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Plasma nitrite reserve and vascular function before and after handgrip training in patients with heart failure

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**PLASMA NITRITE RESERVE AND VASCULAR FUNCTION BEFORE AND AFTER
HANDGRIP TRAINING IN PATIENTS WITH HEART FAILURE**

A Dissertation,

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

In

The Department of Kinesiology

by Daniel P. Credeur
B.S. Louisiana State University
December, 2011

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The Journey has just begun!!

Table of Contents

Acknowledgements.....	ii
Abstract.....	v
Chapter 1: Introduction.....	1
Chapter 2: Brachial Artery Retrograde Flow Increases with Age: Relationship to Physical Function.....	5
2.1 Introduction.....	5
2.2 Methods.....	6
2.3 Results.....	10
2.4 Discussion.....	14
2.5 Conclusion.....	18
Chapter 3: Influence of Brachial Artery Reactivity and Blood Flow Patterns on Physical Function: Modification through Daily Activity.....	19
3.1 Introduction	19
3.2 Methods.....	20
3.3 Results.....	24
3.4 Discussion.....	28
3.5 Conclusion.....	33
Chapter 4: Effect of Handgrip Training with Venous Restriction on Brachial Artery Vasodilation.....	35
4.1 Introduction	35
4.2 Methods.....	36
4.3 Results.....	41
4.4 Discussion.....	47
4.5 Conclusion.....	52
Chapter 5: Plasma Nitrite Reserve and Vascular Function Before and After Handgrip Training in Patients with Heart Failure.....	53
5.1 Introduction	53
5.2 Methods.....	55
5.3 Results.....	61
5.4 Discussion	69
5.5 Conclusion.....	81
Chapter 6: Conclusion.....	82
References.....	85

Appendix A: Review of Literature.....	100
A.1: Introduction.....	100
A.2: Vascular Anatomy and Physiology.....	102
A.2.1: Purpose.....	102
A.2.2: Arterial System.....	102
A.2.3: Venous System.....	105
A.2.4: Summary.....	107
A.3: Vascular Control of Blood Flow.....	108
A.3.1: Purpose.....	108
A.3.2: Hemodynamics through the Vasculature.....	108
A.3.3: Autonomic Regulation of Vascular Tone.....	111
A.3.4: Local Control of Blood Flow.....	117
A.3.5: Summary.....	123
A.4: Assessing Vasoreactivity and Blood Flow.....	125
A.4.1: Purpose.....	125
A.4.2: Macro-Vascular Assessments.....	125
A.4.3: Micro-Circulatory Assessments.....	129
A.4.4: Assessing Regional Blood Flow.....	132
A.4.5: Non-Invasive Assessment Strategy.....	134
A.4.6: Summary.....	135
A.5: Vascular Responses to Exercise.....	136
A.5.1: Purpose.....	136
A.5.2: Components of the Fick Equation.....	136
A.5.3: Local Regulation of Blood Flow during Exercise.....	139
A.5.4: Summary.....	146
A.6: Vascular Adaptations to Chronic Exercise Training.....	148
A.6.1: Purpose.....	148
A.6.2: Adjustments in Fick Equation Components to Exercise Training.....	149
A.6.3: Alterations in Vasoreactivity in Response to Training.....	150
A.6.4: Summary.....	157
A.7: Factors Altering Blood Flow Control.....	158
A.7.1: Purpose.....	158
A.7.2: Aging and Blood Flow Control.....	159
A.7.3: Effects of Disease on Blood Flow Control.....	166
A.7.4: Summary.....	169
A.8: Conclusion.....	170
Appendix B: IRB Approval.....	173
Vita.....	182

Abstract

There is a direct relationship between vascular health and physical function. The controllers of this relationship are unclear, but appear to involve biomechanical and biochemical influences on the vascular wall. **Purpose:** The purpose of this dissertation was twofold: (1) to explore the relationship between vascular health and physical function in three populations (elderly, young and chronic heart failure patients); and (2) to determine the modifying role of physical activity, inactivity and exercise training on controllers of this relationship. **Methods:** Four projects were designed to address 3 key issues in exercise vascular biology, including the influence of the pattern of blood flow on the vasculature; the effects of exercise training with blood flow restriction vascular function; and the influence of exercise training on vascular function in individuals with known disease. **Results:** Projects 1 and 2 indicate an increase in oscillatory shear within the vasculature with aging. This appears to be associated with lower physical function. Those individuals who maintain a higher amount of daily physical activity have more favorable blood flow pattern and higher vascular function. Project 3 indicates that localized exercise training with blood flow restriction dissociates vascular and muscle gains leading to enhanced muscular strength but diminished vascular function. The controller involved in the lower vascular function may be reduced shear stress during exercise. The controller that contributes to greater muscle strength during blood flow restriction remains unknown. Project 4 concludes that localized exercise training significantly improves vascular function and muscular strength in heart failure patients, although the gains are less than in age-matched individuals. The underlying controllers are unclear but may involve localized increases in shear stress and reduced oxidative stress. However, the benefits of exercise training are transient with vascular function returning to pre-training levels within 4 weeks after cessation of the training stimulus. **Conclusion:** These projects confirm a direct relationship between vascular health and physical

function. This relationship is modifiable with physical activity levels and exercise training. It appears that intermittent shear stress, as seen with acute exercise, and oxidative stress serve as important stewards of the relationship between vascular and physical function.

Chapter 1

Introduction

A major threat to the fabric of our society is *Physical Inactivity*. Physical Inactivity is now been recognized as an independent risk factor for modern chronic diseases [1]. The burden of these diseases are profound; cause great human suffering, costs the Nation nearly \$1 trillion in health care expenses per year and contributes to our economic instability [2]. According to the American Heart Association's 2010 update the number one cause of disability and mortality in the United States is cardiovascular disease, in particular coronary artery disease (CAD) [3]. In addition, the Baltimore longitudinal Study on Aging [4] speculate that dysfunction within the peripheral vasculature is a major share-holder in the development of CAD.

Recognizing the importance of sustaining a healthy vascular system this laboratory has provided consistent evidence to support that engaging in moderate amounts of physical activity and exercise training have the ability to rapidly modify and restore vascular function [5-9]. More importantly, this laboratory has shown that maintaining a high degree of vascular function is significantly related to higher physical function in a variety of populations [8-10]. The potential controllers mediating this relationship are not entirely understood, but appear to involve biomechanical (i.e. shear stress) [11] and biochemical (i.e. Nitric Oxide) [12] influences on the vascular wall.

Therefore, the purpose of this dissertation was to explore the potential controllers involved in the vascular adaptive response, and to what extent these controllers mediate the relationship between vascular and physical function. These controllers were explored by four projects revolved around three key issues which have emerged in the field of exercise vascular biology; including the influence of hemodynamic forces on the vasculature; the effects of blood

flow restriction on vascular function; and finally the effects localized exercise training and detraining in patients with chronic heart failure.

There have been an increasing number of reports in the literature examining the potential influence of hemodynamic forces on the vascular endothelium. A healthy endothelium promotes enhanced blood flow control (i.e. vasodilatory and constrictive properties) and protects the vasculature from damage. A recent review by Laughlin and colleagues [11] indicates the importance of the pattern of shear stress on the vascular endothelium [11, 13-14]. For example, areas within the vasculature exposed to low flow and shear stress or a higher reversal of flow direction (i.e. retrograde flow) expresses a pro-atherogenic phenotype (i.e. cellular proliferation and increased atherosclerosis production). However, areas exposed to high unidirectional flow and shear promotes enhanced endothelial function (i.e. increased nitric oxide (NO[•]) production and lower oxidative stress) [11, 15]. Considering this concept, it could be argued that individuals with higher retrograde flow may produce factors which could blunt vasoreactivity and diminish physical function. Therefore, the first key issue that is addressed in this dissertation is the relationship between the shear pattern and physical functionality, and to what extent can this relationship be modified by daily physical activity. Following this introduction, Chapter 2 takes a step toward understanding the importance of the hemodynamic pattern on physical function in the elderly. This investigation continues in Chapter 3 by examining the relationships between daily physical activity, blood flow patterns, vascular and physical function.

Shear stress plays an important role in the vascular adaptive response to exercise. As stated in a recent commentary by Tschakovsky [16], “The notion that mechanical stresses of blood flow can be a stimulus for vascular adaptation is nothing new...” The author is referring to the wealth of knowledge of the role shear stress plays in the adaptive response to exercise.

However, there have been an increasing number of reports in the literature regarding the effects of exercise training with deliberate restriction of blood flow, on physiological adaptations. This form of training, known as “occlusion-training” or Kaatsu, serves as a powerful stimulant for rapid increases in specific metabolic enzymes, muscle mass and strength [17-20]. However, few studies have examined the concurrent vascular responses following restrictive venous blood flow training. Given the consistent evidence that vascular function is linked to muscular strength [10], the second key issue which is addressed is would the muscular benefits seen with occlusion training extend to the vasculature as well. This concept is examined in Chapter four by altering the shear pattern during exercise in a group of healthy young individuals. Collectively, chapters two through four examine the influence of the blood flow pattern and shear on vascular health and physical function. The final project chapter, *Chapter 5*, examines the influence of localized exercise training on vascular function and muscular strength in individuals with known cardiovascular disease.

Exercise training has been shown to enhance functionality in CHF patients, translated through increased muscular strength and improved endothelial function. The precise mechanism through which exercise training induces vascular changes in this population is not entirely understood, but may involve changes to the endothelium and enhanced NO⁻ production [21-22]. Therefore, the third and final important issue which was addressed in this dissertation is whether or not localized exercise training can restore ‘normal’ vascular function in patients with CHF, and if so to what extent this adaptation is NO⁻ mediated. This issue is addressed in Chapter five through an examination into the effects of localized exercise training and detraining on vascular function, and measures of NO⁻ bio-availability (i.e. Nitrite Reserve and Antioxidant status). Furthermore, Chapter five examines the vascular operating range (VOR) which takes into

account the artery's ability to vasodilate and vasoconstrict in response to physiological stimuli (i.e. increased blood flow and cold stress). Forged around a primary objective, this dissertation makes an effort to determine if physical activity and exercise training can preserve vascular health and physical function through modifications in biomechanical and biochemical controllers (Figure 1.1).

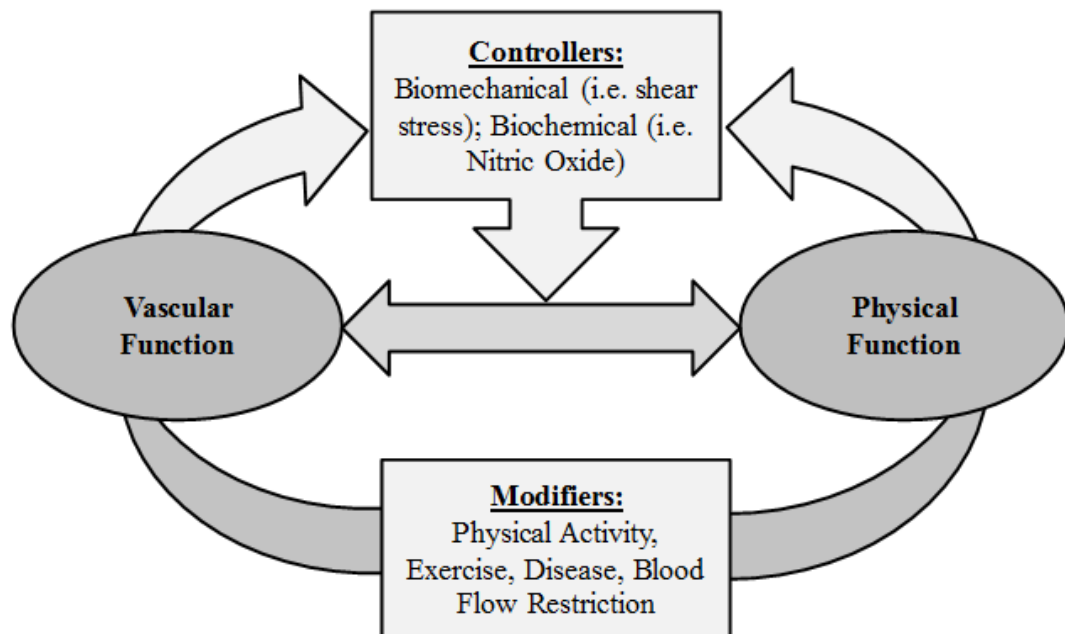


Figure 1.1: Illustration of the primary dissertation purpose. This lab has shown a direct relationship between vascular and physical function. This relationship appears to be mediated through biomechanical and biochemical controllers. The projects attempt to understand the impact of certain modifiers on vascular controllers; including physical activity and exercise, cardiovascular disease and blood flow restriction during exercise. Collectively, these projects make an effort to determine if physical activity and exercise training can preserve and restore vascular health and physical function through modifications in biomechanical and biochemical controllers.

Chapter 2

Brachial Artery Retrograde Flow Increases with Age: Relationship to Physical Function

2.1 Introduction

Aging is associated with alterations in a number of structural and functional properties of large arteries [4, 23-25], including increases in intima-media thickness [26], vascular stiffness [27], reduced endothelial function and altered vascular tone [4, 23-24, 28]. The consequences of these structural and functional alterations are sure to involve changes in blood flow distribution; particularly during conditions of stress (e.g. physical stress). Eventually, changes in blood flow distribution may contribute to reduced tissue perfusion [29], and attenuate physical function [10].

Interestingly, recent evidence suggests the importance of hemodynamic forces on endothelial cell expression. In fact, in a recent review, in vivo shear stress produced during exercise appears to cause beneficial effects on endothelial phenotype [11]. In contrast, oscillatory shear stress produces proatherogenic gene expression [11]. Given the importance of hemodynamic forces on endothelial cell expression, it is certainly of interest to consider work from Green, et al. (2002) who focused on flow velocity patterns with exercise [30]. In the study conducted by Green et al. (2002), it was noted that with increasing intensities of exercise, the retrograde component of the brachial artery flow velocity integral increased. Green et al. (2002) speculated this increase in the retrograde component to be the result of an increase in downstream pressure, which could be attributed to an increase in sympathetic vasoconstriction of inactive vascular beds [31]. Arguably, such apparent changes in the flow velocity patterns, with exercise, may indicate a change in hemodynamic forces on the vascular wall. It is presently unclear to what extent age-related structural and functional alterations of the vasculature influence the kinetics of arterial flow velocities.

Given the evidence in the literature of vascular remodeling [4, 23-24] and increased peripheral resistance with advancing age [28], this study aimed at examining brachial artery antegrade and retrograde flow velocities. Specifically, this study sought to determine the influence of antegrade and retrograde flow velocity on physical function, in the elderly. It was hypothesized that the antegrade and retrograde flow velocity would decrease and increase with age, respectively. Moreover, the magnitude of these changes in antegrade and retrograde velocity would be related to physical function.

2.2 Methods

- **Participants**

Participant sampling was performed via random selection based on voter registration lists and The Medicare Beneficiary Enrollment Data File from the Center of Medicare and Medicaid Services. Methods of recruitment included mail-outs, follow-up phone calls, and in the case of the nonagenarians, a home visit by members of the investigative team to explain the study in detail. Exclusion criteria for the LHAS included individuals scoring below 25 on the mini-mental status exam [32] 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, Tulane University, and The Louisiana State University and Agricultural and Mechanical College.

- **Brachial Artery Imaging and Analysis**

All brachial artery imaging was conducted by the same sonographer using a Toshiba Powervision SSA-380A, in accordance with the Guidelines set forth by the Brachial Artery Reactivity Task Force [33]. Prior to imaging, subjects were instructed to fast and refrain from

exercise for 12 hours, and alcohol for 48 hours. Subjects were advised to rest in a supine position for 20 minutes before baseline imaging was recorded. Forearm occlusion consisted of inflation of a blood pressure cuff, positioned approximately 1 cm distal to the olecranon process, to at least 50 mmHg above systolic pressure for 5 min. Images were obtained at rest, 1 minute prior to and from the final 30 seconds of occlusion, until 5 minutes following the release of the blood pressure cuff. Resting and peak brachial artery flow velocity measurements were obtained using a pulsed Doppler signal at an angle of approximately 60° to the vessel. In addition, blood pressure and heart rate were monitored throughout the imaging process. All ultrasound images were recorded on compact discs for subsequent analysis.

All data was analyzed by the same technician 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 1 minute of resting condition. Peak dilation was defined as the largest diameter following release of the occluding cuff. The value was calculated by the average of 10 images (five seconds) surrounding this highest observable peak. The reproducibility of this technique in our laboratory has yielded average mean differences in brachial artery diameter change for days, testers, and readers of 1.91%, 1.40% and 0.21 mm, respectively, with intra-class correlation coefficients of 0.92, 0.94, and 0.90, respectively [34]. Brachial artery reactivity was defined as the percent change in vessel diameter from rest to post forearm occlusion.

- **Examination of Flow Velocity Signals, Vascular Conductance and Shear Rate**

Flow velocity signals (antegrade & retrograde) were obtained at rest and immediately following release of the blood pressure cuff. Flow velocity values were calculated in a similar

manner to previously published findings [6, 30]. Each flow velocity signal was manually traced using Image Pro Plus 4.0 software. The area of the tracing was considered the mean flow velocity integral (FVI). The antegrade velocity signal was defined as the area of the tracing above the zero line (0 cm/sec). The retrograde velocity signal was defined as the area below the zero line. The FVI was calculated as the average of two consecutive velocity integrals. Examples of FVI tracings are depicted in **Figure 2.1**.

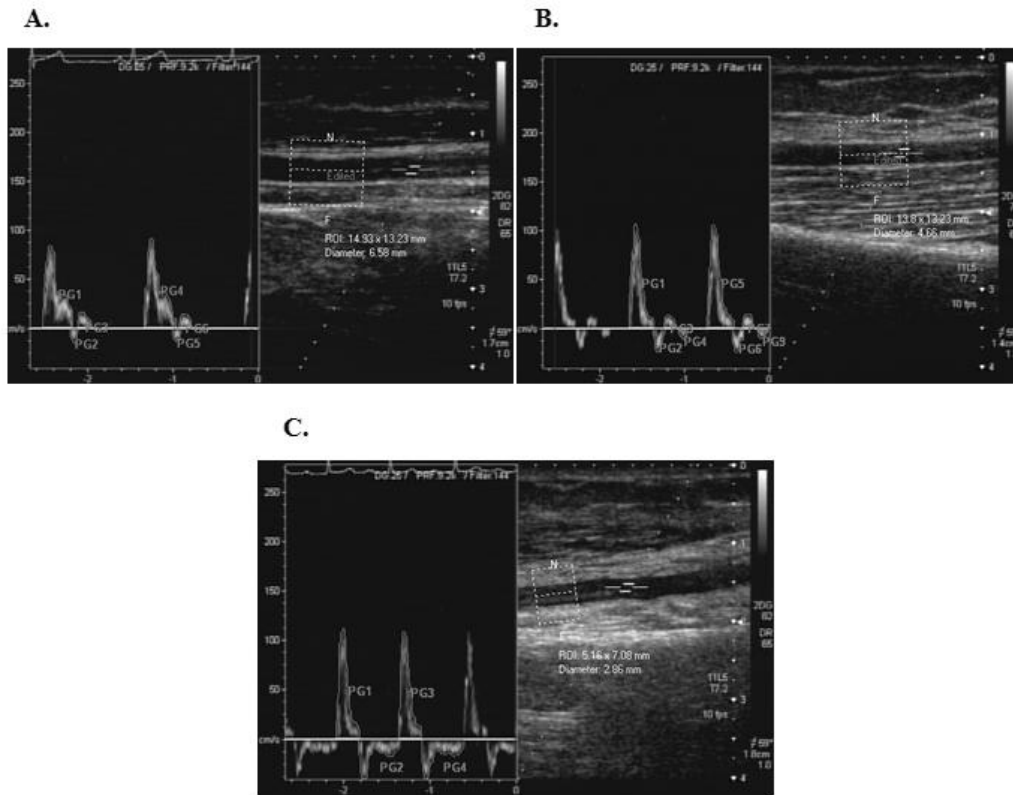


Figure 2.1: Depiction of examples of flow velocity tracings: **(A)** An image from a 35 year old, with an average antegrade and retrograde velocity of 15.95 and 1.01cm/sec, respectively, **(B)** An image from a 48 year old, with an average antegrade and retrograde velocity of 19.65 and 4.09cm/sec, respectively and **(C)** An image from an 82 year old with an average antegrade and retrograde velocity of 19.85 and 12.82cm/sec, respectively.

The mean FVI was then divided by the ejection time (s) from that cardiac cycle to subsequently determine the mean velocity (cm/s). The vessel radius (cm) at rest and immediately

post occlusion was used in the equation, $(\text{mean FVI} \times \text{heart rate}) \times \pi r^2$ to calculate mean blood flows (ml/min) at rest and hyperemia, respectively. The average vascular conductance (mL/min/mmHg) at rest (mean blood flow / mean arterial pressure) was calculated similarly to Wray, et al. (2007) [35]. Finally, the mean wall shear rate, upon release (mean blood flow velocity at hyperemia/Diameter), was calculated according to previously published findings [36].

- **Physical Function Test**

The Continuous Scale of Physical Function and Performance (CS-PFP10) test was used to assess physical function. The scale has been validated elsewhere [37] and is based on the performance of 11 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. The type of task performed determines whether it is quantified by time, distance or weight carried and sometimes all three. For instance, carrying groceries is quantified by both weight and time, at a given distance, 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.

- **Statistical Analysis**

All statistical analyses were performed using SPSS for Windows (version 11.0). Data are presented as mean \pm standard deviation. To examine the influence of age on brachial artery dimensions, flow velocity integrals, and reactivity, and physical function scores, Pearson Product Moment Correlations were used. To determine the relation of flow velocities and physical function, a univariate analysis of variation (ANOVA) was used for specific functional categories

[CS-PFP-10 total score < 25; 25 to < 57; and > 57U]. Finally, a second ANOVA, specifically for the non-agenarians, was used to determine the relation of retrograde velocity and physical function. An alpha level of ($p < 0.05$) was required for statistical significance. Differences between functional categories were evaluated using post-hoc comparisons.

2.3 Results

• Participant Characteristics

Ninety-two individuals were available for these analyses (Age=83±10yrs). The characteristics of these individuals are presented in **Table 2.1**. Of the participants, 43.5% were between the ages of 60 and 80 years, 8.7% ranged from 81-89 years of age and 47.8% made up the non-agenarian group (Individuals ≥ 90 yrs). Mean blood velocity and flow were 10.65±5.21cm/sec and 88±45ml/min. Mean antegrade and retrograde values were 14.24±4.78 and 3.47±2.30cm/sec, respectively. The mean vascular conductance for the group was 0.91±0.49ml/min/mmHg. The average total CS-PFP10 score was 40.15±20.81U, ranging from a low score of 4.11U to a maximum of 81.39U.

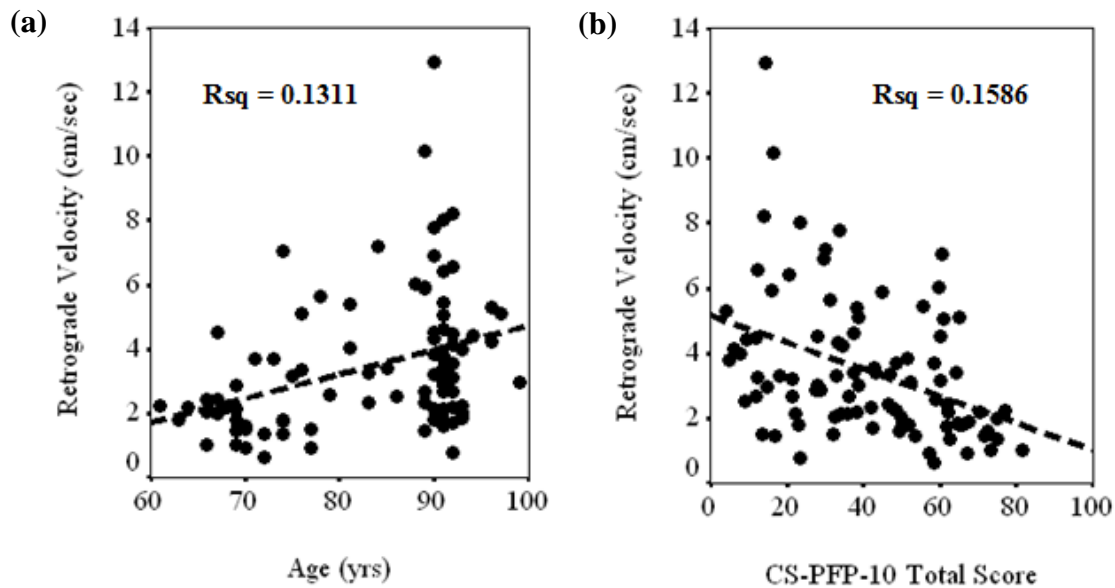
Table 2.1: Participant Characteristics

		Mean	SD
Age	(yrs)	83	10
Mean Arterial Pressure	(mmHg)	98	12
Pulse Pressure	(mmHg)	66	19
Antegrade Velocity	(cm/sec)	14.24	4.78
Retrograde Velocity	(cm/sec)	3.47	2.30
Blood Flow	(mL/min)	88	45
Blood Flow Velocity	(cm/sec)	10.65	5.21
Shear Rate	(s ⁻¹)	160.98	87.21
Brachial Diameter Base	(mm)	4.22	0.74
Brachial Diameter Peak	(mm)	4.4	0.73
BAR	(% change)	3.81	2.98
Vascular Conductance	ml/min/mmHg	0.91	0.49
CS-PFP10 Score	(U)	42.19	20.81

*Values are means ± SD for 92 participants

- **Associations between Age, Brachial Indices and Physical Function**

The association between age, flow velocities, brachial dimensions, vasoreactivity, vascular conductance, shear rate and physical function are presented in **Table 2.2**. Age was inversely related to resting mean blood flow velocity ($r=-0.29$), BAR ($r=-0.35$), vascular conductance ($r=-0.33$) and CS-PFP10 ($r=-0.61$), and directly related to retrograde flow ($r=0.36$) and PP ($r=0.40$). The associations between retrograde velocity and age, and retrograde velocity and physical function are presented in **Figures 2.2(a) and (b)**.



Figures 2.2(a) and (b): (a) depicts the relation between retrograde velocity (cm/sec) and age (yrs); and 2(b) retrograde velocity (cm/sec) and physical function.

- **Blood Flow Velocity and Physical Function Categories**

The results of the ANOVA, comparing retrograde velocity and CS-PFP10 functional categories, revealed a significant main effect ($p=0.03$). Those individuals in the highest functional class had significantly lower retrograde flow velocities, compared to those in the

lower functional classes. No such group differences were noted for the antegrade velocity, or mean blood flow velocity. However, when age was entered in the model as a covariate; the significant differences were lost, indicating a significant age contribution.

- **Blood Flow Velocity and Physical Function Categories in Non-Agenarians**

The results of the ANOVA, comparing retrograde velocity and CS-PFP10 functional categories, in the non-agenarians, revealed a significant main effect ($p=0.015$). As indicated in **Figure 2.3**, those individuals in the highest functional class had a significantly lower retrograde velocity compared to those in the lower functional classes. Again, no such group differences were noted for antegrade velocity, or mean blood flow velocity. Therefore, it appears that the retrograde component of the flow velocity profile increases with no change in the antegrade component, thus reducing the mean blood flow velocity.

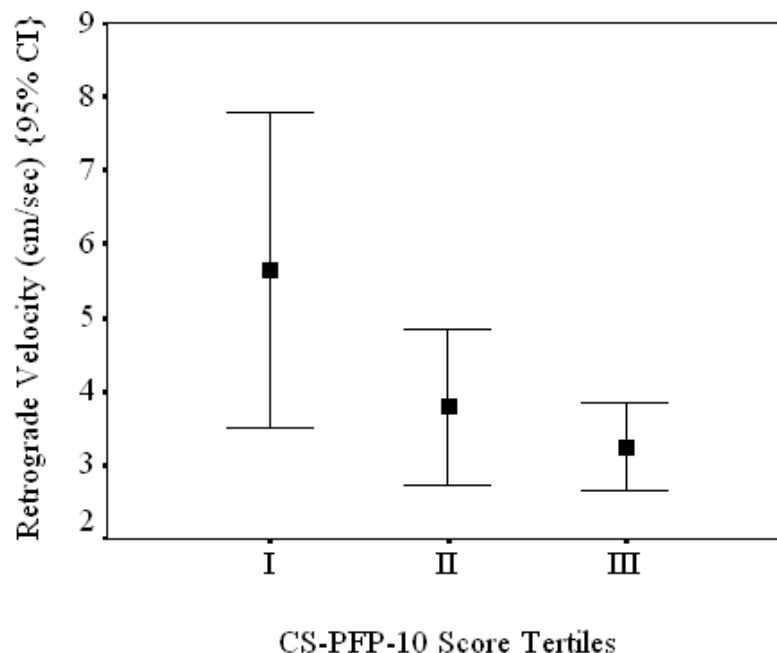


Figure 2.3: Depiction of the retrograde average (cm) per CS-PFP10 score tertiles (I, II & III), among nonagenarians.

Table 2.2: Associations between age, flow velocities, vasoreactivity, vascular conductance and physical function

	Age (yrs)	Antegrade Velocity (cm/sec)	Retrograde Velocity (cm/sec)	Blood Flow Velocity (cm/sec)	BAR %	Shear Rate (s ⁻¹)	Vascular Conductance (mL/min/mmHg)
Antegrade Velocity (cm/sec)	r=-0.17 p=0.115						
Retrograde Velocity (cm/sec)	r=0.36** p=0.000	r=0.26* p=0.011					
Blood Flow Velocity (cm/sec)	r=-0.35** p=0.006	r=0.82** p=0.00	r=-0.23* p=0.027				
BAR %	r=-0.35** p=0.001	r=0.16 p=0.119	r=-0.01 p=0.898	r=0.20* p=0.05			
Shear Rate (s ⁻¹)	r=-0.26* p=0.012	r=0.45** p=0.000	r=-0.02 p=0.816	r=0.47** p=0.000	r=0.59** p=0.000		
Vascular Conductance (mL/min/mmHg)	r=-.33** p=0.001	r=0.49** p=0.000	r=-0.25* p=0.015	r=0.69** p=0.000	r=-0.03 p=0.752	r=0.06 p=0.600	
CS-PFP10 Total Score	r=-0.61** p=0.000	r=0.01 p=0.947	r=-0.35** p=0.001	r=0.23* p=0.025	r=0.25* p=0.017	r=0.11 p=0.278	r=0.26* p=0.010

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

2.4 Discussion

The aim of this study was to examine the flow velocity pattern of the brachial artery, at rest, in a subset of participants from the Louisiana Healthy Aging Study. With advancing age, there was a significant decrease in the average blood flow velocity with a subsequent reduction in basal blood flow. Uniquely, with advancing age, antegrade velocity did not change, while retrograde velocity increased. Furthermore, individuals with greater physical function scores exhibited a lower retrograde velocity, although this was in part influenced by age. Importantly, among non-agenarians, those in the higher tertiles of physical function had significantly lower retrograde velocity, compared to those in the lower tertiles. These data indicate an age-dependent change in flow velocity patterns, which may, in part, contribute to lower physical functionality.

- **Associations between Age, Flow Velocities, Brachial Dimensions and Function**

The overall purpose of the Louisiana Healthy Aging Study is to identify determinants of healthy aging. In so, the study specifically emphasized the recruitment of a large number of non-agenarians, which allows not only for comparisons across age groups, but comparisons within non-agenarians, as well. It is this unique approach that differs from other aging trials. To this extent, nearly 50% of the current study participants were non-agenarians.

The present study did not find an association between age and brachial diameter at rest, which has been previously reported [38]. The average diameter tended to be bigger in participants who were taller ($r=0.47$), larger ($r=0.41$), or males ($r=0.65$). This study also observed that the average brachial arteries of these participants were generally bigger than studies reporting values for individuals under the age of 65years [11, 39]. The lack of an age-dependent enlargement of the brachial artery in the present study may suggest that any such changes may occur before age 60. The age-dependent decline in brachial blood flow and velocity has been

previously reported [40]. In fact, in a recent review, Seals et al. (2008) state that age-associated reductions in blood flow are mediated by corresponding decreases in vascular conductance [41-42], and are closely related to decreases in estimated leg oxygen consumption. Data from the present study confirms a relation between age and vascular conductance ($r^2=0.11$) and strong relationships between vascular conductance and mean blood flow velocity ($r^2=0.47$), and blood flow ($r^2=0.95$).

A unique finding of the present study is that the antegrade velocity did not change, while and retrograde velocity increased, respectively, with age. In fact, the regression equation for retrograde velocity (Retrograde Velocity = $-2.9 + 0.077(\text{Age})$; $p=0.00$) shows an approximate 25% increase per decade after age 65. The fact that retrograde velocity increases significantly with age, suggests those factors that define this signal, change perhaps to a greater extent than those factors that are associated with antegrade velocity (e.g. stroke volume) [43]. Evidence for this stems from a study which showed that an acute increase in local peripheral pressure using strategically placed pressure cuffs resulted in an acute augmentation in the retrograde, but not the antegrade velocity profile in young individuals [44]. Data from the present study confirms an age-dependent increase in pulse pressure, suggesting an imbalance between arterial flow and arterial impedance [45], and a decrease in vascular conductance. Moreover, the present study reveals an association between the retrograde flow velocity and vascular conductance ($r^2=0.06$), which is in line with Bacelli's findings in a cross-sectional study [44].

The mean shear rate values in the current study are quite similar to those observed by Welsch et al. (2008) [35] and Dobrosielski et al. (2009) [6], respectively. Mean wall shear rate was significantly associated with age and BAR. The decline in BAR with advancing age ($\text{BAR} = 11.88 - 0.097(\text{Age})$; $p=0.001$) was similar to a previous report from this laboratory and

Herrington et al. (2001) [38]. The age-dependent decline in BAR, may in part be a reflection of a change in the trigger for vasodilation (i.e. shear rate declined with age), or suggests a change in the mechanisms associated with the vasodilatory responses, including a reduction in the bioavailability or release of nitric oxide [46] or other endothelial derived vasodilators [39].

Finally, the present study shows a very similar decline in physical function scores as previously reported from another cohort from the LHAS. The regression of CS-PFP10 scores against age in the present study ($y = 138.28 - 1.16(\text{Age})$; $p=0.00$) suggests an approximate 1.6% per year decrease in function among adults over 60 years of age. Of further importance is that examination of the CS-PFP10 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.

- **Relation of Blood Flow Velocity and Physical Function**

A second unique finding of the present study is the apparent relation between retrograde velocity and physical function scores. No such associations were noted for antegrade velocity. The evidence for this relationship stems from two analyses, which indicate that individuals in the highest category of physical function exhibited a lower retrograde velocity. In the first analyses, participants were classified as low (below 25), moderate (between 25 and 57), and high (above 57) according to the CSPFP-10 scores. The rationale for this classification is based on the probability of independence graph developed by Cress, et al. (1996) [37]. 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. However, when age was entered into the model the association was no longer significant, indicating an important age influence. Thus a second analysis was

performed on the non-agenarians, which also revealed those with the highest functional scores had a lower retrograde velocity. Perhaps the lower retrograde velocities seen among the non-agenarians in the highest tertile of physical function may indicate that this particular group is still able to counter- balance more efficiently the hemodynamic changes present as we age.

Explanation for the apparent associations may reflect age-dependent alterations in peripheral impedance [30]. The mechanisms by which peripheral impedance increases with age are summarized in **Figure 2.4**, and include a change in autonomic balance toward an increased hyper-adrenergic state, other hormonal influences, or a decrease in the production or effects of Nitric-Oxide (NO) [23]. This increase in peripheral impedance appears to attenuate vascular conductance [47] and increase peripheral resistance, which may limit exercise tolerance [48].

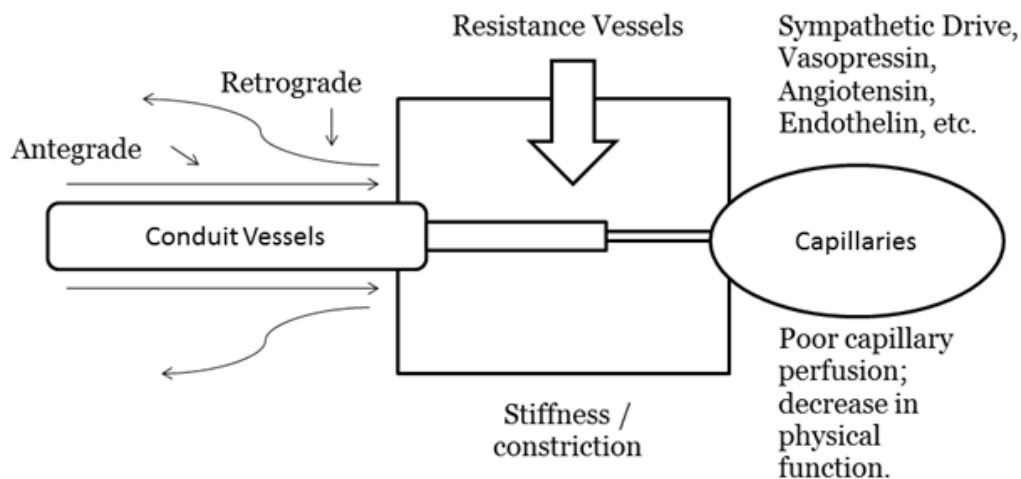


Figure 2.4: depicts the mechanisms by which retrograde velocity integrals may increase with age. Retrograde velocity may be the consequence of wave reflection from peripheral stiffness and constriction, possibly occurring within resistance vessels.

If, indeed, hemodynamic forces may have both beneficial and negative effects on endothelial cell expression, it could be argued that individuals with high oscillatory shear stress produce factors which could blunt vasoreactivity and diminish physical function. One such factor

could be reactive oxygen species [15]. Clearly, future work is needed to examine this possibility more closely, particularly during situations that may trigger oscillatory flow, such as during exercise.

