFMD) in our young group was $\sim 8\%$, which is consistent with the literature in similar populations [7, 238, 239]. Presumably, the initial delay in vasodilation may be dependent upon changes in blood velocity, endothelial stretch receptor stimulation, intracellular signalling and the release of vasoactive substances [235, 240]. Clearly the complex temporal pattern of the post cuff release vasoreactivity curve suggests a significant interplay of many factors that may contribute to the overall vascular response.

Findings from population-based research indicate BAFMD is clearly influenced by the aging process [11, 233]. Celermajer reported a reduction in flow-mediated dilation with increasing age. In men, flow-mediated dilation declined at a rate of 0.21% per year after the age of 40 years. In women, flow-mediated dilation was preserved up till the 5th decade of life, and then declined at a rate of 0.49% per year. Similarly, Herrington et al. [233] report a 0.76% reduction in BAFMD, and approximately a 0.23

mm increase in baseline diameter, for each decade of life after 45 years. The reason for the decline in BAFMD observed may be attributable to reduced nitric oxide-mediated vasodilator response [146] or age related changes in the vascular smooth muscle tone [241]. The structural changes in the vessel may in part explain the progressive increase in baseline diameter seen across the life span. Our results are in agreement with those of Herrington and colleagues. We observed the baseline diameter to be significantly larger (4.4mm vs. 3.6mm, $p=0.001$) and the BAFMD significantly lower in the older adults compared to the younger adults (2.6% vs. 7.7%, $p=0.001$). Importantly, we also found that the mean wall shear rate following occlusion appeared to be greater in the young ($191\pm 64\text{s}^{-1}$) compared to the old ($135\pm 63\text{s}^{-1}$). Although the observed difference was not statistically significant, the p -value was 0.06. Given our study confirms an inverse relation between vessel size and BAFMD, and a direct association between mean wall shear rate and BAFMD, it raises an important issue; is the change in BAFMD merely a consequence of a change in baseline diameter and/or a change in the signal strength for dilation, rather than a reduction in the mechanisms that contribute to the vasoreactivity response?

In an effort to examine this issue more closely we used an ANCOVA, with the BASE diameter and mean wall shear rate as the covariates. The results revealed that the BAFMD remained significantly lower in the Old (see Figure 1.). These findings suggest the blunted BAFMD in the elderly is, in part, due to a reduced physiological (endothelial) response to the vasodilatory stimulus, and not merely a consequence of a larger resting diameter or smaller hyperemic signal. However, these findings do highlight the importance of considering the role of the baseline diameter on BAFMD, and the need to

normalize the reactive hyperemic stimulus for examining BAFMD between groups differing in baseline diameter [61]. Future studies should further examine the mechanisms for the increasing baseline diameter. Some evidence suggests large conduit arteries remodel as a compensation to early atherosclerosis [242]. Other possible mechanisms may include a loss of vascular smooth muscle tone, or a response to an increase in vascular resistance in smaller resistance vessels [3].

In the older group, as in the younger participants continuous monitoring of the vessel diameter throughout the period of reactive hyperemia revealed a biphasic pattern. The reduction in the vessel diameter occurred immediately following the release of the blood pressure cuff, reaching the smallest diameter at approximately 10 seconds, a percentage change from baseline of only 0.4% vs. 2% for the young. The vessel gradually increased in diameter until it reached its peak diameter at approximately 59 seconds (BAFMD=2%). It is important to note that whereas the BAFMD remained significantly lower in the old, when the BASE diameter and mean wall shear rate were incorporated into the model, the magnitude of the drop was no longer statistically significant ($p=0.13$) between the groups. Yet, from the findings it is clear that the biphasic pattern is significantly blunted in the old. While it is understood that reduced nitric oxide-mediated vasodilator response is partly responsible for the diminished BAFMD it is less clear why there is a reduction in the magnitude of the drop in vessel diameter following the release of cuff pressure. However, if the decrease in diameter is a consequence of the myogenic reflex or a decrease in distensibility of the vessel, than these mechanisms may also be influenced by the aging process.

Another unique finding of this study was the apparent inverse association between the magnitudes of change from BASE to NIL diameters and BAFMD. Specifically, individuals with the largest drop in diameter immediately following cuff release had the largest BAFMD. We are unsure as to the exact meaning of this association but believe it ought to be considered when examining the overall reactivity of the conduit vessel. For instance, flow mediated dilation has been traditionally thought of as a tool used exclusively to define endothelial function. Accordingly, diminished response to the flow stimulus is typically viewed as dysfunction of the endothelial cells to produce vasoactive substances such as nitric oxide [243]. The decrease in vessel diameter immediately following the release of cuff pressure observed in the present study is probably not endothelial-mediated, as the drop occurs within 10 seconds. Arguably the immediate response is probably faster than the time needed to release an endothelial substance, like endothelin, that could have a vasoconstricting effect in a large conduit artery. Thus, if we assume that the initial decrease is influenced by factors such as the myogenic reflex or the stiffness of the vessel, it is feasible that these same properties also influence the behavior of the vessel during reactive hyperemia. Consideration of these other factors may ultimately enhance the BAFMD technique and subsequently allow for better understanding of the physiological and structural processes dictating vascular function.

3.4.1 Conclusion

Following forearm occlusion, continuous monitoring of the brachial artery reveals a biphasic pattern, marked by an initial decrease and subsequent increase in brachial artery diameter. This biphasic pattern is blunted in older adults and the magnitude of the initial drop in diameter correlates with the magnitude of the vasodilatory response. These

unique findings may provide additional information regarding the structural and physiological status of the brachial artery following occlusion and may further enhance the use of the BAFMD model.

Chapter 4

The Association Between Flow-Mediated Dilation and Physical Function in Older Men

4.1 Introduction

Optimal physical function is primarily determined by the physiological capacities of the cardiovascular, musculoskeletal, and neurological systems and their integration into coordinated, efficient movements [244]. Thus, impairments in any of these physiological systems may result in functional decline that leads to loss of independence and disability in older adults [18].

Recently, Cress and Meyer [245] examined the relationship between physiological impairment and functional limitations in a group of community dwelling older adults. Physiologic capacity was determined by maximal voluntary performance for aerobic capacity (VO_{2peak}) and muscle strength (maximal torque for knee extensors), whereas physical function was assessed by performance on the Continuous-Scale Physical Functional Performance Test (CS-PFP 10). Using linear regression models the authors identified a threshold score thought to accurately predict functional limitations in these individuals. This threshold score was found to be 57 on the CS-PFP 10 and associated with a VO_{2peak} of $20 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. For those whose functional scores fell below this threshold, a moderate decrease in aerobic capacity was associated with a steeper decline in physical function and a heightened risk of losing independence.

While a decline in VO_{2peak} in older adults is certainly a consequence of a decline in cardiac output [5] it has been suggested that reduced blood flow to the skeletal muscles may also contribute to a reduction in exercise tolerance in older adults [246]. Indeed, limb blood flow and vascular conductance are reduced at rest [247] and during

incremental exercise [162] in older adults compared to their younger peers. It is hypothesized that the age related decline in vasoreactivity of resistance vessels [21] and larger conduits [22] are at least partly responsible for the reduction in limb vascular conductance, and ultimately reduced exercise capacity.

Few studies have examined the associations between vascular reactivity and physical function in the elderly. Interestingly, data describing an association between vascular function and physical function in the elderly have focused on the ankle brachial index. For example, data from the Women's Health and Aging Study suggest that the ankle-brachial index (a clinical measure of peripheral artery disease) predicts risk of disability [248]. Elderly patients who underwent cardioversion for atrial fibrillation, improved ventilatory efficiency and brachial artery flow-mediated dilation, which appeared to contribute to an increase in exercise performance [249]. The identification of the major factors involved in the decline in vascular function that may contribute to the decline in physical functional performance may allow for the development of more appropriate strategies to maintain functional health across the lifespan. Accordingly, the purpose of the present investigation was to examine the relationship between brachial artery flow-mediated dilation (BAFMD), and performance on the 10-item CS-PFP10 (PFP-10) in older adults. We hypothesized that those individuals with lower BAFMD would have lower PFP-10 scores.

4.2 Methods

4.2.1 Participants

Individuals 60 years of age or older, enrolled in the Louisiana Healthy Aging Study were eligible to participate in this study. Sampling of potential participants for the

Louisiana Healthy Aging Study was based on a population-based sampling design strategy that included Medicare Beneficiary Enrollment data provided by the Center for Medicare and Medicaid Services. Potential subjects were subsequently recruited from a 40-mile radius from the Pennington Biomedical Research Center in Baton Rouge, Louisiana. Exclusion criteria for the present study included individuals scoring below 25 on the mini-mental status exam [250] and individuals in American Heart Association Class D (i.e., symptoms of cardiovascular and/or metabolic disease at rest). Each participant signed an informed consent approved by the institutional review boards of the Pennington Biomedical Research Center, The Louisiana State University Health Sciences Center, and The Louisiana State University and Agricultural and Mechanical College.

4.2.2 Vascular Measurements

All brachial artery imaging and analyses were conducted in accordance with the Guidelines set forth by the Brachial Artery Reactivity Task Force [58]. Brachial artery ultrasound measures were obtained with participants in the supine position using a 7.5-MHz linear array transducer prior to, during and following 5 minutes of forearm occlusion. Prior to scanning, the participant was instructed to fast for 12 hours. Baseline ultrasound images were obtained after 20 minutes of supine rest. All images were obtained in the longitudinal view, approximately 4 cm proximal to the olecranon process, in the anterior/medial plane. Image depth was initially set at 4 cm, and gain settings were adjusted to provide an optimal view of the anterior and posterior intimal interfaces of the artery and kept constant throughout. The arm of the participant was immobilized and slightly supinated. Forearm occlusion consisted of inflation of a blood pressure cuff, positioned approximately 1 cm distal to the olecranon process, to 200 mm Hg for 5 min.

Images were obtained at rest, and continuously from the final 30 sec of occlusion until 5 min following the release of the blood pressure cuff. In addition, blood pressure and heart rate were monitored throughout the imaging process. All ultrasound images were recorded on compact discs for subsequent analysis.

Resting and peak brachial artery flow velocity measurements were obtained using a pulsed Doppler signal at an angle of approximately 60° to the vessel. Resting velocities were determined following the initial 10 minutes of supine rest. Peak brachial artery flow velocity was assessed immediately following release of the blood pressure cuff.

Data were analyzed using the Brachial Imager software (Medical Imaging Applications, LLC). Arterial diameters were calculated as the mean distance between the anterior and posterior wall at the blood vessel interface, with the image in diastole, defined as the peak of the r-wave. Base diameter was defined by the average of 30 seconds of data obtained after 10 minutes of resting conditions. Peak dilation was defined (by visual inspection of the arterial diameter curve) as the largest diameter following release of the occluding cuff. Its value was calculated by the average of 10 images (five seconds) surrounding this highest observable peak.

Flow velocity profiles were obtained at rest and immediately following release of the blood pressure cuff. From each velocity profile, the flow velocity integral (FVI)(cm) was manually traced, using Image Pro 4.0 software. The FVI was then divided by the ejection time (s) from that cardiac cycle to subsequently determine the mean velocity ($\text{cm}\cdot\text{s}^{-1}$). At rest, the average of three velocity profiles was used to calculate resting mean velocity (V_{meanrest}). Within ten seconds of cuff deflation, an average of three velocity profiles was used to calculate mean hyperaemic velocity ($V_{\text{meanhyper}}$). The vessel

radius (cm) at rest and immediately post occlusion was used in the equation, $(V_{\text{mean}} \cdot \text{heart rate}) \cdot \pi r^2$ to calculate mean blood flows ($\text{ml} \cdot \text{min}^{-1}$) at rest and hyperemia, respectively. Finally, the mean wall shear rate upon release ($V_{\text{meanhyper}}/\text{Diameter}$) was calculated according to recently published findings [234]

4.2.3 Physical Function Assessment

The PFP-10 was used to assess physical functionality. The scale has been validated elsewhere [251] and is based on the performance of 10 activities of daily living performed at a maximal effort that is safe and comfortable for the individual. Participant instructions and measurement protocols were standardized. Tasks are scored based on data collected on older adults with a wide range of abilities [251]. The type of task performed determines whether it is quantified by time, distance or weight carried. For instance, carrying groceries is quantified by both weight and time whereas transferring laundry from a washer to a dryer is quantified by time alone. Five separate physical domain scores are averaged to yield a total score of between 0 and 100. The physical domains assessed include, upper-body strength, lower-body strength, upper-body flexibility, balance and coordination, and endurance.

The final assessment of the PFP-10 consisted of a Six-Minute Walk Test conducted in an internal hallway, 40 meters in length. This test of exercise tolerance is a useful, simple, noninvasive alternative for assessing physical activity in many patient populations. Participants were instructed to walk up and down the hallway and to cover as much distance as possible in the 6-minute period. The participants were asked to report chest pain, marked dyspnea, or other symptoms. To standardize the protocol, the participants were not coached during the test, but made aware of time remaining to

completion. Details regarding the scoring of the PFP-10 and tasks performed on this test have been published elsewhere [244].

4.2.4 Statistical Analyses

Statistical analyses were performed using SPSS for Windows (version 11.0). Data are presented as means and SD. To examine the associations between BAFMD, age, and the PFP-10 scores, Pearson correlation coefficients were calculated. To further examine the influence of BAFMD on physical function, an ANCOVA was used to compare participants classified as low (below 25), moderate (between 25 and 57), and high (above 57) on the PFP-10 test. The rationale for this classification is based on the probability of independence graph developed by Cress and Meyer [245]. In that study, individuals who scored above the threshold score of 57 were classified as independent, those who scored below 26 rated themselves as limited in physical function, leaving the middle group as a possible “at risk” population. Significance was tested at the 95% confidence level ($p \leq 0.05$).

4.3 Results

4.3.1 Participant Characteristics

Sixty-four (Age = 84 ± 11 yrs) men completed all facets of the study. The characteristics of these individuals are presented in Table 4.1. Twenty percent of the participants were between 61 and 70 years of age, 34% between 71 and 90, and nearly 46% over the age of 90. Nearly 45% of the participants were classified as having stage I hypertension on the basis of their systolic blood pressure. Only, 5% of the study population was considered hypertensive on the basis of diastolic pressure. The average

BMI for the group was 27 ± 3.85 U, with approximately 77% of the individuals above the average category for 60 to 79 year olds.

Table 4.1. Participant Characteristics

		Mean		SD
Age	(years)	83.9	±	10.9
Height	(cm)	172	±	8
Weight	(kg)	80.8	±	13.9
BMI	($\text{kg} \cdot \text{m}^{-2}$)	27.2	±	3.8
Systolic Blood Pressure	(SBP)	141	±	20
Diastolic Blood Pressure	(DBP)	78	±	10
Pulse Pressure	(mmHg)	63	±	17
Resting Heart Rate	(bpm)	66	±	10

4.3.2 Brachial Artery Diameters, Blood Flow and Physical Function

The brachial artery diameters, reactivity, and blood flow responses for the participants are presented in Table 4.2. The average brachial artery diameter at rest during diastole was 4.67 ± 0.60 mm, and increased to a peak diameter of 4.79 ± 0.60 mm within 45 to 90 seconds following occlusion. The average percent change (BAFMD) for the entire group was 2.76%, ranging from -0.92% to 7.97%. The BAFMD was significantly related to age (BAFMD= $-0.06x + 8.18$ years; $r = -0.36$, $p = 0.003$).

The average pre-occlusion blood flow was approximately $68 \pm 45 \text{ ml} \cdot \text{min}^{-1}$, and increased to $595 \pm 301 \text{ ml} \cdot \text{min}^{-1}$ immediately post occlusion. Estimated mean wall shear rate during peak hyperemia was $130 \pm 71 \text{ s}^{-1}$. The association between the estimated mean wall shear rate and BAFMD was highly significant (BAFMD= $0.02x + 0.77$; $r = 0.51$, $p = 0.0001$). Importantly, there was no significant association between the estimated mean wall shear rate and age ($r = -0.19$, $p = 0.13$).

With respect to the domain scores for the PFP-10, the average upper body flexibility (UBF) ($54 \pm 23U$) yielded the highest average score, whereas lower body strength (LBS) ($38 \pm 24U$) was the lowest. The distance walked during the 6-minute walk test averaged $369 \pm 143m$. Thirty-three percent of individuals scored below 300m on the 6-minute walk test. The average score for the Total PFP-10 was $43 \pm 23U$, with 16, 28, and 20 participants scoring below 25U, between 25 and 57U, and above 57U, respectively.

4.3.3 Relations Between Age, Brachial Artery Diameters, Reactivity and PFP-10 Scores

Several statistically significant relationships between BAFMD, age and the individual and Total PFP-10 scores were observed. Notably, PFP-10 scores ($PFP-10 = 173.63 - 1.56(Age)$; $r = -0.75$, $p = 0.001$) and BAFMD ($BAFMD = 8.18 - 0.06(Age)$; $r = -0.36$, $p = 0.003$) declined with age. Moreover, BAFMD was significantly associated with the Total PFP-10 score ($BAFMD = 1.19 + 0.04(PFP-10)$; $r = 0.44$, $p = 0.001$) (See Figure 4.1). This association remained significant even when age was added to the model ($r = 0.29$, $p = 0.03$). There was no significant association between estimated mean wall shear rate and the Total PFP-10 score.

4.3.4 Examination of BAFMD Based on PFP-10 Classifications in Older Men

The results of the ANCOVA comparing BAFMD with the PFP-10 classes revealed significant group differences ($p = 0.001$). Specifically, those in Class III (BAFMD: 4.01% [95%CI: 3.16 to 4.85]) had significantly higher BAFMD than those in Class II (BAFMD: 2.67% [95%CI: 1.95 to 3.38]), which was significantly higher from Class I (BAFMD: 1.44% [95%CI: 0.49 to 2.39]). These differences are depicted in Figure 4.2. When age was entered into the model as a covariate the BAFMD for Class III and

Class II were not statistically different from one another, yet both were significantly greater than Class I. Importantly, there were no significant differences for estimated mean wall shear rate and the PFP-10 classes.

Table 4.2. Brachial Artery Diameters, BAFMD and Blood Flow Parameters

		Mean		SD
Resting Diastolic Diameter	(mm)	4.64	±	0.61
Brachial Artery Peak Diameter	(mm)	4.79	±	0.6
BAFMD	(% change)	2.76	±	2.13
Blood flow at rest	(mL·min ⁻¹)	68	±	46
Hyperemic blood flow	(mL·min ⁻¹)	595	±	301
Mean wall shear rate @ hyperemia	(s ⁻¹)	130.3	±	70.6

4.4 Discussion

The aim of the present study was to examine the possible relation between a measure of vascular function and physical performance in elderly men. The study findings indicate significant relationships between BAFMD and individual items as well as the total scores achieved on the PFP-10. More specifically, individuals in the highest functional class, as defined by the PFP-10 total score, exhibited the highest BAFMD, compared to those in the middle class, who had greater vasoreactivity than those in the lowest functional class independent of age. These findings fit “The Disablement Process” and suggest that lower physical function may in part be a consequence of deterioration of peripheral vascular function. Aging is associated with alterations in a number of structural and functional properties of large arteries, including diameter, wall thickness, wall stiffness, and endothelial function [252]. Consistent with this statement, we also observed a decline in BAFMD with advancing age. This finding confirms work by others [22, 233]. In fact, the regression equation for BAFMD in this study $y = 8.18 - 0.06 (\text{Age})$