2.5 Conclusion

The present study indicates significant relationships between vascular function, physical function, and age. Categorizing participants into functional categories, based on their CS-PFP10 total score, revealed that those in the highest functional group exhibited the lowest amount of retrograde velocity, but this relationship was dependent on age. Interestingly, when individuals within the nonagenarian cohort were grouped into tertiles, they exhibited similar relationships between retrograde velocity and physical function. The increase in retrograde velocity at rest may be in part arterial senescence, which contributes to a decline in physical function.

Chapter 3

Influence of Brachial Artery Reactivity and Blood Flow Patterns on Physical Function: Modification through Daily Activity

3.1 Introduction

Advancing age is considered an independent risk factor for cardiovascular disease and is accompanied with an increase in vascular stiffness and decline in endothelial function [4, 23-24]. These age related vascular changes may also be accompanied by changes in the pattern of blood flow through an artery [49]. Interestingly, recent evidence indicates the importance of hemodynamic forces, in particular the pattern of shear stress on the arterial wall, on vascular endothelial cell expression [11, 13-14]. Areas within the vasculature exposed to low mean shear stress and or a higher reversal of flow direction (i.e. retrograde flow) seem to enhance the expression of a proatherogenic endothelial cell phenotype along with inward vascular remodeling and cellular proliferation. However, areas of high unidirectional shear promote the inverse with enhanced endothelial function [11, 50]. Overtime, such arterial changes may contribute to alterations in blood flow distribution, reduced tissue perfusion [29], and ultimately attenuate physical functionality, especially in the elderly [10, 49].

Physical activity levels (PAL) are significantly reduced with advancing age, implied by a marked decline in total daily energy expenditure (TDEE), which seems to be accelerated beyond the age of 65 years [51-52]. The consequence of this decline in PAL and overall TDEE include detrimental changes in body composition and physical function, defined by a decreased ability to perform activities of daily living (ADL's) such as eating, dressing, transferring, personal hygiene and mobility [10, 52]. In contrast, maintaining a higher PAL with age has been shown to sustain a healthier vascular phenotype [11] and can counter age-related changes in vascular function and ultimately physical function [40].

Therefore the purpose of this investigation is two-fold. First, to examine the associations between BAFMD, antegrade and retrograde flow patterns and physical function, and second to determine the possible modifying role of daily physical activity on these vascular measures and physical function in an elderly cohort. Given the evidence in the literature [23] of vascular aging we hypothesized there would be a decline in BAFMD and an alteration in arterial flow patterns (i.e. higher retrograde flow) with advancing age. Moreover, we wanted to test the hypothesis that those individuals who maintain a higher TDEE, especially through activity (PAL), would have more favorable vascular function, arterial flow patterns and improved physical functionality.

3.2 Methods

Participants in this study represent a subset from the Louisiana Healthy Aging Study (LHAS). Sampling was performed via random selection based on voter registration lists and, “The Medicare Beneficiary Enrollment Data File from the Center of Medicare and Medicaid Services”. Methods of recruitment included mail, follow-up phone calls, and in the case of the non-agenarians, a home visit to explain the study in detail. Exclusion criteria for the LHAS included individuals scoring below 25 on the mini-mental status exam [32] and individuals in American Heart Association Class D (i.e. symptoms of cardiovascular and/or metabolic disease at rest). In addition, individuals with significant dysrhythmias were excluded for the purpose of these analyses. Each participant signed an informed consent approved by the institutional review boards of the host institutions.

- **Brachial Artery Imaging and Analysis**

Brachial artery imaging was conducted by the same ultrasonographer in accordance with the “International Brachial Artery Reactivity Task Force” guidelines [33]. Testing was performed between the hours of 7:00 – 11:00 am. Participants were required to refrain from

caffeine prior to imaging. Subjects were also instructed to fast and refrain from strenuous activity for 12 hours, and alcohol for 48 hours. In addition, subjects completed a 24-hour history questionnaire recalling past meals, drinks, activities, sleep and medications taken. Baseline ultrasound images were obtained after 20 min of supine rest, in a dark, climate-controlled, quiet room (22–24°C) with the participants arm immobilized and slightly supinated and elevated.

Brachial artery imaging was performed using a Toshiba Powervision SSA-380A. Images were obtained in the longitudinal view, approximately 4 cm proximal to the olecranon process, in the anterior/medial plane. Image depth was 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 (**Figure 3.1**). Doppler velocity profiles were collected simultaneously using a pulsed Doppler signal at a corrected insonation angle of 60° to the vessel, with the velocity cursor and gate positioned to sample the volume, mid-artery.

Forearm occlusion consisted of inflation of a pneumatic cuff (E-20 rapid cuff inflator; AG-101 air source; D.E. Hokanson, Bellevue, WA) positioned approximately 1 cm distal to the olecranon process, inflated to 200 mmHg for 5 minutes. Images for vessel diameter and velocity profiles were obtained for 30 seconds at rest, and continuously, from the final 30 seconds of occlusion, until 2 minutes following release of the blood pressure cuff. In addition, heart rate and blood pressure were monitored throughout the imaging process. Heart rate and Blood pressure were recorded using the ECG from the ultrasound and an automated blood pressure device (Datascope-Accutorr 4[®], Mindray DS USA; Mahwah, NJ) prior to, during occlusion and following release of the pneumatic cuff. The ultrasound images were recorded digitally and saved on disc for subsequent off-line analysis. Data were analyzed using Brachial Analysis Tool (Medical Imaging Applications, LLC).

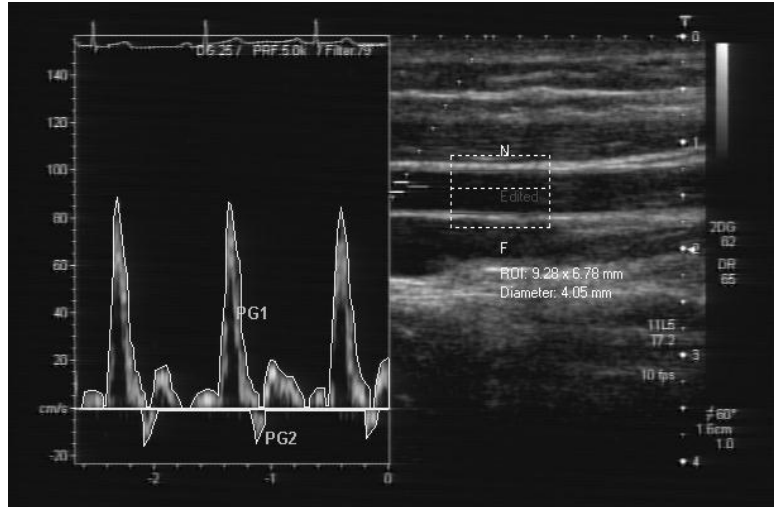


Figure 3.1: Depicts a 2-Dimensional, Doppler Ultrasound Image of the Brachial artery, with the corresponding flow velocity profile. After selecting a particular region of interest (ROI) a Semi-automated, Edge detecting software tracks the near and far luminal walls of the artery. Image Pro[®] software allows for semi-automated tracing of each flow velocity integral.

Arterial diameters were calculated as the mean distance between the anterior and posterior wall of the blood vessel interface, with the image in diastole, defined as the peak of the r-wave from the electrocardiograph and systole defined as peak of the antegrade velocity signal. Brachial diameter at baseline was defined by the average of 30 sec of recorded data. The reproducibility of this technique in our laboratory has yielded average mean differences in brachial artery diameter change for days, testers, and readers of 1.91%, 1.40% and 0.21 mm, respectively, with intra-class correlation coefficients of 0.92, 0.94, and 0.90, respectively [53].

- **Flow Velocity Profiles, Peak Shear Rate and Blood Flow**

Brachial flow velocity profiles were calculated in a similar manner to previously published reports [49]. Each flow velocity signal was traced using Image Pro Plus 4.0 software. The antegrade signal was defined as the area of tracing above 0 cm/sec and the retrograde signal was defined as the area below (**Figure 3.1**).

The antegrade and retrograde ratio (ante-/retrograde ratio) was the quotient of the velocity values, and was used to represent an index of oscillatory flow pattern through the vessel [49]. The flow velocity profiles were then divided by the ejection time (sec) from that cardiac cycle to subsequently determine the mean velocity (V_{mean}) (cm/sec). The mean flow velocity integral (FVI) was calculated as the difference between the antegrade and retrograde flow velocity signals. Peak shear rate following reactive hyperemia was calculated as $4 \cdot \pi r^2 \cdot \text{FVI}$, where 'r' is the blood vessel radius in cm; and the peak 'FVI' during reactive hyperemia; '4' represents a proportionality constant since blood viscosity was not calculated, because blood samples were not drawn during the Ultrasounds. The vessel radius (cm) at rest was used in the equation $(V_{\text{mean}} \cdot \text{heart rate}) \cdot \pi r^2$ to calculate mean blood flow (ml/min).

- **Physical Function Test**

The Continuous Scale of Physical Function and Performance (CS-PFP10) test was used to assess functional ability [37]. This scale which was developed by Cress, (1996) [37] is based on the performance of 11 activities of daily living. Participant instructions and protocols were standardized. 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.

- **Daily Energy Expenditure and Activity Levels**

Total daily energy expenditure (TDEE) was determined by doubly labeled water (DLW) measured over 14 days [52]. An isotope mixture of 2.0 g of 10% enriched H_2^{18}O and 0.12g of 99% enriched $^2\text{H}_2\text{O}$ (Cambridge Isotopes; Cambridge, MA) per kg of estimated total body water (55% of body weight) was consumed. Physical activity level (PAL) was calculated as $\text{TDEE} / \text{Resting Metabolic Rate (RMR)}$; RMR was measured using a Deltatrac II metabolic cart

(Sensormedics, Yorba Linda, CA) and was determined by measuring O₂ consumption and CO₂ production for 30 minutes with the last 20 minutes used to calculate resting energy expenditure.

- **Statistical Analyses**

Statistical Analyses were performed using SPSS version 16.0 for windows. Descriptive statistics are presented as mean \pm SD. To examine relationships between the variables of interest a pearson-product moment correlation was used. To examine the influence of BAFMD and Ante-/Retrograde Ratio on physical function, a multivariate prediction model was performed. To test the hypothesis that physical activity modifies dependent measures (BAFMD, Ante-/Retrograde Ratio and CS-PFP10 score), a multivariate analysis of covariance was performed using PAL tertiles and age and physical height as covariates. Finally, an alpha of ($p \leq 0.05$) was required for statistical significance.

3.3 Results

Fifty-three individuals (Age=83 \pm 11 yrs; males=41, females=28) were available for these analyses. Participant characteristics are presented in **Table 3.1**. Among the participants, 74% had some form of diagnosed cardiovascular disease (e.g. hypertension 63% and congestive heart failure 15%). Other pathologies included arthritis 69%, cancer 10% and osteoporosis 12%. It should be noted that a number of participants were on medications which are presented in **Table 3.2**. However, there were no associations between the type of medications taken, number, incidence of disease and dependent measures of the study.

- **Influence of Arterial Function and Flow Patterns on Physical Function**

Vascular Indices are presented in **Table 3.3**. Brachial diameters and BAFMD were 4.21 \pm 0.7 mm and 3.67 \pm 2.85%, respectively. Antegrade and retrograde flow velocity profiles were 13.42 \pm 4.21 and 3.60 \pm 2.00 cm/sec, respectively. The average ante-/retrograde ratio was

4.67±2.40 U. The average CS-PFP10 score was 43.84±22.00 U and average TDEE and RMR were 2162.50±574.80 and 1289.20±243.89 kcal/day, respectively. Physical activity level (PAL=TDEE/RMR) averaged 1.69±0.26 U. Associations between variables of interest are depicted in **Table 3.4**. Age was inversely related to BAFMD ($r=-0.42$, $p=0.001$), the antegrade/retrograde ratio ($r=-0.59$, $p=0.001$) and CS-PFP10 ($r=-0.73$, $p=0.001$). Moreover, both BAFMD ($r=0.37$, $p=0.002$) and ante-/retrograde ratio ($r=0.61$, $p=0.001$) were strongly associated with CSPFP-10. In addition, a multivariate prediction model, using BAFMD and ante-/retrograde ratio, accounted for 25% of the variance in the CSPFP-10 score.

Table 3.1: Participant Characteristics

	Mean	SD
Age (yrs)	83	11
Height (cm)	164.7	10.3
Weight (kg)	73.9	16.4
BMI (kg/m ²)	27.1	4.5
TDEE (kcal/day)	2162.5	574.8
RMR (kcal/day)	1289.2	243.9
PAL (U)	1.69	0.26
CS-PFP10 (U)	43.84	22.04
Resting Heart Rate (beats/min)	66.3	9.8
Mean Arterial Pressure (mmHg)	97.9	12.4

Table 3.2: Prescribed Medications

Cardiovascular	Number of Participants	Percent (%)
Nitrates	9	10
Angiotensin II Blockers	14	15
Alpha Adrenergic Blockers	17	18
Anti-coagulants	18	19
Calcium Channel Blockers	21	22
ACE Inhibitors	22	23
β-Adrenergic Blockers	26	27
Diuretics	33	35
Others		
Insulin	14	15
Anti-inflammatories	18	19
Anti-depressants	19	20
Statins	32	34

Values are presented as mean ± SD

Table 3.3: Vascular Indices

	Mean	SD
Resting Brachial Diameter (mm)	4.12	0.73
Peak Brachial Diameter (mm)	4.26	0.73
BAFMD (%)	3.56	2.76
Blood Flow (ml/min)	85.33	44.02
Antegrade Velocity (cm/sec)	13.42	4.21
Retrograde Velocity (cm/sec)	3.6	2.04
Antegrade to Retrograde ratio (U)	4.7	2.41
Mean Velocity (cm/sec)	9.7	4.17
Peak Shear Rate (s^{-1})	165.53	79.09

Values are mean \pm SD for 66 participants

Table 3.4: Correlational Matrix

	Age (yrs)	BAFMD	Antegrade Velocity	Retrograde Velocity	Ante-/Retrograde Ratio	Mean Velocity	CS-PFP10
BAFMD (%)	r=-0.42** p=0.001						
Antegrade Velocity (cm/sec)	r=-0.06 p=0.70	r=0.09 p=0.54					
Retrograde Velocity (cm/sec)	r=0.44** p=0.002	r=0.06 p=0.68	r=0.52** p=0.001				
Ante-/Retrograde Ratio (U)	r=-0.59** p=0.001	r=0.10 p=0.50	r=0.04 p=0.78	r=-0.42** p=0.001			
Mean Velocity (cm/sec)	r=-0.29** p=0.02	r=.225 p=0.07	r=-0.8** p=0.001	r=-0.42** p=0.001	r=0.43** p=0.003		
CS-PFP10 (U)	r=-0.72** p=0.001	r=0.37** p=0.002	r=-0.21 p=0.15	r=-0.42** p=0.001	r=0.61** p=0.001	r=0.11 p=0.37	
PAL-TDEE/RM R (U)	r=-0.43** p=0.001	r=0.45** p=0.001	r=-0.14 p=0.001	r=-0.14 p=0.34	r=0.35* p=0.01	r=0.05 p=0.71	r=0.51** p=0.001

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed).

- **Influence of Physical Activity on Vascular and Physical Function**

Three separate ANCOVA's revealed that those in the highest tertiles of daily physical activity had a greater BAFMD (Low PAL: $2.18 \pm 1.82\%$; Mod PAL: $3.52 \pm 2.70\%$; High PAL: $4.97 \pm 2.80\%$, $p = 0.03$), more favorable blood flow patterns (Low PAL: $4.08 \pm 1.30U$; Mod PAL: $3.60 \pm 1.70U$; High PAL: $6.54 \pm 2.93U$, $p = 0.05$) and a higher CS-PFP10 score (Low PAL: $31.02 \pm 18.90U$; Mod PAL: $38.80 \pm 18.74U$; High PAL: $62.37 \pm 15.93U$, $p = 0.003$) (**Figure 3.2**).

3.4 Discussion

This investigation examined the associations between vascular reactivity, flow patterns and physical function, and determined the possible modifying role of daily physical activity in a subset of participants from the Louisiana Health Aging Study. With advancing age, there was a decline in endothelial function (BAFMD), an alteration in arterial flow patterns with higher retrograde flow at rest, and a lower antegrade to retrograde ratio and a decline in physical function (CS-PFP10). Importantly, those individuals who maintained higher daily energy expenditure through activity had a more favorable vascular function and greater physical functionality.

- **Influence of Age on Vascular and Physical Function and TDEE and PAL**

The age associated decline in BAFMD observed in the present study is in agreement with previously published reports [4, 10, 54]. Dobrosielski et al. [54] compared BAFMD responses between a similarly aged group of men and young controls. In that study, the average BAFMD in the elderly men was 2.6%, compared to 7.7% in the young controls. In addition, Welsch et al. [10] examined BAFMD in a similar group of elderly men and found that the average percent change in BAFMD for their group was 2.67%, ranging from -0.92 to 7.97% [10]. The difference in BAFMD in the present study could stem from the fact that the resting brachial diameters are

significantly smaller, in part due to the fact that there were a large percentage of females included in the current analyses.

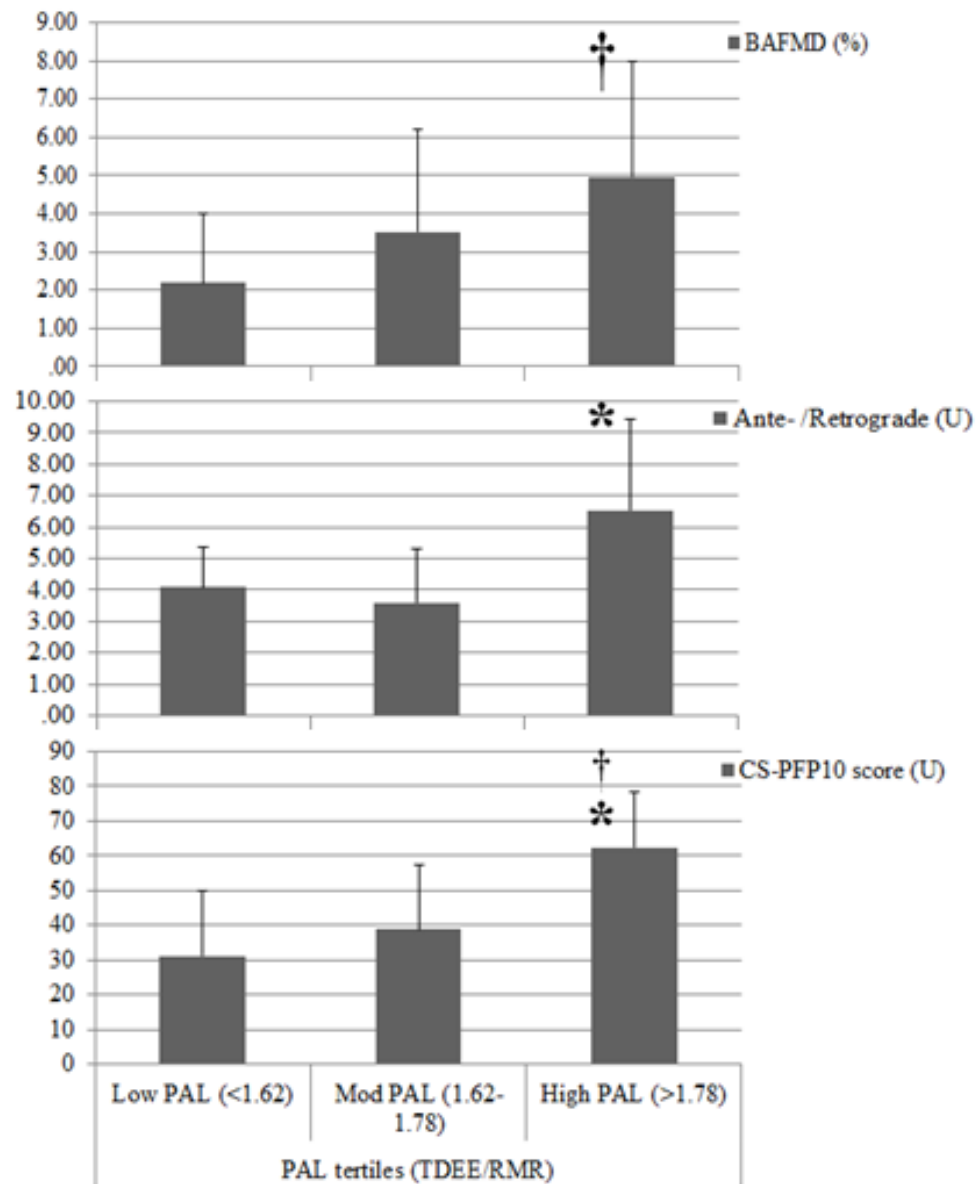


Figure 3.2: Depicts Brachial Artery Flow Mediated Dilation (BAFMD), Ante-/Retrograde Ratio and CS-PFP10 scores per PAL tertile (Low PAL: <1.62; Mod PAL: 1.62-1.78; High PAL: >1.78) († $p \leq 0.05$ vs. lowest tertile; * $p \leq 0.05$ vs. lower tertile).

In addition, no individuals were reported to have endothelial dysfunction, as defined as a decrease in diameter in response to forearm ischemia [33]. Some potential mechanisms to explain the reduction in BAFMD with advancing age include, but are not limited to, a reduced synthesis and/or release of nitric oxide [10] or other endothelial-derived dilators [39]. In addition, reduced BAFMD may be the result of increased formation of endothelium derived vasoconstrictor factors [55]; or production of reactive oxygen species [55].

The age related increase in the resting retrograde velocity profile observed in the present study is in agreement with Credeur et al. [49] who have shown that the retrograde component of the flow velocity profile increases with age. In addition the retrograde velocity profile seems to outweigh the magnitude of the antegrade signal, signifying a greater oscillatory flow pattern and through the artery. The increase in retrograde velocity at rest may be the result of wave reflection due to increased peripheral vascular resistance, due to higher sympathetic tone [13, 49].

The CS-PFP10 test used to measure physical function pertains to the execution of a combination of basic and instrumental activities of daily living. The CS-PFP10 scoring system is based on a continuous scale with scores between “0” and “100”, with “0” representing an individual who is completely dependent on others for survival and “100” representing an individual who is highly independent and able to function on their own [37]. Previous work from the LHAS has indicated an approximate 1.6% per year decrease in function among adults over the age of 60 [10]. The rate of decline in the present sub-sample from LHAS Cohort is representative of the entire study sample to date [10, 56].

With advancing age, there also appears to be a marked decline in TDEE, so noted by Johannsen et al. [52]. The present study confirms this notion. In addition a large percentage of the decline in TDEE with advancing age has been attributed to a reduction in the amount

physical activity by which older individuals participate in throughout the day. Potential reasons for the decline in activity levels seen in older adults are unclear, and due to the cross-sectional design of the present study, they remain ambiguous. However, previous work in this area has speculated that the decline in TDEE and more importantly PAL could be the consequence of environmental [52], psychological [57] and physiological factors [51] such as age related declines in muscle mass.

- **Influence of Arterial Function, Flow Patterns, TDEE and PAL on Physical Function**

Previous investigations from our laboratory have indicated the importance of vascular indices, particularly BAFMD, on measures of physical functionality in the elderly [10, 49, 54]. These findings are intriguing with respect to bridging the gap between pathology and disability within the context of the “The Disablement Process” paradigm [58]. This model suggests that disablement begins with some pathology/injury (i.e. cardiovascular disease) or defect resulting in impairment at the tissue, organ, and/or system level [10]. Interestingly, 74% of the population in this study had some form of cardiovascular disease, which is well known to influence functionality among older adults, possibly through its effects on cardiorespiratory function, fitness and overall functional reserve [59].

Traditionally, the age associated decline in cardiorespiratory fitness is ascribed to a reduction in stroke volume, and subsequent reduction in cardiac output for any given workload [51]. Previous evidence has indicated that age-associated changes in local blood flow have a major impact on functional capacity in the elderly [60]. For example, Poole et al. [61] 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 [62]. 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 [62]. Alternatively, age-related blunting of endothelium-mediated vasodilation has also been identified as another potential mechanism underlying differences in vascular control [63]. Finally, impaired functional sympatholysis observed in older adults may also attenuate blood flow and subsequently influence exercise performance and overall physical functionality [47].

The present study indicates an apparent relation between the ante-/retrograde ratio and physical function scores. The overall contribution of altered arterial flow patterns on physical function is unclear but may contribute to further reductions in arterial function and muscle blood flow. For example, with advancing age, there appears to be no significant change in the antegrade velocity ($p=0.70$) profile but an increase in the retrograde velocity ($p=0.002$). The result of this decrease in mean velocity would impair overall blood flow through the tissue. As previously mentioned, the increase in retrograde flow with advancing age can be attributed to an increase in peripheral impedance, possibly the result of a change in autonomic balance toward an increased hyper-adrenergic state, other hormonal influences, or a decrease in the production or effects of nitric oxide [4, 13, 49]. The increase in peripheral impedance may attenuate peripheral blood flow and increase peripheral resistance, which may limit exercise tolerance and physical function [47].

Habitual physical activity plays a considerable role in the development and maintenance of physical function throughout life [64]. In accord, the present study confirms a direct relationship between TDEE and PAL, and CS-PFP10 scores. Physical activity is a behavioral aspect which can be modified and adequate amounts is associated with improved health,

functional status, and longevity. Encouraging a physically active lifestyle, especially among the elderly, has become an imperative vital public health goal [64-65]. However, low physical activity has been shown to contribute to a reduced health-related quality of life in older persons [52], and several studies have shown that older adults who report lower levels of physical activity are at greater risk for mortality than those reporting moderate or high activity [52, 65].

- **Modifying Role of Physical Activity on Vascular Indices and Physical Function**

Maintaining habitual physical activity throughout life has been shown to promote a positive vascular phenotype [40] and can counter the age related declines in vascular function. Consistent with previous work, these findings indicate that individuals who maintain higher activity levels with advancing age, display a positive vascular phenotypic expression {higher BAFMD [21]} and greater physical function [66]. Uniquely, the present study identifies a relationship between physical activity levels and arterial flow patterns. Individuals who expended more energy through activity, had a higher ante-/retrograde ratio (lower retrograde in relation to antegrade) possibly suggesting less vascular resistance and oscillatory flow within the arterial system. Adults who are habitually active throughout their life appear to have smaller or no age-associated increases in large elastic artery stiffness and reductions in vascular endothelial function. Overall, the present study reinforces this notion, indicating that the maintenance of physical activity throughout life enhances both vascular and physical function.

3.5 Conclusion

The data from the present study concludes that advancing age is associated with a decline in vascular function, an increase in retrograde flow, a decline in total daily energy expenditure and reduced physical activity levels. The age related vascular and activity changes are associated with lower physical function. However, higher daily energy expenditures, especially through

activity, contribute to a positive vascular phenotypic expression and greater physical function.

Promoting physical activity among the elderly may enhance functional capacity, in part mediated through vascular adaptations.

Chapter 4

Effect of Handgrip Training with Venous Restriction on Brachial Artery Vasodilation

4.1 Introduction

There have been an increasing number of reports in the literature regarding the effects of exercise training with deliberate restriction of venous blood flow, on skeletal muscle adaptations. This form of training, known as “occlusion-training” or Kaatsu, serves as a powerful stimulant for rapid increases in specific metabolic enzymes, muscle mass and strength [17-20]. In fact, the muscle adaptations seen with restrictive venous blood flow training protocols suggests the improvements can be accomplished with much lower intensities of exercise, which may represent an alternative method of training for individuals intolerant to higher intensity training protocols. Interestingly, few studies have examined the concurrent vascular responses following restrictive venous blood flow training protocols.

Findings from our laboratory have consistently reported that regional specific resistance training results in large conduit artery adaptations [5-7]. In addition, our data show a direct association between vascular and physical function (e.g. muscular strength) [10]. The underlying trigger for such adaptations and associations are believed to be muscular contraction-induced increases in local shear forces which contribute to vascular modifications including endothelial mediated dilators [21]. Given the consistent evidence that vascular function is linked to muscular strength [10], we anticipate that the muscular benefits with occlusion training would extend to the vasculature as well.

Thus, the purpose of the present study was to examine the effects of 4 weeks of handgrip exercise training combined with or without restricted venous blood flow on handgrip strength and brachial artery dimensions and vasodilation. We hypothesized that handgrip exercise training

with venous restriction would result in superior strength gains and vasoreactivity, compared to the non-restricted arm.

4.2 Methods

Twelve participants (Age = 22 ± 1 yr; male = 5, female = 7) were selected from the Kinesiology student body, at Louisiana State University. Before initiation of the study, subjects completed a medical history/health habits questionnaire. In addition, all subjects were familiarized with the equipment and experimental procedures. Exclusion criteria were any diagnoses or evidence of cardiovascular, metabolic, orthopedic, and/or neurological disease; active infection; risk for adverse responses to exercise; and/or taking any medication which may affect cardiovascular function. Each participant signed an informed consent approved by the institutional review board of the Louisiana State University and Agricultural and Mechanical College.

- **Assessment of Handgrip Strength**

Handgrip strength was measured using a hydraulic hand dynamometer (Baseline[®]; Irvington, NY). The subject was asked to perform a max voluntary contraction (MVC), standing with the dynamometer at ones side and gripping the dynamometer as hard as they could, for 3 seconds. This was repeated 3 times for each hand. The average of the 3 trials for each hand was considered to be the maximum voluntary handgrip strength. Forearm circumference was examined using a weighted measuring tape positioned 10 cm distal to the midpoint between the lateral epicondyle and olecranon process. All pre-training assessments were performed within the week prior to commencement of training. Handgrip strength trials were performed 5 minutes following ultrasound assessments. These tests were performed before, and following the final

week of training. The right arm was assessed first each time and subjects were allowed one minute of rest between handgrip trials.

- **Assessment of Vascular Function and Blood Velocity Profiles**

All brachial artery imaging was conducted by the same ultrasonographer in accordance with the “International Brachial Artery Reactivity Task Force” guidelines [33]. Testing was performed between the hours of 7:00 – 11:00 am. Participants were required to refrain from caffeine prior to imaging. Subjects were also instructed to fast and refrain from strenuous activity for 12 hours, and alcohol for 48 hours. In addition, subjects completed a 24-hour history questionnaire recalling past meals, drinks, activities, sleep and medications taken. Baseline ultrasound images were obtained after 20 min of supine rest, in a dark, climate-controlled, quiet room (22–24°C) with the participants arm immobilized and slightly supinated and elevated. An additional 10 minute of rest was given prior to imaging the opposite arm. The right arm was imaged first in each case.

All brachial artery imaging and velocity profiles were obtained using a Hewlett-Packard Sonos 2000 (Bloomfield, CT) Doppler ultrasound, with a 7.5-MHz linear array transducer. Images were obtained in the longitudinal view, approximately 4 cm proximal to the olecranon process, in the anterior/medial plane. Image depth was 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. Doppler velocity profiles were collected simultaneously using a pulsed Doppler signal at a corrected insonation angle of 60° to the vessel, with the velocity cursor positioned to sample the volume, mid-artery.

Forearm occlusion consisted of inflation of a pneumatic cuff (E-20 rapid cuff inflator; AG-101 air source; D.E. Hokanson, Bellevue, WA) positioned approximately 1 cm distal to the

olecranon process, inflated to 200 mmHg for 5 minutes. Images for vessel diameter and velocity profiles were obtained for 30 seconds at rest, and continuously, from the final 30 seconds of occlusion, until 2 minutes following the release of the blood pressure cuff. In addition, heart rate and blood pressure were monitored throughout the imaging process. Heart rate and Blood pressure were recorded using the ECG from the ultrasound and an automated blood pressure device (Datascope-Accutorr 4[®], Mindray DS USA; Mahwah, NJ) prior to, during occlusion and following release of the pneumatic cuff. The ultrasound images were recorded digitally and saved on disc for subsequent off-line analysis.

An examination of the blood velocity profile during handgrip exercise was also performed. In one arm, a cuff was placed on the forearm, approximately 4 cm distal to the antecubital fossa. It should be noted that only 6 individuals participated in the experiment to examine blood velocity patterns during exercise, with restricted venous blood flow, after completion of the study. Three females and 3 males were selected for the blood velocity during exercise experiment. The purpose of this additional experiment was to assess the impact of partial forearm vascular occlusion on brachial artery blood velocity patterns. It should be noted that the magnitude of blood flow was not pertinent to this particular investigation, only the pattern of the velocity profiles (e.g. ante- and retrograde profiles). Additionally, when performing the Doppler ultrasound imaging of the brachial artery during handgrip exercise, the blood pressure cuff was placed distal to the position that was used during the actual training intervention. This distal position was chosen to facilitate the placement of the ultrasound probe. The training session followed the same protocol as the study (See *Exercise Training*, section). A total of five, 10 sec ultrasound images were recorded in each arm; at rest, during 5, 10, 15 and 20 min of exercise.

- **Data Analysis**

Off-line analyses of diameters were analyzed similar to previously published reports [6, 10], using a semi-automated edge-detecting software, Brachial Analyzer (Medical Imaging Applications, LLC; Coralville, IA). The reproducibility of this technique in our laboratory has yielded average mean differences in brachial artery diameter change for days and testers of 1.91% and 1.4%, respectively, with intra-class correlation coefficients of 0.92 and 0.94, respectively [34]. 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 electrocardiograph. Resting diameter was defined by the average of 30 seconds of data obtained after 20 minutes of resting conditions. Peak dilation was defined as the largest diameter following release of the occluding cuff. Finally, brachial artery flow mediated dilation (BAFMD) was defined as the percent change in vessel diameter from rest to peak diameter post forearm occlusion.

Blood velocity profiles were analyzed similar to previously published reports [6, 49]. Each profile was traced using Image Pro Plus 4.0 software (Media Cybernetics; Bethesda, MD). The antegrade component was defined as the area of tracing above 0 cm/s from the Doppler ultrasound scale and the retrograde component was defined as the area below. The velocity profiles were then divided by the ejection time (s) from that cardiac cycle to subsequently determine the mean velocity (cm/s). The mean velocity (V_{mean}) during baseline was calculated as the difference between the antegrade and retrograde velocity components. To establish an estimate of oscillatory flow patterns, a ratio was taken between the antegrade and retrograde flow velocities (ante-/retrograde ratio) [49]. Shear rate ($4 * V_{\text{mean}} \text{ (cm/s)} / \text{diameter (cm)}$) was measured at 10 second intervals during reactive hyperemia up to the point of maximum vessel

diameter and plotted against time (s). A trapezoidal model was then used to calculate area under the curve (AUC) above baseline [6, 67]. During a handgrip training session three blood velocity profiles were traced within 2 minutes of each time point (at rest, during 5, 10, 15 and 20 min of exercise), prior to a muscular contraction and averaged to determine flow velocity patterns during each time point of exercise.

- **Exercise Training**

Exercise training involved gripping a hydraulic hand dynamometer (Baseline[®]; Irvington, NY) and contracting the forearm at a rate of 15 times*min⁻¹ (1 contraction every 4 sec) at the pace of an electronic metronome, and a resistance of 60% of MVC. The intensity was marked on the gauge of the handgrip dynamometer using a dry-erase marker. The subjects were asked to train for 20 min, 3 days*week⁻¹ for 4 weeks, at Louisiana State University, under the supervision of a lab technician. Throughout the study, the participants were positioned facing two small mirrors to allow for a visual reference of the handgrip dynamometer. Subjects trained both hands, at the same time. However, for one of the limbs the pneumatic blood pressure cuff was placed on the upper arm, 4 cm proximal to the antecubital fossa. The decision which arm would receive the occlusion during training was randomized to avoid a dominant or non-dominant hand bias.

During the training this blood pressure cuff was partially inflated (80 mmHg) to ensure venous occlusion. Although some arterial inflow may have been restricted, the purpose was to induce venous pooling within the forearm vasculature. Previous work utilizing restricted venous blood flow in the legs, have indicated that the application of 100 mmHg was significant enough to restrict venous blood flow and cause venous pooling in the thighs distal to the cuff [68]. The current study examined venous restriction in the forearm, and the application of 100 mmHg

caused discomfort and exercise intolerance during handgrip training. An occlusion pressure of 80 mmHg was tolerable and sufficient, given that the average resting diastolic blood pressure in the EXP arm for the participants was ~71 mmHg. Subjects were allowed to take 1 min rest periods, after the completion of 5 minutes of training while the cuff remained inflated, but were encouraged to progress through each session.

- **Statistical Analysis**

Statistical analyses were performed using SPSS for Windows (version 17.0). Data are presented as mean \pm standard deviation. To determine the effects of the four weeks of handgrip exercise training on handgrip strength and BAFMD, a two (EXP arm vs. CON arm) by two (pre-training vs. post-training) repeated-measures ANCOVA was performed, using the baseline (pre-training) measures as the covariate. To determine gender differences, a 2-way MANOVA (females vs. males, dependent measures; magnitude of change for MVC and BAFMD (%) following exercise training) was performed for both the EXP and CON arms. To examine the change in blood velocity patterns (ante-/retrograde ratio and shear rates) during exercise training, a subsequent two (EXP arm vs. CON arm) by five (Velocity at rest, 5, 10, 15 and 20 minutes of exercise) repeated-measures ANOVA was performed. Differences between means were evaluated using a post-hoc LSD test. An alpha level of $p \leq 0.05$ was required for statistical significance.

4.3 Results

Twelve participants completed all facets of this study. All individuals were free of symptoms indicative of chronic illness. No one was taking any vascular medication that may influence the results. The baseline characteristics of these individuals are presented in **Table 4.1**. Resting systolic and diastolic blood pressure for the participants (pre and post training) averaged

116±9 / 71±8 and 113±8 / 71±7 mmHg, respectively. The average height for males and females in the present study was 185.93±4.89 cm and 168.37±6.8 cm, respectively. The average weight for the males and females was 94.09±13.02 kg and 68.7±10.95 kg, respectively.

Table 4.1: Participant Characteristics

	Mean	SD
Age (yrs)	22	1
Height (cm)	175.68	10.54
Weight (kg)	79.38	16.88
BMI (kg/m ²)	25	3.3
Resting Heart Rate (beats/min)	66	6
Pulse Pressure (mmHg)	45	11

Values are mean ± SD for 12 participants

- **Handgrip Strength and Forearm Circumference**

All subjects completed a total of twelve training sessions. There were no significant strength or circumference differences between arms prior to training. The results of the ANCOVA for handgrip strength (covariate=baseline MVC), revealed an increase in strength for the CON arm (8.32%, $p=0.05$) and the EXP arm (16.17%, $p=0.05$). These results are illustrated in **Figure 4.1** as the mean change in handgrip strength (kg) between study arms, following 4 weeks of handgrip exercise. It should be noted that there was no significant gender difference between males and females for the magnitude of change in handgrip strength ($p=0.36$). Following training, there was also a significant increase in forearm circumference for the CON

(1.62%: Pre=24.80 vs. Post=25.20 cm, $p=0.05$) and EXP arms (2.42%: Pre=24.80 vs. Post=25.40 cm, $p=0.05$).

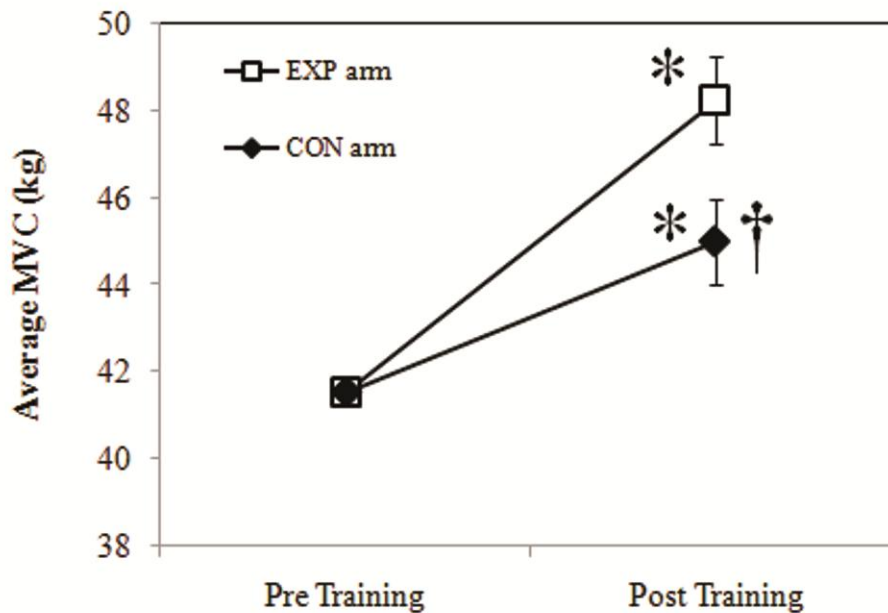


Figure 4.1: depicts the mean change in handgrip strength at baseline (pre-training) and following 4 weeks (post-training) of exercise. Data are presented as mean \pm SE. *Significant vs. baseline; †Significant vs. EXP Arm, $p \leq 0.05$.

- **Assessment of Vascular Function and Blood Velocity Profiles**

Values for vascular diameters are presented in **Table 4.2**. No significant differences in baseline brachial diameter, BAFMD or velocity profiles were noted between arms prior to training. The average baseline brachial diameters for males were; EXP arm = 4.21 ± 0.34 mm and CON arm = 4.29 ± 0.25 mm, and for females; EXP arm = 3.45 ± 0.42 mm, CON arm = 3.54 ± 0.6 mm. **Figure 4.2** presents the results from the ANCOVA for BAFMD, pre- and post-training. BAFMD increased in the CON arm (24.19%, $p=0.0001$; Absolute change: pre-training= 0.22 ± 0.01 mm vs. post-training= 0.29 ± 0.11 mm), and decreased in the EXP arm (30.36 %, $p=0.0001$; Absolute change: pre-training= 0.27 ± 0.07 mm vs. post-training= 0.19 ± 0.06 mm).

There were no significant gender differences for the magnitude in change in BAFMD ($p=0.11$). **Figure 4.3** presents the actual BAFMD values, between study arms, for the individuals following 4 weeks of handgrip exercise. Mean values for blood velocity profiles and shear AUC, pre- and post-training, are presented in **Table 4.3**.

Table 4.2 : Vascular diameters at baseline and following 4 weeks of exercise

EXP Arm	Baseline (Pre-training)	Week 4 (post-training)
Rest Diameter (mm)	3.76 ± 0.54	3.84 ± 0.66
Peak Diameter (mm)	4.04 ± 0.53	4.03 ± 0.68
CON Arm		
Rest Diameter (mm)	3.85 ± 0.61	3.87 ± 0.62
Peak Diameter (mm)	4.07 ± 0.6	$4.35 \pm 1^*$

Values are mean \pm SD for 12 participants. *Significant vs. baseline, $p \leq 0.05$

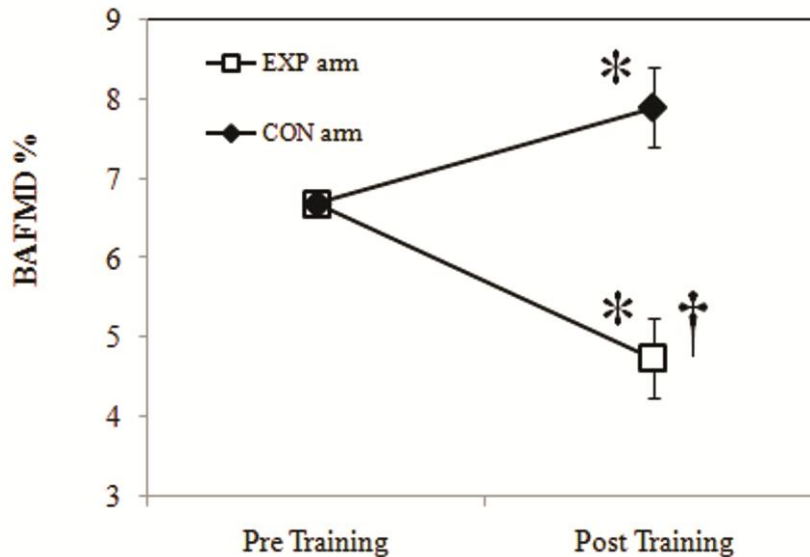


Figure 4.2: depicts the mean change in BAFMD at baseline (pre-training) and following 4 weeks (post-training) of exercise. Data are presented as mean \pm SE. *Significant vs. baseline; †Significant vs. CON Arm, $p \leq 0.05$.

An examination of the blood velocity pattern during exercise revealed upon inflation of the cuff, prior to exercise, the retrograde flow velocity component increased (Pre-inflation=1.27 cm/s vs. Post-inflation=13.49 cm/s, $p=0.03$), and antegrade velocity decreased (Pre-inflation=17.96 cm/s vs. Post-inflation=14.68 cm/s, $p=0.05$). Consequently, the ante-/retrograde ratio decreased significantly (Pre-inflation=14.14 cm/s vs. Post-inflation=1.09 cm/s, $p=0.05$) following cuff inflation in the EXP arm. Furthermore, the retrograde flow velocity component remained for the occluded arm, with no such evidence in the non-occluded arm. **Figure 4.4** illustrates the mean antegrade and retrograde shear rate (s^{-1}) values during the training session.

Table 4.3: Blood velocity profiles (resting) and shear stimulus (post cuff release) at baseline and following 4 weeks of exercise

EXP Arm	Baseline (Pre-training)	Week 4 (post-training)
Antegrade Velocity (cm/sec)	16.45 \pm 5.85	18.64 \pm 6.41
Retrograde Velocity (cm/sec)	3.88 \pm 4.01	2.72 \pm 3.24
Ante-/Retrograde Ratio (AU)	15.25 \pm 19.93	10.38 \pm 6.99
Vmean (cm/sec)	12.58 \pm 7.23	15.92 \pm 6.97
Shear Stimulus (AUC)	5472.86 \pm 1216.90	6776.69 \pm 1313.19*
CON Arm		
Antegrade Velocity (cm/sec)	19.47 \pm 8.71	14.68 \pm 6.27
Retrograde Velocity (cm/sec)	3.43 \pm 3.38	3.38 \pm 2.25
Ante-/Retrograde Ratio (AU)	24.90 \pm 38.8	15.5 \pm 27.53
Vmean (cm/sec)	16.04 \pm 10.91	11.29 \pm 6.73
Shear Stimulus (AUC)	6579.24 \pm 1475.69	7664.13 \pm 2568.81*

Values are mean \pm SD for 12 participants. * Significant vs. baseline, $p \leq 0.05$

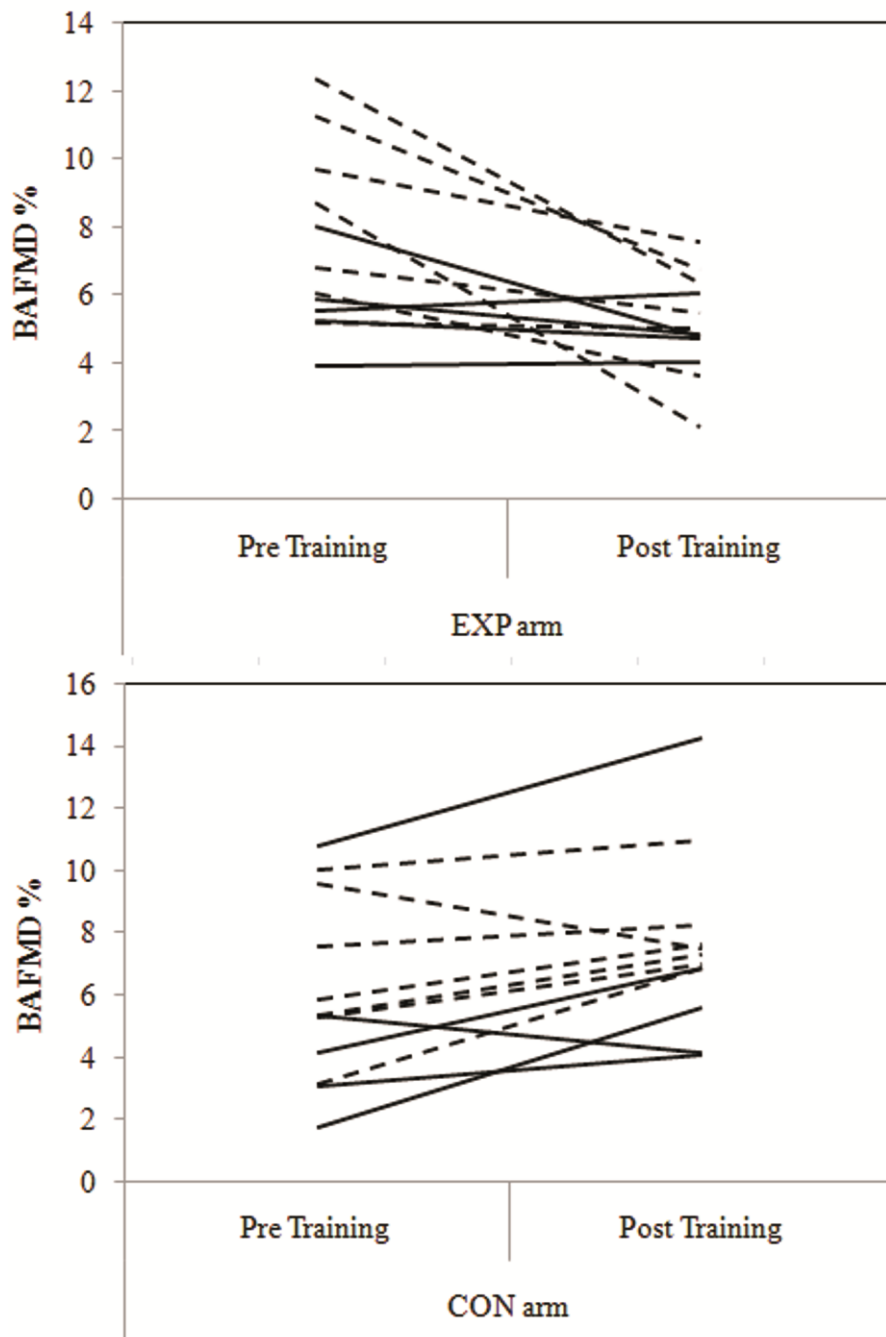


Figure 4.3: Depicts the individual changes in BAFMD at baseline (pre-training) and following 4 weeks (post-training) of exercise. Solid lines represent male participants, while dashed lines represent female participants.

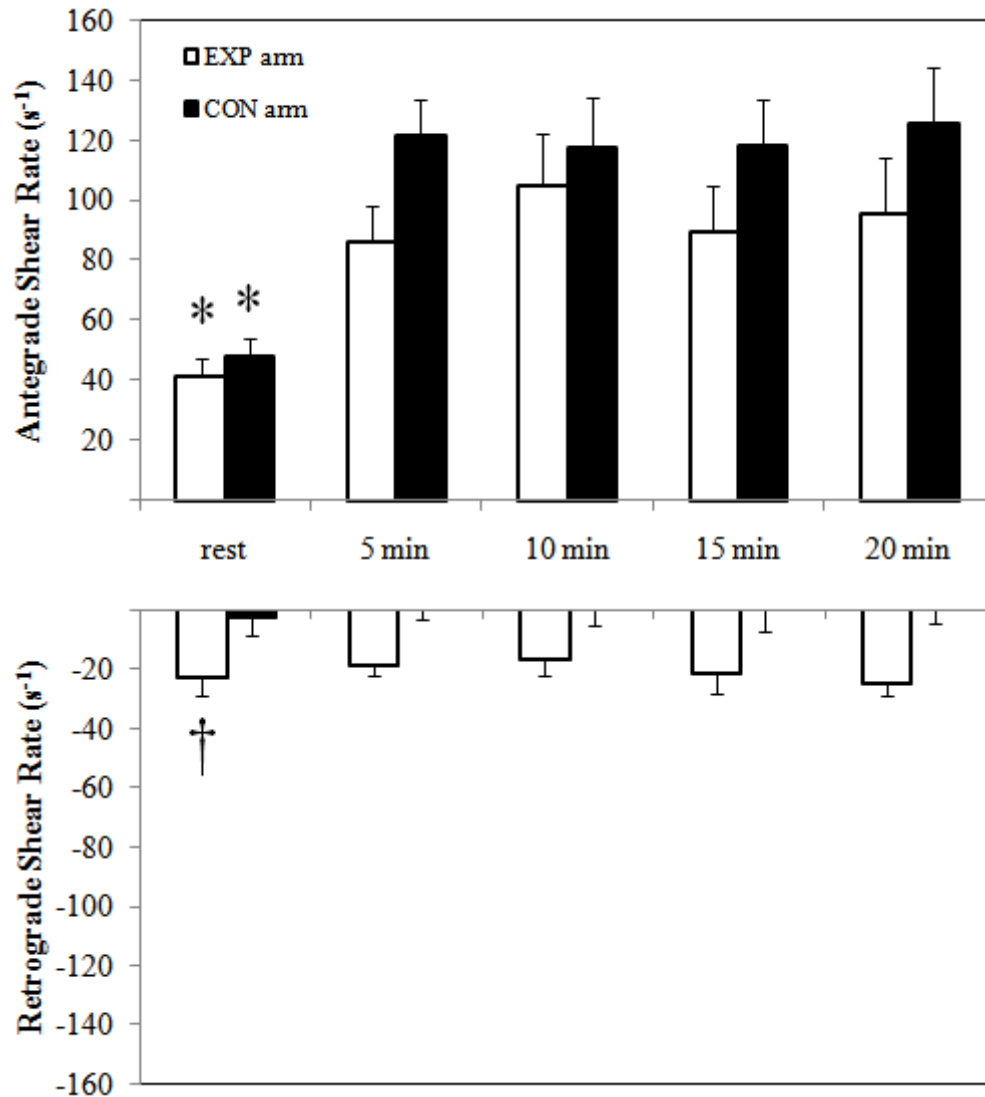


Figure 4.4: Depicts an examination of Ante- and Retrograde shear (s⁻¹) in the brachial artery, at rest and during 20 minutes of handgrip exercise, in the EXP and CON arm. *Significant vs. ante- and retro- shear rates during exercise; †Significant vs. CON at rest, $p \leq 0.05$.

4.4 Discussion

The present study examined the effects of 4 weeks of handgrip exercise training, coupled with and without restricted venous blood flow, on handgrip strength and brachial artery vasodilation. The data indicate that forearm exercise training combined with restricted venous

blood flow results in a significantly different change in muscular strength (superior strength gains) and vascular function (reduced vasodilation) compared to the non-restricted arm.

- **Handgrip Strength and Forearm Circumference**

The present study observed significant improvements in handgrip strength and forearm circumference following regional specific handgrip training. These results compare to others who have observed the effects of regional specific exercise on handgrip strength changes [5, 7]. Alomari and Welsch (2007) reported a 14.5% improvement in grip strength following 4 weeks (5 sessions per week) of handgrip exercise training [7]. Interestingly, the present study reports a significantly greater improvement in grip strength in the EXP arm compared to the CON arm. Previous studies have shown that combining resistance training with restricted venous blood flow results in significant improvements in muscle size and strength [18-20]. Consistent with this statement, the present study confirms a 50% greater improvement in handgrip strength in the EXP arm compared to the control. Mechanisms which could have contributed to this increase in strength are not entirely understood, but have been proposed to be the consequence of an upregulation in specific growth factors (e.g. IGF-I) [17] specific metabolic enzymes (e.g. creatine phosphokinase- CPK) [17], the result of hormonal changes (e.g. increase in growth hormone) [17], and/or a preferential recruitment of larger, fast motor units [18, 69].

- **Vascular Function and Blood Velocity Profiles**

Brachial artery resting diameter, prior to training, was similar between arms and to previous reports from our laboratory [5, 54], and significantly associated with BAFMD prior to training ($r=0.57$, $p=0.04$). Moreover, the average vasodilatory response following 5 minutes of occlusion, prior to training, were also similar between arms, and in agreement with Dobrosielski et al. (2006), who reported a BAFMD value of $7.7\pm3.5\%$ in healthy adults (Age= 28 ± 8 yrs)[54].

The present data indicate that following 4 weeks of handgrip exercise training BAFMD improved 24.19% in the CON arm. In fact, 10 of 12 arms showed improvements after training, indicating the consistency of the adaptive response. However, the observed improvement is less than previously reported by Allen et al. (2003). In that study, BAFMD improved 62% following 4 weeks of handgrip exercise training using a protocol of 20 min*day⁻¹, 5 days*week⁻¹ [5]. The discrepancy between studies could in part be the result of a significantly lower volume of training, in the present study (~50% less).

Typically, improvements in BAFMD with exercise training are believed to be secondary to the changes in shear stress induced by the muscular contractions during the acute bouts of exercise [21]. The muscular contraction induced changes in shear stress are thought to alter the endothelial machinery involved in vasodilatory pathways, including increased nitric oxide production, eNOS, PGI₂, anti-oxidant defenses, and a reduction in reactive oxygen species, adhesion molecules, and vasoconstriction factors (e.g. endothelin-1) [21-22, 70]. We also acknowledge that the improvement in BAFMD, in the present study, may also be the result of endothelial independent changes in resistance vessel function and microcirculation [21] as the relevant shear stimulus was higher at week 4 in both the CON and EXP arm compared to the pre-training measures. The fact that in the EXP arm BAFMD declined after training despite higher shear AUC is intriguing and suggests a significant alteration in the vasodilatory response to a larger trigger.

Uniquely, the present study observed a significant reduction in BAFMD in the EXP arm. The reduction was apparent in 9 of 12 arms, and appeared to be greatest in those who had the highest pre-training BAFMD, suggesting those individuals “suffered” to a greater extent than those who had lower vascular function prior to training. The fact that the change in vascular

function is completely in the opposite direction of the CON arm is quite intriguing. Interestingly, a recent study examined oxidative stress in response to partial vascular occlusion of the upper arm in young men [71]. Goldfarb et al. (2008) found an elevation in oxidative stress, as defined by a glutathione ratio and plasma protein carbonyls, in response to moderate intensity resistance training, as well as, with prolonged vascular occlusion (e.g. 20 mmHg less than resting diastolic pressure) [71]. The present study utilized a similar occlusion pressure and exercise training intensity, which could have resulted in an elevation in oxidative stress within the EXP arm. This is important considering the inherent effects of oxidative stress on endothelial function [70, 72].

An additional explanation for the decline in BAFMD observed in the EXP arm stems from the hypothesis that regular exposure to exercise induced increases in endothelial “shear stress” is the primary signal for a positive expression of endothelial function [11, 21-22, 70, 73]. A reduction or alteration of the shear stimulus during exercise may compromise the vascular adaptive response. In fact, in the present study, upon inflation of the cuff and prior to exercise in the EXP arm, the retrograde velocity component increased and the antegrade velocity component decreased. Consequently, the ante-/retrograde ratio decreased significantly following cuff inflation in the EXP arm. The fact that handgrip exercise with restricted venous blood flow has such a large retrograde component, in comparison to antegrade, suggests that there may be a significant change in oscillatory flow patterns and shear stress [11, 14, 49, 74]. Such a shear stress is thought to promote a proatherogenic phenotype and oxidative stress within the endothelium, resulting in a decline in vascular function [11, 15].

The examination of flow velocity patterns during exercise should be interpreted within the constraints of potential limitations. Our study utilized a non-invasive measure of blood velocity and flow. It is difficult to establish an estimate of oscillatory flow patterns within a

vessel with 2 dimensional imaging. However, it is interesting to see that these patterns change when exposed to an increase in resistance (e.g. restricted venous blood flow). The finding that the flow patterns are changed during restrictive flow conditions are in agreement with Tinken et al. (2009), who reported that during handgrip exercise, cuffed arm retrograde flow was higher than in the non-cuffed [14]. In fact, BAFMD was not significantly changed in the cuffed arm in response to acute bouts of heating, handgrip, and cycling [14]. In another study, Thijssen et al. (2009) reported that acute alterations in flow velocity patterns, at rest, resulted in a reduction in subsequent measures of BAFMD. In that study, it was noted that with increasing occlusion pressures (50-75 mmHg), there was a dose response reduction in reactivity of the brachial artery [74]. The apparent reduction in reactivity seen with Thijssen et al. (2009) may also have been, in part, the consequence of a change in pressure exerted upon the arterial wall [75]. Interestingly, Padilla et al. (2009) observed a decline in vascular reactivity in the brachial artery when acutely exposed to an increase in hydrostatic pressure [75].

- **Clinical Relevance and Future Recommendations**

Finally, from a clinical perspective, it is critical to understand that alterations in regional blood flow patterns, due to vascular disease (peripheral vascular disease), may affect the exercise adaptation. Perhaps, a change in regional flow patterns in patients with peripheral arterial disease (PAD) may explain why they experience an acute inflammatory response with a subsequent reduction in BAFMD, following an acute bout of exercise [76]. Given this evidence, future studies should investigate the effects of moderate intensity exercise training with partial vascular occlusion on markers of oxidative stress (e.g. peroxynitrite, superoxide, and reactive oxygen species) and endothelial function (e.g. flow mediated dilation). In addition, given the milieu of

hemodynamic forces exerted upon the arterial wall, future investigations should continue to target the underlying mechanisms involved in vascular responses and adaptation.

4.5 Conclusion

These data indicate that forearm exercise training combined with restricted venous blood flow results in a significant increase in muscular strength, coupled with a significant decrease in vascular function (reduced vasodilation). The contrasting change in vascular function following exercise training with venous blood flow restriction in the forearm may, in part, be the consequence of significant alterations in blood flow patterns during handgrip exercise.

Chapter 5

Plasma Nitrite Reserve and Vascular Function Before and After Handgrip Training in Patients with Heart Failure

5.1 Introduction

The number one cause of disability and mortality in the U.S. is cardiovascular disease (CVD) with the main contributor being coronary artery disease (CAD) [3]. Advances in cardiovascular care have allowed CAD patients to live longer, but have a tendency to develop more severe chronic heart failure (CHF). The cardinal symptom of the CHF patient is exercise intolerance [77-78] which is thought to be the consequence of mal-adaptation in the periphery, including increased vascular stiffness, and decreased vasodilatory function [4].

Localized, short-term endurance exercise training typically results in significant improvements in vascular function in the trained limb(s) of young [8], elderly men [6] and those with known disease (i.e. CHF) [79]. Given the ability of the vasculature to respond favorably to an exercise stimulus is preserved, even in those with a compromised vascular system, is important in regards to disease management and maintenance of physical function. However, the precise mechanism(s) through which exercise training induces vascular changes in CHF is not entirely understood, but appears to involve changes to the endothelium lining of arterial blood vessels [21-22].

It has been proposed that repetitive increases in shear stress, associated with muscle contractions during exercise, signals the formation of a beneficial endothelial phenotype; including increased nitric oxide (NO^-) production and lower oxidative stress [11, 15]. Unfortunately, it has remained challenging to measure changing levels of NO^- *in vivo* considering that NO^- is converted almost immediately to nitrite (NO_2^-), subsequently nitrate (NO_3^-) [80]. Recent work by Kelm et al. [81] have demonstrated the importance of nitrite as a

measure of NO⁻ function in humans. Kelm et al. [81] established a significant relationship between vascular function (i.e. Brachial Artery Flow-Mediated Dilation; BAFMD) and plasma nitrite reserve (i.e. plasma nitrite difference from pre- to post-reactive hyperemia), but not nitrate or total Nitrogen-oxides (NO⁻_x). Previous works from this laboratory, as well as others, has established the potential clinical importance of BAFMD as a physiological reflection of endothelial function in healthy and diseased populations [9, 54, 82-83]. However, it is currently not known if changes in BAFMD, seen with localized endurance exercise training, coincide with changes in plasma nitrite reserve. If indeed such a relationship exists, it could be hypothesized that localized endurance exercise training indeed alters NO⁻ function in the human vasculature.

Therefore, the purpose of this study was to examine the influence of regional specific, hand-grip training and training cessation on measures of vascular stiffness, brachial artery reactivity and Plasma Nitrite Reserve in individuals with and without (CON) known CHF. It was hypothesized that training would result in an increase in vascular reactivity and a decrease in regional vascular stiffness, in both groups. In addition, it was hypothesized that training would result in an increase in the plasma nitrite reserve, and that these changes would coincide with an increase in vascular reactivity in both groups.

An additional objective was added to this the study which involves the comparison of antioxidant status between CHF and CON subjects. Given the important link between pro- and antioxidants, and NO⁻ [84], it was believed this information could provide further insight in the effects of handgrip training on vascular biology. It was hypothesized that CHF patients would have significantly lower total antioxidants levels compared to the CON subjects. Furthermore, it was hypothesized that there would be a direct relationship between total antioxidant status, vascular function and nitrite reserve.

5.2 Methods

- **Study Design**

The study is a prospective design consisting of four weeks of handgrip exercise training followed by four weeks of training cessation. Major dependent variables of study include blood levels for nitrite, BAFMD and cold pressor test responses (CPT), measures of vascular stiffness (Pulse Wave Velocity; PWV) and antioxidant capacity (Total Antioxidant Levels). Secondary measurements of study include estimated shear rate area under the curve (AUC), maximal handgrip strength (MVC) and a 6-minute walk test. Variables were examined prior to training (Study Visit 1; SV1), at the end of 4 weeks of training (Study Visit 2; SV2) and following 4 weeks of training cessation (Study Visit 3; SV3).

- **Participant Selection**

Participants were recruited from Louisiana Cardiology Associates, at Our Lady of the Lake Hospital, and the local Baton Rouge community. Eligible CHF patients included men and women at least 18 years of age with a diagnosis of CHF; including an ejection fraction $\leq 40\%$; NYHA Classification II and III; non-smokers and not pregnant. Eligible participants for the control group include those with no known chronic ailments.

- **Assessing Vascular Stiffness**

Prior to ultrasound imaging, radial (carotid to radial) and distal (carotid to foot) PWV measurements were performed using the SphygmoCor System by AtCor Medical[®]. Briefly, pulse wave measurements were obtained non-invasively upon the neck, radius and below the medial malleolus of the foot using a pressure tonometer. These pressure waves were linked to the R-

wave from an ECG to determine transit time. The distance between sites was measured by tape starting proximally from the carotid pulse to the suprasternal notch, and distally from the suprasternal notch down to the either the radial or posterior tibial artery sites. Pulse wave velocity was subsequently calculated as the quotient of distance over transit time (m/s).

- **Brachial Artery Imaging**

All brachial artery imaging was conducted by the same sonographer in accordance with guidelines set forth by the Brachial Artery Reactivity Task Force to assess BAFMD [33, 85]. Prior to testing visits, subjects were instructed to fast and refrain from alcohol, caffeine and strenuous activity for 12 hours. Baseline ultrasound images were obtained after 20 minutes of supine rest with the subject's arm immobilized and slightly supinated. Images were examined using a 7.5-MHz linear array transducer (Acuson- Sequoia[®]). Images were recorded in longitudinal view, approximately 4 centimeters proximal to the olecranon process, in the anterior/medial plane. Image depth was 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. Doppler velocity profiles were collected simultaneously using a pulsed Doppler signal at an angle of approximately 60° to the vessel, with the flow-velocity cursor set mid-vessel. Following baseline ultrasound measurements, a pneumatic forearm cuff positioned approximately 1 cm distal to the olecranon process was inflated to 200 mmHg for five minutes. Images were obtained continuously from the final 30 seconds of cuff occlusion until 2 mins following release of the blood pressure cuff. Allowing a 5 mins recovery period, additional baseline imaging was performed for 1 min prior to the Cold Pressor Test (CPT). In brief, the opposing foot from the imaged arm was placed in an ice bath for 1 min, during which time

images were recorded continuously until 1 min following removal of the foot from ice water. All ultrasound images were recorded digitally and saved to discs for subsequent off-line analysis.

- **Assessing Physical Function**

Tests of physical function were performed at each study visit following vascular testing. Each participant performed 3 maximum-voluntary handgrip contractions (MVC) on the right and left arms using a handgrip dynamometer (Baseline[®]). The MVC was performed to assess isometric strength and to establish a training intensity for the intervention. Lastly, each participant underwent a 6-min walk test. This test was administered using a standardized protocol outlined by the American Thoracic Society [86]. Briefly, cones were placed 30m apart along a well-lit indoor hallway. Participants walked as far as possible in 6-min by walking back and forth around the cones. The distance covered was recorded to the nearest meter.

- **Vascular Analysis**

Brachial diameters were analyzed using a semi-automated edge-detecting software, Brachial Analyzer (Medical Imaging Applications, LLC; Coralville, IA). Arterial diameters were calculated as the mean distance between the anterior and posterior wall of the blood vessel interface with the image in diastole, defined as the peak of the r-wave on the electrocardiograph. Resting diameter was defined by the average of 30 seconds of data obtained following the 20 minutes of supine rest. Peak dilation was defined as the largest diameter following release of the occluding cuff. Finally, brachial artery flow mediated dilation (BAFMD) was defined as the percent change in vessel diameter from rest to peak response post cuff release.

Responses to CPT were analyzed similarly to BAFMD. A pre-CPT diameter was determined from the average of 30 sec of data prior to placing of the subject's foot in ice water. Diameters were analyzed during the 1-min of CPT and also for an additional minute following

the removal of the foot from ice water. The minimal diameter and CPT response were recorded as a percent change from the pre-CPT diameter. Collectively, the BAFMD and CPT responses were combined to establish a vascular physiological operating range identified as VOR (**Figure 5.1**).

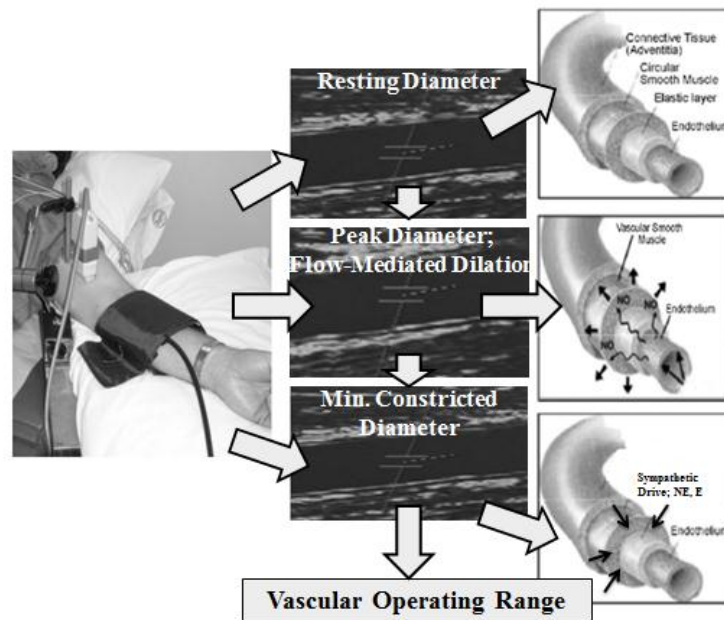


Figure 5.1: Depiction of the Vascular Operating Range (VOR). The vasoactive substance NO^- is believed to stimulate this brief rise in diameter, subsequently increasing blood to the forearm and reducing the shear stress upon the arterial wall. The minimal diameter achieved is recorded as the CPT response. This CPT response has been proposed to be a consequence of increased sympathetic nervous system activity, eliciting a vasoconstrictive response.

Blood velocity profiles were traced using Image Pro Plus 4.0 software (Media Cybernetics; Bethesda, MD). The antegrade component was defined as the area of tracing above 0 cm/s from the Doppler ultrasound scale and the retrograde component was defined as the area below. The velocity profiles were then divided by the ejection time (s) from the corresponding cardiac cycle to determine the mean velocity (cm/s). The mean velocity (V_{mean}) during baseline

was calculated as the difference between the antegrade and retrograde velocity components. Shear rate ($4 * V_{\text{mean}} \text{ (cm/s)} / \text{diameter (cm)}$) was measured at 10 second intervals during reactive hyperemia up to the time of maximum vessel diameter and plotted against time (s). A trapezoidal model was then used to calculate area under the curve (AUC) above baseline [6, 67].

- **Measurement of Plasma Nitrite Reserve and Antioxidant Capacity**

On each study visit, a total 20mL of blood was drawn from an antecubital vein of the arm opposing the ultrasound scan. One sample of 10mL was collected before inflation of the blood pressure cuff while the second sample was collected within 1 min following cuff release. Blood samples were divided into three 1.5 mL microcentrifuge capsules, pre-treated with 5 μL of Heparin (1000 U/mL). The samples were then centrifuged at 8,000-G for 2 minutes. The separated plasma was removed using sterile transfer pipettes and placed into additional capsules. The plasma capsules were snap frozen using liquid nitrogen and transferred to a -80°C freezer, awaiting future analysis. The reproducibility of this technique was tested among 8 participants outside of the present study. An independent T-test indicated no significant difference in resting plasma nitrite between tests performed one week apart (Test 1; nitrite= $126.95 \pm 37.67 \text{ nM}$; Test 2; nitrite= $120.62 \pm 44.17 \text{ nM}$, $p > 0.05$). The intra-class correlation coefficient between tests was 0.96.

The measurement of plasma nitrite reserve has been previously explored by Rassuf, et al. [80]. Pre- and Post-reactive hyperemia nitrite samples were defrosted, kept on ice and quantified by chemiluminescence within 30 mins following defrosting, using an Ionic-Sievers NO^- Analyzer (NOA-280; Sievers Instruments; Boulder, CO). Plasma samples were transferred in 10 μL increments to a reducing chamber consisting of reductant potassium iodide in acetic acid. This reductant has the potential to convert nitrite to NO^- but is insufficient to reduce any higher NO_x^- such as nitrate and thus is specific to nitrite. Plasma nitrite reserve was calculated as the

difference between post- and pre-reactive hyperemia nitrite levels. For measures of total antioxidants (mM/mg protein), samples were run in commercial assay kits from Cayman Chemicals.

- **Exercise Training**

Subjects trained for 20 min; 3 days per week for 4 weeks at their homes, under the supervision of a lab technician. Training involved gripping a hydraulic hand dynamometer (Baseline®; Irvington, NY) and contracting the forearm at a rate of 15 hand contraction*min⁻¹ (1 contraction every 4 sec) set to the pace of an electronic metronome. Both hands were trained at the same time with an intensity of 60% MVC. The intensity was marked on the gauge of the handgrip dynamometer using a dry-erase marker, and participants were positioned facing two small mirrors to allow for a visual reference of the gauge.

- **Statistical Analyses**

All statistical analyses were performed using SPSS for Windows (version 16.0). Descriptive statistics are presented as means \pm SD. Independent T-tests were performed to compare pre-training hemodynamic, vascular, and physical and biochemical measures between CHF and CON subjects. To determine the effects of the localized exercise and detraining on PWV and BAFMD, a 2 (Groups: CHF and CON) by 3 (Study Visits) ANOVA for repeated measures was performed. A second ANOVA for repeated measures was performed to detect changes in plasma nitrite levels pre- and post-reactive hyperemia and a 2 (Groups: CHF and CON) by 3 (Study Visits) ANOVA for repeated measures was performed to detect changes in plasma nitrite reserve following training and detraining, respectively. Differences between means were evaluated using a post-hoc LSD test. An alpha level ≤ 0.05 was required for statistical significance.