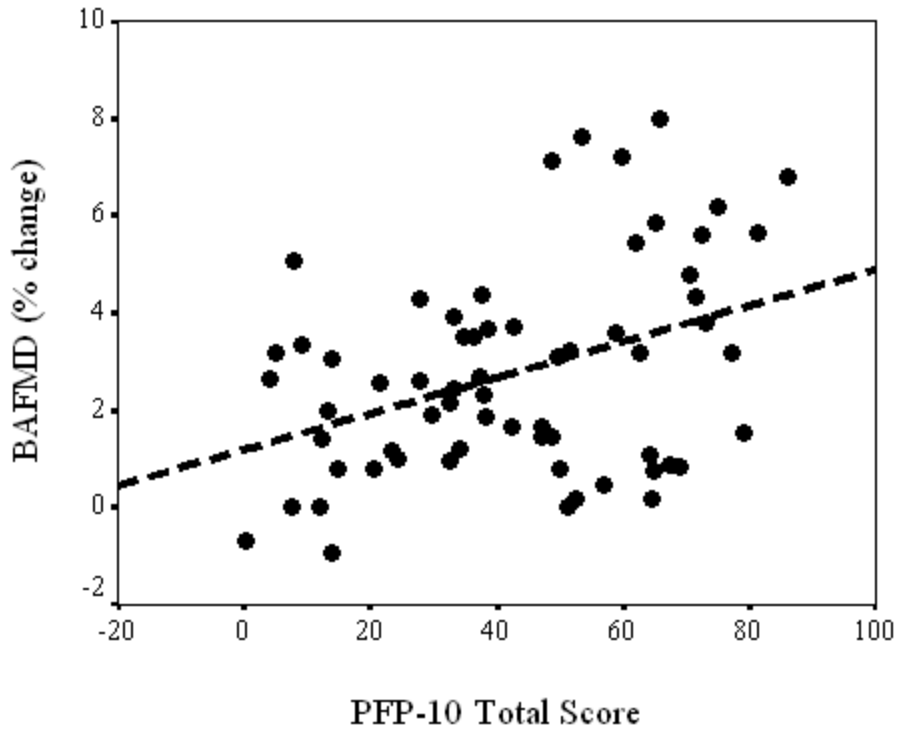


Figure 4.1. The relation between Total PFP-10 Score and BAFMD in older men

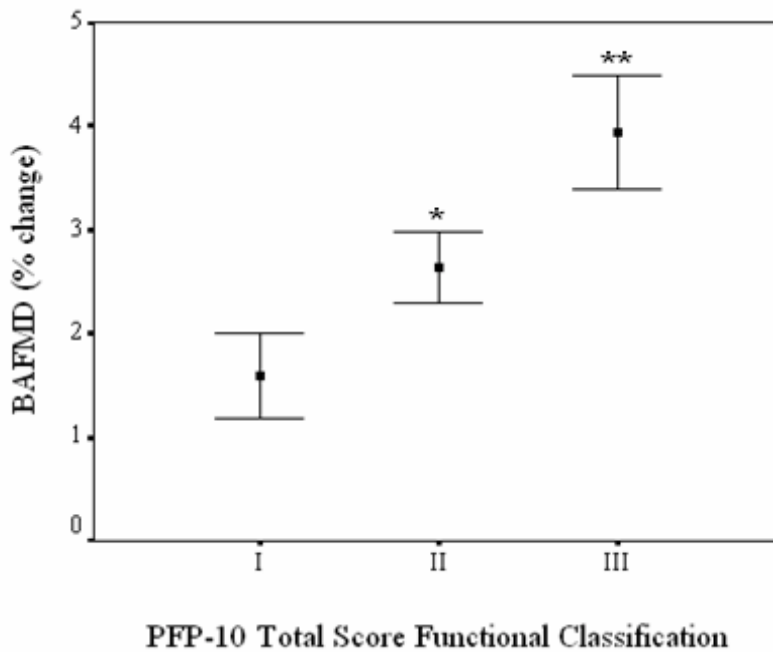


Figure 4.2. Brachial Artery Flow Mediated Dilation per PFP-10 Category. Axis Label: I= PFP-10 score < 26; II= PFP-10 score 26-57; III= PFP-10 score > 57. * significantly different from 1; ** significantly different from 1 and 2.

is very similar to that reported by Herrington et al. [233]. In contrast, no association between age and brachial artery diameter was noted, as has previously been observed [233]. This suggests that the lower BAFMD, in the present study, may indeed be a consequence of changes within the mechanisms associated with the reactivity response, rather than merely a consequence of differences in vessel size [253]. These potential mechanisms include: reduced synthesis and/or release of nitric oxide [243] or other endothelial-derived dilators [235]. Reduced BAFMD may be the result of increased formation of endothelium derived vasoconstrictor factors [254]; or production of reactive oxygen species [254].

However, we must remain cautious in our speculations regarding possible mechanisms to explain lower BAFMD, as our findings do show a strong relationship between the estimated shear rate at peak hyperemia and BAFMD. This implies that the magnitude of vasoreactivity is dependent on the degree of the shear stimulus [25].

The PFP-10 test measures physical function as it pertains to the execution of a combination of basic and instrumental activities of daily living. Task performance reflects the person's ability as each task is performed at maximal effort within the person's judgment of comfort and safety. Insofar as the PFP-10 scoring system is based on a continuous scale with scores between "0" and "100", the regression of PFP-10 scores against age in the present study ($y = 173.63 - 1.56(\text{Age})$) suggests an approximate 1.6% per year decrease in function among adults over 60 years of age. This rate of decline in the present sub-sample of the Louisiana Healthy Aging Study Cohort is representative of the entire study sample to date [255]. Of further importance is that examination of the PFP-10 component scores suggests a similar decline across the

individual components of the functional tests with age, suggesting that no one particular domain of functional fitness is responsible for lower overall functional performance with advancing age.

The major focus of this paper was to examine the relationship between the brachial artery indices and physical function scores. The data are consistent with the stated hypothesis that individuals with greater BAFMD have higher scores on the physical functional tests independent of age. More specifically, when individuals were categorized based on their PFP-10 total score, those in the highest functional class, exhibited the highest BAFMD, compared to those in the middle class, who had greater vasoreactivity than those in the lowest functional class. Importantly, these findings cannot be entirely explained through differences in estimated mean wall shear rate since the proposed signal for dilation of the artery was similar between the three functional classes. Secondly, even when age was added to the model a main effect for BAFMD was observed. These findings strongly suggest that alterations in vascular reactivity (defined by BAFMD) may contribute to a decrease in physical function in elderly men. These findings are particularly interesting in light of recent data that clearly indicate that lower cardiorespiratory fitness is a significant predictor of dependence, in the elderly [256].

Traditionally, the reason for the decline in cardiorespiratory fitness is ascribed to a reduction in stroke volume, and subsequent reduction in cardiac output for any given workload [156]. Presently, there is accumulating evidence that age-associated changes in local blood flow could have a major impact on functional capacity in the old [20]. For example, Poole et al. [162] observed that older men had significantly attenuated leg blood flows during incremental cycle-ergometer exercise compared to younger men. Similarly,

basal limb blood flow to the leg was found to be 26% lower and vascular resistance 45% higher in older men compared to younger men despite similar cardiac outputs in the two groups [257]. These age-related changes in muscle blood flow could result from age differences in several mechanisms, such as an increase in tonic vasoconstriction due to elevated sympathetic nerve activity [108]. Alternatively, age-related blunting of endothelium-mediated vasodilation has also been identified as another potential mechanism underlying differences in vascular control [21]. Finally, impaired functional sympatholysis observed in older adults may also attenuate blood flow [115] and subsequently influence exercise performance.

The relationship between BAFMD and PFP-10 scores is also intriguing with respect to bridging the gap between pathology and disability within the context of the “The Disablement Process” paradigm [18]. This model suggests that disablement begins with some pathology/injury or defect resulting in impairment at the tissue, organ and/or system level. Cardiovascular disease is well known to influence functionality among older adults, possibly through its effects on cardiorespiratory capacity (and therefore functional reserve) [256]. While the present investigation does not include quantification of cardiovascular disease per-se, the present data support the use of BAFMD as a measure of impairment that may quantify the influence of cardiovascular disease on functional ability/limitations in older adults.

Another unique finding of the present study is that individuals with PFP-10 scores above the threshold score of 57 had the highest BAFMD in comparison to those with scores below 57. In fact, all but one individual in the lowest functional class had a BAFMD below 4%, versus 12 out of 20 individuals, or 60% in the highest functional

score exhibiting a BAFMD over 4%. This is particularly intriguing given the work by Cress et al. [245] who reported that individuals below the 57 threshold were more likely to report functional limitations, and at risk for the need of dependent care. It is also intriguing as it raises the question whether vasoreactivity is in fact modifiable in those who are ‘non-responders’ (those with BAFMD less than 2%), and if particular attention should be given to ‘moderate-responders’ (those with BAFMD between 2% and 5%), as they may be at greatest risk for loss of independence.

According to the most recent predictions by the National Institute on Aging, the United States population aged 65 and over is expected to double in size within the next 25 years. It is estimated that in 2030, almost 1 out of every 5 Americans -- some 72 million people -- will be 65 years or older. Moreover, the age group 85 and older is now the fastest growing segment of the U.S. population. Although, the proportion of older Americans with a disability fell significantly from 26.2 percent in 1982 to 19.7 percent in 1999, many are disabled and suffer from chronic conditions. In fact, 14 million people age 65 and older reported some level of disability in Census 2000, mostly linked to a high prevalence of chronic conditions such as heart disease or arthritis.

Expanding the current knowledge regarding predictors of “healthy” aging is critical in the creation of preventive and compensatory interventions to improve the functional lifespan, thereby reducing the demands placed on families and communities. The results of the present study are clinically relevant insofar as they extend our understanding of the influence of cardiovascular health on functional fitness. In addition to providing clinicians and scientists with information about the severity of cardiovascular disease, measures of brachial artery flow mediated dilation also have

potential to provide a relatively simple and non-invasive approach for identifying individuals at risk of losing independence. Furthermore, from the results of this study, it is hypothesized that interventions, such as physical training, aimed at preserving or improving vasoreactivity may compress morbidity and prolong functional lifespan.

We remain cautious in our interpretations considering the limitations inherent to a cross sectional design. It is also recognized that the lack of longitudinal data regarding vascular status, physical function and many other important factors that contribute to the “successful” aging of an individual are not accounted for in the present study. Our intention is to continue our efforts along these lines to account for these shortcomings in future studies. However, we do believe the present observations contribute to the existing literature as it identifies several unique aspects that warrant further discussion and research.