5.3 Results

- **Baseline Participant Characteristics**

Ten CHF (4 males and 6 females; average age 62 ± 8 yrs) and 10 CON subjects (4 males and 6 females; average age 55 ± 5 yrs) completed all facets of the study. Heart Failure patients were selected according to their NYHA classification, ejection fraction and etiology of CHF. Classifications for NYHA (groups I-IV) indicated 4 class II and 6 class III patients. Average ejection fraction (EF%) for the group was $30 \pm 4\%$, ranging from a low of 25 to a high of 40%. There was a wide range in the etiology of the CHF diagnoses. The majority of the patients were classified with ischemic-cardiomyopathic (N=6). Other diagnoses included right heart failure (N=1), hypertensive heart failure (N=2) and unknown cause (N=1). Patients were on various cardiovascular medications including nitrates (54%), beta-blockers (63%), ACE-inhibitors (18%), glycosides (27%) and other blood pressure lowering drugs (53%). It should be noted that there were no changes in the amount and or types of medications taken over the course of the study. Finally, CON subjects were free from overt chronic ailments and were on few medications (i.e. anti-inflammatory (N=2), multivitamins, statins (N=1) and blood pressure lowering medication (N=1).

- **Comparison of Pre-training Hemodynamic, Vascular, Biochemical and Functional Measures**

Table 5.1 summarizes the pre-training values of study participants. The results of independent T-tests revealed differences in resting HR (CHF= 71 ± 16 ; CON= 64 ± 7 bpm; $p=0.09$), BMI (CHF= 29.12 ± 3.1 ; CON= 24.74 ± 3.87 kg/m²; $p=0.05$), and aortic diastolic pressure (CHF= 72.50 ± 8.23 ; CON= 80.00 ± 8.52 mmHg; $p=0.08$). There were no significant differences in baseline brachial diameters between groups (CHF= 4.28 ± 0.9 mm vs. CON= 4.18 ± 0.74 mm). Baseline BAFMD values were significantly higher in the CON (CHF= $2.98 \pm 1.49\%$;

CON=6.21±1.21%; p=0.01). In addition, absolute BAFMD (CHF=0.12±0.05 vs. CON=0.25±0.07mm; p=0.05) was significantly different at baseline.

Table 5.1: Pre-training Values

	CHF		CON	
Physical Indices	Mean	±SD	Mean	±SD
Pulse Pressure (mmHg)	52.8	19.4	48.3	9.4
BMI (kg/m ²)	29.12	3.1	24.74	3.87
Combination MVC (R + L; kg)	57.8†	15.13	72.75	17.6
6-min Walk Distance (m)	342.41†	100.9	444.17	48.42
Vascular Indices				
Base Diameter (mm)	4.28	0.9	4.18	0.74
Peak Diameter (mm)	4.4	0.87	4.6	0.84
Minimal Diameter (mm)	3.91	0.84	4.12	0.75
VOR (%)	6.57†	1.19	8.69	1.48
BAFMD (%)	2.98†	1.49	6.21	1.21
CPT (%)	-3.38†	0.83	-2.46	0.62
Shear (AUC)	3658.45†	331.96	7002.37	2376.32
PWV Radial (m/s)	7.64	2.26	7.3	1.82
PWV Distal (m/s)	8.81	1.11	8.69	1.9
Nitrite Base (nM)	541.67†	462.74	133.84	201.97
Nitrite Post Hyperemia (nM)	566.51†	517.62	136.66	194.1
Nitrite Reserve (nM)	24.8	124.6	2.82	18.167
Total Antioxidants (mM/mg)	24.81†	6.22	31.29	7.98

† Denotes significant difference between groups (p≤0.05).

The change in heart rate during the CPT was significantly greater in CON (CHF=6±10bpm vs. 17±10bpm; p=0.03). The average percent change in the brachial diameter during the CPT was significantly greater for CHF patients (CHF=-3.38±0.83%; CON=-2.46±0.62%; p=0.05). There was also a significant greater change in the absolute diameter during the CPT (mm) between groups (CHF=0.21± 0.17; CON=0.1± 0.03mm; p=0.05). There also was a significant difference in the baseline VOR (%) between groups (CHF=6.57±1.19%; CON=8.69±1.48%; p=0.05). No significant differences were reported for PWV.

Plasma nitrite levels were significantly higher in the CHF patients (CHF=541.67±462.75 vs. CON=133.84±201.97nM, p=0.05). These differences were also noted after 5 minutes of occlusion (CHF=566.51±517.62 vs. 136.66±194.10nM). However, no differences were noted for

plasma nitrite reserve (CHF=24.84±124.66 vs. CON=2.82±18.17nM). Total Antioxidant levels were significantly higher in CON subjects (CHF = 24.81±6.22; CON = 31.29±7.9 mM/mg protein; p=0.05). In regards to the functional measures, CHF patients produced significantly less force on the handgrip test (CHF = 57.80±15.13; CON = 72.75±17.6kg; p=0.05). In addition, the CHF patients walked less on the 6 minute walk test (CHF =342.41±100.90; CON = 444.17±48.42m; p=0.007).

- **Effects of Handgrip Training and Detraining on Vascular and Physical Function**

Values for vascular indices and physical function across study visits are presented in **Table 5.2**. Results from the repeated measures ANOVA indicated no significant differences in resting brachial artery diameter, across the study visits. However, handgrip training resulted in significant improvements in BAFMD for both groups (CHF=30.76±58.29%; CON; 35.53±29.18%; p=0.01). Following training cessation BAFMD returned to pre training values in both groups (CHF= 2.9±1.08% [~19% reduction]; CON=7.28±4.08 [~16% reduction). Average values for BAFMD across study visits are depicted in **Figure 5.2**; individual BAFMD changes are depicted in **Figure 5.3**.

No changes in the CPT response was noted following handgrip training or detraining (p=0.75). The repeated measures ANOVA revealed a significant main effect for changes in the absolute VOR (mm) across study visits (p=0.05). Changes in the absolute VOR (mm) following training and detraining are depicted in **Figure 5.4**. Handgrip training resulted in a significant and similar increase in the combination MVC (~5.2%) for both CHF and CON groups, while detraining restored pre-training values for CHF patients. There was significant difference reported in the six minute walk test distance. Finally, no significant differences were reported in the types or amounts of daily activity engaged in per day by study participants.

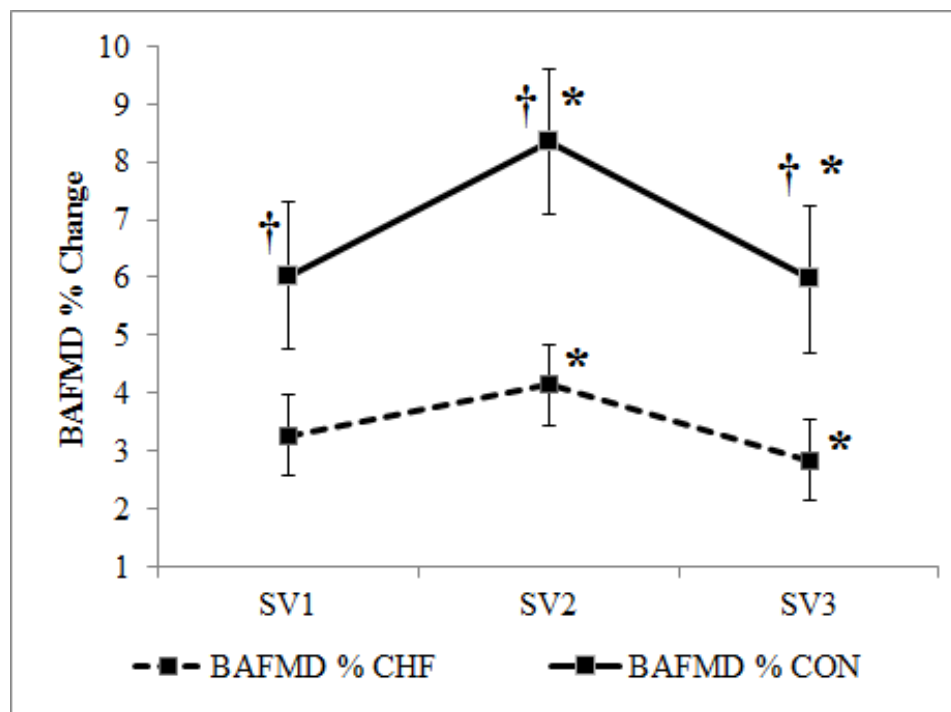


Figure 5.2: Depiction of the mean change in BAFMD at baseline (SV1), following 4 weeks of exercise (SV2) and following 4 weeks of detraining (SV3). Data are presented as mean \pm SE. (*Significant vs. previous study visit; †Significant vs. group ($p \leq 0.05$)).

Table 5.2: Effects of Handgrip Training on Vascular and Physical Function

CHF				CON		
Vascular Function	SV1	SV2	SV3	SV1	SV2	SV3
Base Diameter (mm)	4.28±0.9	4.21±0.89	4.55±0.68	4.18±0.74	4.16±0.65	3.94±0.72
BAFMD (%)	2.98±1.49†	3.75±1.56†*	2.9±1.08†*	6.21±1.21	8.02±1.75*	5.97±1.54*
CPT (%)	-3.38±0.83†	-3.12±1.88	-2.76±1.72	-2.46±0.62	-2.58±0.85	-2.03±0.8
VOR (%)	6.57±1.19†	7.34±1.42†*	5.56±1.8†*	8.69±1.48	10.86±1.71*	7.89±1.86*
Shear (AUC)	3658.45±332†	4927.84±2317.5*	3329.7±801.6†*	7002.37±2376.32	7274.9±1097.5*	8742.15±3669.61*
PWV Radial (m/s)	7.64±2.26	8.53±0.87	8.61±2.02	7.3±1.82	7.78±2.23	7.73±1.93
Nitrite Base (nM)	541.67±462.74†	362.36±315.83	394.59±349.20	133.84±201.97	320.81±363.34	253.90±250.03
Nitrite Post Hyp.(nM)	566.51±517.61†	375.91±376.94	398.08±360.92	136.66±194.10	314.41±364.0	285.88±286.19
Nitrite Reserve (nM)	24.84±124.66	13.56±278.82	3.50±85.5791	2.82±18.17	-6.39±27.82	31.9863±40.81
Total Antioxidants (mM/mg)	24.81±6.22†	22.95±4.027†	19.55±7.17†	31.29±7.98	30.01±17.13	43.14±10.52
Physical Function						
Combination MVC (kg)	56.1±16.29†	58.67±19.31*	56.28±18.19	65.96±13.14	69.8±12.98*	64.79±11.19*
6-min Walk (m)	342.41±100.9†	412±109.3†*	383.13±109.5	444.17±48.42	446.3±46.4	433.3±119.04

*Significantly Different from Previous Study Visit ($p \leq 0.05$)†Significant Group differences ($p \leq 0.05$)

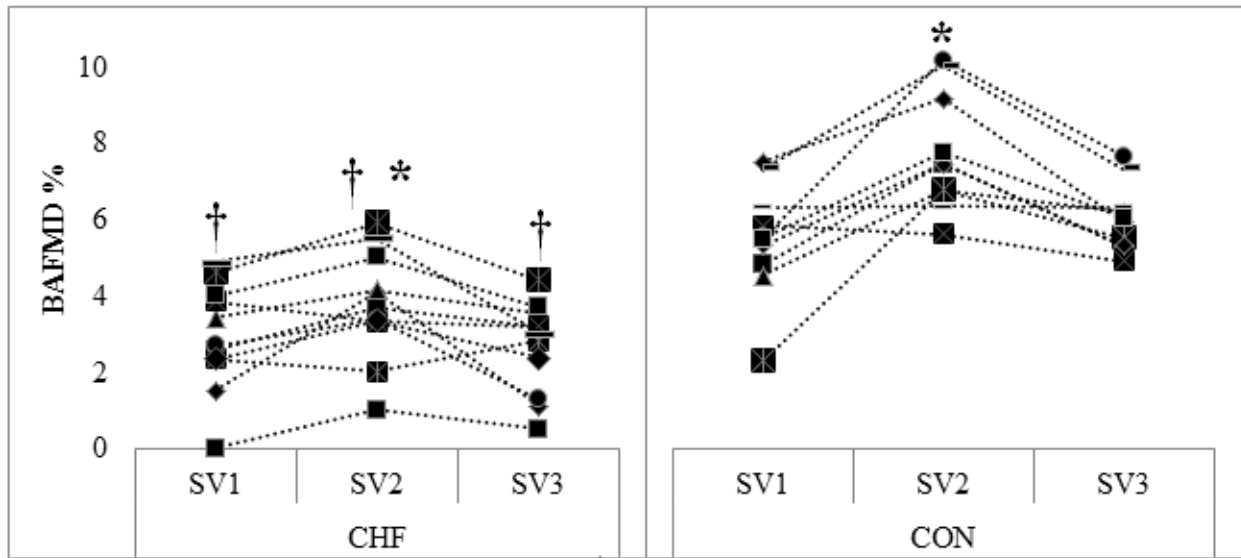


Figure 5.3: Depiction of the individual BAFMD changes in CHF and CON subjects from baseline (SV1), following 4 weeks of handgrip exercise training (SV2) and following 4 weeks of detraining (SV3). († Indicates a significant group difference; * Indicates a significant difference from SV1).

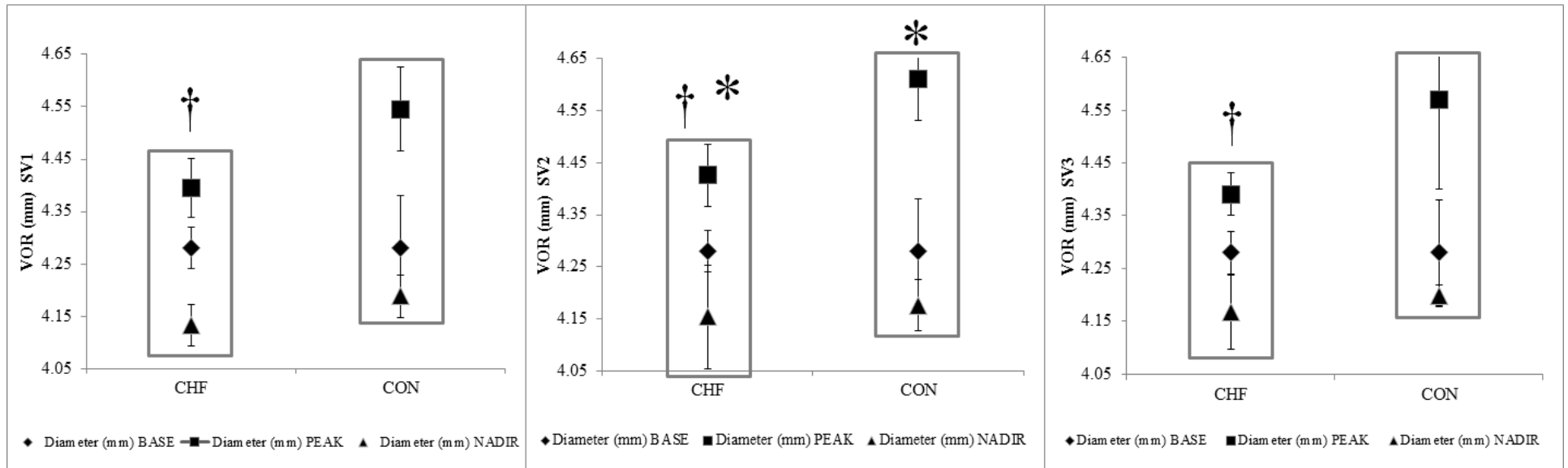


Figure 5.4: Depiction of the absolute Vascular Operating Range (VOR; mm). The VOR (mm) depicts the combined absolute BAFMD and CPT responses (mm). Average absolute VOR for CHF and CON subjects are depicted at baseline (SV1), following 4 weeks of handgrip exercise training (SV2) and following 4 weeks of detraining (SV3). Average absolute BAFMD and CPT range values are depicted by the solid squares (PEAK) and triangles (NADIR), respectively. Average BASE diameter is controlled for at 4.28 ± 1.0 mm. († Indicates a significant group difference in absolute VOR; * Indicates a significant difference in absolute VOR from SV1).

- **Effects of Handgrip Training and Detraining on Plasma Nitrite Reserve and Total Antioxidants and Their Relationship to Vascular Function**

For plasma nitrite levels and nitrite reserve, no significant differences were reported across the study visits. In addition, there was no significant effect of training or detraining on total antioxidant capacity. The results from the ANOVA for repeated measures indicated no significant relationship between changes in VOR and the plasma nitrite reserve in response to handgrip training and detraining, respectively. However, when all observations for total antioxidants were compared against BAFMD, a significant relationship was found ($r^2=0.47$; $p=0.01$) (**Figure 5.5**).

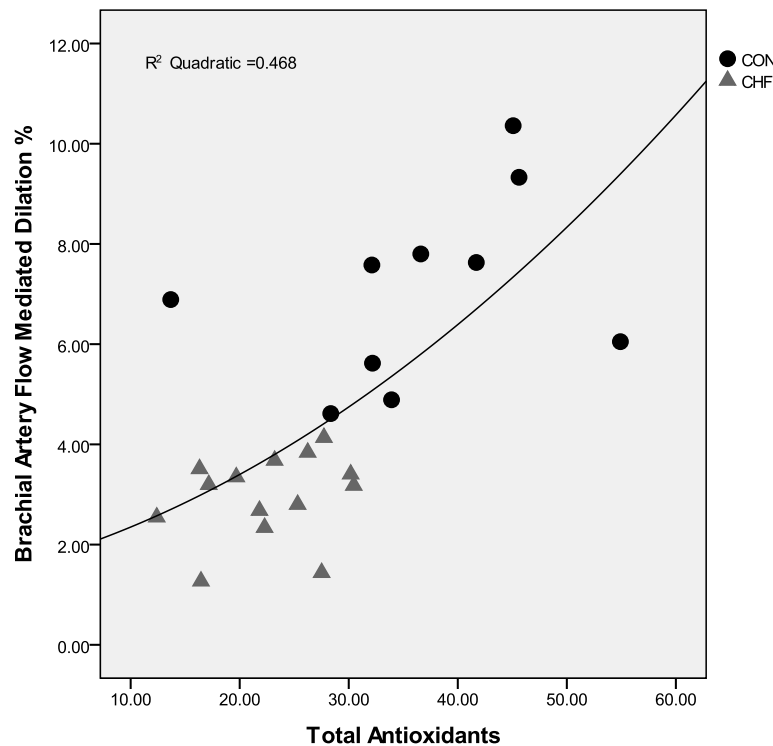


Figure 5.5: The figure shows the relationship between pre occlusion anti-oxidant status for visit 1-3 and brachial artery flow mediated dilation. There is a significant difference in total anti-oxidant concentration between the control and heart failure groups, with the heart failure patients having significant lower concentrations.

5.4 Discussion

The results of the present study confirm that 4 weeks of handgrip exercise training significantly increases handgrip strength, and improves BAFMD and VOR in both CHF and CON subjects. Removal of the training stimulus returned strength measures and vascular function towards pre-training levels. No changes were noted for regional vascular stiffness, nitrite, or nitrite reserve across the study visits. Consequently the present findings do not support that regional specific training changes vascular function secondary to changes in nitrite or nitrite reserve.

- **Comparison of Pre-training Hemodynamic, Vascular, Biochemical and Functional Measures**

The results of the present study indicate significant differences in resting HR and aortic diastolic pressure between CHF and CON subjects, but not for measures of vascular stiffness (PWV). The lower aortic diastolic blood pressure and lack of differences in vascular stiffness could be due to the efficient management of the CHF subjects. Cardiovascular risk factors and complications may be reduced in this population by optimal pharmacotherapy, potentially masking physiological differences [3, 87]. The current results indicate that these patients appear to be well managed in regards to their disease state. There was also a significant difference in the HR change during the CPT between groups, with a blunted response in the CHF patients. The CPT is utilized as a non-invasive means of eliciting and studying a sympathetically mediated pressor reflex [88]. Importantly, the significant rise in HR during CPT observed in the CON subjects indicates that the desired pressor reflex was achieved [88-89]. For example, Hassellund et al. [88] have recently reported an approximate 10 unit rise in HR in response to a 1-minute hand CPT in healthy men, compared to the approximate 17 unit rise in HR in the CON subjects of the present study. The blunted HR response to CPT seen in the CHF patients has been

previously explored [89]. Grassi et al. [89] indicate an approximate 7 unit rise in CHF patients, compared to the approximate 6 unit rise in patients of the present study. Furthermore, Grassi et al. [89] point out that basal muscle sympathetic nerve activity (MSNA) (i.e. peroneal nerve) and plasma norepinephrine concentrations are significantly higher with increasing severity of CHF. Although not entirely understood, Grassi et al. [89] conclude that the higher MSNA, albeit lower HR responses, seen with increasing severity of CHF may be due to a loss of the sympathetic reflex sympathetic. This loss of sympathetic reflex seen in the CHF patients may also be the result of a down regulation in β -adrenergic receptor (i.e. β -1 adrenergic receptor) number due to heightened basal sympathetic nervous system activity [90]. However, this concept warrants further investigation. An alternative explanation is that certain medications may have influenced HR responses to the CPT, as 63% of patients were on Beta-blockers.

Contrary to the blunted HR response, the CHF patients in the present study exhibited a greater vasoconstriction response for pre-training CPT. Previous reports have indicated that CHF patients exhibit a blunted vasoconstriction response to the CPT compared to healthy controls, albeit in a different conduit artery [91]. The fact that the vasoconstriction response to CPT is higher in the CHF patients of the present study is unique. The rationale for the elevated vasoconstriction response to CPT observed in the present study, is not fully understood, but may again stem from the potential influence of certain medications taken. For example, Noll et al. [92] have reported that medications such as nitrates and other blood pressure lowering drugs (i.e. captopril and lisinopril) have the ability to augment the pressor reflex while normalizing blood pressure. Considering this finding, approximately 75% of the present CHF population was using some form of the aforementioned drugs. Finally, in consideration of the Vascular Operating Range, the vasodilatory effects of these drugs, in theory, may be pushing the resting

diameter of a CHF patient's artery higher within their VOR, allowing for the potential for greater vasoconstriction and less vasodilation. Once again, this theory is visualized in **Figure 5.4**, where the resting diameter of the CHF patient's artery is consistently lower within their VOR compared to CON subjects.

The present study also reports a blunted vasodilatory response in the CHF patients compared to CON subjects, which is in agreement with previously published reports [79, 93-95]. There are several factors which could contribute to the observed vascular dysfunction in this population including reduced endothelial synthesis and release of relaxing factors (i.e. NO⁻, prostaglandins and EDHF), an increase in circulating vasoconstrictor agonists (i.e. ANG-II, vasopressin and endothelin) and increased oxidative stress [93]. Also, impaired resistance vessel function may alter the blood flow and shear response through the vessel, thus reducing the stimulus for flow-mediated vasodilation [93, 96]. Alternative explanations that may contribute to the differences observed in BAFMD of CHF patients may include microcirculatory changes (e.g. reduced capillaries, or pre-sphincter responsiveness to metabolic dilators) [97], a change in fiber type (preferential atrophy of type-I fibers compared to type II-b) [98] or muscle mass (atrophy due to chronically high levels of cortisol, catecholamines etc.) [99], consequent to their disease state.

Uniquely, the present study identifies significant differences in plasma nitrite levels between CHF and CON subjects, with an approximate 5-fold higher pre- and post-reactive hyperemic nitrite value. In contrast, no significant differences in plasma nitrite reserve were noted. Average plasma nitrite levels seen in the CON subjects appear to be in agreement with the values reported by Rassaf et al. [80]. In that study, nitrite levels were examined among healthy young individuals (average age=25 yrs) and those with cardiovascular disease risk factors

(average age=62 yrs). Resting plasma nitrite levels were significantly higher in the risk factor group (approximately 115nM vs. 60nM in controls). The nitrite levels for the CON group in the present study are similar to those reported for the risk factor group in the study by Rassaf et al. [78]. Although the CON subjects in the present study were free of overt disease, there may have been some hidden factors that could influence plasma nitrite levels.

The higher levels of nitrite observed in the CHF patients is a unique finding, and is, in fact, the first report to examine plasma nitrites in this population. An explanation for the higher nitrite levels seen in the CHF population may be the effect of certain exogenous variables, beyond endothelial derived NO⁻ synthase (e-NOS) influencing the total nitrite pool. For example, Rassaf et al. [80] indicate that approximately 70% of circulating plasma nitrite is derived from NO⁻ synthases (NOS), with the remainder ingested in the form of nitrite and nitrate containing foods (i.e. processed meats, preservatives and nitrate containing foods). In the present study a 3-day food recall to determine how much and what types of foods were consumed, did not reveal changes across the study visits, suggesting diets remained similar for the participants. A more likely explanation for the higher nitrite levels concerns the effects of certain medications. For example, nitrates are known to affect the total nitrite pool and 50% of the CHF patients were taking a form of nitrate.

While the present study did not control for medications, there were no changes in the amount or types of medications taken by the participants over the course of the study. Finally, other sources of NO⁻ could influence the total nitrite pool. There are three known iso-forms of human NOS; e-NOS, neuronal (n-NOS) and inducible (i-NOS) which catalyze NO⁻ production from L-Arginine [100] . As previously discussed, e-NOS may be activated by biomechanical stresses on the vascular wall. Neuronal-NOS have been shown to be present throughout the

central and peripheral nervous system, synthesizing NO⁻ in response to glutamate; however, i-NOS may be activated by cytokines in macrophages and hepatocytes [100]. Chronic heart failure patients have elevated circulating levels of inflammatory cytokines [101-103]. Therefore, considering the previously discussed reports on NOS, it could be argued that the circulating nitrite pool may include varying sources of NO⁻ production, not limited to the e-NOS isoform.

Another unique finding in the present study is a significant correlation between antioxidant status and BAFMD and VOR. Importantly, the present data report a significant lower total antioxidant capacity in the CHF patients. These data are in agreement with Demirbag et al. [104] who indicate a significant correlation between the potency of oxidative stress and the severity of cardiomyopathy. The fact that measures of vascular reactivity are directly related to total antioxidant capacity may indicate that patients with CVD exhibit an imbalance between pro- and antioxidants, favoring greater oxidative stress [104] which may contribute to lower vascular reactivity [105].

Finally, there are differences in measures of physical function reported when comparing CHF patients to controls. The lower strength (MVC) and endurance (six minute walk) measures seen in the CHF patients is a hallmark reflection of their physiological decline. Patients with CHF experience a number of physiological compensations including increased muscle atrophy [99], reduced left-ventricle function [24], neuro-humoral changes [78] and endothelial dysfunction [4, 78], all contributing to exercise intolerance and reduced functionality [78]. These changes in the body develop chronically in an effort to sustain cardiac output in the failing heart.

- **Effects of Handgrip Training and Detraining on Vascular and Physical Function**

This study confirms a significant increase in BAFMD in both the CHF and CON groups. The changes in BAFMD following handgrip training and detraining, seen in the CHF subjects of

the present study are in agreement with previously published reports [79, 96]. Hornig et al. [79] have reported that handgrip exercise training can restore normal artery function in CHF patients; however gains are quickly lost following a period of detraining. It should be noted that, Hornig et al. [79] did report on the radial artery. This artery typically has a smaller diameter, often yielding a larger vasodilatory response compared to larger conduits. Therefore, the improvement in FMD with training may be less. In fact, Hornig et al. [79] report an approximate 13% increase in FMD, compared to the 30% improvement observed in brachial artery of the present CHF population.

The present study also reports a significant increase in the shear stimulus (AUC) following training in both groups. The shear stimulus is considered an important trigger for vasodilation in response to local increases in blood flow [11, 67]. Therefore, a potential mechanism responsible for the improvement in BAFMD observed in the CHF patients is a greater shear stimulus (Shear AUC) following forearm ischemia in response to the handgrip training. The localized endurance training may have enhanced blood flow capacity through the forearm, possibly through increased capillary density and pre-capillary sphincter sensitivity to metabolites [97, 106]. Therefore, in response to local ischemia, a greater vasodilation in resistance vessels and the microcirculation would reduce vascular resistance in the forearm, subsequently leading to a greater increase in blood flow and shear following cuff release.

The changes in BAFMD following handgrip training in the CON subjects of the present study are in agreement with previously published reports from the current laboratory [5-6, 8-9]. Four weeks of handgrip exercise training typically results in a significant improvement with an approximate 40% increase in BAFMD following training in healthy young [5, 8-9] and elderly populations [6]. The responsible mechanisms are thought to include an increase in the shear stimulus during reactive hyperemia and an up-regulation of various endothelial derived relaxing

factors, most notably NO^- [21], in response to the chronic exposure to increases in shear stress during rhythmic muscle contractions [39]. The present study attempted to examine whether NO^- played a significant role in the vascular modification. However, the present data does not support this hypothesis. Alternative pathways that may have contributed to the changes in BAFMD will be discussed in the section entitled: *Effects of Handgrip Training and Detraining on Plasma Nitrite Reserve and Total Antioxidants and Their Relationship to Vascular Function*.

Uniquely, the present study presents the Vascular Operating Range (VOR). The VOR represents a physiological range in which a vessel responds to both vasodilatory and constrictor agonists (i.e. shear stress, Ach, NE, etc.). The present study reports that handgrip training and detraining has the ability to significantly increase and decrease the VOR, respectively. The theory of the VOR is depicted in **Figure 5.4**. This figure presents the vessel's range for absolute BAFMD and CPT responses about a resting diameter. Considering this concept, the CHF patient's resting vessel diameter is set higher within their VOR, favoring greater vasoconstriction and less vasodilation. However, the CON subject's vessel is set lower within their VOR, favoring greater vasodilation and less constriction. **Figure 5.5** illustrates the vessel's responsiveness within the respective VOR for the CHF and CON subjects. An explanation for a higher set resting diameter in CHF patients is that they exhibit less 'basal' vessel tone, which may be a consequence of vasodilatory medications (**Figure 5.5**).

In certain physiological cases the VOR may favor greater dilation in endurance trained muscle [107-108]. Additionally, the vessels ability to vasoconstrict may be accentuated in non-trained or inactive vascular beds [108-109]. For example Lash et al. [109] have reported in rats that 8 weeks of endurance exercise training enhances alpha-adrenergic vasoconstriction of larger

feed arteries supplying the spinotrapezius muscle, which is largely inactive during standard treadmill running.

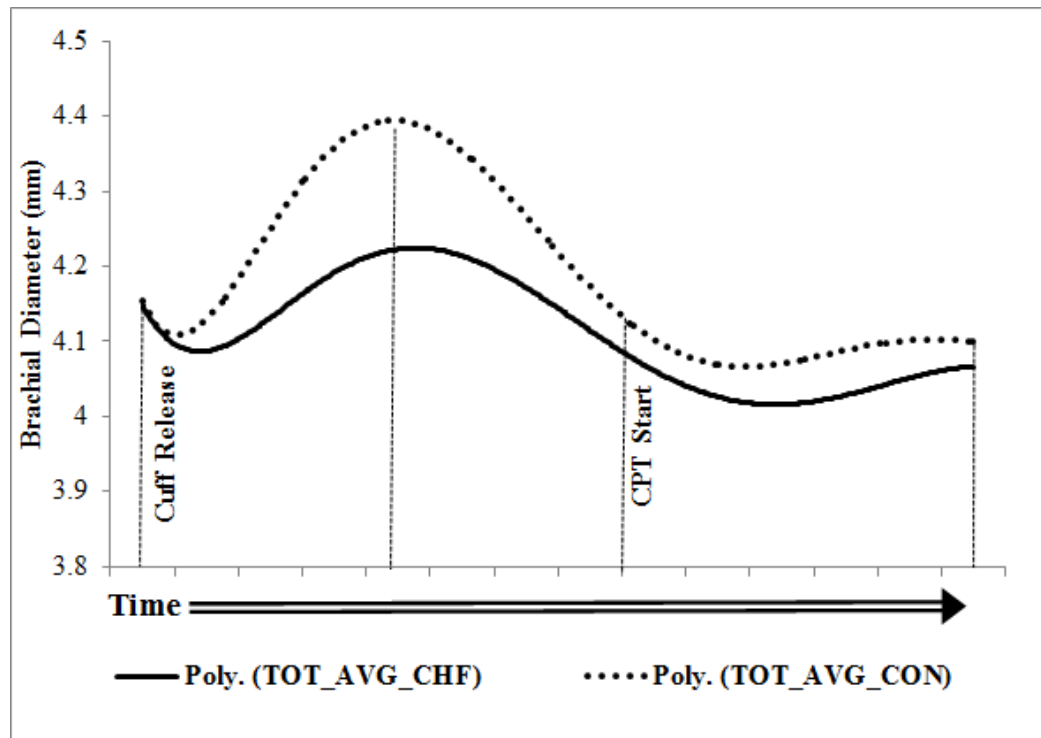


Figure 5.5: Depicts a representation of the Vascular Operating Range (VOR) in both CHF patients and CON subjects. The resting diameter in patients (CHF) may be higher within their VOR, favoring a greater vasoconstrictive responsiveness (Polynomial Function controlled for an average resting diameter).

The ability of an artery to vasoconstrict is important in regards to blood flow distribution during exercise. At the onset of exercise, sympathetic nervous system activity increases in order to accelerate heart, perfusion pressure and cardiac output [110]. In addition, increased adrenergic activity increases vasoconstriction of peripheral arteries and veins [111]. However, local factors in the exercising tissue may override sympathetic nervous system activity through increased vasodilation (i.e. NO^- , adenosine, H^+). This phenomenon has been termed functional

sympatholysis and contributes to blood flow redistribution to contracting skeletal muscle during exercise [111-112]. The fact that the artery's vasoconstrictive response may be reduced raises the question of whether this vasoconstrictive reflex can be increased in non-active tissue (i.e. viscera and non-trained skeletal muscle) following training in order to improve blood flow redistribution during activity.

Finally, the fact that normal vascular function can be restored in CHF patients, but detraining reverses these gains, has significant clinical relevance and reinforces the importance of engaging in habitual physical activity [40]. The localized handgrip training also increased handgrip strength (MVC) for both CHF and CON groups, which is in agreement with previous studies in CHF patients [79], elderly [6] and younger populations [5, 8]. Therefore, regional vascular and muscle function appear to be modifiable in a variety of populations. No significant differences were reported for the six minute walk test in response to training. This is understandable and reinforces the principle of 'specificity of training'; that one should perform moderate intensity, rhythmic contractions to upper and lower body muscle groups. This would provide a 'well-rounded' muscle and vascular stimulus thus enhancing strength and systemic vascular function. In theory, this enhancement would allow for greater strength and blood flow capacity through the trained skeletal muscle, subsequently increasing exercise capacity and tolerance.

- **Effects of Handgrip Training and Detraining on Plasma Nitrite Reserve and Total Antioxidants and Their Relationship to Vascular Function**

The present study indicates no significant training effect on the plasma nitrite reserve. Previous studies have reported significant increases in total plasma nitrite and nitrate levels following prolonged endurance training, but there is very little evidence to support a training effect on plasma nitrite reserve. For example, Zaros et al. [113] indicate that 6 months of

endurance exercise results in a significant increase in total plasma nitrite and nitrate levels in hypertensive, post-menopausal women. Furthermore, Allen et al. [12] have established a significant relationship between plasma nitrite, disease state and fitness status. In that study, plasma nitrite reserve (measured as the nitrite difference from peak exercise and resting values) was directly related to the severity of cardiovascular disease and functional capacity. However, the present study was the first to examine changes in the plasma nitrite reserve method as described by Rassaf et al. [80] in response to localized training. Additionally, it could be argued that a localized exercise stimulus applied to a small muscle mass may not provide a significant enough stimulus to detect changes in the circulating biomarker.

Plasma nitrite reserve has been proposed to reflect NO⁻ mediated endothelial function in humans [12, 80, 114]. However, the present study indicates no significant relationship between plasma nitrite reserve and vascular function in both CHF patients and CON subjects. Rassaf et al. [80] reported significant relationships between plasma nitrite reserve and BAFMD in healthy young subjects, but not in patients with cardiovascular disease risk factors. The present study examines a similar patient population, wherein the CON subjects of the present study were in the same age range, possibly indicating some age-dependent effect on nitrite levels. Furthermore, the patient population of the current study presents more severe cardiovascular disease and endothelial dysfunction. Therefore, it remains difficult to generate an accurate interpretation of the nitrite responses in the CHF patients due to the lack of evidence and comparative data in the literature. If in fact plasma nitrite reserve reflects endothelial function, then one could argue that any localized increases in NO⁻ (i.e. in response to an increase in shear stress) would be detected by the measure. However, given that there are a number of exogenous variables which can affect plasma nitrite (i.e. diet and medications) this measure may contain excess background nitrite in

certain populations. Therefore, future investigations should continue to investigate and control exogenous variables when studying the relation of plasma nitrite to endothelial function.

Nevertheless, it can be hypothesized that changes in vascular function following exercise training in CHF may not be entirely NO^- dependent. Hornig et al. [79] have reported that following administration of N-monomethyl-L-arginine (L-NMMA), an e-NOS inhibitor, radial FMD was significantly reduced but not completely impaired. This finding supports the argument for additional mechanisms involved in the vasodilatory response. A review by Laughlin [39] highlights alternative pathways involved in endothelial-dependent vasodilation. In response to a mechanical stimulus (i.e. increased shear stress and circumferential wall stress) the vasodilatory response is not entirely NO^- dependent, but rather involves additional signaling pathways including prostacyclin and endothelium hyperpolarizing factor [11, 39]. These factors also appear to be influenced by an individual's health and training status [39, 107, 115]. This important concept eludes to the potential for additional mechanisms involved in the vascular adaptive response which may not be reflected by changes NO^- and subsequently plasma nitrite reserve [115].

The overall contribution of NO^- to the vasodilatory response in CHF still remains unclear. While NO^- has been reported to stimulate smooth muscle relaxation [116], the dedicated path of endothelial derived NO^- may not solely suit vasodilation. For example, high concentrations of pro-oxidants (i.e. superoxide) in blood may consume viable NO^- thus producing additional radicals (i.e. peroxynitrite) [84]. The body defends oxidative damage through increases in antioxidants (i.e. superoxide dismutase). Interestingly, the present study indicates that total antioxidant capacity is higher among CON subjects, compared to CHF patients. In addition, there is a significant relationship when examining BAFMD and total antioxidants amongst the CHF

and CON subjects (**Figure 5.5**). Therefore, examining the balance between pro- and antioxidants in relation to plasma nitrite reserve may provide insight into additional mechanisms involved in the vascular adaptive response to exercise.