We also recognize our inability to identify possible mechanisms for the changes in BAFMD and the relationship to the functional measures in this population. The lack of a mechanistic approach in the current study prevents more sophisticated speculation regarding the development of possible preventive or compensatory interventions for the elderly. However, given our and other researchers findings that exercise training can improve vasoreactivity [14, 21] we hypothesize that physical training interventions may be an excellent way to maintain and/or improve vascular function and contribute to the preservation of functional ability and independence in the elderly. Our efforts along these lines are continuing in our present studies.

The present study indicates significant relationships between vascular function, defined by brachial artery flow mediated dilation and individual items as well as the total

scores achieved on the PFP-10. More specifically, when individuals were categorized based on their PFP-10 total score, those in the highest functional class, exhibited the highest BAFMD, compared to those in the middle class, who had greater vasoreactivity than those in the lowest functional class. These findings fit “The Disablement Process” and suggest that lower physical function may in part be the consequence of some pathology, injury, or defect in peripheral vascular function, which contributes to functional limitations and ultimately contribute to loss of dependence and disability in the elderly.

Chapter 5

Time Course for Vascular Improvements with Localized Training in Elderly Men

5.1 Introduction

Advancing age is associated with a decline in peak oxygen consumption (peak VO_2) [19, 183, 258]. However, it is less clear to what degree central and/or peripheral components of the cardiovascular system or skeletal muscle metabolism contribute to this decline. A reduction in the vasodilatory capacity of large conduits and resistance vessels represents one factor which may compromise blood flow delivery to active muscle tissue [259]. The vasodilatory response of a blood vessel is partly mediated through the endothelium. This single layer of cells reacts to elevations in wall shear by releasing vasoactive substances, which cause smooth muscle relaxation. Brachial artery flow-mediated dilation (BAFMD) in response to five minutes of forearm occlusion is a popular non-invasive tool to assess global vascular health because it is associated with endothelial dependent function and linked to vasoactivity in the coronary arteries[8, 236]. BAFMD is reduced in individuals with cardiovascular risk factors [15, 252] and with advancing age [22].

Exercise training has emerged as an effective therapy to improve vascular function in the young and in those with disease [260]. Additional evidence suggests that the BAFMD model is a sensitive biomarker capable of detecting changes in the vascular system following training [24, 217-220, 223]. For example, Allen et al. [24] found a 60% improvement in BAFMD following four weeks of localized handgrip training. Also, chronic heart failure patients who engaged in long term exercise training experienced

improved vasodilatory function of trained [216] and untrained limbs [261] which was associated with enhanced exercise capacity, due perhaps to a decrease in peripheral vascular resistance or enhanced coronary perfusion [216].

Few investigations, have demonstrated the restorative capacity of exercise in the old [21, 23]. In particular, it is not clear whether vascular function is modifiable in the oldest old (those 75 years of age and beyond). Furthermore, we are aware of only one study which examined the time-course of vascular adaptations to exercise training in older individuals [23]. Given data from other studies indicating that vascular adaptations may occur within a week after initiating training it is important to examine whether this occurs in the elderly [24, 38, 262]. Moreover, we have previously observed a biphasic pattern of the vasoreactivity curve which is considerably blunted in elderly [263], suggesting mechanical and structural factors influence the vessel behavior during hyperemia. The influence of exercise training on the features of the vasoreactivity curve has not been documented. Ultimately, such information may lead to better understanding of the manner in which the vasculature adapts to exercise stress and may contribute to the development of appropriate strategies which counter the decline in vascular function commonly observed with advancing age.

Accordingly, the purpose of this study was to examine the influence of unilateral handgrip exercise program on BAFMD. A second purpose was to examine the effects of exercise training on the features of the vasoreactivity curve in elderly adults. We hypothesized that four weeks of handgrip exercise training would result in an increase in the magnitude of reduction in vessel diameter following cuff occlusion and improved BAFMD in the trained arm only. Additionally, given the recent findings from our lab

[24, 263] and others [38, 262] we anticipate that these modifications will occur early in the training program.

5.2 Methods

5.2.1 Participants

Men over the age of 70 years were recruited to participate in this study. Exclusion criteria for the study included the following; (1) smokers, (2) known alcohol or drug abuse problems, (3) heart attack or stroke in the last 3 months, or changes in a resting ECG, (4) American Heart Association Class D (i.e., symptoms of cardiovascular and/or metabolic disease at rest), (5) poorly controlled high blood pressure or diabetes (i.e., change in medication within the last 6 months), (6) known blood clotting disorders, (7) known blood vessel aneurysm (weakness or enlargements), (9) myasthenia gravis, (10) known acute infection and/or significant emotional distress, (11) adult dementia or other neurological impairment, (12) inability to see or hear. The current study was reviewed and approved by the Pennington Biomedical Research center Institutional Review Board.

5.2.2 Experimental Design

The study was a prospective design consisting of four weeks of isometric handgrip exercise training of the non-dominant arm. The dominant arm of the participant served as the control. BAFMD, features of the vasoreactivity curve and blood flow parameters served as the major endpoints of this study. In addition, hemodynamic measures, maximal voluntary contraction (MVC) and forearm circumference were also assessed.

5.2.3 Experimental Protocol

The PASE physical activity questionnaire was used to estimate the level of physical activity participation of each participant before training [264]. Participants forearm circumferences was assessed using a weighted measuring tape approximately 10 cm distal to the midpoint between the lateral epicondyle and the olecranon process. Maximum voluntary contraction was evaluated using a dynamometer (Baseline®). The participants stood upright while bending forward slightly and were instructed to grip at a maximum effort for three seconds. The average of three consecutive trials was used to determine MVC. Brachial artery and blood flow parameters were assessed in the trained and untrained arms pre-training and at the end of each week of the study period.

All brachial artery imaging and analyses were conducted in accordance with the Guidelines set forth by the Brachial Artery Reactivity Task Force [58]. Brachial artery ultrasound measures were obtained with participants in the supine position using a 7.5-MHz linear array transducer prior to, during and following five minutes of forearm occlusion. Prior to scanning, the participant was instructed to fast for 12 hours. Baseline ultrasound images were obtained after 20 minutes of supine rest. All images were obtained in the longitudinal view, approximately four cm proximal to the olecranon process, in the anterior/medial plane. Printed ultrasound images were used to ensure accurate comparisons over time. Image depth was initially set at four cm, and gain settings were adjusted to provide an optimal view of the anterior and posterior intimal interfaces of the artery and kept constant throughout. The arm of the participant was immobilized and slightly supinated. Forearm occlusion consisted of inflation of a blood pressure cuff, positioned approximately one cm distal to the olecranon process, to 200

mm Hg for five minutes. Images were obtained at rest and continuously from the final 30 seconds of occlusion until five minutes following the release of the blood pressure cuff. In addition, blood pressure and heart rate were monitored throughout the imaging process. All ultrasound images were recorded on compact discs for subsequent analysis.

Resting and peak brachial artery flow velocity measurements were obtained using a pulsed Doppler signal at an angle of approximately 60° to the vessel. Resting velocities were determined following the initial 10 minutes of supine rest. Peak brachial artery flow velocity was assessed immediately following release of the blood pressure cuff.

Data were analyzed using the Brachial Imager software (Medical Imaging Applications, LLC). Arterial diameters were calculated as the mean distance between the anterior and posterior wall at the blood vessel interface, with the image in diastole, defined as the peak of the r-wave. Base diameter was defined by the average of 30 seconds of data obtained after 10 minutes of resting conditions. Peak dilation was defined (by visual inspection of the arterial diameter curve) as the largest diameter following release of the occluding cuff. Its value was calculated by the average of 10 images (five seconds) surrounding this highest observable peak. The pre release diameter (PRE) was defined as the average of 20 seconds of data collected during occlusion, prior to release of the blood pressure cuff. The initial decrease in arterial diameter following cuff release was quantified as the average of 10 images surrounding the smallest diameter (NIL). The magnitude of the initial decrease following occlusion was defined as the absolute (mm) and percent change in vessel diameter from BASE to NIL. Finally, BAFMD was defined as the absolute (mm) and percent change in vessel diameter from BASE to PEAK.

Flow velocity profiles were obtained at rest and immediately following release of the blood pressure cuff. From each velocity profile, the flow velocity integral (FVI)(cm) was manually traced, using Image Pro 4.0 software. The FVI was then divided by the ejection time (s) from that cardiac cycle to subsequently determine the mean velocity ($\text{cm}\cdot\text{s}^{-1}$). At rest, the average of three velocity profiles was used to calculate resting mean velocity (V_{meanrest}). Within ten seconds of cuff deflation, an average of three velocity profiles was used to calculate mean hyperaemic velocity ($V_{\text{meanhyper}}$). The vessel radius (cm) at rest and immediately post occlusion was used in the equation, $(V_{\text{mean}}\cdot\text{heart rate})\cdot\pi r^2$ to calculate mean blood flows ($\text{ml}\cdot\text{min}^{-1}$) at rest and hyperemia, respectively. Finally, the mean wall shear rate (SR@10) upon release ($V_{\text{meanhyper}}/\text{Diameter}$) was calculated according to recently published findings [234].

Exercise training involved 20 minutes of handgrip exercise at an intensity of 60% MVC. The handgrip dynamometer was squeezed at a rate of one contraction per four seconds for four consecutive days of each week. These sessions were monitored by members of the research team. Maximum voluntary contractions were assessed on a weekly basis in order to ensure the relative training intensity would be maintained over the course of the study.

5.2.4 Statistical Analyses

All statistical analyses were performed using SPSS for Windows (version 11.0). Paired t-tests were used to detect pre- and post-training changes for forearm circumference, MVC and resting hemodynamics. Relationships between BAFMD, SR@10 and hyperemic blood flow were examined using Pearson Product Moment Correlations. To determine the effect of the four week intervention on the outcome

variables a two (trained arm vs. non-trained arm) by two (pre vs. post) repeated-measures ANOVA was performed. To determine the time course of the changes in BAFMD across the training protocol a repeated measures ANOVA was used. Differences between means were evaluated using LSD. Finally, differences in the features of the vasoreactivity curve of the trained arm were assessed using a two (pre vs. post) by three (feature: Rest, Nil, Peak) ANOVA. An alpha level ≤ 0.05 was considered statistically significant. Data were presented as mean \pm standard deviation.

5.3 Results

5.3.1 Participant Characteristics

Twelve older men (81 ± 5 years) completed all facets of the study. All individuals were free from symptoms indicative of chronic illness although 2 individuals were taking ACE inhibitors, 3 were on cholesterol lowering medications and 2 were taking β -blocking drugs. The average PASE score of this sample indicate that these individuals were moderately active in comparison to a group of elderly [265]. The pre-training characteristics of these individuals are presented in Table 5.1.

Table 5.1. Participant Characteristics

Variable		Pre-Training
Age	(years)	81 \pm 4.8
Height	(cm)	176 \pm 5.8
Weight	(kg)	77 \pm 12
BMI	($\text{kg}\cdot\text{m}^{-2}$)	24.9 \pm 3.9
PASE score	(AU)	165 \pm 86

Values are means \pm SD for 12 participants

5.3.2 Forearm Circumference, Handgrip Strength and Hemodynamics

All subjects completed a total of sixteen training sessions. Maximal voluntary contraction increased 6.2% in the trained arm only, but failed to reach statistical

significance ($p=0.102$). No significant change was observed in the forearm circumference of either arm (See Table 5.2). Also, the results of a paired samples t-test for pre- and post-training measures for cardiovascular hemodynamics indicate no significant change in systolic blood pressure (Pre: 133 ± 11 mmHg vs. Post: 131 ± 10 , $p=0.083$) and diastolic blood pressure (Pre: 76 ± 6 mmHg vs. Post: 75 ± 6 mmHg, $p=0.686$) or resting heart rate (Pre: 61 ± 9 beats·min⁻¹ vs. Post: 59 ± 10 beats·min⁻¹), $p=0.315$).

Table 5.2. Forearm Circumference and Handgrip MVC at Pre- and Post-Training

	Trained Arm		Untrained Arm	
	Week 0 (Pre)	Week 4 (Post)	Week 0 (Pre)	Week 4 (Post)
Arm Circumference	(cm) 25.9 ± 2.3	26.1 ± 2.1	26.1 ± 1.9	26.5 ± 1.8
MVC	(kg) 32.4 ± 7.0	34.4 ± 6.7	35.7 ± 9.0	35.3 ± 7.2

Values are mean \pm SD for 12 participants

5.3.3 Effects of Exercise Training on flow-mediated dilation

The vascular parameters of the trained and untrained arm over the course of the training program are presented in Tables 5.3 and 5.4, respectively. Analysis of variance revealed no difference in resting brachial artery diameter between arms at pre-training and no significant change from pre-training values were observed over the course of the study. The 2 (trained arm vs. control arm) X 2 (pre vs. post training) repeated measures ANOVA revealed a significant arm by week interaction whereby BAFMD improved 45% in the trained arm only ($p=0.05$)(See Figure 5.1). No significant differences were observed in pre-training BAFMD between arms or between pre and post- training BAFMD in the untrained arm.

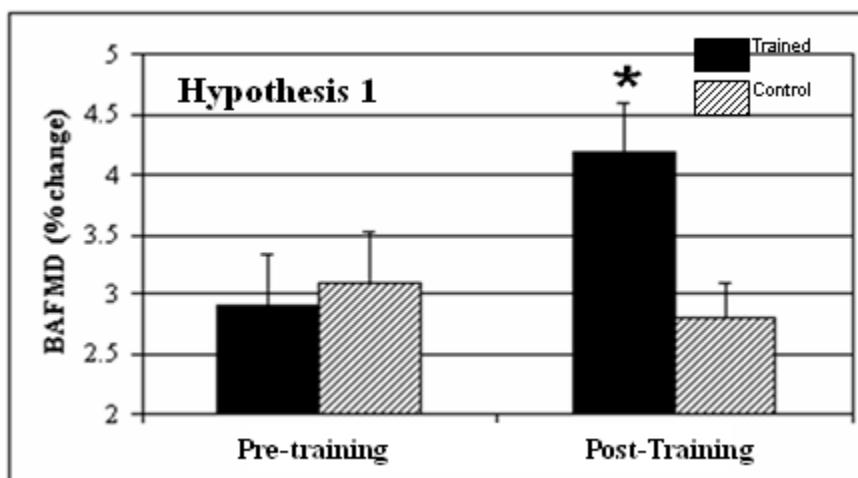


Figure 5.1. Mean value of percent change in BAFMD at pre and post-training. Values are mean \pm SE = $p < 0.05$ as compared with pre-training and the control arm.

5.3.4 Time course changes of flow-mediated dilation

The time course of change in BAFMD is presented in Figure 5.2. The repeated measures ANOVA revealed a significant within trial difference for percent change in BAFMD ($p=0.012$) across the five visits. Subsequent analysis indicated a statistically significant difference in BAFMD by the second week of training compared to pre-training ($p=0.024$). In addition, BAFMD at weeks three and four were different from pre-training (all p 's < 0.07). No differences were noted between weeks two and four. Furthermore, no differences were noted in BAFMD in the untrained arm over the course of the intervention.

5.3.5 Effects of Exercise Training on Features of the Vasoreactivity Curve

Immediately following forearm occlusion a decrease in the diameter (0.2%) of the brachial artery is observed. Subsequently, the vessel diameter increases and reaches a peak diameter at approximately 45 to 60 seconds after release of the forearm occlusion.

However, the magnitude of the decrease in vessel diameter immediately following cuff release, i.e., the absolute change in vessel diameter from rest to Nil, was not significant

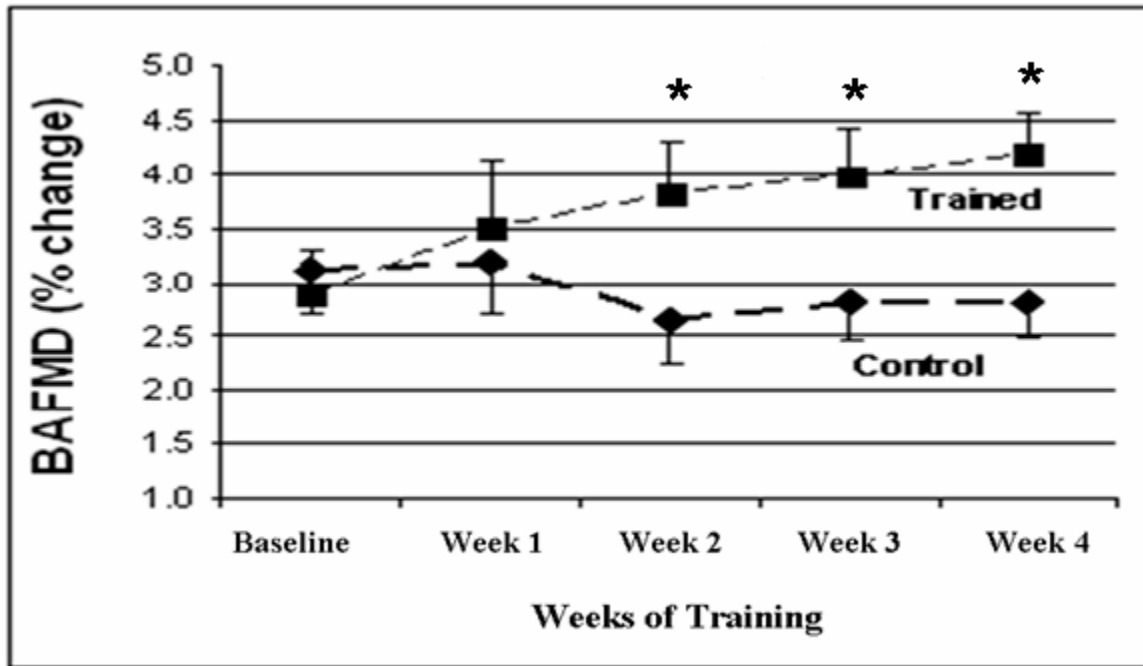


Figure 5.2. Group mean values ± SE for percent change in BAFMD across the training program. * $p < 0.05$ as compared with pre-training.

for either arm at pre-training. No change in this variable was observed over the course of the training program for the trained arm (Figure 5.3). The magnitude of the rise in vessel diameter following cuff release, i.e., the absolute change in vessel diameter from resting diameter to peak diameter, was significant ($p=0.001$), and similar for both arms at pre-training. Following the training intervention, the magnitude of the increase in vessel diameter from the resting diameter was significantly greater for the trained arm compared to the untrained arm ($p=0.05$).

5.3.6 Effects of Exercise Training on Blood Flow Parameters

Estimates of blood flow at rest and during the first 10 seconds of reactive hyperemia for the trained and control arms are presented in Tables 5.5 and 5.6,

respectively. In addition, the estimated wall shear rates at 10 seconds post cuff occlusion (SR@10) are presented in these tables. Pearson product moment correlations revealed a significant relationship between SR@10 and BAFMD ($r=0.341$, $p<0.001$) (See Figure 5.4) across all visits. A 2 (trained arm vs. control arm) X 2 (pre vs. post) repeated measures ANCOVA, with resting brachial artery diameter as a covariate, revealed a significant arm by week interaction whereby increases were observed for SR@10 ($p=0.021$) and hyperemic blood flow ($p=0.022$) in the trained arm only. A repeated measures analysis revealed significant changes in SR@10 ($p=0.011$) and hyperemic blood flow ($p=0.015$) across the five visits in the trained arm. Subsequent comparisons indicated a statistically significant difference in SR@10 at week three ($p=0.024$) and post- training ($p=0.043$) compared to pre-training. Similarly, hyperemic blood flow at week three ($p=0.048$) and post-training ($p=0.025$) was higher in comparison to pre-training. No significant differences from pre-training levels were noted in either of the variables after the first or second week of training. Furthermore, no differences were noted in SR@10 or hyperemic blood flow in the untrained arm over the course of the intervention.