- **Clinical Relevance**

The improvement in BAFMD observed in the CHF subjects is an important clinical finding in regard to the functional outcomes of this population. For instance, a symptom often experienced by CHF patients is severe intolerance to exercise and physical activity [117-118]. This intolerance has largely been considered to be the result of poor vasodilatory behavior within the exercising skeletal muscle [118]. Normal vascular function can be restored in CHF patients, but detraining reverses these gains, reinforcing the importance of engaging in habitual physical activity [40]. Performing moderate intensity, rhythmic contractions to upper and lower body muscle groups may provide a ‘well-rounded’ vascular stimulus thus enhancing systemic vascular function. In theory, this enhancement would allow for greater blood flow capacity through the trained skeletal muscle and subsequently increase exercise tolerance.

- **Study Limitations**

This study presents several unique findings, yet, we remain cautious in our interpretations due to potential limitations inherent of clinical trials. One particular limitation remains at the etiology of disease and its progression. While a majority of the diagnosed CHF observed in the present study is of ischemic-cardiomyopathic origin; there were additional subjects classified by hypertensive and other idiopathic-induced heart failure. These characteristics often make defining a true CHF population challenging for the researcher. In addition, there will always be an influence of certain medications on physiological outcomes. In the present study, the CHF populations as well as some CON subjects were on a variety of medications. Once again, it

remains challenging to control for exogenous variables which may influence physiological outcomes. The nitrite values of CHF patients reported were approximately 5-fold higher than CON subjects. In this regard, it has been previously reported that approximately 70% of circulating plasma nitrite may be induced by endothelial-derived NO⁻ synthase (e-NOS) activity, while the remainder of that being ingested in the form of nitrite- and nitrate-containing products (i.e. processed foods and medications) [80]. In conclusion, there is potential for exogenous factors to affect the total nitrite pool *in vivo*.

- **Future Direction**

This study is the first to report on plasma nitrite levels in CHF. In addition, this study is the first to examine the effects of localized exercise training on plasma nitrite reserve. Future studies should continue examining the effects of extended training periods; varying volumes; and total body training programs as an intervention on changes in plasma nitrite reserve. Finally, we should continue to investigate the controllers involved in the exercise-induced vascular adaptive response in patients with known disease.

5.5 Conclusion

The results of the present study confirm that 4 weeks of handgrip exercise training significantly improves BAFMD and VOR in both CHF and CON subjects. However, removal of training stimulus returns BAFMD to pre-training levels. Additionally, the present study uniquely reports vascular reactivity as defined by a physiological operating range (VOR) which appears to be modified by training and detraining, respectively. No changes were noted for regional vascular stiffness, nitrite, or nitrite reserve across the study visits. Consequently the present findings do not support that regional specific training changes vascular function secondary to changes in nitrite or nitrite reserve.

Chapter 6

Conclusion

The purpose of this dissertation was to explore the relationship between vascular and physical function and to determine the modifying role of physical activity, inactivity and exercise on the biomechanical and biochemical controllers of this relationship. It has been speculated by Lakatta et al. [4] from the Baltimore Longitudinal Study on Aging that dysfunction within the peripheral vasculature is a major share-holder in the development of CVD. The studies in this document conclude with data to support that physical activity and exercise have the ability to rapidly modify peripheral vascular function. However, removal of the training stimulus or physical inactivity reverses these gains and promotes vascular dysfunction. The modifying effects of physical activity and inactivity on vascular function have become a focus in the field of exercise vascular biology. The current document explores 3 key issues which have emerged in the field including the pattern of blood flow, the effects of training with vascular occlusion, and the effects of training in individuals with cardiovascular disease.

The first key issue addressed was the relationship between the blood flow pattern and physical functionality in the elderly. Chapter 2 presents a new finding in that chronic alterations in the pattern of blood flow through an artery, as seen with aging, is associated with one's physical function. Furthermore, Chapter 3 concludes that daily physical activity promotes vascular health through enhanced vasoreactivity, a more favorable blood flow and shear pattern and improved physical function. Participating in daily physical activity throughout life appears to promote higher vascular and physical function.

The second key issue addressed includes an exercise training methodology which involves deliberate vascular occlusion of the trained limbs. This occlusion or kaatsu training has

been reported to significantly increase muscle size and strength over non-occluded limbs [17]. However, it was unclear of the effects of occlusion training on vascular function. Chapter 4 concludes that performing exercise training with vascular occlusion diminishes the positive effects on the vasculature, secondary to enhanced muscular strength. This is an intriguing finding in that muscular and vascular gains are dissociated in response to the occlusion training. The controllers of this response are unclear, but appear to include a reduction in or alteration of the shear stimulus, or an increase in oxidative stress in response to the localized ischemia [71].

The third and final key issue addressed involves the localized exercise training effects on vascular function in patients with chronic heart failure. Localized handgrip training has been shown to improve vascular function in CHF patients [79]. However the potential controllers of this response were unclear. Chapter 5 reports that the brachial arteries of CHF patients express reduced vasodilatory behavior but modestly greater vasoconstrictive responsiveness compared to control subjects. Furthermore, it appears that the vascular physiological operating range, or VOR, can be modified by localized handgrip endurance exercise training to promote greater vasodilation in both the CHF and control groups. Chapter 5 concludes that short term localized training does indeed restore regional vascular control, but that this adaptation may not be NO⁻ dependent. The changes in vascular function did not reflect changes in plasma nitrite reserve, suggesting the potential for other mechanisms involved in the vascular adaptive response. Finally, removal of the training stimulus returned vascular measures to pre-training levels.

Together these studies present novel findings and methodology in the field of vascular biology. Maintaining a high degree of physical activity throughout life preserves higher vascular and physical function (**Figure 6.1**). Furthermore, this dissertation reinforces the notion that physical inactivity reduces vascular health and physical function. In closing, these projects

expand upon the current literature regarding the positive effects of exercise and the negative consequences associated with physical inactivity on overall health and functionality. It is our duty as Kinesiologists, exercise researchers and clinicians to continue to promote the notion that physical inactivity KILLS and physical activity SAVES lives through enhanced vascular health and physical functionality.

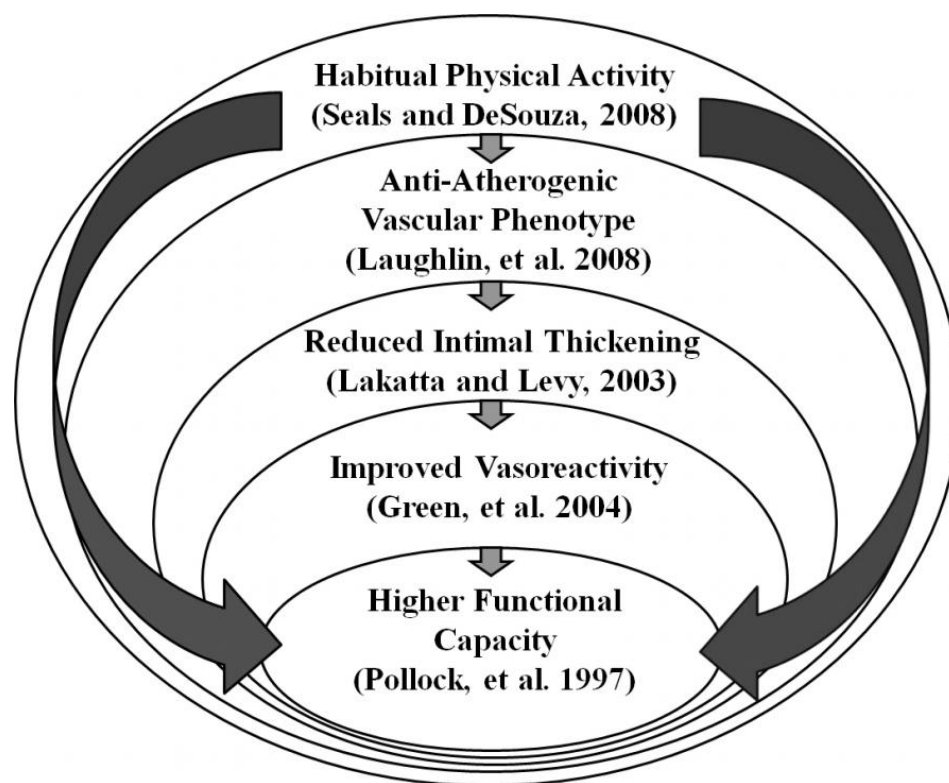


Figure 6.1: Depiction of a rationale for the effects of habitual physical activity and exercise training on vascular and physical function. Physical activity promotes vascular health defined by reduced intimal thickening and increased vasoreactivity. A healthy vascular phenotype promotes a higher functional capacity and improved quality of life.

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Appendix A: Review of Literature

Vascular Control of Blood Flow: Interplay between Vasoactive Properties

A.1 Introduction

According to the American Heart Association's 2010 update, the number one cause of disability and mortality in the United States is owed toward cardiovascular disease (CVD), in particular coronary artery disease (CAD) [3], and it is estimated that 81 million American adults have one or more types of the disease. Additionally, it is estimated that CVD will contribute to ~34 % of all deaths in the United States by the end of the year [3], with a health care cost estimate of ~\$500 billion. The significance of these statistics relates to the health outcomes of these individuals. Most treatment strategies for cardiovascular disease management are pharmacological and a popular medication prescribed to patients is in the form of vasodilators [3]. However, as exercise specialists, researchers and clinicians, we must identify and treat the root of the problem which is not only an inability to vasodilate, but inadequate redistribution of blood flow, which involves an intriguing interplay between peripheral vasoconstriction and local vasodilation.

As we know, the cardiovascular system is an intricate system which serves as a transport for oxygen and nutrients to various tissue, as well as assisting in the removal of metabolic waste [119]. Additionally, the vasculature transports and produces hormones and also assists in immune function by transporting cells to defend the body [119]. The vasculature functions as a closed circuit along a pressure gradient, and is divided among specific compartments serving the purpose of supplying adequate blood flow to metabolically active tissue. During exercise, the

vasculature is challenged to quickly redistribute blood flow towards contracting skeletal muscle. This redistribution of flow is critical in the maintenance of tissue homeostasis and performance [31]. This can be visualized in **Figure A.1**, where blood flow to various organ systems of rationed between organ systems; note that trained individuals experience greater blood flow to the active muscle in comparison to their sedentary counterparts, as well as patients with mitral stenosis [31]. The redistribution of blood flow is governed by autonomic regulation and vasoactive properties, making up a balance between vasoconstrictive and vasodilatory responses, respectively.

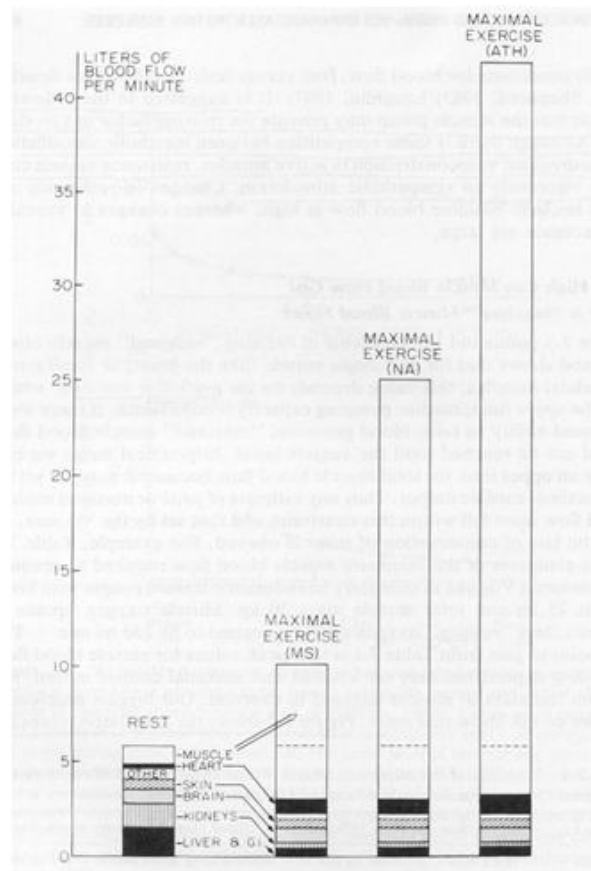


Figure A.1: Depicts a generalized distribution of cardiac output during rest and maximal exercise in athletes (ATH) non-athlete controls (NA) and in patients with mitral stenosis (MS). Taken with permission from [31].

When considering the functional limitations of the cardiovascular system of an elderly or diseased patient, most treatment strategies have targeted only one aspect of their demise, which is an inability to vasodilate in the periphery. However, blood flow distribution involves an interplay between vasoactive properties. Therefore, the purpose of this literature review will be to examine the balance between vasodilation and vasoconstriction, as it relates to blood flow distribution, and discuss how this balance is altered in response to exercise training in both health and disease.

A.2: Vascular Anatomy and Physiology

- **A.2.1 Purpose**

Adequate blood flow distribution is critical in achieving homeostasis. The vasculature is not uniform in nature, but is divided among several branches serving the purpose of controlling the distribution of blood it receives from the heart. Therefore, the purpose of this chapter will be to examine the anatomy and physiology of each vascular compartment, starting with the arterial system and progressing into the venous system.

- **A.2.2 Arterial System**

The arteries are the distributing vessels of the body. It is these vessels which ensure adequate delivery of blood to various tissues (e.g. all major organs, as well as skeletal muscle, skin, etc.), as well as maintaining blood pressure, particularly perfusion pressure, at rest and during conditions of physical stress (e.g. exercise) [110]. The walls of the arteries consist of three layers; the tunica adventitia, the tunica media and the tunica intima [120]. The tunica adventitia, the outermost layer, provides stability, protection and anchors the vessel to the surrounding tissue [120-121]. This layer consists of a collagen-rich extracellular matrix produced by a population of fibroblasts [121]. The high levels of collagen present help to prevent the vessel

from rupture during conditions of extremely high pressure [121]. The adventitia also gives rise to the vasa-vasorum [120-122] which provides nourishment and oxygen to the cells in the vessel wall [121]. The middle layer, or tunica media, consists of smooth muscle cells embedded in a matrix of elastin and collagen [121]. Elastin is a distensible tissue which acts to distribute stresses evenly throughout the vessel wall [121]. The tunica media is of importance because it is responsible for acutely changing the diameter of the vessel by dilating (vasodilation) or constricting (vasoconstriction) its smooth muscle cells in response to chemical [112], mechanical [123] or neuro-humoral controlling mechanisms [116]. Finally, the innermost layer, the tunica intima (endothelium), is lined with a single layer of endothelial cells [120-121]. The endothelium is of particular importance in mediating the actions of the tunica media (vasodilation or – constriction) and is also the barrier between blood and smooth muscle [110, 124]. The different layers of the artery are shown in **Figure A.2**.

The arteries can be classified as elastic or muscular. Elastic arteries (e.g. aorta) are large and initially receive blood from the left ventricle under high pressure [120]. They act as low resistance pathways to conduct blood down the vascular tree. The walls consist of both smooth muscle and an elastic laminae which enable them to resist the constant fluctuations in volume and pressure during each cardiac cycle. In addition, large elastic arteries are capable of distending and storing the ejected volume of blood (i.e. ventricular contraction) and then discharging it down the vascular tree. This phenomena is known as the *Windkessel-Effect* [121] and helps to reduce the after load on the heart and maximize blood flow into the arterioles [121]. The muscular arteries (e.g. brachial and popliteal) are smaller than elastic arteries and immediately distal [120]. These arteries serve as conduits to the vascular beds of the major organs, including skeletal muscle and skin. The walls of these vessels are less elastic and contain

predominately smooth muscle cells. Wall thicknesses and caliber of these vessels also decrease distally until they become arterioles.

The arterioles control blood flow through the capillary beds in such a way that each capillary is provided with the correct amount of blood to serve the needs of the tissues [125]. The pre-capillary arterioles (terminal arterioles) are equipped with circularly arranged smooth muscle cells (pre-capillary sphincters) which control capillary flow and tissue perfusion [120]. The smallest blood vessels are capillaries and are just large enough for single erythrocytes to flow through [120]. These vessels are responsible for the exchange of gases and nutrients at the tissue beds and are therefore very permeable. Essentially the walls of capillaries contain a thin basal membrane and/or just endothelial cells with clefts between them [120].

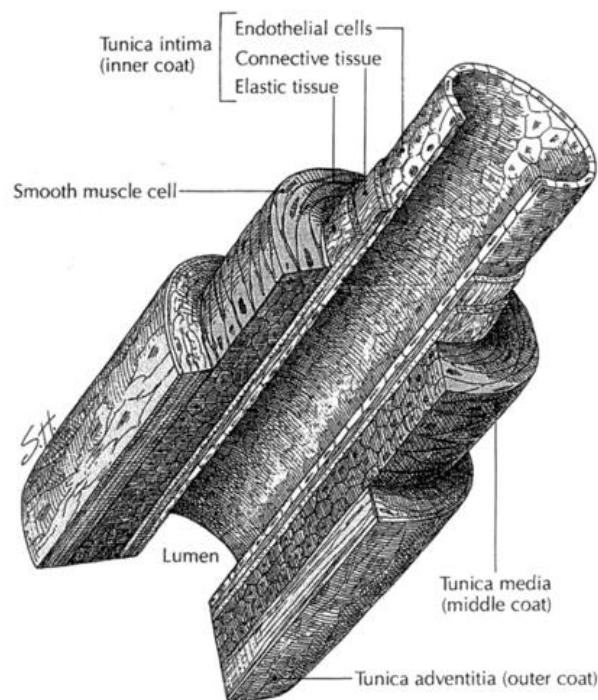


Figure A.2: Depicts a typical elastic artery; including different segments of a vessel. Taken with permission from [126].

Capillaries can be categorized into two types, true capillaries and metarterioles. The metarterioles act as a direct through-way from arterioles to venules. Surrounding this route are networks of true capillaries (exchange vessels) forming a bed to better supply the adjacent tissues. True capillaries descend from the terminal arterioles. Aforementioned, the terminal arterioles have a sphincter muscle situated at each branch point from the metarterioles which can be open or closed depending on the needs of the tissue [110, 120]. True capillaries then drain back into the metarterioles. There are two general methods by which substances pass through the capillary walls. Lipid soluble materials such as oxygen (O_2) and carbon dioxide (CO_2) diffuse rapidly [120], while water soluble substances are filtered through intercellular pores of the capillary wall. The process of filtration- absorption, 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 re-absorption [110].

A.2.3: Venous System

Veins carry blood away from the tissues and deliver it back towards the heart [120]. The smallest of these vessels are the post-capillary venules, which are essentially the distal end of the capillaries [120]. Post capillary venules join and flow into larger venules which in turn join to form veins. The walls of veins are more compliant than arteries and consist of a relatively weak media of smooth muscle and an adventitia with some smooth muscle cells (**Figure A.3**). For a given increase in pressure the blood volume in veins increase to a greater extent than arteries [119]. For this reason, veins are typically known as ‘volume stores’ or ‘capacitance vessels’ because they serve as reservoirs for blood volume [31]. At rest veins can hold approximately 60-

80% of the total blood volume [31]. Uniquely, veins are equipped with valves or folds in the intima, which prevent back-flow of blood returning to the heart. These valves are of particular importance from transition to supine and upright posture [31] by preventing blood from pooling into the periphery [31]. 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’ [50] which will be discussed in detail in latter sections of this review. By now having a better appreciation of the anatomy of the vasculature we can now review the dynamics of blood flow through the vessels, as well as the mechanisms involved in controlling blood flow distribution.

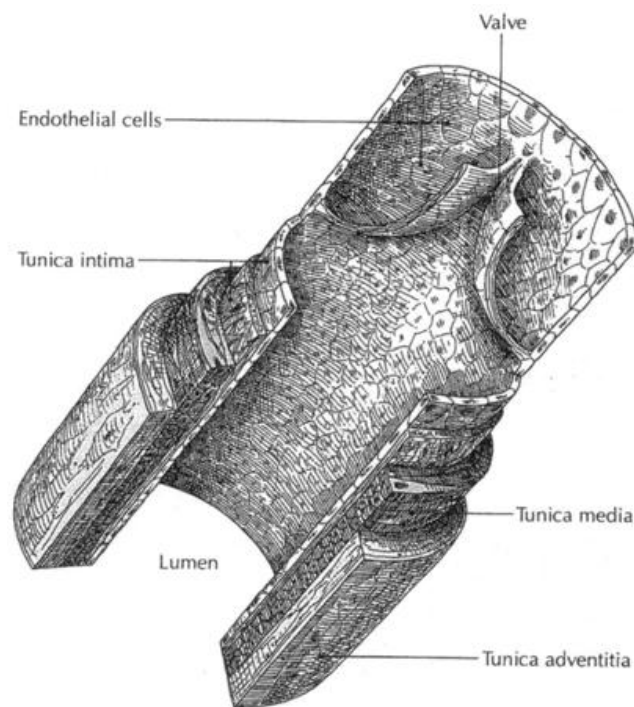


Figure A.3: depicts a typical Vein. Shown longitudinal cut for a medium sized vein. Emphasized the valves and different layers compose the venous vessel. Taken with permission from [126].

A.2.4: Summary

The main types of blood vessels are arteries and veins. There are two great arteries which emerge from the heart; the aorta from the left ventricle and the pulmonary artery from the right ventricle [120]. These large elastic arteries then divide into medium sized arteries which feed various vascular beds. These feed arteries then divide into smaller arterioles which are often called resistance vessels. The arterioles play an important role in controlling pressure and redistributing blood flow [127]. As arterioles enter a tissue they branch into a network of tiny vessels called capillaries where the exchange of substances between blood and tissues occur [110]. Finally, the capillaries merge to form venules and then veins which conduct blood back through the vena cava and into the right ventricle (**Figure A.4**).

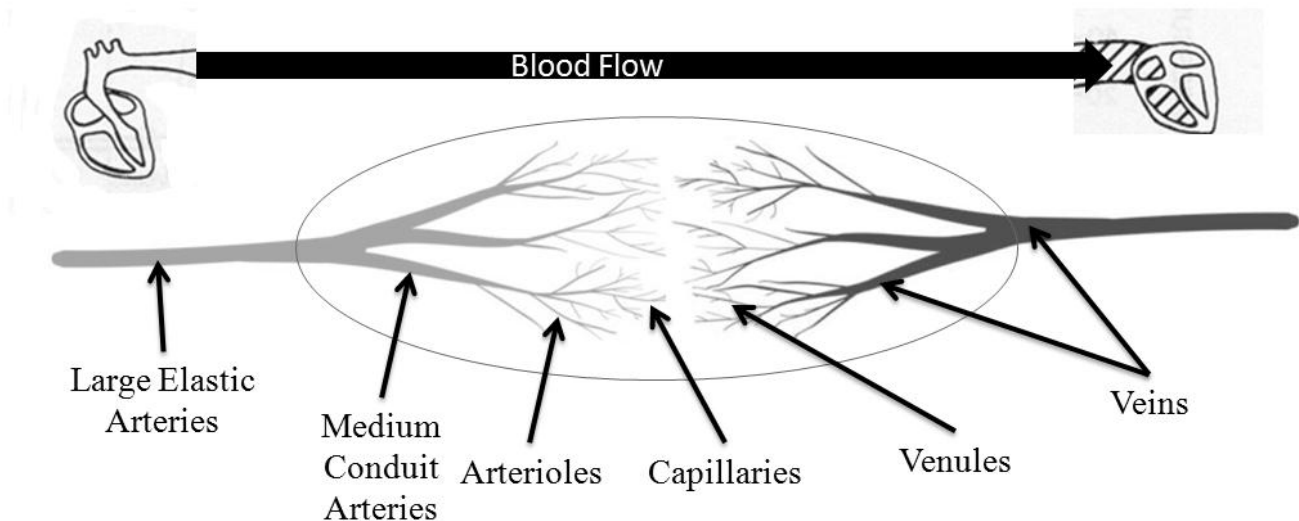


Figure A.4: Depicts a generalized layout of the peripheral vasculature. Blood exits the left ventricle and travels down the vasculature into systemic circulation and returns to the right atrium. The Hierarchy of blood vessels is as follows: Arteries, arterioles, capillaries, venules and Veins.

A.3 Vascular Control of Blood Flow

- **A.3.1: Purpose**

The distribution of blood flow through the vasculature is regulated by changes in vascular resistance. Aforementioned, the vasculature functions as a closed circuit along a pressure gradient. A generalized view is that blood flow follows the path of least resistance and is directed from the high pressure arterial side, through each vascular compartment and ending on the low pressure venous side (e.g. vena cava). The purpose of this chapter will be to evaluate the hemodynamics of blood flow through each vascular compartment, as well as the controlling mechanisms involved in altering vascular resistance, and subsequently blood flow distribution.

- **A.3.2: Hemodynamics through the Vasculature**

The control of blood flow is governed primarily by 3 basic principles: 1. Blood flow to tissues is controlled in relation to tissue needs; 2. Cardiac output is controlled by the sum of all the local tissue flows; 3. Arterial pressure is controlled independently of local blood flow and cardiac output control, respectively [110]. Blood flow through the vasculature depends in part on the difference in pressure (ΔP) at two ends of the system [110]. This pressure, within a blood vessel, refers to the force against its wall measured in millimeters of Mercury (mmHg). Blood pressure is directly related to the product of stroke volume and wall tension but inversely related to the vessel diameter [119]. Blood flow displaces from areas of high pressure or resistance to areas of low pressure. Consequently, if ΔP is equal to zero, then there is no flow. As mentioned previously (2.1.2: *Venous System*), approximately 60% of total blood volume is collected in the low pressure venous system at rest. This is important as it ensures an adequate pressure gradient (ΔP) across the tissue beds served by the vasculature [119].

As stated by Wiggers [128], the heart can pump only as much as it receives. The heart can be influenced by several factors such as local metabolites, nervous or hormonal stimulation but ultimately acts to propel the blood received from the vena cava (~0 mmHg) to the aorta (~100 mmHg) [119]. Under resting conditions, the systolic blood pressure (SBP) of muscular arteries (e.g. brachial artery) averages approximately 120 mmHg and the diastolic blood pressure (DBP) averages approximately 80 mmHg [110]. The difference between the systole and diastolic pressures is known as the ‘pulse pressure’ or driving pressure (PP mmHg) [31, 110]. The mean arterial pressure (MAP) is maintained at approximately 100 mmHg throughout the day [129]. The mean arterial pressure (MAP mmHg) within the body can be calculated as:

$$\text{MAP mmHg} = \text{DBP} + [(\text{PP mm Hg}) / 3]$$

When pressure drops below normal reflexes are evoked throughout the body in order to maintain adequate perfusion. The mechanisms behind these reflexes will be discussed in the next section. A general estimation of blood flow through a vessel can be calculated by a derived form of *Ohm’s Law*, which is presented below:

$$Q = \frac{\Delta P}{\text{TPR}}$$

In effect, this formula states that blood flow (Q) is directly proportional to the change in pressure (ΔP) but inversely proportional to vascular resistance (TPR) [110]. To estimate vascular resistance the equation for *Ohm’s law* is simply inverted so that vascular resistance is proportional to ΔP and inversely related to Q. Theoretically, the higher the blood flow to an area, the lower the vascular resistance but this can be dependent on the vessels distensibility [119].

When blood flows at a steady rate through a vessel the flow is in streamline layers called ‘laminar flow’ [119]. During laminar flow the velocity of blood flow in the center of the vessel is greater than that towards the intima. Blood velocity slows down towards the intima because of

adherence to the vessel wall [119]. When the flow of blood and its velocity become too great, or when it passes on obstruction (e.g. narrowed lumen, bifurcation, turn), the flow may become turbulent or oscillatory in nature. Turbulent flow is disorderly rather than streamlined, meaning that it flows in all directions. The tendency for turbulent blood flow can be calculated using *Reynold's number* which is presented below:

$$\text{Re} = \frac{v d \rho}{\eta}$$

Re is Reynold's number, v is the mean velocity of blood flow (measured in cm/sec), d is the vessel diameter and ρ is the density of the blood, and η represents the viscosity of the blood [119]. In some cases turbulent flow may arise when the velocity of blood flow is high (e.g. during reactive hyperemia), the pulsatile nature of the flow (e.g. antegrade and retrograde flow), a sudden change in vessel diameter (e.g. sympathetic vasoconstriction) and in large vessels (e.g. aorta and pulmonary artery). Interestingly, Laughlin et al. (2008) have indicated that complex flow profiles within recirculation zones and/or oscillatory flow patterns increase proatherogenic gene expression in local endothelial cells [11]. Additionally, oscillatory flow patterns also seem to increase with advancing physiological age [49], primarily, the consequence of alterations in vascular stiffness (**Figure A.5**). Aforementioned, when considering non-pulsatile flow through a vessel, flow is determined by the pressure difference between the two ends of the vessel, but is inversely related to the total peripheral resistance, which can be generalized using *Poiseuille's law*...

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

The letter r represents the radius of the vessel, l is the vessel length and η is the viscosity of the blood. Proportionality constants (π and 8) have been added to Poiseuille's equation [119].

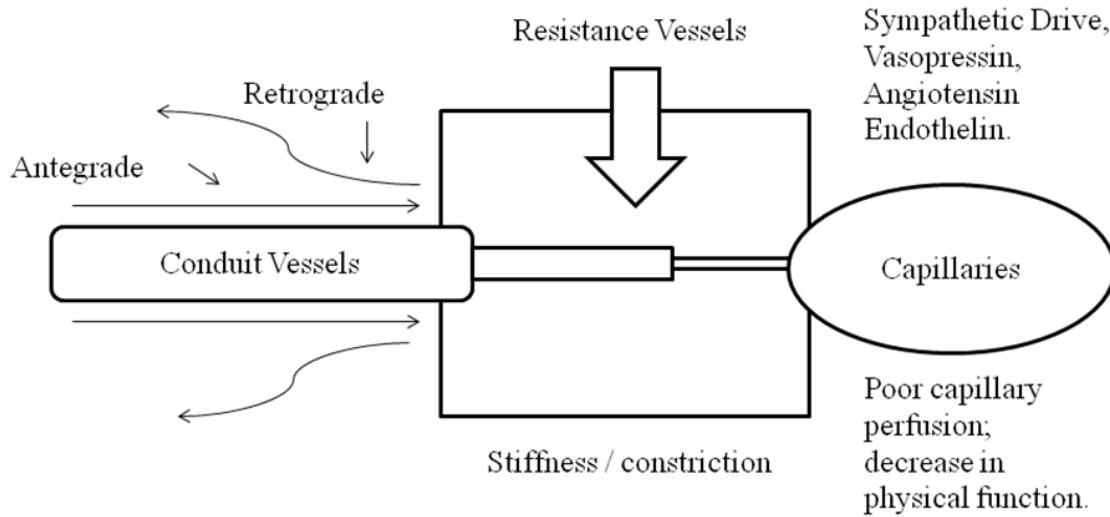


Figure A.5: Depicts an illustration of proposed mechanisms underlying an increase in retrograde flow with aging. An increase in peripheral resistance, maybe reflected by a higher retrograde blood flow.

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 [119]. According to Poiseuille's law, a unit increase in the vessel radius results in an increase in a 4-fold increase in blood flow (e.g. 4-fold increase in vessel radius causes a 256-fold increase in blood flow) [110]. Given the importance of vessel diameter and blood pressure in the control of blood flow distribution the following sections of this review will highlight the controllers of vascular tone and pressure.

- **A.3.3: Autonomic Regulation of Vascular Tone**

Neural control of circulation is governed primarily by the autonomic nervous system [110] which is composed of both a sympathetic (thoracic-lumbar) and a parasympathetic (cranial-sacral) division [31, 119]. Both divisions of the autonomic nervous system innervate the heart, however the peripheral vasculature has an extensive network of sympathetic innervation

with very little parasympathetic innervation [110]. This is of particular importance in the control of blood flow. The sympathetic division innervates the entire arterial tree down to the arterioles as well as small venules, up into larger veins [31]. Capillaries and terminal arterioles have little innervations but respond primarily to local controlling mechanisms (e.g. changes in tissue metabolism) [110]. The sympathetic division innervates the vasculature via postganglionic neurons which insert into the adventitia of the vessel and at the junction between the adventitia and the media [110].

The primary neural transmitter involved in blood flow control of the sympathetic division is norepinephrine (NE) released from ‘adrenergic’ nerve terminals [119]. Other neural transmitters of the autonomic nervous system include acetylcholine (Ach) released from, ‘cholinergic’ nerve terminals of the parasympathetic division and Epinephrine (E) released from the adrenal glands (adrenal medulla) [119]. The adrenal medulla is sympathetically innervated via preganglionic neurons [119]. Importantly, only the adrenergic fibers are tonically active and contribute to resting vascular (Basal) tone [31]. The types of receptors on smooth muscle cells associated with corresponding nerve terminals include Alpha (α)- and Beta (β)-adrenergic receptors and muscarinic, cholinergic receptors [111, 119]. The α and β receptors can be further divided into α_{1-2} and β_{1-2} . The α_{1-2} receptors are concentrated in the smooth muscle of arteries and veins and respond primarily to NE by eliciting smooth muscle cell contraction with subsequent vasoconstriction. In an interesting study performed by Anderson and Faber (1991), a differential sensitivity of arteriolar α_1 and α_2 adrenergic constriction was found in response to muscular contraction [130]. The α_2 constriction of both large and small arterioles were significantly attenuated at all frequencies of muscle contraction whereas large arteriolar α_1 constriction was reduced only during high frequency muscle contractions [130]. Although this study utilized rat

cremaster muscle, the findings suggests a differential sensitivity of α -adrenergic receptor subtypes to metabolic inhibition with α_2 receptors having a greater inhibitory response.

Additionally, in vitro experiments in animals have displayed a heterogeneity of NE concentrations in differing blood vessel segments [111, 131-132], which is exemplified in **Figure A.6**. The varying degree in catecholamine concentration within different vessel segments appears to relate to the size of the vessel and its function [132]. The β_1 receptors are found primarily in the heart, and concentrated around the myocardium, sino- and atrioventricular nodes, and the Purkinje fiber system [119]. These receptors respond to NE and E by dilating the coronary vessels and increasing heart rate, conduction velocity and contractility of the heart. The β_2 receptors are located in smooth muscle of arteries and veins but appear to be more concentrated in coronary and skeletal muscle arterioles. When stimulated by NE or E these receptors cause relaxation of smooth muscle. Interestingly, the adrenergic response to NE by α_1 receptors seems to be more pronounced than E to β_2 receptors [119]. **Figure A.7** presents a generalized distribution of receptor subtypes in different regions of the vasculature. The cholinergic receptors of the parasympathetic branch are scattered throughout the vascular tree ranging from the arterioles of skeletal muscle, the heart, brain, some larger blood vessels and other organs (**Figure A.6**). All endothelial cells seem to inherit muscarinic receptor types and elicit vasodilation of smooth muscle when stimulated [22, 116, 133-134]. These receptors in cardiac tissue respond to Ach by eliciting vasodilation, slowing the rate of contraction of the myocardial tissue and also by inhibiting NE release from sympathetic nerve endings [119]. When considering blood flow distribution to different organ systems (e.g. brain, splanchnic, pulmonary), the rate of flow control may be different from that of skeletal muscle. For example, cerebral blood flow has been shown to be auto-regulated by the brain, and is sensitive to changes

in the partial pressure of CO_2 (PCO_2) thus controlling its delivery blood flow independent of peripheral factors (e.g. contracting skeletal muscle) (**Figure A.7**) [135].

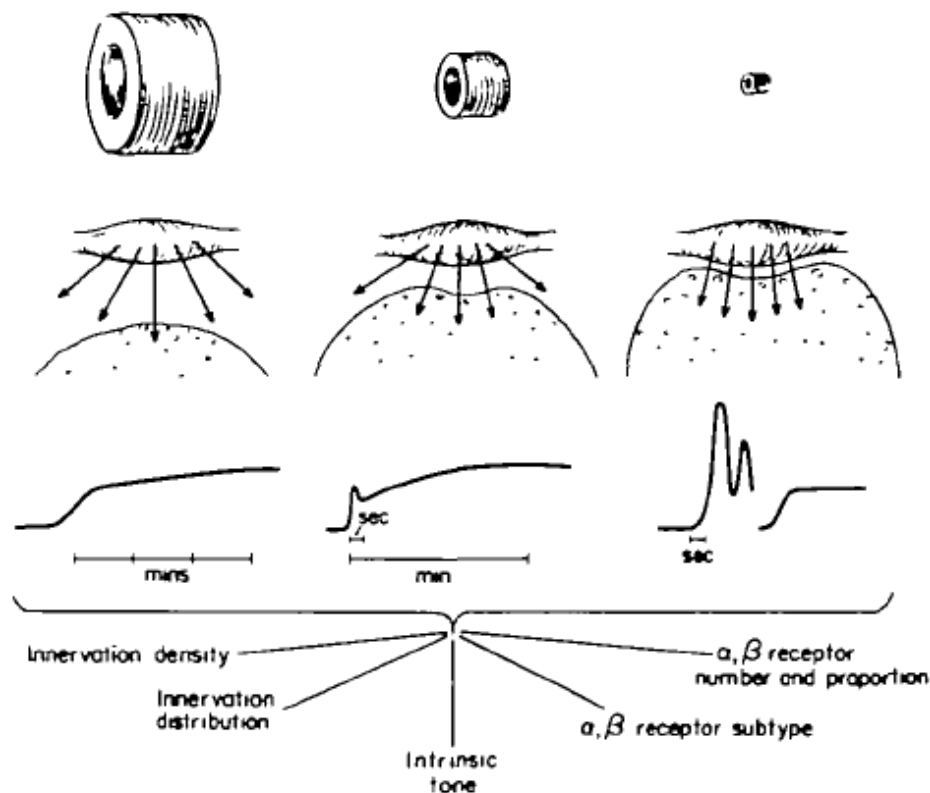


Figure A.6: Depicts a representation of three vascular neuroeffector patterns showing (from above downward) vessel size, neuroeffector relationships and distribution of released transmitter, and contractile response to NE. The pattern on the extreme left is that seen in an elastic artery and on the right that for a small muscular artery or arteriole. The middle pattern is found in vessels of intermediate size. Responses of the three vessels are influenced by a number of factors including innervations density, innervation distribution, intrinsic tone, α - and β -receptor number and proportion, and α - and β -receptor subtype. Taken with permission from [132].

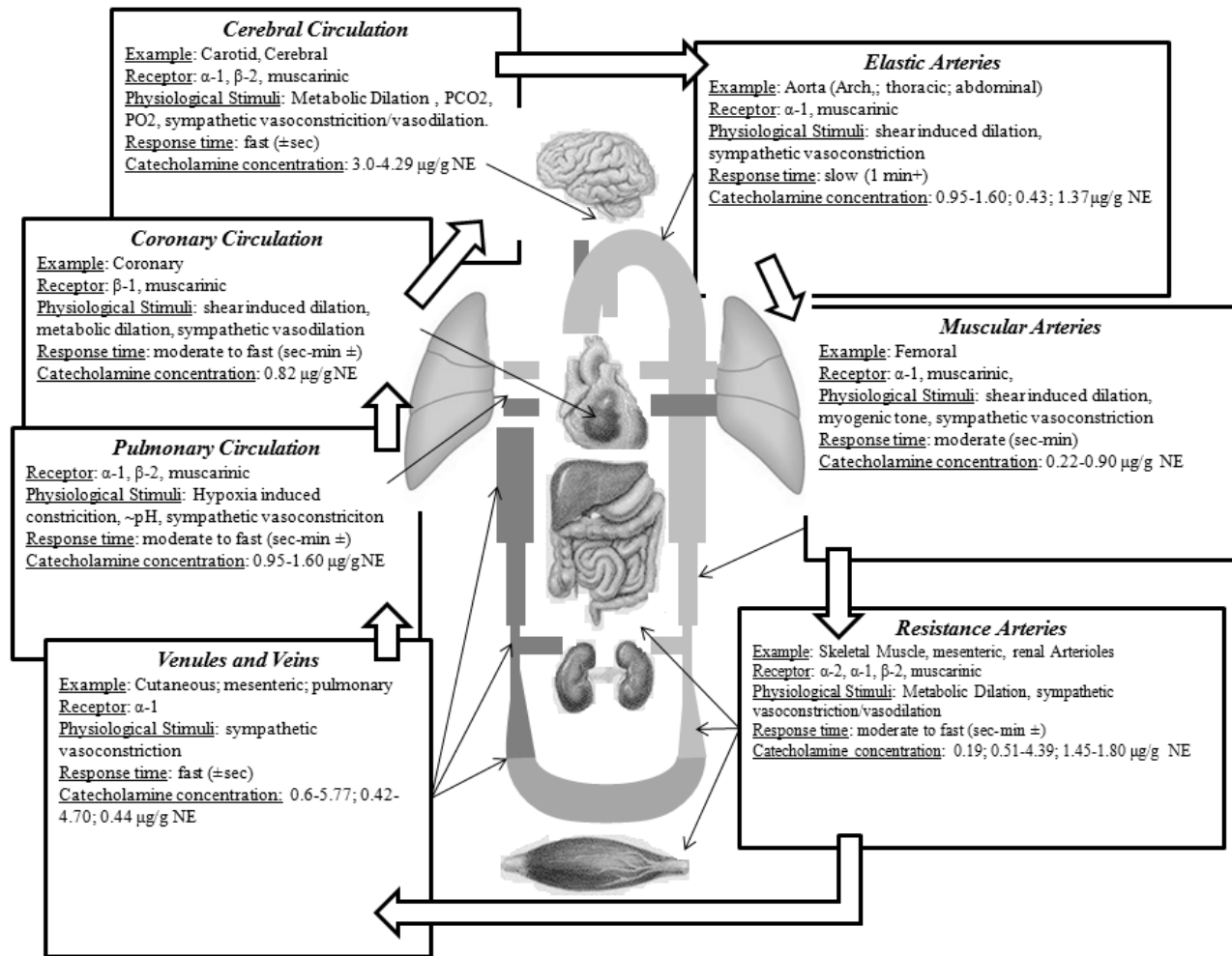


Figure A.7: Depicts the heterogeneity of differing vascular beds; including example of blood vessels [120], predominate receptor subtype upon smooth muscle [31, 132, 136], physiological stimuli [21-22, 31, 111, 135] involved in controlling diameter size and concentration of NE [111, 131]. The gradation from light to dark down the vasculature represents the transition from distributing to capacitance vessels. It should be noted the NE concentrations were taken [111, 131], from in vitro experiments in dogs.

Moderate to high intensity exercise has been shown to moderately increase cerebral blood flow despite large increases in systemic sympathetic nerve activity and arterial blood pressure [135]. The underlying mechanisms behind this cerebral auto-regulation of arterial inflow is unclear but may, in part, be the consequence of an increase in sympathetic nerve activity in the brain and/or a collapsing of the jugular vein (which has been shown to occur in the upright posture) further increasing the resistance to flow in the brain [137]. Interestingly enough, the distribution of blood flow within the cerebral circulation seems to follow a similar degree of functional sympatholysis to that of systemic circulation [135], thus redistributing its flow to areas of higher activation and metabolic rate (e.g. dynamic movement has been associated with elevations in blood flow to the supplementary and primary sensorimotor areas of the cerebrum) [138]. After having a greater appreciation for the autonomic control of the vasculature, it is now appropriate to discuss reflexes involved in blood flow and pressure control.

Short term regulation of blood pressure can be achieved by the baroreflex [110, 129, 134]. Baroreceptors, located in the aortic arch and the internal carotid arteries, can respond to acute changes in pressure [110]. For example, an increase in pressure causes a nerve propagation to the tractus-solitarius in the medulla of the brainstem, stimulating inhibition of the vasoconstrictor center (sympathetic) with a subsequent excitation of the vagal center (parasympathetic) [110]. The resulting cardiovascular responses are inhibition of sympathetic vasoconstriction, with a subsequent increase in vasodilation of the peripheral vascular beds and veins, as well as, a decrease in heart rate and contractility [110]. As a consequence of this baroreflex, blood pressure begins to fall. On the other hand, a significant drop in arterial pressure causes a withdrawal of baroreceptor signals to the medulla and a reversal of effects [110]. The baroreflex is of particular importance when moving from supine to upright posture. The passive

effects of gravity causes a drop in venous return to the heart [31] with a subsequent reduction in blood pressure [31]. Humans have adapted to compensate for this orthostatic response by equipping venous valves in the periphery, and stimulating an orthostatic reflex (baroreflex) to maintain blood pressure [31].

- **A.3.4: Local Control of Blood Flow**

The cardiovascular control centers are directed toward maintaining blood pressure while local regulation of blood flow is directed toward meeting the metabolic demand of the tissue [110]. At the onset of exercise, muscle afferents (e.g. mechanoreceptors and chemoreceptors) send signals to the cardiovascular control centers to elevate heart rate through vagal withdrawal and increase systemic blood pressure by sympathetic vasoconstriction, thus increasing the driving pressure of blood flow [110]. However, local controlling mechanisms act towards meeting the metabolic demand of the tissue. The importance of local metabolic control is most apparent in the resistance arteries [125]. Local metabolic dilation acts to ‘shunt’ blood flow from inactive vascular beds towards contracting skeletal muscle [31, 136, 139]. Metabolites (e.g. adenosine, adenine nucleotides, CO_2 , K^+ , phosphate ions, lactate and Krebs cycle intermediates) which are released from active muscle fibers, diffuse through the interstitial space to act on adjacent smooth muscle cells causing vasodilation, overriding sympathetic nerve activity and opening pre-capillary sphincters with a subsequent increase in blood flow through the exercising vascular bed [110]. Remensnyder et al. (1962) termed a phenomenon known as functional sympatholysis in which local metabolites (e.g. H^+ , Adenosine, P_i^- , NO^-) produced in contracting skeletal muscle appear to override the sensitivity of vascular endothelium and smooth muscle to catecholamines released during sympathetic stimulation, thus contributing to an increase in blood flow through the contracting skeletal muscle [112]. The endothelium also appears to be an

important mediator in the control of blood vessel radius and subsequently the redistribution of blood flow.

Two general classes of stimuli elicit an endothelial-dependent response, including chemical and physical stimuli, by synthesis or release of vasoactive and thromboregulatory factors which include NO⁻, endothelial derived hyperpolarizing factor (EDHF), bradykinin, thromboxane, endothelin-1 and tissue-type plasminogen activator [21-22, 97, 134]. In addition, chemical or pharmacological stimuli through NE, Ach and bradykinin release stimulates specific endothelial cell receptors. For example, NE stimulates α 2 receptors, bradykinin stimulates a bradykinin receptor (e.g. B₁) receptor and Ach stimulates muscarinic receptors [22, 116]. Researchers Furchgott and Zawadzki (1980), by chance, were the first to recognize the importance of the endothelium in controlling vascular smooth muscle tone by observing that an unrubbed endothelium is necessary for vasodilation of an arterial ring in response to Ach [116] (**Figure A.8**). It appears that from these results a disruption or injury to the endothelium abolishes its mediating response of Ach on smooth muscle relaxation. This finding has important clinical significance in that chronic disease and aging are associated with endothelial dysfunction. Appropriate strategies at restoring normal endothelial function is imperative to physical functionality of a patient.

A physical stimulus such as the shear force of blood upon the endothelium, produced during muscular contractions, can lead to endothelium dependent vasodilation [22]. The shear is thought to activate specific stretch activated potassium (K⁺) channels located on the surface of the endothelial cell, thus resulting in a signal amplification leading to an increase in intracellular Ca²⁺ concentration [140]. This is an important mechanism in the regulation of the radius in large conduit vessels and skeletal muscle arteries during physical stress [22]. An increase in

intracellular Ca^{2+} concentration is believed to be the trigger for vasodilation by the activation of three of the previously mentioned pathways; prostacyclin (PGI-2), NO^- and EDHF (**Figure A.9**) [22]. The endothelial derived isoform of Nitric Oxide Synthase (e-NOS) is responsible for the metabolic conversion of the substrate L-arginine to L-citrulline and subsequent NO^- release from the endothelial cells [140].

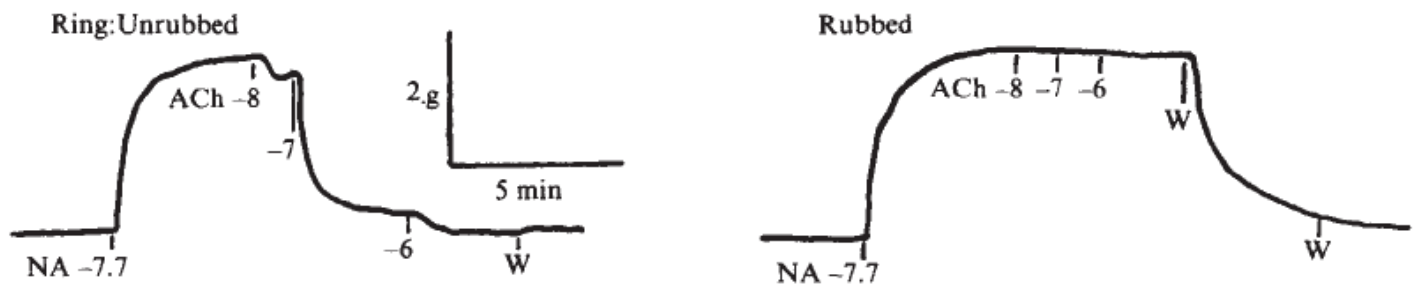


Figure A.8: Loss of relaxing response of preparations of rabbit aorta to ACh after rubbing of the intimal surface. Modified and taken with permission from [116].

The free radical NO^- appears to have a large influence on vascular tone under normal conditions. The vasodilator activity of NO^- occurs in the vascular smooth muscle, due to its interaction with guanylate cyclase [141] to produce cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP) [22, 142]. The cGMP activates additional protein kinases leading to further Ca^{2+} release in the endothelial cell, but increased Ca^{2+} uptake by the smooth muscle sarcoplasmic reticulum and subsequently relaxation [22]. The increased endothelial intracellular calcium concentration can also activate phospholipase-A2 to release arachidonic acid (AA), which is converted by the enzyme cyclo-oxygenase to PGI-2 [143]. The vasodilatory capacity of PGI-2 in vascular smooth muscle is by the activation of the enzyme adenylate

cyclase to convert ATP to adenosine 3'-5'-monophosphate (cAMP) with a subsequent activation of additional protein kinases [22, 143].

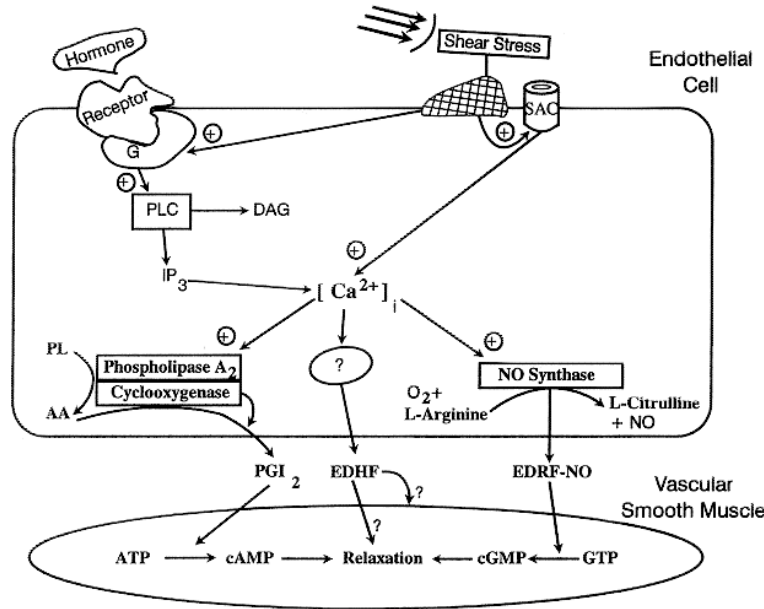


Figure A.9: A simplified model of receptor-signal transduction processes in endothelial cells leading to the release of putative endothelium-derived prostacyclin (PGI₂), endothelium-derived hyperpolarizing factors (EDHF), and endothelium-derived relaxing factor, nitric oxide (EDRFNO). Vasoactive substances (hormone) such as: norepinephrine, UK14304, acetylcholine, bradykinin, and substance-P, bind to their specific endothelial cell membrane receptor that is coupled by a G protein to phospholipase C (PLC). Activation of PLC leads to hydrolysis of phosphatidyl inositol biphosphate producing two intracellular second messengers: inositol trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ signals calcium release from intracellular stores. Increased intracellular free calcium ([Ca²⁺]_i) activates three processes: 1) phospholipase A₂ to release arachidonic acid (AA) which is converted to prostacyclin by cyclooxygenase; 2) nitric oxide synthase (NO synthase) to produce EDRF-NO; and 3) release of unidentified EDHFs by as yet to be established pathways (?). As illustrated, flow-induced vasodilation results from increased shear stress which induces increased [Ca²⁺]_i via stretch activated channels (SAC) or G-protein mediated mechanisms. PGI₂ is believed to signal relaxation of vascular smooth muscle by stimulating adenylate cyclase which produces cAMP (adenosine 3'-5'-monophosphate) from ATP (adenosine triphosphate). EDRF-NO is believed to signal relaxation in vascular smooth muscle by stimulating guanylate cyclase which produces cGMP (guanosine 3'-5'-cyclic monophosphate) from GTP (guanosine 5'-triphosphate). As illustrated by the process whereby EDHF is produced and mechanism by which EDHF causes hyperpolarization are not established. Taken with permission from [22].

The endothelium controls vascular tone not only by releasing NO⁻ and PGI-2 but also through additional pathways through hyperpolarization of smooth muscle cells. This concept eluded towards the term ‘endothelium-derived hyperpolarizing factors’ (EDHF) [144]. However, this acronym includes different mechanisms; arachidonic acid metabolites produced from cyclo-oxygenase, lipo-oxygenase and cytochrome P-450 pathways, H₂O₂, H₂S and other peptides can be released by endothelial cells [144]. EDHF appears to be released by the stimulation of endothelial muscarinic receptors and may act via the activation of a K⁺ channel [145]. The factors which activate K⁺ channels hyper-polarize the vascular smooth muscle cells, subsequently contributing to smooth muscle relaxation. EDHF may become an important contributor to vasodilation when NO⁻ and PGI-2 are reduced such as in certain disease states (e.g. atherosclerotic, hyper-cholesterolemic or ischemic conditions) [146].

The blood vessels themselves are capable of eliciting a vasoactive response, independent of neural, humoral and metabolic controllers. This “myogenic” reflex, or “Bayliss effect,” named after the discoverer is most pronounced in muscular arteries and arterioles where it may protect the capillary beds from over-perfusion [119, 123, 134]. The stimulus for the myogenic reflex is uncertain but it may be a response to an increase in tension on the vessel wall and/or to stretch on the smooth muscle myofibrils [123]. The wall tension theory in a blood vessel can be supported by La Place’s Law...

$$\dots T = P_t * r$$

In a blood vessel, the circumferential wall tension (T) is usually measured as the product of the transmural pressure [$P_t = \text{internal pressure } (P_i) - \text{external pressure } (P_e)$] and the vessel radius I. If wall tension increases due to a rise in intravascular flow and pressure, a reduction in the vessel radius can counteract this increased wall tension and create feedback loop to achieve transmural

homeostasis [123, 134]. In general, the increase in vessel wall tension activates ion channels, causing depolarization, an influx of Ca^{2+} , through stretch-operated channels and/or a cascade of second messenger pathways, which would then result in smooth muscle contraction [123].

Blood pressure can be maintained on a moderate basis (hours to days) through hormonal regulation of blood volume [129]. Over time, a significant reduction in blood pressure, as may occur with prolonged hypotension, stimulates the posterior pituitary gland to release a hormone called vasopressin [129]. Vasopressin release can also be stimulated by a decrease in blood volume (hypovolemia), particularly an increase in plasma electrolyte concentration (hemo-concentration) [129]. This hormone is of particular importance, because it has a direct effect on the kidneys and the peripheral vasculature. This hormone travels to the kidney and increases the permeability of the collecting ducts and tubules to water. This allows for water to be reabsorbed, thus conserving blood volume and returning adequate perfusion pressure [110].

Over time (days to weeks), the primary control of blood pressure is through the renin-angiotensin-aldosterone system [110]. The enzyme renin is stored and released by the juxtaglomerular cells in the kidney in response to a drop in blood pressure and a decrease in renal flow [110]. Most of the enzyme enters renal blood and passes out of the kidney to circulate in the periphery where it acts on the substrate angiotensinogen. The result is an amino-peptide known as angiotensin-I. Angiotensin-I is a mild vasoconstrictor, which can be catalyzed into Angiotensin-II in the lung, by way of Angiotensin Converting Enzyme (ACE) [110]. Angiotensin-II (ANG-II) is a powerful vasoconstrictor acting on the peripheral vasculature, especially the resistance vasculature, to elevate blood pressure. In addition, ANG-II acts on the kidneys to cause sodium (Na^+) and water retention, as well as acting on the adrenal glands,

which release aldosterone, subsequently increasing salt and water reabsorption by the kidneys [110].

- **A.3.5: Summary**

In summary, each vascular compartment serves a particular purpose in the redistribution of blood flow from rest to dynamic exercise by vasoconstriction of non-exercising beds, and vasodilation of arteries in response to increases in blood flow and tissue metabolism in the active skeletal muscle. The elastic arteries are low resistance pathways which transfer the blood ejected from the ventricle under high pressure towards the periphery. The conduit vessels transport the blood to a particular region in the periphery and respond predominately to hemodynamic forces (e.g. shear stress) by releasing endothelial derived relaxing factors (NO^- , PGI-2, EDHF) which act on the adjacent smooth muscle cells. The arterioles within the feed the vascular beds of a tissue and have a high degree of basal tone. These vessels respond predominately to neuro-humoral stimulation (e.g. NE, Ach, ANG-II) and serve as a major controller of vascular resistance and redistribution of flow. The exchange of substances between blood and tissue occurs in the capillaries. These vessels respond predominately to metabolic influences (e.g. Adenosine, H^+ , K^+) by opening terminal arterioles and allowing greater blood flow to the exercising muscle. The venules and particularly the veins serve as the volume stores for blood at rest and are heavily innervated by $\alpha 1$ -adrenergic receptors. The veins can quickly increase blood return to the heart and into arterial circulation through their unique valve orientation, the pumping effect of the contracting skeletal muscle, as well as increasing their tone in response to an increase in sympathetic nerve activity. Each of these vascular domains serves the purpose of meeting the supply of blood to the metabolic demand of the tissues. A summary of vascular responsiveness through each domain is depicted in **Figure A.10**.

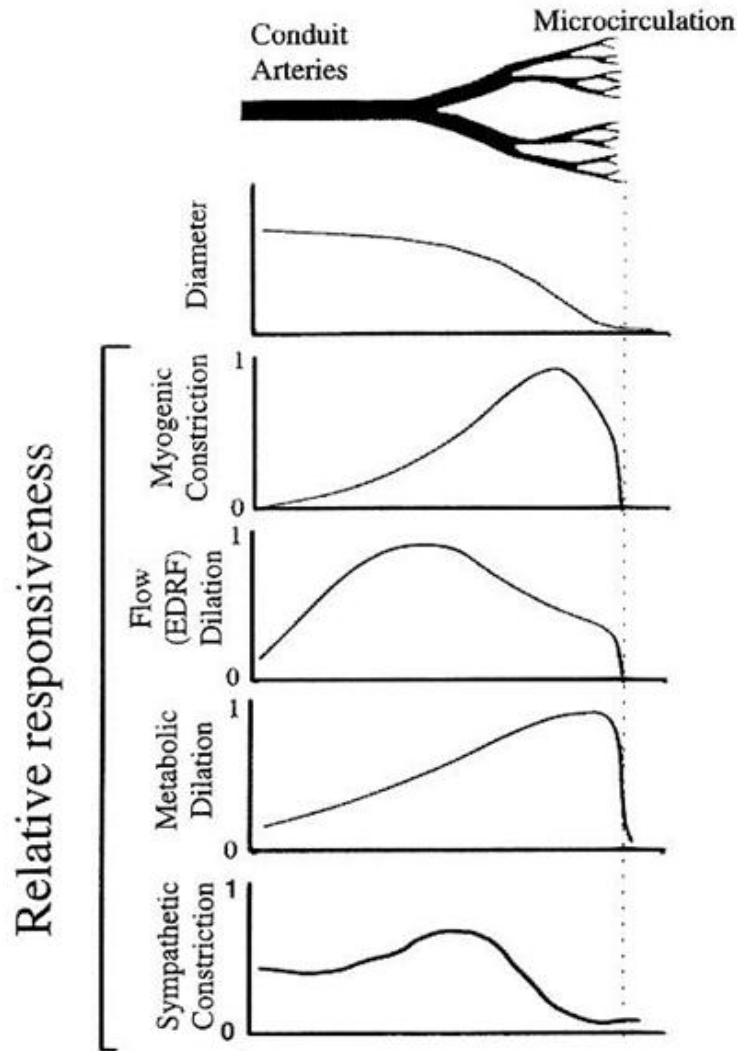


Figure A.10: Schematic illustrating the relationship between the relative responsiveness of segments of the arterial tree to vascular control systems and the diameter of each arterial segment. The top panel illustrates the arterial tree and the second panel reflects changes in diameter observed on progression from the conduit arteries down to the resistance arteries and arterioles. The last four panels illustrate the relative responsiveness of the segments of the arterial tree respectively to: myogenic reactivity, flow-induced vasodilation, metabolic vasodilator signals, and sympathoamines. Taken with permission from [142].

A.4 Assessing Vasoreactivity and Blood Flow

- **A.4.1 Purpose**

Following the discussion of vascular structure, function and its controlling mechanisms it is now appropriate to introduce the various assessment tools used in measuring vascular physiology and blood flow. Although a number of techniques are currently being used in cardiovascular research, only those tools relevant to this review will be discussed. The purpose of this chapter will be to review both non-invasive and invasive techniques used in humans and animals to assess the macro-vasculature (e.g. elastic and conduit vessels), the micro-circulation (e.g. resistance and exchange vessels) as well as regional blood flow distribution (combination of all vascular compartments).

- **A.4.2: Macro-Vascular Assessments**

A popular methodology for measuring macro-vascular function, non-invasively, is through Doppler Ultrasonography. Doppler Ultrasonography is a very functional measure in that it has the capability of examining the structure and physiology of large conduit vessels, as well as blood flow and velocity. In general, a pulsed ultrasound wave is emitted through the examined tissue (i.e. bicep; *brachial artery*) and the crystal within the probe used to detect the returning ‘echo’ is briefly activated; a *time gate* [147]. Depending upon the length of time between signal emission and receiver activation, the depth of the structure from which the returning signal enters the gate can be estimated. As this length is varied, a three dimensional map of the vessel can be constructed [147]. For the assessment of blood flow, the frequency of the reflected ultrasound wave is dependent upon the speed of the blood from which the waves have been reflected. The transmitted waves are reflected by passing erythrocytes in the vessel lumen [148] and can be

visualized in real time, in Duplex mode, which combines ultrasonic imaging coupled with Doppler flow measurements [149]. This allows investigators to determine blood velocity and direction.

Perhaps the most widely used application of the Doppler Ultrasound in vascular research has been to examine conduit arteries of the peripheral vasculature and their responses to various physiological stimuli. Aforementioned, arteries and veins contain a wide range of receptor types (*as mentioned in Chapter 2*) mediating smooth muscle contraction (α -adrenergic), relaxation (β -adrenergic) and endothelial function (*Nitric Oxide*). A multitude of studies have utilized Doppler Ultrasonography to obtain an image of the brachial artery at rest and following a period of forearm occlusion (Nitric Oxide dependent) and/or during a cold pressor test (Sympathetic nervous activity) [150] (**Figure A.11**). Previous work has reported an increase in blood pressure and heart rate during a cold stimulus [28] along with decreased blood flow to the peripheral vasculature, indicating a sympathetic, vasoconstrictive response [151]. The percent change in vessel diameter between resting, peak (following arterial occlusion) and minimum (following cold stimulus) can serve as a surrogate marker of vascular function (vasoreactivity) and is thought to be a potential marker for coronary artery disease [152]. This protocol was developed by Celermajer and colleagues in 1992, because the brachial artery could be easily imaged by conventional ultrasound and the vessel itself is of similar size to the major coronary arteries [152]. Therefore, they reasoned it may give useful insight in atherosclerosis. Of course this model is of limited consequence to systemic cardiovascular control due to its small muscle mass and blood flow requirements. Recent studies have used Doppler Ultrasonography to study the leg vasculature [153-154] and several factors have been known to influence vasoreactivity, including time of day of testing [155], relationship to meals [156-157], effect of smoking [158] and more

recently magnitude and direction of the shear stress imposed on the vessel wall [11, 15, 21, 73-74]. This lead to the publication of “The Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery,” in 2002 [33]. The purpose of these guidelines was in part to discuss the technical and interpretive limitations of the technique and provide a universal standardized approach.

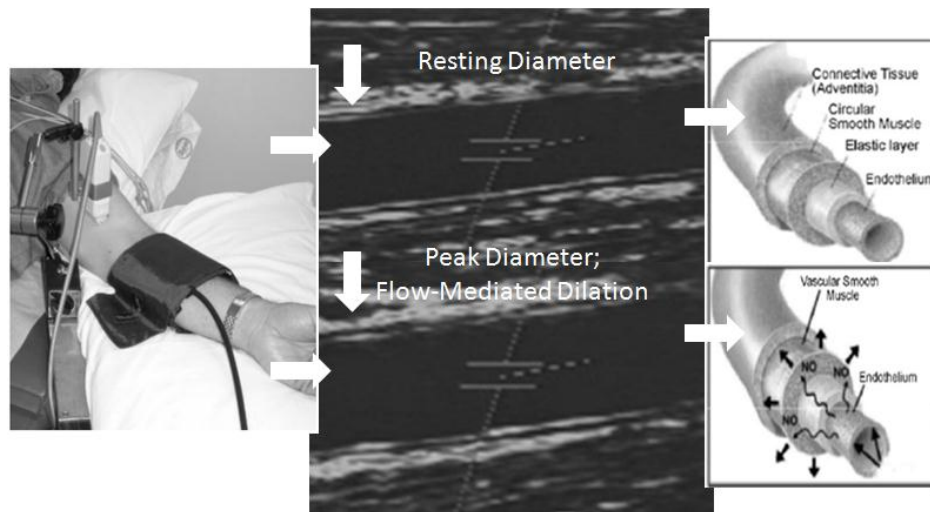


Figure A.11: Depiction of the Brachial Artery Flow Mediated Dilation model applied to the arm. The Ultrasound probe is positioned over the brachial artery, approximately 2 inches above the antecubital fossa. An occluding cuff is placed approximately 1 inch below the antecubital fossa. After 20 minutes of supine rest, a baseline (Resting Diameter) image is recorded. The occluding cuff is then inflated to a supra-systolic pressure to insure arterial occlusion for a period of 5 minutes. Following release of the cuff pressure, images are recorded for 2 minutes, and the peak diameter achieved is used to express a percent change from the resting diameter. The vasoactive substance NO- is believed to stimulate this brief rise in diameter, subsequently reducing the shear stress upon the arterial wall and increasing blood flow to the forearm.

Aforementioned, shear induced vasodilation stimulates the formation and release of vasoactive substances, particularly NO^- , resulting in smooth muscle relaxation [22]. The measurement of NO^- poses difficulty because the molecule is rapidly oxidized to form Nitrite

(NO₂⁻) and further nitrate (NO₃⁻) [12]. Venous plasma NO₂⁻ has been shown to be a marker of regional NO⁻ production, following pharmacological (i.e. ACH infusion) and physiological (i.e. acute exercise) stimuli [12, 114, 159].

An additional, non-invasive method used in evaluating the integrity of large conduit arteries is measuring the pulse wave velocity (PWV). This measurement is based on the factor that the pressure pulse generated by ventricular ejection travels through the vasculature (i.e. arterial tree) at a certain speed. The speed at which this pulse travels can be altered by the elastic properties of the blood vessel and provides an index of distensibility and arterial compliance. For example, the higher velocities indicate greater vascular resistance [160]. The pulse wave-forms can be recorded simultaneously at two different sites along the vascular tree (e.g. carotid/ankle) using a Doppler Ultrasound probe or using Applanation Tonometry [161-162]. The velocity can be derived from these waveforms by measuring the time delay (transit time) and the distance between the two sites [161], which can be quantified using a tape measure.

An examination into the stability and reproducibility of the Doppler Ultrasound measure was exemplified by Welsch and colleagues [53]. Data were collected from a group of men (mean age 41 years) free from overt signs of disease, acute medical conditions or pharmacotherapy with known vascular effects [53]. The subjects were instructed to fast and refrain from exercise 12 to 48 hours to control for any external influences. Ultrasound imaging was conducted at the same time of day, following 15 minutes of supine rest in a quiet room. The study design was such that intra-class correlation coefficients and covariance parameter estimates could be made between and within days for ultrasound technicians, image readers and subjects. Average mean percent artery dilation differences for days and testers were 1.91% and 1.40%, respectively [34]. 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 [34].

Additional techniques are available to measure macro-vascular function invasively, but have been restricted to animal studies. Experiments using animals (e.g. rat, swine, mouse, etc.) have allowed researchers to address factors influencing human disease and performance by the use of in-vitro and in-vivo preparations. A common methodology for assessing functional and mechanical properties of blood vessels are arterial ring preparations [163]. Because smooth muscle cells are oriented in circular fashion along the intima-media, a convenient means of measuring contractile and relaxant tension is using an arterial ring preparation [116, 163-165]. The general concept of this technique is as follows; two opposing supports (e.g. hooks, pins or wires) are inserted through the vessel, or the vessel is slipped over them. One of these supports is attached to a micrometer, allowing control of vessel circumference. The other support is attached to a force transducer for the measurement of tension development. The entire preparation is kept under *near* physiological conditions; at a temperature of 37° C, pH 7.4 and aerated with an O₂/CO₂ mixture [163]. Various physiological (i.e. electrical stimulation) and pharmacological (i.e. catecholamine infusion) treatments can be administered to the vessel and the vasoactive responses can be recording via force transducer readings [163] (**Figure A.12**). Following the discussion of macro-vascular assessment techniques will be a review of methodology assessing microcirculatory function.

- **A.4.3: Micro-Circulatory Assessments**

The microcirculation plays a crucial role in the interaction between blood and tissue both in the physiological and pathophysiological states [166]. The gold standard for assessment of

microcirculation has been considered intravital microscopy (IVM). However, this technique cannot be performed in patients because there is a need for the injection of fluorescent dyes and transillumination [166].

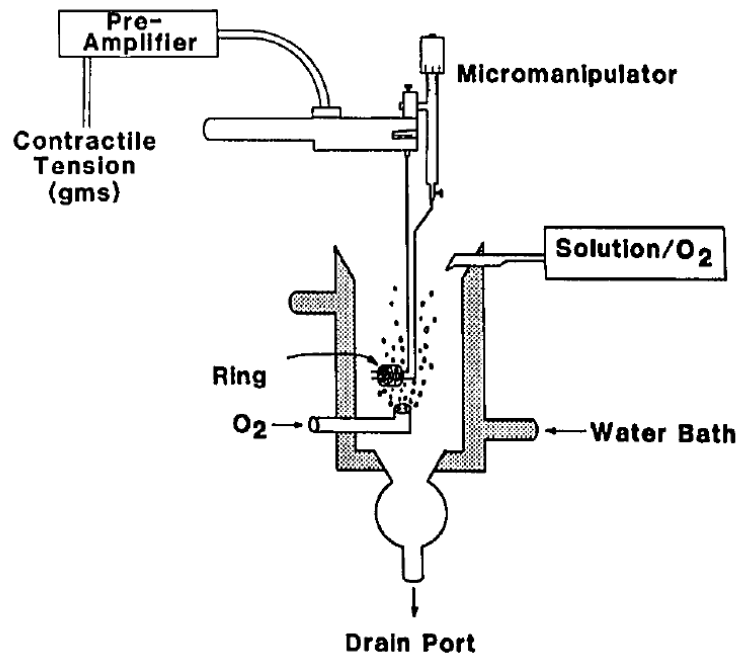


Figure A.12: Depiction of arterial ring preparation. Two opposing supports (e.g. hooks) are inserted through the vessel lumen, or the vessel is slipped over them. One of these supports is attached to a micrometer (micromanipulator), allowing control of vessel circumference. The other support is attached to a force transducer for the measurement of tension development. The entire preparation is kept under *near* physiological conditions; at a temperature of 37° C, pH 7.4 and aerated with an O₂/CO₂ mixture [163]. Various physiological (i.e. electrical stimulation) and pharmacological (i.e. catecholamine infusion) treatments can be administered to the vessel and the vasoactive responses can be recording via force transducer readings [163].

Recent advances have eluded toward non-invasive imaging of capillaries [166]. This technique is called Orthogonal Polarization Spectral Imaging (OPS). In general, OPS imaging involves a series of light reductions (**Figure A.13**). First, an application light, or *incident light*, is polarized onto a lens, then projected through a beam splitter and into the examined tissue (i.e.

skin flap) [166]. Most of the reflected light from the tissue will remain polarized and cannot pass through the analyzer (Orthogonal polarizer). The light that more deeply penetrates the tissue will scatter and depolarize. This depolarized wave of light can pass through the analyzer. This depolarized light illuminates the absorbed material of the foreground and projects it through a magnifier and video camera for visualization of the tissue structure (i.e. capillaries) [166].

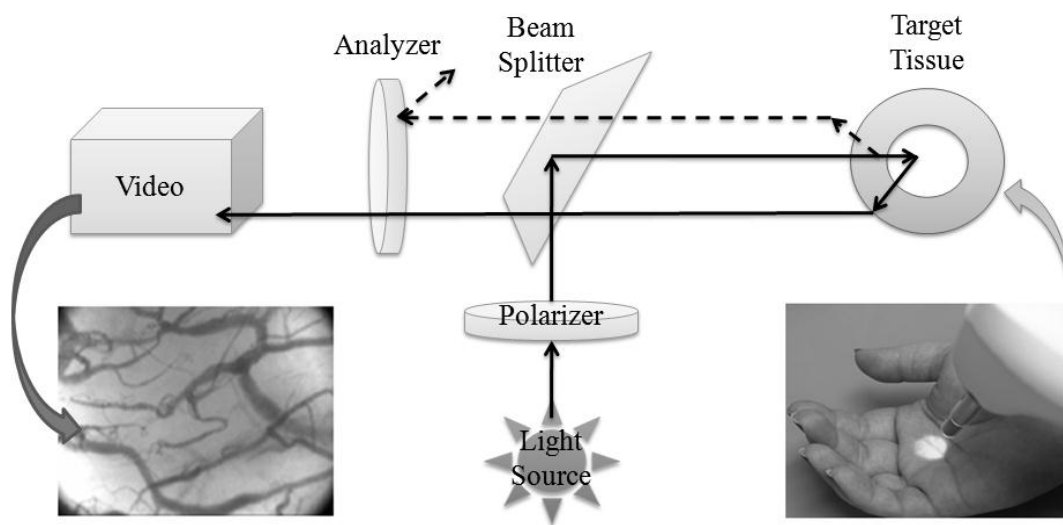


Figure A.13: Depiction of OPS imaging. Incident light is projected through a polarizing plane. The light is refracted towards the target tissue. The reflected ‘polarized’ light waves pass through the splitter and are reflected off of the analyzer and the deep, scattered ‘depolarized’ light waves pass through the analyzer, magnified and projected via video camera.