5.4 Discussion

To our knowledge, this is the first study to examine the effects of four weeks of unilateral handgrip training on BAFMD and the pattern of the vasoreactivity curve in elderly men. The findings indicate that training produces significant improvements in BAFMD of the trained arm only, and these improvements are observed within two weeks of training onset. In contrast to previous work, there was no significant decrease in vessel diameter immediately following cuff release either before or after training. Lack

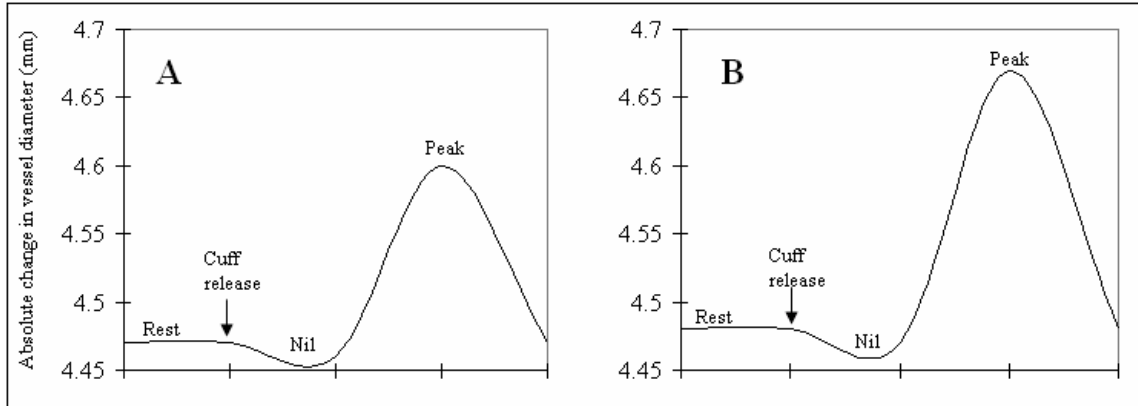


Figure 5.3. A) Vasoreactivity curve of the brachial artery following forearm occlusion at Pre-training in the trained arm. B) Vasoreactivity curve of the brachial artery following forearm occlusion at Post-training in the trained arm.

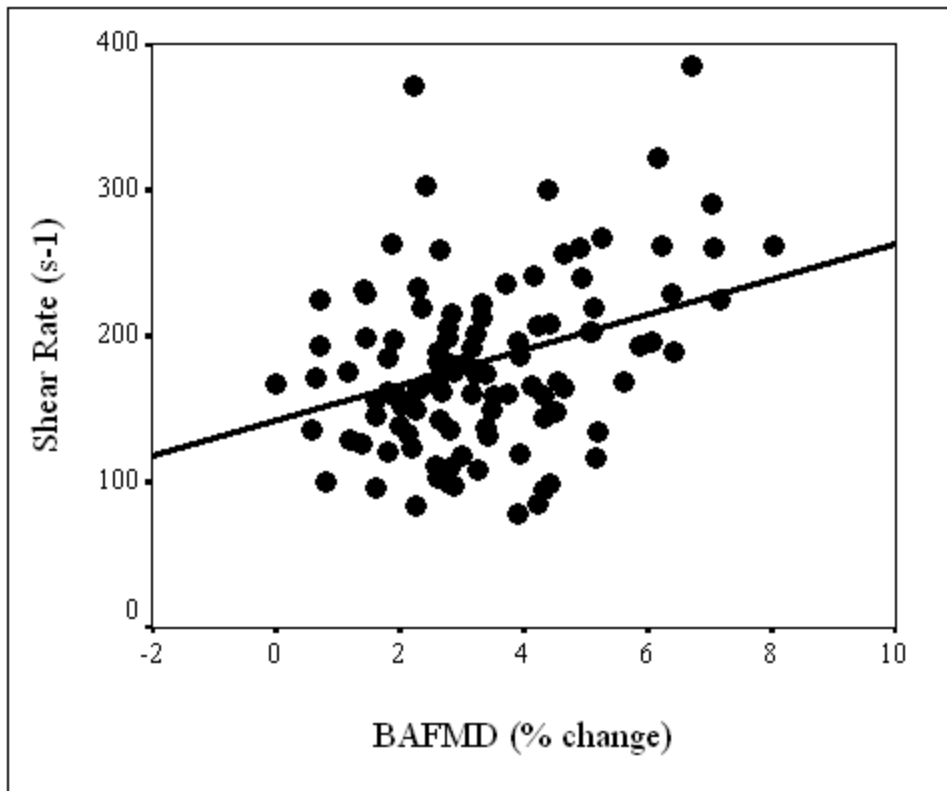


Figure 5.4. Relationship between shear rate at 10 seconds (SR@10) and subsequent vasodilatory response of the brachial artery (BAFMD)

Table 5.3 Trained Arm Vascular Parameters Throughout the Training Program

	Week 0 (Pre)	Week 1	Week 2	Week 3	Week 4 (Post)
Rest Diameter (mm)	4.47 ± 0.55	4.48 ± 0.54	4.47 ± 0.55	4.47 ± 0.55	4.48 ± 0.55
Nil Diameter (mm)	4.46 ± 0.57	4.48 ± 0.53	4.48 ± 0.53	4.48 ± 0.56	4.47 ± 0.56
Peak Diameter (mm)	4.60 ± 0.55	4.62 ± 0.52	4.64 ± 0.53	4.64 ± 0.54	4.67 ± 0.55
BAFMD (%)	2.9 ± 1.5	3.5 ± 2.3	3.8 ± 1.7	4.0 ± 1.6	4.2 ± 1.4
BAFMD (absolute) (mm)	0.13 ± 0.06	0.14 ± 0.06	0.17 ± 0.06	0.17 ± 0.06	0.18 ± 0.05

Values are means ± SD for 12 participants

Table 5.4. Control Arm Vascular Parameters Throughout the Training Program

	Week 0 (Pre)	Week 1	Week 2	Week 3	Week 4 (Post)
Rest Diameter (mm)	4.64 ± 0.55	4.65 ± 0.55	4.67 ± 0.54	4.66 ± 0.52	4.66 ± 0.52
Nil Diameter (mm)	4.65 ± 0.55	4.64 ± 0.53	4.67 ± 0.54	4.65 ± 0.52	4.66 ± 0.54
Peak Diameter (mm)	4.78 ± 0.57	4.8 ± 0.55	4.8 ± 0.56	4.8 ± 0.54	4.79 ± 0.53
BAFMD (%)	3.1 ± 1.5	3.2 ± 1.7	2.6 ± 1.4	2.8 ± 1.3	2.8 ± 1.2
BAFMD (absolute) (mm)	0.14 ± 0.07	0.15 ± 0.07	0.12 ± 0.07	0.13 ± 0.06	0.13 ± 0.06

Values are means ± SD for 12 participants

Table 5.5. Trained Arm Vascular and Blood Flow Parameters Throughout the Training Program

	Week 0 (Pre)	Week 1	Week 2	Week 3	Week 4 (Post)
Resting Blood Flow (ml·min ⁻¹)	118 ± 48	140 ± 85	137 ± 78	104 ± 42	121 ± 54
Hyperemic Blood Flow (ml·min ⁻¹)	679 ± 200	769 ± 207	739 ± 156	800 ± 204	852 ± 185
SR@10 (s ⁻¹)	167 ± 40	188 ± 44	185 ± 56	201 ± 59	217 ± 74

Values are means ± SD for 12 participants

Table 5.6. Control Arm Vascular and Blood Flow Parameters Throughout the Training Program

	Week 0 (Pre)	Week 1	Week 2	Week 3	Week 4 (Post)
Resting Blood Flow (ml·min ⁻¹)	99 ± 47	120 ± 65	111 ± 51	123 ± 43	123 ± 43
Hyperemic Blood Flow (ml·min ⁻¹)	829 ± 186	811 ± 156	762 ± 156	764 ± 197	775 ± 135
SR@10 (s ⁻¹)	183 ± 74	179 ± 56	162 ± 51	165 ± 59	173 ± 66

Values are means ± SD for 12 participants

of changes in the vessel diameter immediately following cuff release may imply structural and/or mechanical limitation in the elderly that are insensitive to a short regional training stimulus.

The BAFMD response in these individuals (ranging from 2.9%-4.2%) is similar to what has been observed in older adults[23]and is consistent with an age-related decline in flow-mediated dilation [22]. The precise mechanism of flow-mediated dilation is not completely understood but it is generally believed to reflect the capacity of the endothelial cells to produce vasoactive substances, e.g., nitric oxide (NO), which causes smooth muscle relaxation [243]. Thus, a diminished vasodilatory response to hyperemic blood flow observed in advancing age may indicate impaired NO bioavailability [266]. More recently however, it has been suggested that this decline could reflect a reduction in the magnitude of post occlusion wall shear rate [25].

5.4.1 Unilateral Response

The magnitude of change in BAFMD (45%) observed in the current study is lower in comparison to the approximate 60% improvement observed in a group of younger men using a similar study design [24]. Allen et al. [24] observed these unilateral improvements without concurrent changes in other factors known to influence arterial reactivity, i.e., central hemodynamic factors, indices of heart rate variability, hematologic markers or changes in cardiorespiratory fitness, suggesting that the improvements were locally mediated. While we did not assess all of these factors in the current study we have no reason to believe a similar exercise protocol would elicit changes in these factors in an aging model. Thus, the direct repetitive shear stress imposed on the vessel wall during exercise training may have lead to increased bioavailability of NO [267] resulting

in improved vasoreactivity of the brachial artery. Alternatively, the training could have led to changes in the vascular bed of the forearm which subsequently increased the blood flow stimulus (See section 5.4.4 Blood Flow and Shear Rate Changes with Exercise).

In light of the current findings it appears that elderly men retain their ability to respond to an exercise training protocol and therefore exhibit preserved vascular plasticity. While the improvements in BAFMD were slightly lower than what has been previously observed in younger men it may be inappropriate to make direct comparisons between the two samples. Of note, while the relative intensity of the exercise training was the same (60% MVC) between studies, the older men were training at a lower absolute intensity (approximately 20kg in old vs. 26 kg in young). Importantly however, the strength gains of the trained arm in both groups were nearly identical (6.2% improvement in MVC for elderly vs. 6.3% improvement in MVC for young). Also, it should be pointed out that the resting brachial artery diameter of the trained arm in the present study (4.47 mm) is much higher than what Allen et al. [24] reported in younger men (3.38 mm). The importance of this finding is underscored by the observations of others who report an inverse relationship between flow-mediated dilation and vessel size [7, 233] and a direct relationship between baseline diameter of the brachial artery and age[233].

We are aware of only two studies which have examined the effects of exercise training on vascular reactivity in older adults. Desouza et al. [21] observed that three months of aerobic exercise training, consisting mainly of walking, resulted in a 30% improvement in acetylcholine induced vasodilation in the forearm. These investigators

did not assess conduit vessel function, per se, which precludes making direct comparisons with the current investigation.

More recently, Wray [23] observed a significant improvement in BAFMD following six weeks of single-leg knee extensor training performed three times per week. The exercise regimen combined short, high-intensity (5-10 minutes at 70-95% of WR_{max}) intervals, with longer, low-intensity (15-45 minutes at 40-65% of WR_{max}) intervals. Their findings are in agreement with others who show improved vasodilatory capacity with exercise training in untrained limbs [54, 223, 268] but appear to contradict the current findings and those by others which show increased vasodilatory function of vessels in the trained limb only [24, 218]. While these differences are perplexing, Green et al [54] recently suggested that anterograde/retrograde oscillations in blood flow through a resting limb may actually provide a significant shear stimulus for vasodilation [54]. It is worth noting that the study was limited to six individuals (mean age of 71 years) whose baseline BAFMD response was approximately 1%. Thus, the present findings extend our current knowledge of exercise training effects on vascular function to those living into their ninth decade of life. Consequently, exercise therapy should be promoted in the elderly as a means to counter the age-related decline in vascular function [252].

5.4.2 Time Course

Given the significant increase in BAFMD in the trained arm only, a second objective was to determine the time course of this vascular response. We observed a statistically significant improvement in the trained arm BAFMD after only eight days of training (approximately 30%), with a gradual improvement observed over the remaining

weeks. Interestingly, rapid improvements in vasodilatory function with short term exercise training has been well documented in animal models [262, 269] and is attributed to the increased production and bioactivity of NO [270] or other vasodilators [271], producing a buffer to exercise induced shear stress.

Very few studies have examined the time course for vascular adaptation in humans. Allen et al. [24] observed a significant change in BAFMD following four days of handgrip training in young men, while Alomari et al. [272] recently found a 13% improvement in regional hyperemic blood flow within one week of handgrip training. Evidence of the rapid alterations in BAFMD of the trained arm only in the present study extend these prior findings to the vasculature of the elderly and lend support to the recommendation by the American College of Sports Medicine position statement for a well-rounded exercise program [273]. However, the sizable improvement in BAFMD after six weeks of lower leg training observed by Wray and colleagues [23] cloud this issue and should not be disregarded. Clearly, future efforts should be focused toward determining the optimal mode and volume (intensity, duration, frequency) of exercise needed to further stimulate vascular adaptations in the elderly.

5.4.3 Vasoreactivity Curve

Another unique aspect of the current study is the examination of the impact of exercise training on the brachial artery vasoreactivity curve. We have previously described the biphasic pattern of this curve elsewhere [263] and postulate that the initial decrease in diameter observed upon cuff release is partly due to mechanical and/or physiological factors, i.e., myogenic reflex which is modified by structural properties, i.e., elastin/collagen ratio, of the vessel. Furthermore, given the magnitude of the

decrease is inversely associated with BAFMD, we speculate that the structural properties of the vessel directly influence the vasodilatory response. It is well known that vessels become less compliant with age [67] and undergo changes in structure [68], perhaps due to alterations in elastin/collagen ratio, and this may account for blunted vasoreactivity curve observed in older men. Since exercise training modifies BAFMD and induces vascular remodeling [274] we hypothesized that four weeks of localized training would result in a greater magnitude of drop and subsequent rise, possibly indicating alterations in the structural components of the vessel.

In the present study, we did not observe a significant decrease in vessel diameter upon cuff release at pre-training. We speculate that a myogenic reflex is absent in the brachial artery of this group or the vessel has become too stiff to experience any elastic recoil in response to the sudden change in pressure induced by cuff release. Furthermore, while exercise training resulted in an improved vasodilatory response no alteration in the decrease in diameter was observed perhaps indicating that the structural components do not necessarily inhibit the flow-mediated dilation as we initially thought. Finally, we observed no significant change in resting brachial artery diameter over the course of the study. It has been shown that vessel enlargement occurs with exercise training in order to normalize the peak shear stimulus on the vessel wall [275]. Training studies of much longer duration in humans i.e., 8-12 weeks, have observed this phenomenon in the aorta [276] and femoral arteries [277]. More recently however, Rakabowchuk and colleagues [278] observed increased brachial artery diameter following just 6 weeks of resistance training in young men, which they attributed to physical structural changes of the vessel. Given the results of our present findings it is possible that the transient shear stresses

resulting from localized handgrip training were not high enough to induce vascular remodeling in an older group, or perhaps the time course for these structural changes is much longer than four weeks. This issue warrants further investigation.

5.4.4 Blood Flow and Shear Rate Changes with Exercise

The flow-mediated response is determined to a large degree by the magnitude of the shear stress imposed on the vessel wall [25]. Consequently, diminished BAFMD does not necessarily reflect poor endothelial function. Recognizing this important argument we quantified the shear stimulus using shear rate (velocity / diameter) as a surrogate measure.

Consistent with previous findings [61] we found a significant relationship between shear rate and BAFMD. In addition we observed a significant change in the shear rate in the trained arm only. Increased blood velocity through the vessel certainly appears to have contributed to the increase in BAFMD. Indeed, the concomitant increase in hyperemic blood flow may support this speculation. Based on these findings it is possible that the localized handgrip protocol did not alter brachial artery endothelial function per se, rather the improved BAFMD could be mediated through an elevated shear stimulus due to improvements in the vasodilatory capacity of the resistance vessels or changes in the microcirculation [279]. It should be noted however, that the magnitude of change in shear rate from pre to post training (approximately 30%) may not entirely account for the approximate 45% change observed in BAFMD. Furthermore the increase in BAFMD was observed before a significant change in shear rate. Together these findings suggest that the vasodilatory capacity of the brachial artery was improved independent of modifications occurring in the vessels of the forearm.

Previous handgrip studies, which have examined regional blood flow using strain gauge plethysmography, a technique which reflects resistance vessel function, have shown improvements in forearm vasodilatory capacity between 20% and 30% suggesting the adaptations in the vasculature include other aspects of the circulation [225, 280]. An alternate explanation for the increased hyperemic flow is that the training increased capillary density. Capillary proliferation has been observed in young men who undergo weight training of much longer duration (12 weeks) [281] but it is not known whether this adaptation would be observed in four weeks in elderly. However, Suzuki et al. [282] observed an increased capillary cross-sectional area after only one week of exercise training in the rat, which they attributed to the recruitment of “pre-existing” capillaries. Finally, increased forearm muscle mass would increase metabolite accumulation during ischemia. In fact, we recently found a positive relationship between BAFMD and lean muscle mass ($r=0.305$, $p=0.001$) in a group of elderly individuals (unpublished findings). However, it is unlikely that a training protocol of the current length would induce changes in muscle hypertrophy. The lack of significant change in the forearm circumference of the trained arm would support this argument.

5.4.5 Practical Implications

We have previously discovered that vascular function is significantly related to measures of physical function in older adults. These prior findings fit “The Disablement Process” [18] and suggest that lower physical function may in part be the consequence of some defect in peripheral vascular function, which contributes to functional limitations and ultimately contribute to loss of dependence and disability in the elderly. Given our current findings, it appears that exercise training is an effective strategy to improve

vascular function in the elderly. Future efforts should focus on how/if exercise induced improvements in vascular function contribute to the preservation of functional ability and independence in the elderly.

5.4.6 Conclusion

In conclusion, a localized short term exercise program resulted in significant improvements in BAFMD of the trained arm of elderly men compared with the control arm. Furthermore, measurable changes were observed following just eight days of exercise training suggesting these modifications take place very rapidly. On the contrary, lack of changes in the vessel diameter immediately following cuff release may imply structural and/or mechanical limitations in the elderly that are insensitive to a short-term regional training stimulus. Importantly, the positive association between shear rate and BAFMD and the significant change in shear rate over the course of the intervention suggests the improvements observed in BAFMD may be partly mediated by modifications in resistance vessel function.

Chapter 6

Conclusion

The purpose of this final chapter is to summarize and connect the findings and conclusions from this dissertation work. The initial study investigating the biphasic nature of the vasoreactivity curve revealed a blunted pattern in elderly adults. Specifically, the decrease in vessel diameter immediately following cuff release and the subsequent BAFMD are significantly less in elderly men compared to their younger counterparts. In addition, the magnitude of this decrease was inversely associated with BAFMD suggesting that the factors responsible for the initial decrease may also influence the vasodilatory capacity of the vessel. The exact mechanisms responsible for this initial decrease in diameter could not be elucidated using the BAFMD protocol but is worthy of future attention.

The second study was a cross-sectional examination of the relationship between vascular function, utilizing our BAFMD protocol, and a measure of physical function in a group of older men. Physical function was measured using the Continuous Scale Physical Function Performance Test (PFP-10). This study reported a positive association between BAFMD and the PFP-10 Total score, independent of age. Furthermore, individuals scoring above a threshold score of 57 on the PFP-10 exhibited the highest BAFMD compared to those who scored below this threshold. These findings fit “The Disablement Process” and suggest that lower physical function may in part be the consequence of some impairment in peripheral vasodilatory function, which compromise blood flow delivery to active skeletal muscle. This may result in functional limitations and loss of independence in the elderly. Future longitudinal studies are needed

to confirm the relationship between vascular function and physical function and to determine whether exercise interventions can maintain and/or improve vasodilatory function and contribute to the preservation of functional ability in the elderly.