A wavelength of the emitted light (550 nm) was chosen to achieve optimal imaging of the microcirculation because at this wavelength oxy- and deoxy-hemoglobin absorb the light equally. Thus, the blood vessels of the microcirculation can be visualized by OPS imaging [166]. Orthogonal Polarization Spectral Imaging has been validated against conventional capillary microscopy at rest and during venous occlusion in healthy subjects [167], demonstrating that

OPS images provide similar values for RBC-velocity and capillary diameter as those measured by conventional capillary microscopy [167].

- **A.4.4: Assessing Regional Blood Flow**

Blood flow distribution through the vasculature involves interplay between local vascular controlling mechanisms, adequate blood flow delivery and return. In order to understand the mechanisms of vascular control as it related to health, disease and performance, one must examine the vascular tree thoroughly. Following the review of techniques for assessing macro- and micro-circulatory function, it is now appropriate to examine gross measures of regional blood flow. Venous occlusion plethysmography, or strain-gauge plethysmography, has been a popular tool in the assessment of regional blood flow (e.g. limbs) [148], in both health and disease [168]. When considering blood flow to a particular limb (e.g. forearm), the general idea behind plethysmography is measuring the accumulation of blood. By placing a collecting cuff around the upper arm and inflating it to a pressure less than diastolic will obstruct venous outflow without significantly altering arterial inflow [168-169]. Thus, the limb swells and the volume of the limb increases. The subsequent change in volume is most commonly recorded with the use of mercury strain gauges [169]. The thin mercury filled tube is wrapped around the experimental limb and a small electric current is passed through it. The tube is stretched when the limb expands during venous occlusion, thus reducing the diameter of the tubing and increasing the electrical resistance providing a voltage change which can be recorded [148]. 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. Strain-gauge plethysmography is a highly reproducible measure following reactive hyperemia and during 5 minutes of forearm occlusion [169-170]. The advantages of

using venous occlusion plethysmography is that it is relatively easy to use, non-invasive and low running costs [148]. Due to artifact from motion and of contracting a skeletal muscle accurate exercise blood flow measurements are limited to recording during rest intervals [148]. Regional blood flow can be measured accurately during exercise by using several different types of indicators which can be divided into intravascular indicators (e.g. indo-cyanine green dye, Evans-blue dye, labeled microspheres, and labeled albumin), freely-diffusible indicators that pass through cell membranes (e.g. ^{133}Xe , heat, N_2O and H_2 gas) and small hydrophilic extracellular indicators (e.g. sucrose, EDTA and insulin) which distribute in the interstitial space via small pores in capillary walls [148]. A majority of indicator methods have been restricted to animal studies. However, indocyanine green is an ideal tracer for human studies because it is non-toxic, soluble, has a relatively short half-life, and binds 95% to plasma proteins [171].

Near-Infrared Spectroscopy (NIRS) is another valid tool to assess regional muscle blood flow and is based upon the principle that near-infrared light (700-1000nm) passes through tissues with relative ease [172]. The amount of light recovered after illuminating the tissue depends upon the degree of scattering by the tissues and the amount of absorption by chromospheres in the tissue. Currently, only hemoglobin, myoglobin and cytochrome-c oxidase are known to effect near-infrared light absorption [172]. Using tracers, which absorb near-infrared light, it is possible, by using the Fick principle, to produce flow estimates. The rate of accumulation of a tracer in a tissue is the rate of inflow minus the rate of outflow. In conjunction with NIRS, Indocyanine green can be introduced into the venous circulation, where it is assumed to be evenly-mixed during passage through the heart and lungs, before entry into the arterial and tissue microcirculation. Blood can then be drawn from an artery to and when the amount of tracer and blood flow in the injection artery are known, the blood flow in the tissue region of interest can be

estimated from the peak of the recorded dye curves. When the curve reaches maximum height, the measured tracer concentration will reflect blood flow in that region [171]. NIRS is sensitive for detecting progressive changes in tissue perfusion introduced by incremental exercise and for assessing perfusion differences between tissue regions [172]. Drawbacks to NIRS include the high cost of the equipment and the current limitation of a trade-off between light penetration to deeper tissues and spatial resolution. Additionally, comparisons of human forearm blood flow using NIRS and venous occlusion plethysmography show good correlation [173], making it a valid and reliable measure.

- **A.4.5: Non-invasive Assessment Strategy**

In order to appreciate the techniques discussed in assessing vascular physiology one must apply them to a model. For example, a majority of studies have focused on the vasculature of a particular limb; for example the arm. We can assess both macro-vascular and micro-circulatory function, as well as regional blood flow (**Figure A.14**). By using Doppler Ultrasound, one can assess vascular reactivity in the conduit artery in response to localized forearm ischemia, induced by occluding downstream from the point of examination then releasing the occlusion. Additionally, an autonomic reflex, such as vasoconstriction can be assessed in response to a cold stimulus, such as placing ones hand in cold water for a period of time (cold pressor test). Blood draws can be taken from an antecubital vein to assess regional markers of NO^- (i.e. nitrite levels) and oxidative stress. Forearm regional blood flow can be assessed using strain-gauge plethysmography. Finally, microcirculatory function can be examined using OPS imaging of a skin flap in the hand or along the nail bed. Collectively, these assessments provide a regional investigation into one's vascular health and functionality.

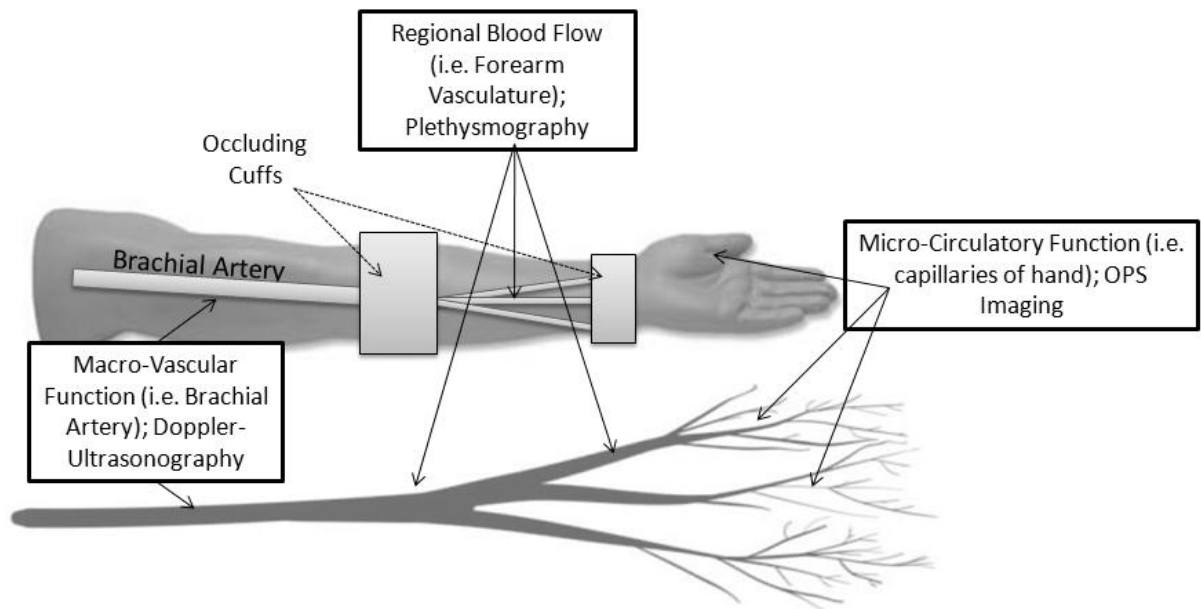


Figure A.14: Depiction of a vascular assessment model in humans. The chosen limb's (arm) vasculature can be assessed on a macro- and micro- level. Macro-vascular physiology (e.g. changes in diameter in response to flow induced shear and cold stimulus) can be assessed using Doppler Ultrasonography. Micro-circulatory function (e.g. capillary density, size and blood flow responses to shear and cold stimulus) can be assessed using OPS imaging of phalangeal skin flaps on the hand. Regional blood flow and venous capacitance of the forearm vasculature can be estimated using venous occlusion-plethysmography. The occluding cuff below the brachial artery is used to induce flow-mediated dilation and venous occlusion while the wrist cuff is used to occlude arterial flow to the hand.

• A.4.6: Summary

In summary, there are a number of research tools available to assess vascular responsiveness to various physiological stimuli. Vasoreactivity, in response to a period of ischemia, induced by occluding downstream from the point of examination then releasing the occlusion, and an autonomic reflex, such as placing ones hand in cold water for a period of time (cold pressor test) can be assessed on a macro-vascular (Doppler Ultrasonography of Conduit vessels) and micro-vascular (OPS imaging of arteriolar and capillary structure and flow) level. From both a clinical and research point of view, it is important to evaluate these physiological

responses at rest. However, in order to truly appreciate the role of the vasculature in physical function, one must stress the system. Following this review of assessments of vascular function and regional blood flow, it is now appropriate to discuss vascular responses to physical stress.

A.5 Vascular Responses to Exercise

- **A.5.1: Purpose**

Central cardiovascular control mechanisms are primarily for the maintenance of systemic perfusion whereas peripheral or local vascular control mechanisms are primarily for the maintenance of tissue homeostasis. Most of the vascular effects of exercise are related to supplying adequate oxygen and nutrients to working muscles [174] and an acute bout of exercise challenges the vasculature to quickly increase and sustain the delivery of blood to the contracting skeletal muscle [175]. This can be accomplished by an increase in cardiac output, decreases in vascular conductance in the skin and inactive regions (e.g. viscera) and an increase in vascular conductance to active (contracting) skeletal muscle [175]. This redistribution in blood flow is controlled by the balance between global sympathetic vasoconstriction and local vasodilatory factors. Therefore the purpose of this chapter will be to examine how the system responds to acute dynamic exercise, in order to quickly redistribute blood flow to the contracting skeletal muscle. The chapter begins with an examination into the general response of the central cardiovascular system, followed by peripheral vasoactive responses involved in the redistribution of blood flow.

- **A.5.2: Components of the Fick Equation**

Central cardiovascular function can be summarized with the Fick Equation...

$$\text{VO}_2 = \text{HR} * \text{SV} * \text{a-vO}_2 \text{ diff...}$$

...where VO_2 is oxygen uptake, HR is heart rate, SV is stroke volume and a-v O_2 diff is the arterial-venous oxygen concentration difference [31]. Central cardiovascular responses traditionally pertain to Q (L/min). Cardiac output (Q) is the product of HR and SV. Adjustments in either of these variables will influence Q. The arterio-venous oxygen difference (a-v O_2 difference) is considered a peripheral feature, because of its heavy peripheral influence. In consideration of cardiac rate, during dynamic exercise HR increases with exercise intensity and oxygen consumption (VO_2) [31, 176]. Typically, heart rates range from 70 beats/min at rest to approximately 180-200 beats/min at peak oxygen uptake (VO_2 peak) [176]. This increase is controlled by a combination of parasympathetic nervous withdrawal and sympathetic nervous stimulation. The sympathetic nerve activity begins to rise when cardiac parasympathetic (vagal) withdrawal is nearly complete, at a heart rate of ~100 beats/min [177].

Stroke volume has been considered a large determinant between an individual's Q and VO_2 peak [31, 176]. On average SV ranges from ~70 ml during upright rest to as high as 140 ml during exercise. Normal values for cardiac output at rest are approximately 5 L/min and increases to approximately 25 L/min during exercise in healthy adults [31]. In elite endurance athletes however, exercise stroke volumes can be as high as 200 ml. This increase can occur despite the reduction in diastolic filling time from 0.55 sec at rest (70 beats/min) to 0.12 sec during maximal exercise (195 beats/min) [31]. There are a number of factors which influence SV. These include intrinsic factors such as cardiac contractility and extrinsic factors such as preload (e.g. venous return) and after load (e.g. peripheral vascular resistance) on the heart. Increases in cardiac contractility can be attributed to an increase in heart rate, by a reduction in the diastolic relaxation phase and thereby reducing the time available for calcium clearance from the cell [110]. Additionally, increased activity of cardiac sympathetic nerves and increases in

plasma NE and E may also increase cardiac intropy [110]. Ventricular filling pressure or preload is the degree of stretch on the muscle fibers of the ventricular wall prior to systole. According to the Frank-Starling mechanism this is positively correlated with stroke volume, because as a muscle fiber is stretched it causes an increase in tension and ability to exert force. Aortic pressure is the pressure to be overcome by the left ventricle in order for blood to be pumped out of the heart. This is known as after load and determined largely by peripheral vascular resistance [31].

Another important predictor of VO_2 peak is the a-v O_2 diff. During dynamic exercise, total body a-v O_2 diff increases with exercise intensity and VO_2 . Typically, a-v O_2 diff ranges from 5 ml O_2 /100 ml blood at rest to approximately 15 ml O_2 / 100 ml blood at VO_2 peak [31]. These values are dependent upon factors such as the arterial oxygen carrying capacity (hematocrit), the ability of the respiratory system to load oxygen in the pulmonary capillaries, as well as maximal oxygen extraction by the fed tissues [174]. During intense exercise, there is a large increase in a-v O_2 diff, which is the result of a decreased mixed venous oxygen content [174]. An arterial oxygen content of approximately 20 ml O_2 /100 ml blood does not change with increasing exercise intensity but venous content falls considerably until approximately 85% of the oxygen is extracted at VO_2 peak.

Intense exercise also increases the hemo-concentration in the vasculature caused by an increase in arterial pressures, a plasma volume shift from the interstitium into circulation and a redistribution of cardiac output from tissues that extract small amounts of O_2 (e.g. liver and kidneys), to tissues that extract a large amount of O_2 (e.g. cardiac and skeletal muscle) [174]. During dynamic exercise, the working tissues also become more effective at extracting oxygen. At rest a small percentage of the total capillaries in a given tissue are open at any time, the

muscle fiber to capillary ratio is low and oxygen diffusion distances to feeding the muscle fibers are relatively high [106]. During dynamic exercise, the increase in muscle metabolism and oxygen demand causes a fall in tissue oxygen pressure (PO_2) with a subsequent increase in the diffusion gradient from blood to the tissue, resulting in a greater muscle fiber to capillary ratio and higher red blood cell transit time through the exchange vessels [106].

- **A.5.3: Local Regulation of Blood Flow during Exercise**

As previously mentioned, the control of exercising muscle blood flow is a careful orchestration of many parts and factors. At rest, approximately 5-10 mL of blood are distributed per 100 g of quiescent skeletal muscle [178]. Additionally, approximately 60% of the body's blood volume remains in the capacitance vessels [31]. During high intensity exercise active skeletal muscle blood flows have been reported to increase 150-500 mL/min/100 g of muscle tissue [178]. During the transition from rest to work (e.g. exercise) blood flow increases very rapidly in the exercising limb, quickly reaching a plateau at around 5-7 sec of exercise (*Phase 1*: **Figure A.15**) [179]. At ~15-20 sec following the onset of exercise a second but slower increase in skeletal muscle blood flow and velocity is observed which progresses toward a new steady state level (*Phase 2*: **Figure A.15**) [179]. The initial hyperemic response (*Phase 1*) has been considered to be the consequence of the mechanical interactions between contracting and relaxing skeletal muscle and blood vessels [175]. During a muscular contraction limb venous pressure significantly falls causing an increase in the arterio-venous pressure gradient across the vascular bed [31]. The term “muscle pump” refers to contraction-induced propulsion of blood from skeletal muscle veins, which facilitates venous return to the heart and a subsequent increase in perfusion of skeletal muscle (**Figure A.16**) [178].

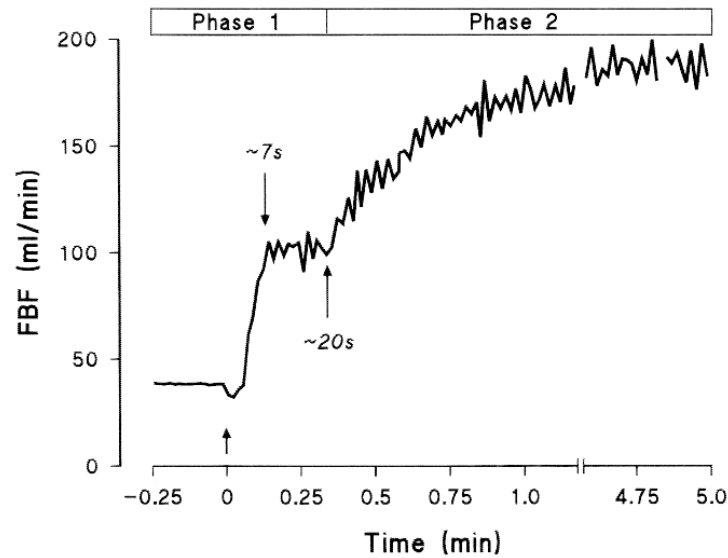


Figure A.15: The Rapid Biphasic Increase in Limb Blood Flow at the Onset of Exercise
Limb blood flow increases rapidly at the onset of exercise in a biphasic manner. An initial plateau following 5-7 s of exercise (Phase 1) gives way to a more slowly increasing segment following ~20 s (Phase 2) toward a new steady-state level. *FBF=Forearm Blood Flow*. Taken, with permission, from [179].

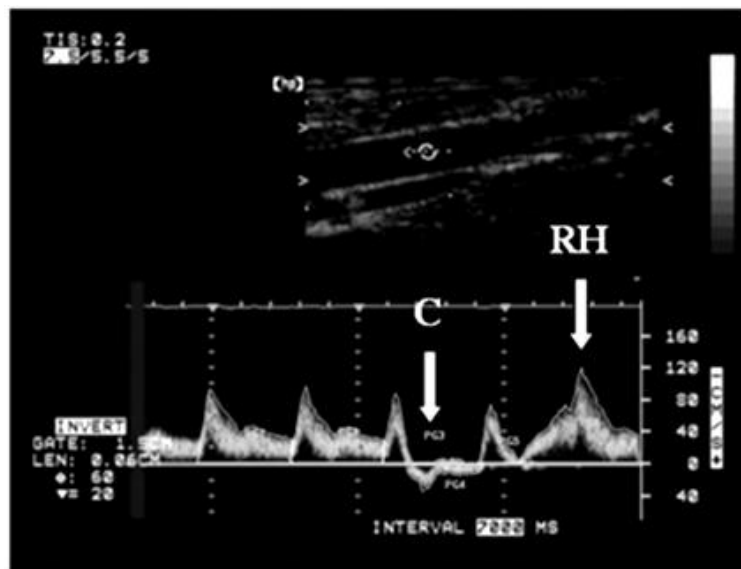


Figure A.16: Depiction of an ultrasound image of a brachial artery (top) and the blood flow responses to a single handgrip contraction (C). Note the increase in retrograde flow, with subsequent increase in blood flow, reactive hyperemia (RH).

The muscle pump is proposed to act via two mechanisms. First, the contraction phase of skeletal muscle causes compression of the veins [178]. Due to the valve orientation in the venous system, blood can only flow towards the heart. The refilling of this pump mechanism occurs at muscle relaxation as blood refills the veins [175, 178-179]. Second, the muscle pump alters the pressure gradient across capillaries by lowering venous pressure in the venules and deep small veins during the relaxation phase of the contraction cycle [31, 178] (**Figure A.17**). The veins are attached to the muscle tissue that surrounds them and are therefore pulled open when this muscle relaxes. Pressure is lowered by this increase in venous volume and by the fact that it should already be empty from the previous contraction [178].

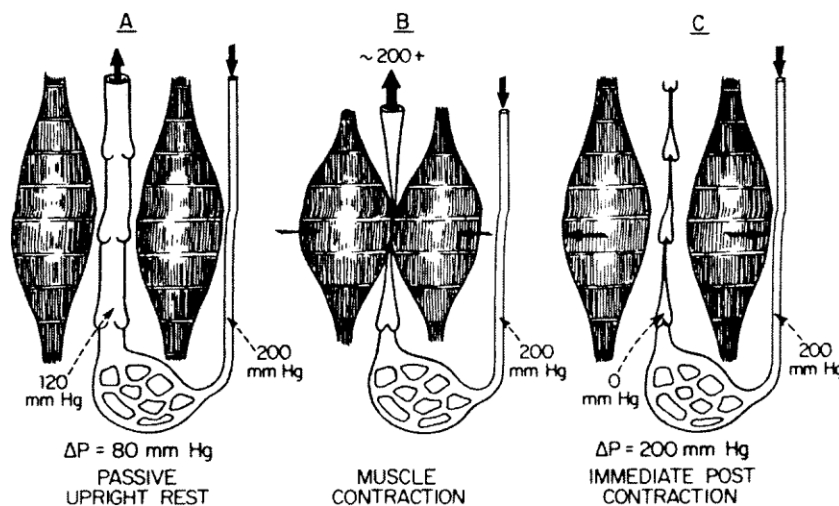


Figure A.17: Illustration of muscle pump effect on venous return and muscle blood flow to active muscle tissue. A: hemodynamic conditions for a skeletal muscle of lower limb of a subject in upright posture, at rest. DP, change in pressure. B: effects of muscle contraction on vasculature. Muscle contraction forces blood out of venous segments, increasing venous driving pressure toward the heart, and blocks arterial inflow. C: conditions believed to exist immediately after muscle contraction. Note that pressures in small veins fall to zero and that, because of venous valves, refilling must occur from the arterial system through the capillary bed. The net effect of the muscle pump is to increase driving pressure for blood flow through muscle and to enhance venous return to the heart. Taken, with permission, from [31].

This could be interpreted as sequential filling of venous compartments following contraction, from the first venous valves in the small veins to the systemic veins. If the next contraction is sufficiently delayed the venous bed will completely refill. If not, venous pressures will remain decreased for the next relaxation phase [178]. The two actions of the muscle pump are only effective during rhythmic contractions, whereas isometric contraction results in an increase in resistance to flow [174-175, 178]. The large increase in blood flow that follows 15-20 sec (*Phase 2*) of early exercise hyperemia, has been suggested to be under feedback regulation by local metabolic factors and endothelial mediated factors [179]. It could be hypothesized that the increase in blood velocity and shear stress in response to *Phase 1*, would stimulate the production and release of prostaglandins (PGI-2) and NO⁻ [145]. There is normally a close relationship between skeletal muscle metabolic rate and muscle perfusion. Having considered mechanical contributions of the muscle pump we must now consider substances released from the tissues or blood which could initiate vasodilation.

In addition to increasing venous return at the onset of exercise, muscular contractions also increase shear stress and alter the flow patterns within the artery in such a way that it triggers the production and release of vasoactive substances (e.g. NO⁻) from the endothelium and diffuse into the smooth muscle causing vasodilation and further increases in blood flow through the contracting muscle [179]. However, recent evidence suggests that the vasodilatory response can begin in *Stage 1* in conjunction with the muscle pump. The dilatory response to muscle contraction may not be uniform along the vascular tree, and may occur to a greater extent in the small arterioles [106]. From distal sites along the skeletal muscle vascular tree, the dilatory response may ascend proximally, from terminal arterioles which govern total flow into the muscle bed, towards larger segments, predominately through endothelial and smooth muscle

cell-to-cell communication, soon following the first muscular contraction [180]. Following repeated muscular contractions, a variety of substances with vasodilator properties are also released from active skeletal muscle including ions and metabolites [181].

The mechanisms for metabolic dilation must involve either a direct effect on vascular smooth muscle tone or by interference with adrenergic constrictor influences, which was discussed in *chapter 3* [179]. Metabolites from active skeletal muscle diffuse into the interstitium and resistance arteries, increasing capillary recruitment [175]. The increased blood flow and oxygen extraction that result from metabolic vasodilation increases oxygen supply to a level compatible with tissue oxygen demand [175]. The most likely signal for early induced vasodilation is potassium ions (K^+) [175]. K^+ is released into the interstitial fluid as a result of skeletal muscle repolarization following contraction and its accumulation can mirror hyperemia to that muscle [175]. Hydrogen Ion (H^+) production is another potential mediator of early hyperemia [175] and can act by reducing the pH of the interstitial space and within the arterioles, thus stimulating terminal arterioles to open, allowing fresh blood to enter, while removing the excess ions. In addition, Adenosine releases from hydrolyzed ATP has also been proposed to contribute to the hyperemic response [175].

In conjunction with vasodilatory responses, an overlooked property of the vasculature is the arteries ability to vasoconstrict. When considering blood flow distribution to inactive beds during exercise, the response is to decrease, thus allowing greater redistribution of flow to the exercising regions. For example, a classic study by Qamar and Read (1987) examined the effects of acute, moderate intensity, treadmill exercise on superior mesenteric blood flow, as measured by Doppler Ultrasound. After treadmill exercise, superior mesenteric artery blood flow was significantly attenuated [182] (**Figure A.18**). In addition, Musch and Poole (1996) examined

blood flow responses in rat spinotrapezius muscle (inactive) to treadmill running and found that regional blood flow, as assessed by radioactive microspheres, was significantly reduced during exercise [183].

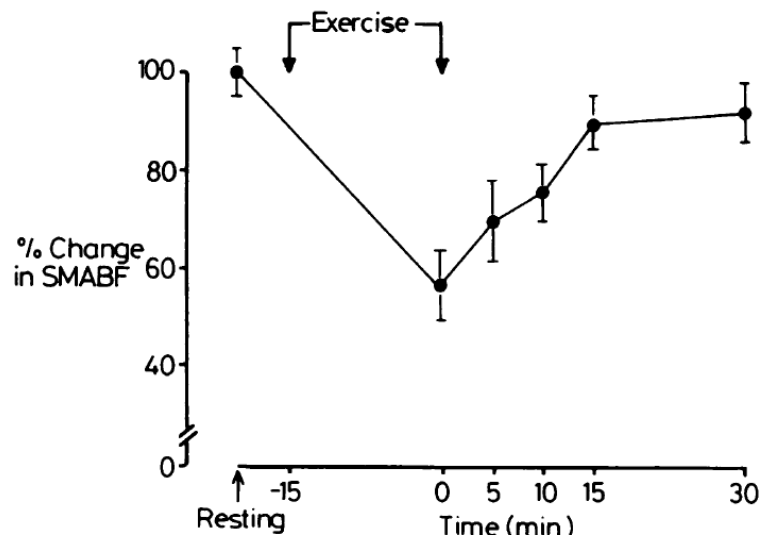


Figure A.18: Percentage change in superior mesenteric artery blood flow (SMABF) after treadmill exercise in 16 normal subjects. Taken with permission from [182].

The most likely explanation for this reduction in blood flow to the non-exercising regions, is that during rest, sympathetic tone is relatively low but contributes to basal vascular resistance (along with myogenic effects), yet during dynamic exercise, sympathetic outflow increases dramatically resulting in an elevation in circulating NE [177] (**Figure A.19**).

This is at first advantageous, in that the maintenance of ventricular filling pressure depends not only on active mobilization of blood volume out of veins but also on the extent to which blood volume is redistributed away from compliant beds (e.g. mesenteric circulation) by vasoconstriction [177]. All vascular beds receive sympathetic stimulation, including the active skeletal muscle [106]. However, the concept that muscular activity may alter the responsiveness

of the vasculature to vasoconstrictor substances has been referred to as “*functional sympatholysis*” [112].

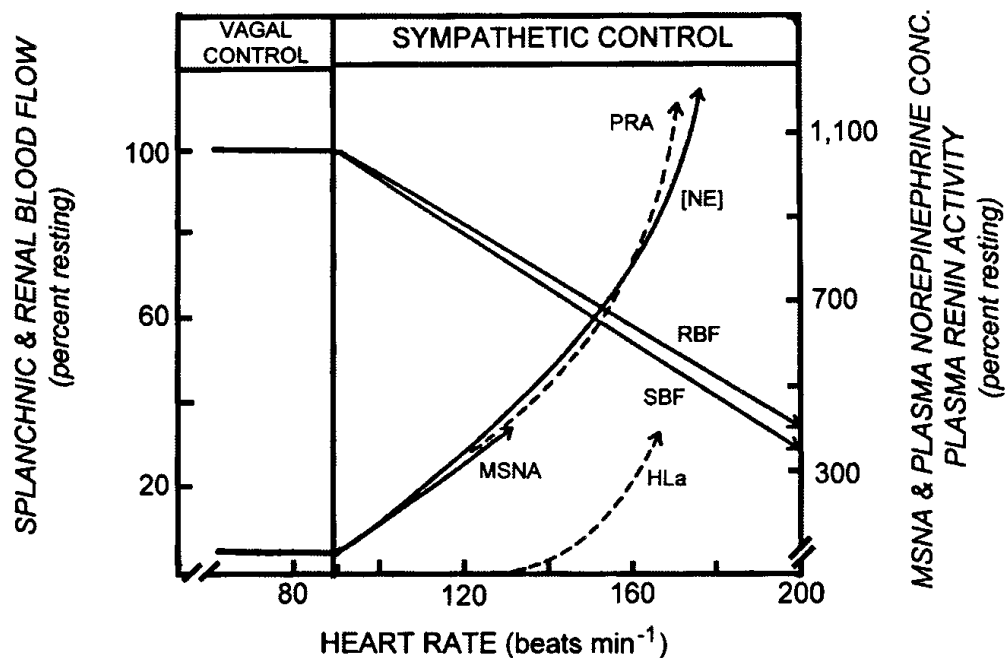


Figure A.19: Illustration of effects of increasing exercise intensity on sympathetic control of blood flow and vascular conductance in humans, reflected in measurements of heart rate. Note that at rest, when heart rate is low, the heart is under vagal (parasympathetic) control. Sympathetic nervous system activity increases as vagal control is withdrawn. Sympathetic nervous activity increases with increasing exercise intensity as reflected in decreases in splanchnic (SBF) and renal blood flow (RBF) to vascular beds, increased plasma norepinephrine concentrations ([NE]), increased plasma renin activity (PRA), and increased muscle sympathetic nerve activity (MSNA). Blood lactate concentration (HLa) does not increase until exercise intensity is 50–60% of maximal (heart rates of 130–140 beats/min). Taken, with permission, from [31], as adapted from Laughlin [174] and Rowell [177].

In general, the term functional sympatholysis describes the interaction between a vasoconstrictor stimulus (e.g. sympathetic activation) and a vasodilator stimulus (e.g. muscle contraction). The net effect of this interaction or the degree of sympatholysis, will vary depending on both the strength of the vasoconstrictor stimulus and the intensity of the muscle contraction [111]. As discussed in Chapter 1, differences in the degree of sympatholysis between arteries of different caliber have alluded to the concept of heterogeneity of distribution of α -1 and α -2 receptors within the microcirculation [111, 132]. Large resistance arterioles are served by both α -1 and α -2 receptors, whereas small nutrient arterioles are served by predominantly α -2 receptors [130]. This becomes more intriguing when we consider the α -2 receptors are much more sensitive to metabolic inhibition than the α -1 receptors [130, 184]. Therefore the response to sympathetic nerve stimulation and the susceptibility of sympathetic vasoconstriction to inhibition by muscle fiber activity appears greatest in distal versus proximal arterioles [130, 184].

- **A.5.4: Summary**

In summary of this section, maximal oxygen consumption (VO_2 peak) is dependent on cardiac output (Q), the ability to redistribute blood flow to contracting skeletal muscle and the maximal ability of the tissue to extract and utilize oxygen (a-v O_2 difference) from the delivered blood. It appears that the vasoactive response of redistributing blood occurs with the first muscular contraction (e.g. muscle pump) and propagates through each vascular compartment. Vasoactive metabolites are released within the resistance vasculature further contributing to this vasodilator system. The amplification of this vasodilator system could be ascending metabolic influences on larger proximal arteries and/or shear induced release of NO^- and prostaglandins (e.g. PGI-2) [179]. A summary of the different phases of the acute hyperemic response are

depicted in **Figure A.20**. Further increases in the rate of contractions and/or duration, increases sympathetic nerve activity, resulting in an elevation in heart rate, an increases in the vasoconstrictor system and a redistribution of blood flow from inactive towards metabolically active vascular beds (functional sympatholysis) [112].

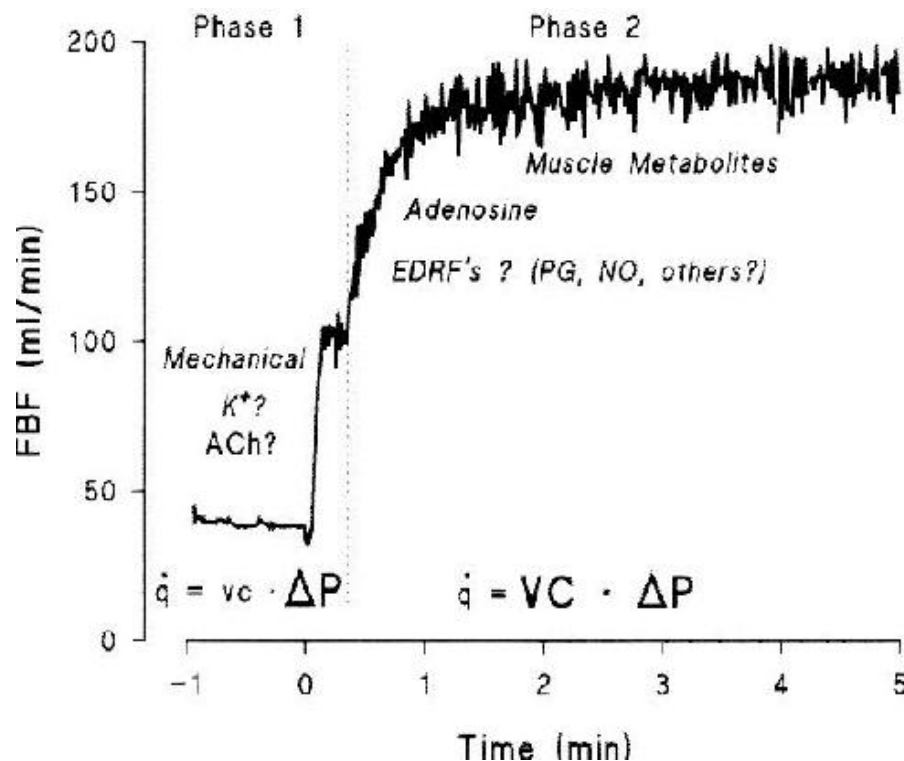


Figure A.20: Working hypothesis for the control of limb blood flow adaptation following the onset of contractions in humans. Limb blood flow (q) is the product of vascular conductance (VC) and the pressure gradient across the capillary bed (ΔP). In Phase 1 the flow response in the first 5-7 s of exercise is determined primarily by a change in ΔP by the muscle pump in combination with a smaller change in vascular conductance resulting from vasodilation. The mechanism of the early dilation is unknown but may involve either potassium ion (K^+) or acetylcholine (Ach). Greater feedback regulation of the flow response is likely to begin in Phase 2 where adenosine, endothelial-derived dilators, and metabolic factors may exert greater changes in vascular conductance. PG, prostaglandins; NO, nitric-oxide. Taken with permission from [179].

The degree of vascular responsiveness among similar vascular compartments of differing organ systems may not be homogenous. For example, blood flow to skeletal muscle and the heart increase dramatically during high intensity exercise, simultaneously, renal and splanchnic blood flow decreases. This can be, in part, be achieved by an increase in metabolic rate and vasodilation within the contracting muscle; second, by vasoconstriction via activation of α -adrenergic receptors, of the splanchnic and renal circulation [185]. This high vasoconstrictive response is the consequence of dense sympathetic (adrenergic) innervation of the splanchnic and renal, and mesenteric vasculature [132]. If we revisit **Figure A.7**, within *Chapter 1*, we can visualize that there is some heterogeneity within the vasculature that we should appreciate. It appears to be very advantageous to increase the vasoconstrictive responsiveness of a splanchnic or renal vascular bed but not in regularly active skeletal muscle. In order to determine whether these vasoconstrictive and vasodilatory responses can change, the focus of this next chapter will be to examine vascular adaptations to chronic exercise training.

A.6 Vascular Adaptations to Chronic Exercise Training

- **A.6.1: Purpose**

In response to repeated exposure to exercise, as with exercise training, the vasculature must adapt in order to ensure adequate delivery of blood flow to the contracting skeletal muscle. This adaptation can be achieved through changes in the vasodilatory and vasoconstrictive behavior of the vasculature. The purpose of this chapter will be to discuss how the vasculature adapts and alters its responsiveness to chronic exercise training. The chapter will begin with central cardiovascular adjustments to training, and progress into peripheral adaptations, including alterations in vasodilation and constriction.

- **A.6.2: Adjustments in Fick Equation Components to Exercise Training**

When considering central cardiovascular adjustments to chronic exercise training, repetitive bouts of exercise challenges the cardiovascular system to increase the delivery of oxygen and metabolic substrates toward, as well as the removal of metabolic by-products from the active skeletal muscle [186]. The increase in VO_2 peak following exercise training is accompanied by classic adaptations in central and peripheral mechanisms including exercise tolerance, skeletal muscle oxidative capacity, cardiac output, cardiac hypertrophy, bradycardia, and arterial and venous capacitance [22].

Approximately 5.5 liters of blood is needed to supply the body at rest [110]. Sedentary individuals have a resting stroke volume of approximately 75 ml. In athletes, such as a marathon runners, stroke volume can be as high as 105 ml at rest [110]. In sedentary individuals resting HR tends to be approximately 70 beats per minute, however because the conditioned athletes SV is much larger (~40 beats per minute) [31]. Normal heart rate values at peak exercise are similar for both sedentary individuals and athletes at approximately 200 beats per minute. The current literature suggests that central myocardial adaptations relate to changes in contractility, as well as increases in end-diastolic, ventricular volume (preload) [176]. Normal values for Q during peak exercise in sedentary individuals is approximately 25 l/min whereas in endurance athletes it can be as high as 42 l/min [187]. The higher peak cardiac outputs seen in trained individuals are thought to be related to peak stroke volume [31] which is, in part, the result of greater venous return (preload), myocardial contractility and a decrease peripheral resistance or after load on the heart. Adaptations in a- vO_2 diff generally relate to peripheral vascular adaptations and intrinsic changes in skeletal muscle metabolism, together allowing the tissue to become more efficient in extracting and utilizing the delivered O_2 [31].