Prior to determining whether exercise training contributes to improved functional capacity through improved vascular function it is necessary to establish whether vascular function is modifiable in the elderly. It is well established that exercise training improves vascular function in clinical populations but the effects of exercise training on individuals over the age of 75 who are free from overt cardiac illness is not clear. In order to determine whether exercise training had a direct effect on vascular function, similar to what has been seen in a younger population, the final study employed a localized handgrip exercise intervention to determine the magnitude and time-course of BAFMD improvements in a group of elderly men. A second objective was to determine the effects of exercise training on the biphasic pattern of the vasoreactivity curve.

Uniquely, a significant improvement in BAFMD of older adults was observed following unilateral handgrip training. Furthermore, measurable changes were observed following just eight days of exercise training suggesting these modifications take place very rapidly. Importantly, the positive association between shear rate and BAFMD and the significant change in shear rate over the course of the intervention suggests the improvements observed in BAFMD may be partly mediated by modifications in resistance vessel function.

Together, the above findings have allowed the author to establish a link between physical function and vascular function in elderly men. This finding has clinical relevance considering that the age-related decline in physical function and disability is

often a consequence of impairments of the cardiovascular system. Based on the findings from the last project exercise training is one therapeutic strategy which may improve vascular health. Thus, older men should be encouraged to engage in a comprehensive exercise training program. Future efforts should focus on identifying the mechanisms which account for modifications in vascular function and how/if exercise induced improvements in vascular function contribute to the preservation of functional ability and independence in elderly men and women. Answers to these questions may help to reduce the health care costs associated with treating older adults who have lost their physical independence.

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Appendix: Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title of Study: The Effects of Hand Grip Exercise Training on Brachial Artery Vasoreactivity in Older Adults

What you should know about a research study

- We give you this consent form so that you may read about the purpose, risks and benefits of this research study.
- The main goal of research studies is to gain knowledge that may help future patients.
- You have the right to refuse to take part, or agree to take part now and change your mind later on.
- Please review this consent form carefully and ask any questions before you make a decision.
- Your participation is voluntary.
- By signing this consent form, you agree to participate in the study as it is described.

1- Who is doing the study?

Investigator Information:

Principal Investigator: Michael A. Welsch, Ph.D
225-578-9143

Medical Investigator: Frank L. Greenway, M.D.
Day Phone: 225-763-2576
24-hr. Emergency Phone Nos:
225-763-2576 (Weekdays 7:00a.m.-4:30 p.m.)
225-765-4644 (After 4:30 p.m. and weekends)

Co-Investigators: Devon A. Dobrosielski, M.S.
225-578-2074

Dr. Welsch directs this study, which is under the medical supervision of Dr. Greenway.

We expect about 15 people from 1 site will be in this study. The study will take place over a period of 16 weeks. Your expected time in this study will be 5 weeks.

2- Where is the study being conducted?

This study is being conducted at the Pennington Biomedical Research Center.

3- What is the purpose of this study?

The purpose of this study is to examine how 4 weeks of hand grip exercise training will affect the function of the blood vessels in your forearm.

4- Who is eligible to participate in the study? Who is ineligible?

Inclusion criteria:

You are eligible to participate if:

- you are 70 years of age or older.
- you are willing to participate in the 5 week program.
- you currently do not smoke.
- you are willing to fast and refrain from exercise and alcohol for 12 hours.

Exclusion Criteria:

- Persons who have known alcohol or drug abuse problems.
- Heart attack or stroke in the last 3 months, or change in a resting ECG.
- Significant heart disease (known signs or symptoms of heart disease, i.e. severe shortness of breath, nausea, dizziness, chest pain or profuse sweating at rest or with low to moderate activity).
- Poorly controlled high blood pressure.
- Poorly controlled diabetes.
- Known blood clotting disorders.
- Known blood vessel aneurysm (weakness or enlargement).
- Uncontrolled asthma or emphysema.
- Myasthenia gravis (neuromuscular disorder characterized by fatigue).
- Known acute infection and/or significant emotional distress.
- Adult dementia or other neurological impairment.
- Inability to see or hear.

5- What will happen to you if you take part in the study?

If you choose to participate in this study you will be involved for a total of 5 weeks. You will come to Pennington Center on Fridays for testing. On Monday through Thursday for 4 weeks, you will do handgrip training at the St. James Fitness Center. You will then refrain from exercise Monday through Thursday for the 5th week and come to the Pennington Center on Friday for assessment.

Fridays at PBRC (length of visit- approximately 1 hour)

Brachial Artery Test- You must fast and refrain from exercise and alcohol for 12 hours prior to the scanning. Once you have arrived at the Pennington Center you will be asked to lie down on an examination bed. A technician will then place 3 electrodes on your chest and 1 blood pressure cuff on each arm. The electrodes are used to measure your heartbeat. The cuffs are used to provide pressure to the arms to allow for the measurement of blood pressure and blood flow, so that we may test how your arteries work. After all the equipment is in place you will be given a 15 minute rest period. During this time an ultrasound technician will be taking pictures of a blood vessel in your arm. After a clear picture has been found, the width of your blood vessel will be measured before and after inflating one of the forearm pressure cuff for 5 minutes. Throughout the procedure, your heart rate and blood pressure will also be recorded. Following this, we will remove the cuffs, place them on the opposite arm and repeat the brachial artery test on your other arm.

Maximal voluntary handgrip strength test- You will be asked to squeeze a handgrip device and hold it tightly for 3 seconds. You will repeat this 3 times for each hand.

Monday-Thursday at the St. James Fitness Center- (length of visit- 20 minutes)

After the first PBRC assessment on Friday, you will be asked to perform handgrip training on Monday through Thursday. This involves gripping and releasing the handgripper at a rate of 15 times per minute at a resistance set at 60 percent of your last measured maximum (performed the previous week). You will be asked to gradually work up to 20 minutes of handgrip exercise per session. The handgrip exercise will be done at St. James Place under the supervision of one of the study investigators. The training session on Monday through Thursday will be held at times most convenient for you. No training will take place the last week of the study.

6- What are the possible risks and discomforts?

-No risks are associated with squeezing the handgrip device.

-Inflation of the blood pressure cuff on the forearm during the Brachial Artery Test may cause some temporary discomfort in the forearm and hand. If the discomfort is too severe you may terminate the procedure immediately by asking the technician to stop the test. Temporary numbness and tingling in the hand similar to the sensation of having your hand “fall asleep” may occur.

-There is no known risk to the use of ultrasound to measure the size of the blood vessel. There is no risk to blocking blood flow in the artery through inflation of the blood pressure cuff. Not a single permanent adverse event has been associated

with this procedure in over 30,000 examinations reported in the medical literature.

-In addition to the risks listed above, the procedures may have unknown, unforeseen or unanticipated side effects. There is always the possibility that you will have a reaction that is currently not known and not expected.

7- What are the possible benefits?

We cannot promise any benefits from being in the study. However, participation in this study may assist the investigators in gathering information leading to better treatments for cardiovascular disease.

8- If you do not want to take part in the study, are there other choices?

You have the choice at any time not to participate in this research study. If you choose not to participate, any health benefits to which you are entitled will not be affected in any way.

9- If you have any questions or problems, whom can you call?

If you have any questions about your rights as a research volunteer, you should call the Institutional Review Board Office at 225-578-9143 or Dr. Claude Bouchard, Executive Director of PBRC at 225-763-2513. If you have any questions about the research study, contact Dr. Michael Welsch (PI) at 225-578-9143. If you have a research-related injury or medical illness, you should call Dr. Frank Greenway during regular working hours. After working hours and on weekends you should call the answering service at 225-765-4644. The on-call physician will respond to your call.

10- What information will be kept private?

Every effort will be made to maintain the confidentiality of your study records. However, someone from the Food and Drug Administration, the Pennington Biomedical Research Center, and Louisiana State University may inspect and/or copy the medical records related to the study. Results of the study may be published; however, we will keep your name and other identifying information private. Other than as set forth above, your identity will remain confidential unless disclosure is required by law.

11- Can your taking part in the study end early?

Dr. Greenway or any of the investigators or the study sponsor can withdraw you from the study for any reason or for no reason. You may withdraw from the study at any time without penalty. Possible reasons for withdrawal include inability or unwillingness to complete the required testing or training. The sponsor of the study may end the study early.

12- What if information becomes available that might affect your decision to stay in the study?

During the course of this study there may be new findings from this or other research which may affect your willingness to continue participation. Information concerning any such new findings will be provided to you.

13- What charges will you have to pay?

None.

14- What payment will you receive?

None.

15- Will you be compensated for a study-related injury or medical illness?

No form of compensation for medical treatment or for other damages (i.e., lost wages, time lost from work, etc.) is available from the Pennington Biomedical Research Center. In the event of injury or medical illness resulting from the research procedures in which you participate, you will be referred to a treatment facility. Medical treatment may be provided at your expense or at the expense of your health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.) which may or may not provide coverage. The Pennington Biomedical Research Center is a research facility and provides medical treatment only as part of research protocols. Should you require ongoing medical treatments, they must be provided by community physicians and hospitals.

16- HIPAA

Records that you give us permission to keep, and that identify you, will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in records disclosed outside of Pennington Biomedical Research Center (PBRC). For records disclosed outside of PBRC, you will be assigned a unique code number.

17- Signatures

(Note: Signatures of volunteer and person administering informed consent must appear on the same page.)

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the study investigators. I agree with the terms above and acknowledge that I have been given a copy of the signed consent form.

With my signature, I also acknowledge that I have been given either today or in the past a copy of the Notice of Privacy Practices for Protected Health Information.

Signature of Volunteer

Date

Date of Birth of Volunteer

Signature of Person Administering Informed Consent

Date

Michael A. Welsch
Principle Investigator

Frank L. Greenway
Medical Investigator

The study volunteer has indicated to me that the volunteer is unable to read. I certify that I have read this consent form to the volunteer and explained that by completing the signature line above the volunteer has agreed to participate.

Signature of Reader

Date

Vita

Devon Dobrosielski is a native of New York. He received his bachelor's degree from The Pennsylvania State University in May of 1999 and his master's degree from Wake Forest University in May of 2001. He currently resides in Baton Rouge with his wife Meredith. At the August 2007 commencement ceremony he will be awarded the Doctor of Philosophy in kinesiology from the graduate school at Louisiana State University.