- **A.6.3: Alterations in Vasoreactivity in Response to Training**

Vasoreactivity, as defined by an artery's responsiveness to a vasodilatory and vasoconstrictor stimulus, is mediated through the actions of the endothelium, vascular smooth muscle cells, and connective tissue elements in its wall [121, 142, 188-189]. Vascular smooth muscle is the final common pathway for vasoactive control of diameter of the arteries, veins, vascular resistance, and therefore, distribution of blood flow. As was discussed in *Chapter 2*, a non-invasive measure of vascular health and function is endothelial-dependent dilation (EDD) in response to ischemia. Exercise training has shown to increase maximal blood flow capacity, and improve EDD in conduit and resistance vessels, in both humans and animals [5, 21, 142]. For example, Volianitis and colleagues found that arm vascular conductance in elite rowers [190] was greater than in average fit subjects [106]. Furthermore, Allen et al. (2003) have reported a significant improvement in EDD following 4 training sessions, and the improvements continued up until 4 weeks of training [5]. Interestingly, these vascular adaptations were specific only to the trained limb, with no significant improvements in the untrained limb. This is an interesting finding, in that the distribution of vascular adaptations seem to occur only in regions of increased activity (e.g. increased metabolic rate and blood flow) [106, 154], however this area has recently been debated. Green et al. (1996) have investigated regional specific adaptations in tennis players and have reported higher vasodilator and blood flow capacity in preferred, versus non-preferred, limbs [191]. However, additional reports have observed significant forearm vasodilator and blood flow capacity after lower limb training [83, 192]. Recent investigations have alluded to the fact that localized exercise training, involving a large muscle mass, will alter the pattern of blood flow and shear stress through the non-exercised limb or region [11, 49, 83, 193] in such a way, that it may modulate endothelial function. For example, training a large

muscle mass, such as the legs, may in fact alter the arterial flow pattern of the brachial artery in such a way that it mimic exercise training itself [194-195]. However the magnitude of changes seen in inactive, or less active, vascular beds seems to be less than that if the region were trained directly. The repetitive exposure to contraction induced shear stress, through repeated training sessions, seems to be the trigger for conduit artery modification [21-22, 83].

It has been high-lighted in several reviews that in response to intermittent episodes of high shear stress, endothelial derived relaxing factors, particularly NO^- , are released from the endothelium and contribute to smooth muscle dilation [11, 21-22, 107]. Chronic exercise training has been shown to up regulate the Nitric Oxide dilator system and the first event appears to be an increase in NO^- production and bioactivity. Shortly thereafter, appears to be an up-regulation in endothelial derived nitric oxide synthase (e-NOS). Following long-term exercise training (up to 8 weeks and beyond), vascular remodeling may occur as a result of a chronic increase in vessel diameter which ‘structurally-normalizes’ the shear stress imposed by muscular contractions [21], thus NO^- function returns toward baseline levels (**Figure A.21**).

The underlying mechanism for vascular remodeling in response to chronic exercise training is unclear, but appears to be NO^- mediated. A potential answer to this question may be found in a recent review by Martinez-Lemus et al. (2008). The review encompasses micro-vascular remodeling however, it is reported that the vascular wall exhibits a remodeling continuum, allowing the vascular wall to rapidly modify its structural characteristics in response to its environment, through the re-positioning of vascular smooth muscle cells, in order to achieve ‘tensional homeostasis’ [196]. For example, in-vivo high flow and shear seems to stimulate ‘outward hypertrophic’ remodeling (increase in diameter). However in-vivo low flow seems to stimulate inward remodeling.

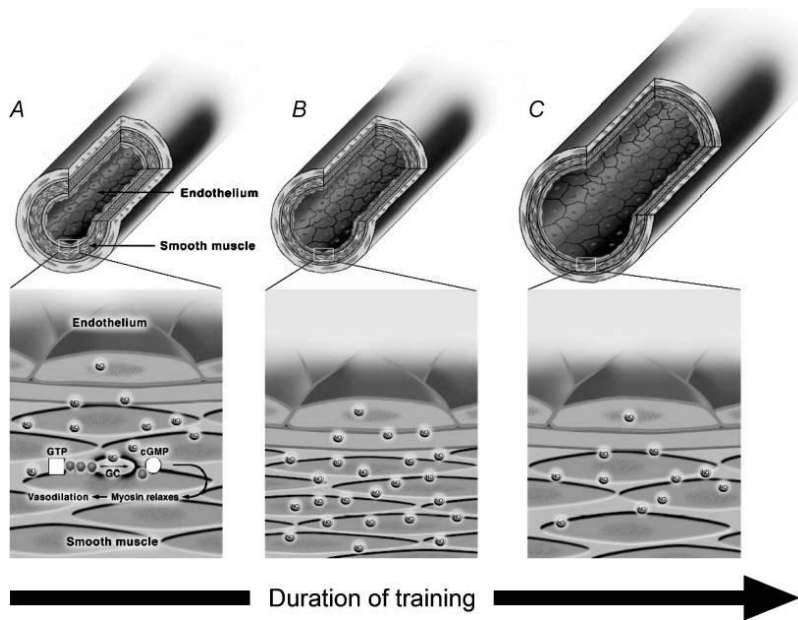


Figure A.21: In the untrained vessel (A), basal release of NO causes subjacent smooth muscle cell vasodilation which acts to homeostatically regulate wall shear. In response to medium-term exercise training (B), acute increase in shear stress, associated with repetitive exposure to increased flow during bouts of exercise, stimulates increased endothelial NO production and consequent vasodilation. Up-regulation of the NO-dilator system, including eNOS expression, occurs to buffer increased shear stress. Following long-term exercise training (8), structural adaptation occurs, possibly in part due to NO-mediated remodeling, resulting in chronic increase in vessel caliber which ‘structurally normalizes’ shear stress. NO function returns towards baseline levels. Taken with permission from [21].

Interestingly, if the vasculature remodels and alters its function in response to the environment from which is imposed upon it, then an alteration in the shear rate may manipulate any adaptations that may occur to training. For example, recent data from our laboratory examined the effects of 4 weeks of bilateral handgrip exercise, while partially occluding blood flow in one of the upper arms. Vascular function was assessed in the brachial artery by examining the vasodilatory response to ischemia (BAFMD). Following training, BAFMD was significantly reduced in the cuffed arm while improvements were observed in the non-cuffed arm [8]. More recently, Tinken et al. (2010) had a group of individuals perform a similar exercise intervention for up to 8 weeks and found no significant improvements in BAFMD in the cuffed arm. The authors attributed the differing vascular responses in the limbs to alterations in the shear pattern imposed on the arterial wall [197]. The results from these studies [8, 197] demonstrate that exercise-induced changes in shear provide a principal physiological stimulus to adaptation in flow-mediated endothelial function in response to exercise training in humans.

There also appears to be changes in the micro-circulation in response to training, consisting of greater capillary recruitment and an increase in capillary density [106-107]. The mechanism seems to involve new capillary recruitment and/or the formation of new vessels. Some proposed signals for the formation of new vessels have been an increased release of specific growth factors, particularly Vascular Endothelial Growth Factors (VEGF), an increased metabolic rate or 'hypoxia', within the muscle interstitium, as well as increases in flow and shear (NO⁻ mediated) [106, 198]. This hypothesis has been explored by Hellsten and colleagues, who have reported an increased release of VEGF and increased e-NOS expression in human muscle tissue in response to knee extensor exercise [199]. Resistance artery remodeling has also been

proposed to occur in response to increases in blood flow and shear rate through the vessel [107] as well as enhanced sensitivity to metabolites, particularly adenosine [106].

In consideration of vasoconstrictive responsiveness in exercise trained regions, it is surprising that very few studies have addressed the effects of training on vascular responsiveness to adrenergic stimuli. In humans, some evidence has suggested that training does not alter sympathetic mediated constriction at rest, but reduces the response in a trained limb during moderate intensity exercise [35]. This would make sense, in that adaptations within the trained muscle should be balanced towards greater vasodilation, but a greater –constrictor response should be observed in the untrained limb or region. Interestingly enough, Laughlin and colleagues [106] have observed heterogeneity in vasoconstrictor responsiveness, of the resistance vasculature of rat gastroc-nemius muscle, in response to training. They reported that high intensity, interval sprint training decreased the vasoconstrictor responses of gastroc- feed arteries to phenylephrine, but increased the constrictor responsiveness of fast-twitch (white) fiber arterioles [106]. In addition, Donato and colleagues have reported the endurance training in rats decreases the vasoconstrictor responsiveness of gastrocnemius arterioles to NE [200]. These data, collectively, indicate the each type of skeletal muscle (e.g. fast red, fast white and slow oxidative) may alter the intrinsic responsiveness of its vasculature to accommodate greater redistributions of flow via different recruitment patterns during exercise [106, 200].

An examination into an artery's vasodilatory and constrictor properties may provide insight into the ability of the vasculature to distribute blood during times of high demand, such as occurs during acute exercise. In an attempt to investigate this hypothesis, our laboratory has recently observed a direct relationship between arterial reactivity (i.e. magnitude of change in brachial diameter following occlusion and a cold stimulus) and aerobic fitness (VO₂ Peak). In

highly trained athletes, the vascular operating range (absolute change in brachial diameter following occlusion and a cold stimulus) was significantly greater than that of normal controls and even greater from that of heart failure patients. A well-adapted vasculature, defined by a wide vascular operating range, appears to promote higher aerobic fitness (**Figure A.22**).

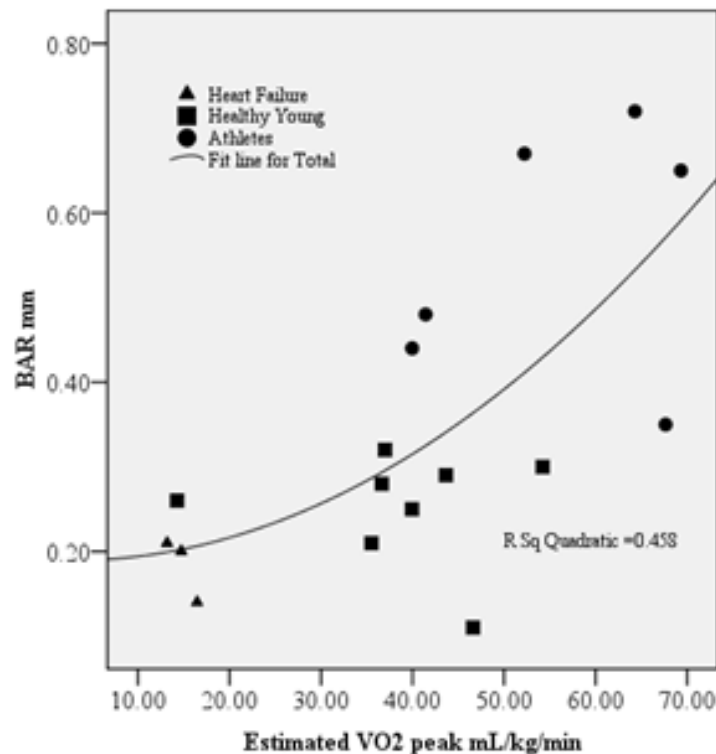


Figure A.22: Presentation of the vascular operating range (VOR) across fitness levels in Healthy Young, Heart Failure and Athletes.

When considering the vascular responsiveness to training in the non-exercised regions (e.g. splanchnic, renal and mesentery), it could be hypothesized that the vasculature in these regions may alter its vasoconstrictive responsiveness to accommodate a greater redistribution of blood flow to the exercising limb. McAllister et al. (1996) examined the responses of visceral

conduits (e.g. mesenteric, renal, splanchnic and hepatic arterial rings) in swine following chronic exercise training and found no change in endothelial function (as measured by vasodilatory responses to sodium nitro-prusside and adenosine). However, contractile responses of the renal splanchnic arteries to NE did decrease, along with no significant changes in vasoconstrictor responses to NE in the superior mesenteric and hepatic arteries [185, 201]. This is an interesting finding in that contractile responsiveness only changed in the renal and splanchnic regions. Greater splanchnic and renal blood flows during acute exercise following training may potentially be beneficial in regards to metabolism. An increased splanchnic blood flow in the trained state may confer benefits for glucose metabolism during prolonged exercise [185], while increases in renal blood flow will assist in the filtration process and the removal of metabolic waste. Another train of thought could be that total blood volume also increases with exercise training. An increase in blood volume can be observed within 24 hours following exercise and can increase an additional 10% above pre-training by 1 to 4 days of training [202]. After approximately 2-3 weeks of training, erythrocyte volume expansion occurs [202]. With higher blood volumes, there is likelihood that all vascular beds may increase maximal blood flow capacity. Another possibility is that sympathetic nervous system outflow is less to the vasculature of these beds in the trained state. Also, given the same absolute workload, the overall intensity (e.g. % VO_2 peak) may be lower following exercise training, and if we refer back to **Figure A.5**, sympathetic outflow (i.e. plasma NE concentrations) is directly related to the relative exercise intensity [177]. This explanation appears likely as splanchnic and renal blood flow during acute exercise decreases to a lesser extent from resting flows after a period of endurance exercise training [185]. Furthermore, improved vasodilatory responses of active

vascular beds have been observed and may result in greater reductions in splanchnic and renal flow.

- **A.6.4: Summary**

In summary, exercise-induced vascular adaptations include increases in maximal blood flow capacity, which may be dependent on the alterations in vasodilatory and vasoconstrictive properties of large arteries, arterioles and capacitance vessels. The relative contributions of these adaptations and distribution throughout the vascular tree are not uniform. Depending on the mode, intensity, duration of training and region exercised the mechanisms can be activated to differing degrees in order to achieve homeostasis and adequate perfusion. Exercise-induced changes in vasodilatory behavior include increases in EDD of large conduits and arterioles. The signals for this adaptation stem from muscular contraction-induced increases in shear as well as increases in tissue metabolism. **Figure A.23** below summarizes endothelial dependent changes to exercise within each vascular domain, as well as in different regions of the vasculature.

The vasoconstrictor properties of the artery, in conjunction with vasodilatory properties, assist in redirecting blood flow during exercise and appear to be altered with training. Changes in vasoconstrictor responsiveness have been observed in both exercised and non-exercised vascular beds. The available information indicates that training decreases vasoconstrictor responsiveness in a trained limb, or region, allowing greater blood flow into the metabolically active tissue. Vascular responsiveness of non-exercising tissues, such as of the kidney and splanchnic circulation, appears to be modified in response to training, owing to increases in blood flow following training to splanchnic and renal circulation, which is likely an adaptation to assist in metabolism and blood glucose regulation in response to endurance training. When comparing overall vascular reactivity (vasodilatory and constrictive responsiveness) between groups of

trained, un-trained and diseased individuals, a higher vascular operating range is associated with greater overall health and fitness, and is important for the redistribution of blood flow during exercise.

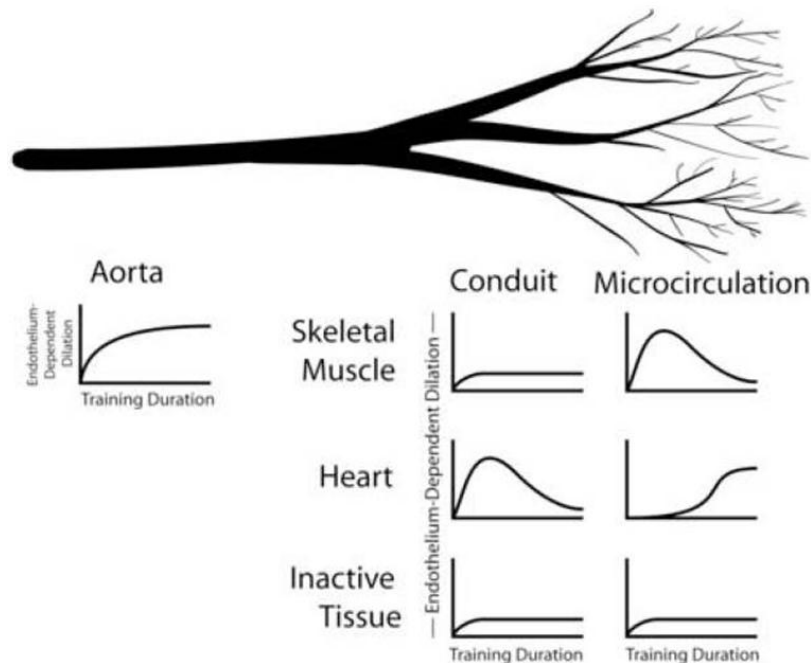


Figure A.23: Interaction between organ, vessel size, and training duration on the increase in endothelial-dependent dilation resulting from exercise training. A small deflection above baseline indicates small or inconsistent increases in endothelial function. A large deflection above baseline indicates larger and consistent increases in endothelial function. Taken with permission from [107].

A.7 Factors Altering Blood Flow Control

• A.7.1: Purpose

Previous sections of this review have highlighted on the importance of the vasculature's ability to balance vasodilation and vasoconstriction in the control of blood flow. Importantly, having a high degree of vasoreactivity translates into greater blood flow to exercising muscle and

a higher functional capacity. In consideration of the other chapters, there are factors which may compromise blood flow distribution. An unhealthy lifestyle including excess caloric intake, lack of exercise, smoking and excessive alcohol consumption increases ones risk of developing disease [203] and prolonged physical inactivity, in particular, has many detrimental effects on the cardiovascular system [204]. It is proposed that humans inherited genes that were evolved to support a physically active lifestyle and it is further postulated that physical inactivity in sedentary societies directly contributes to multiple chronic health disorders [205]. A potential mechanism by which physical inactivity mediates the progression of cardiovascular disease is likely to originate as a disturbance on the vascular wall (endothelium).

Aging and diseases of the cardiovascular system have been associated with a decline vascular and physical function, and exercise intolerance. These limitations seem to stem from a vascular system that is incapable of shunting blood flow to active vascular beds. Therefore, the purpose of this chapter will be to discuss instances where the control of blood flow may be restricted, and how exercise may be the key to unlocking these restrictions.

- **A.7.2: Aging and Blood Flow Control**

The proceeding chapter in this section will discuss the factors associated with aging, in particular, ‘sedentary’ aging. Aging is influenced by a number of different factors, including genetics, environment and lifestyle. Depending on any alteration in these factors, the rate of aging may be exacerbated or reduced. Lakatta et al. (2003), in their work taken from the Baltimore Longitudinal Study on Aging, as well as data from our laboratory, have indicated the effects of aging on the vasculature and include a decline in EDD of conduit vessels in response to arterial occlusion [10, 206], an alteration in the hemodynamic forces imparted on the

endothelium [49], an increase in vascular thickness and stiffness (as measured Carotid Intima-media thickness-CIMT and PWV) [4], and an altered regulation of vascular tone [25]. **Table A.1**, taken from Lakatta et al. summarizes the age related changes in vascular structure and function. The consequence of these age related vascular changes may be a shift in the vasoactive balance towards higher vasoconstriction at rest, which could limit tissue perfusion [29] with a subsequent decline in physical functionality (i.e. VO₂ Peak) [4, 10, 23-24, 51].

Table A.1: Summary of the age related changes in vascular structure and function. Taken with permission from [4].

Relationship of Vascular Human Aging in Health to Vascular Diseases

Age-Associated Changes	Plausible Mechanisms	Possible Relation to Human Disease
Vascular structural remodeling		
↑ Vascular intimal thickness	↑ Migration of and Ý matrix production by VSMC Possible derivation of intimal cells from other sources	Promotes development of atherosclerosis
↑ Vascular stiffness	Elastin fragmentation ↑ Elastase activity ↑ Collagen production by VSMC and ↑ Cross linking of collagen Altered growth factor regulation/tissue repair mechanisms	Systolic hypertension Left ventricular wall thickening Stroke Atherosclerosis Left ventricular hypertrophy
Vascular functional changes		
Altered regulation of vascular tone	↓ NO production/effects	Vascular stiffening; hypertension Early atherosclerosis
Reduced physical activity	Learned lifestyle Frailty	Exaggerated age changes in some aspects of vascular structure and function, eg, arterial stiffening Negative impact on atherosclerotic vascular disease, hypertension and heart failure

VSMC indicates vascular smooth muscle cell.

The vascular endothelium, in aging, is a key element in maintaining vascular homeostasis [4, 40]. Data from our laboratory, as well as others, has indicated a reduction in EDD with advancing age (**Figure A.24**). Importantly, this reduction in vascular function in the elderly is also related to a decline in physical function (**Figure A.25**).

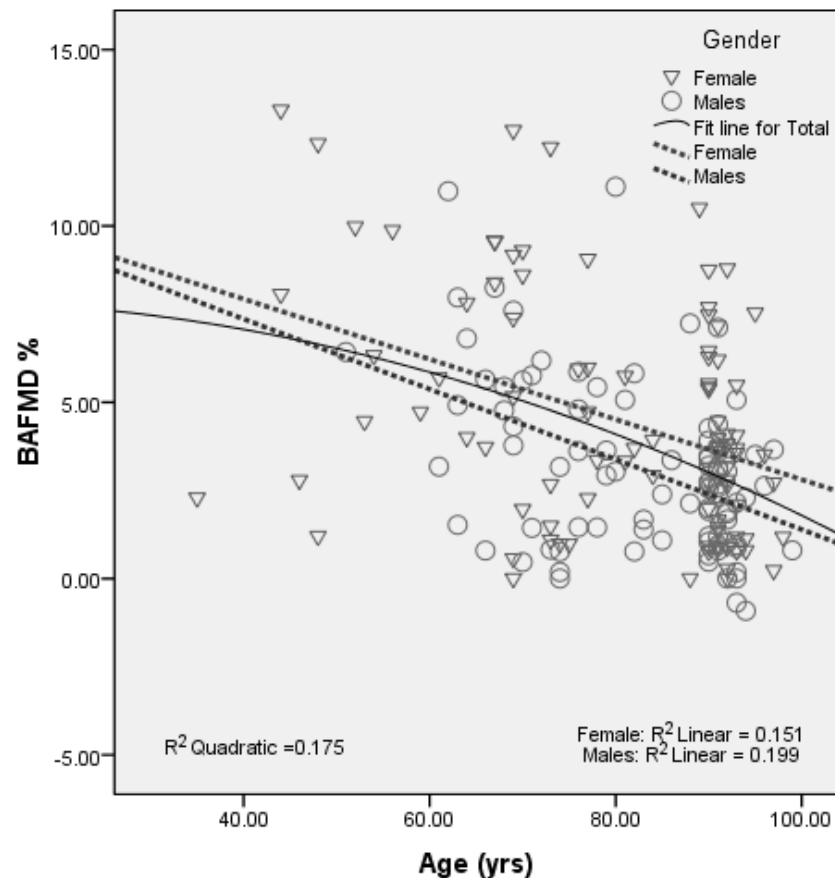


Figure A.24: Depicts the age associated decline in brachial artery flow mediated dilation (BAFMD).

One potential mechanism for the age associated decline in endothelial function is a reduction in the bioavailability of NO^- . Van der loo and colleagues found that e-NOS expression and activity in rat abdominal aorta increased in an aged dependent manner while NO^- mediated vascular

endothelial function declined with age [207]. In addition, levels of NO^- were lower in the old and middle aged animals compared with those from young rats indicating that the decreased NO^- production in the aged animals was not due to a reduced expression of e-NOS. The authors attributed this reduced NO^- bioavailability to a higher level of oxidative stress and activity of superoxide (O_2^-) [207]. In general, the free radical O_2^- , which is formed by the oxidation of components of the electron transport chain, neutralizes NO^- by forming peroxynitrite (ONOO^-). In situations of high oxidative stress, more NO^- may be neutralized before contributing to the vasodilatory process.

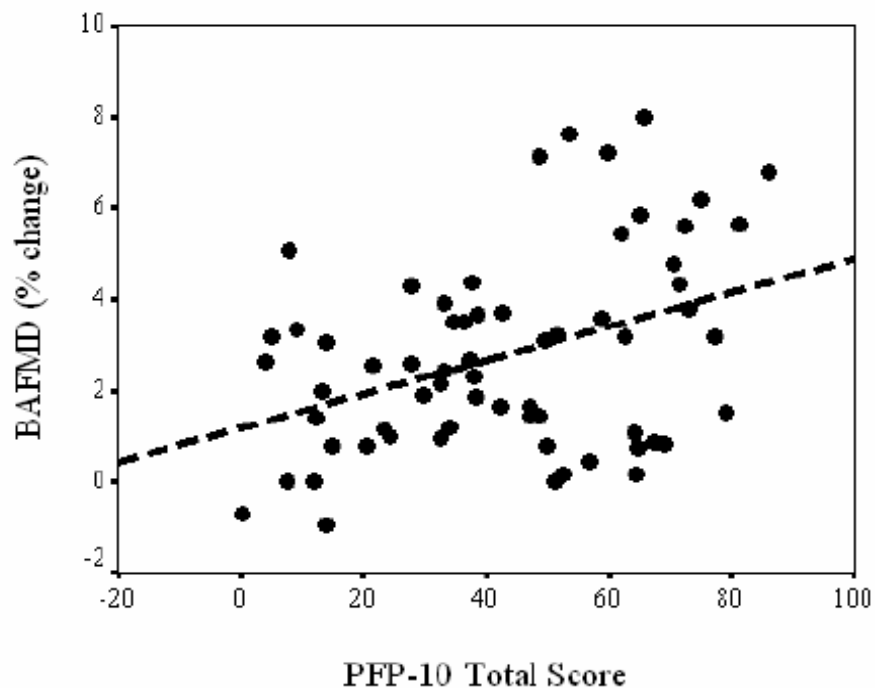


Figure A.25: The relation between Total PFP-10 Score and BAFMD in older healthy men. Taken with permission from [10].

Large elastic artery stiffness and thickening has been shown to increase with advancing age [208], and post-mortem studies have indicated that aortic wall thickening consists mainly of intimal thickening [4, 26]. **Figure A.26** depicts age related increase in carotid intima media thickness with advancing age.

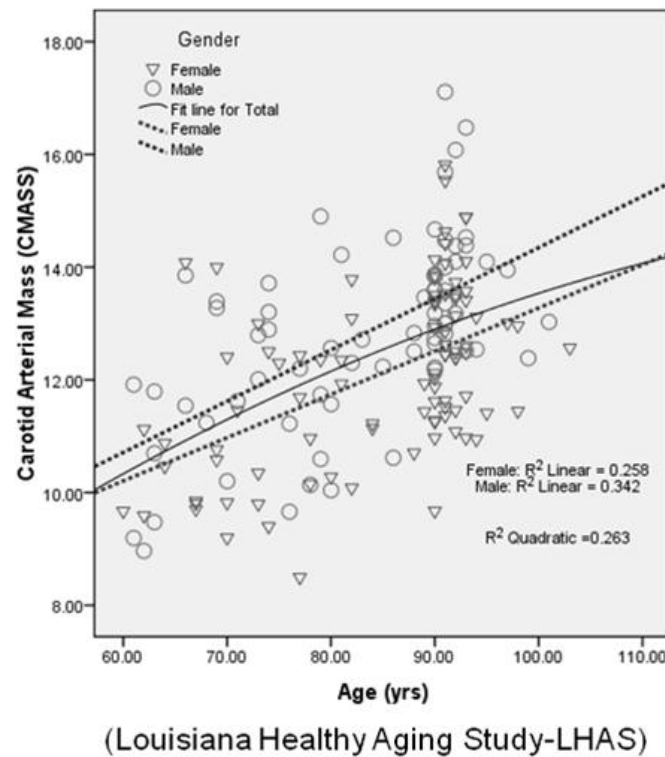


Figure A.26: Age associated increase in carotid arterial mass (CMASS) from individuals in the Louisiana Healthy Aging Study (LHAS).

This can be the consequence of changes in the composition of the arterial wall. For example, in an aged artery, the thickened intima exhibits an increased expression of inflammation, a deterioration of the micro fibrils, a reduction in elastin content and an increased calcification of the extracellular matrix [4, 23]. The consequence of these structural changes may be a loss of much of the artery's elastic properties [23, 209]. In addition, an accumulation of interstitial cell

adhesion molecules and growth factors, as well as vascular smooth muscle cell hypertrophy are believed to be important mechanisms in mediating arterial stiffness and thickening with age [210].

The alterations in vascular function and structure may also alter the hemodynamic pattern of blood flow in such a way that the flow may become turbulent or oscillatory in nature. In a recent review by Laughlin et al. (2008), higher oscillatory flow patterns were associated with a proatherogenic expression on the vasculature (i.e. greater oxidative stress) [11, 15]. Interestingly, Credeur et al. (2009) observed a higher retrograde component (backwards flow) of the flow velocity profile in comparison to the antegrade component in a group of elderly [49]. The ratio of flow patterns was used as an index of oscillatory flow. Interestingly, this retrograde component was directly associated with a decline in physical functionality in this population. Whether the higher retrograde profiles observed directly contributes to the decline in vascular and physical function, or a reflection of the age associated vascular changes has yet to be determined.

With advancing age, there is also an increase in sympathetic neural activity which may alter vascular tone and contribute to an increase in vascular resistance (decrease in vascular conductance) at rest and during exercise [211]. A higher adrenergic activity and a reduction in vasodilator function may reduce the ability to redistribute blood flow during exercise. For example, Kenney and Ho (1995) examined the effect of age on regional blood flow (skin, splanchnic and renal) responses to upright exercise in young and older fit subjects in a warm (36°C) environment. The subjects were matched for VO_2 peak to further isolate age. The results of their study indicated, that at 60% VO_2 peak, forearm blood flow was significantly lower in the old subjects in each environment (22 and 36°C). Splanchnic blood flow fell at the low intensity then decreased to a greater extent at 60% VO_2 peak in young compared to older subjects. Renal

blood flow was lower at rest in the old group, remained relatively constant at 35% VO_2 peak, then decreased by 20-25% in both groups at 60% VO_2 peak. At 60% VO_2 peak, 27 and 37% more total blood flow was redistributed away from renal and splanchnic circulations in the young compared to the older group at 22 and 36°C, respectively. It was concluded that the greater increase in skin blood flow in young subjects is partially supported by a greater redistribution of blood flow away from splanchnic and renal vascular beds [212].

The reduction in blood flow and vascular conductance to active regions with aging has been attributed to increased sympathetic α -adrenergic tone [62]. For example, basal limb blood flow decreases in a linear fashion between the ages of 25 and 65 [213]. An additional study by Ho and colleagues [214], reported that healthy ‘fit’ older men do shunt more blood flow away from their splanchnic and kidney circulations than ‘un-fit’ older subjects, but less than younger control subjects during exercise and/or heat stress, even though there were higher plasma NE levels in the aged group [214]. This may contribute to the reduction in blood flow redistribution seen in aging. Whether exercise training can improve vasoconstrictive responsiveness (e.g. less adrenergic activity rest and greater redistribution) to exercise or not, in the elderly, has yet to be determined, but available evidence appears to favor the former. Additionally, increases in vascular resistance, seen with aging, have also been linked to an increased bioactivity of local vasoconstrictor molecules (e.g. endothelin-1), as well as reduced NO^- bioavailability [215].

In contrast to age, regularly performed (habitual) physical activity with advancing age is associated with a more favorable arterial structure and enhanced vascular function [40]. Endothelial dependent vasodilation is greater in middle aged and older men who perform aerobic exercise compared with sedentary groups [40, 63]. The greater vascular function seen in aerobically exercised older men may, in part, be mediated by enhanced NO^- bioavailability

[216], secondary to reduced oxidative stress [217]. Additionally, data from our laboratory has examined a timeline of changes in BAFMD in response to localized handgrip training in the elderly. Dobrosielski et al. (2009) indicate that flow mediated dilation is modified with training in the elderly with evidence of a significant improvement in BAFMD after 2 weeks of training [6] (**Figure A.27**).

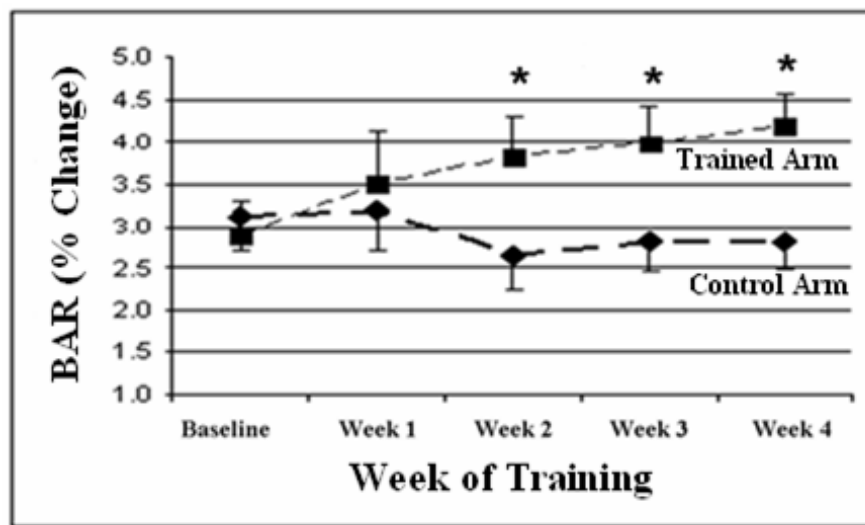


Figure A.27: Group mean values \pm SE for percent change in BAR across the training program. * $p < 0.05$ as compared with pre-training. Taken with permission from [6].

In addition, Vaitkevicius and colleagues [48] 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_2 Peak [48].

- **A.7.3: Effects of Disease on Blood Flow Control**

Cardiovascular diseases (CVD) have been the leading form of disease globally and accounts for approximately 20% of all mortality [218]. In the United States in 1995,

approximately 54% of the population died of cardiovascular disease and stroke [219]. Two common manifestations of these diseases are coronary artery disease (CAD) and peripheral artery disease (PAD). Patients who experience CVD share a common underlying problem of reduced muscle blood flow with subsequent exercise intolerance, which is largely attributable to disorders of the arteries [40]. The current thinking in the pathogenesis of CVD stems from a disturbance in the vasculature and the development of atherosclerosis. The process of atherosclerosis development is initiated by “injury” to the intima or endothelium [220]. There are several possible explanations for the injury, but can be grouped into mechanical (e.g. circumferential stress and blood pressure), physiological (e.g. inflammation, trauma) and chemical stressors (e.g. toxic chemicals) [176].

Metabolic diseases, such as diabetes mellitus, have also been associated with a decline in vascular reactivity. Diabetes mellitus (DM) is a group of chronic metabolic conditions, all of which are characterized by elevated blood glucose levels resulting from the body’s inability to produce insulin or resistance to insulin action, or both [221-222]. Diabetes Mellitus is also associated with an increased risk for CAD, stroke and PAD, and a diminished quality of life. Altered endothelial function (e.g. endothelial dysfunction) has been considered a potential contributor to vascular complications in DM [223]. It is unclear as to what underlying mechanism is involved in endothelial dysfunction in uncontrolled DM, but is more likely to be the result of several, such as hyperglycemia induced oxidative stress [224], activation of protein kinase-C [225], and formation of advanced glycosylation end products (e.g. glycosylated hemoglobin) [226-227]. Interestingly, among individuals with Type 2 DM, there is a reduction in exercise capacity (VO_2 Peak) [228] which may, in part, be the consequence of a reduction in blood flow and/or vascular conductance to the exercising muscle [227].

In chronic heart failure, it has been documented that there is a severe limitation in exercise tolerance [118]. It is thought that this intolerance to exercise may, in part, be due to inadequate skeletal muscle blood flow with consequent muscular fatigue. A majority of studies suggests this limitation is a consequence of vasodilatory properties within the working muscle. However, data from Wilson et al. (1986) paints a slightly different picture. In their study, they examined the effects of acute forearm exercise and ischemia on vasodilatory behavior in CHF patients, and found that the forearm vasodilatory and blood flow response was not intrinsically impaired compared to healthy age-matched controls [118]. In a subsequent study, Wilson et al. (1989) noted that during acute lower limb exercise, there was a significant increase in blood flow to the leg, with no significant change in non-leg flow (forearm, splanchnic regions) [229] (**Table A.2**).

Table A.2: Table taken with permission from [229].

Effect of Maximal Bicycle Exercise on Blood Flow to Nonexercising Tissues				
	Supine	Upright	20 W	Maximal
Non-leg flow (l/min)	3.1±1.1	2.9±1.0	3.1±1.3	2.8±1.4
Non-leg vascular resistance (units)	31±12	34±16	40±25	45±25*
VO ₂ (ml/min)	249±39	283±42	637±124*	850±307*
Cardiac output (l/min)	3.7±1.1	3.4±0.9	5.6±1.6*	6.8±2.5*
Leg flow (l/min)	0.3±0.1	0.3±0.1	1.3±0.5*	2.0±1.0*
Pulmonary wedge pressure (mm Hg)	23±8	21±10	27±10*	31±9*
Mean arterial pressure (mm Hg)	86±10	86±10	94±12*	98±14*
Heart rate (beats/min)	86±12	93±16	111±19*	121±20*

(n=28). Exercise data is given for the 20-W work load, a submaximal work load achieved by all patients, and the maximal work load.

*p<0.02 versus upright rest.

The authors interpreted from these results [229], that in contrast to normal subjects, heart failure patients do not develop major redistributions of blood flow from non-exercising beds to exercising muscle. Given the evidence [118, 229], one could hypothesize that a mechanism by which there is a limitation in exercise tolerance in heart failure, is an inability to adequately redistribute blood flow from non-exercising regions to exercising skeletal muscle. Surprisingly, there is little evidence to support if chronic exercise training can improve the ability to redistribute blood flow in CHF patients. Exercise training has been shown to improve EDD in the coronary circulation and in skeletal muscle [230] in patients with CAD heart failure [95]. Whether these adaptations involve changes in the responsiveness of both trained and non-trained vascular beds, relating to greater redistributions in blood flow has yet to be determined.

- **A.7.4: Summary**

In summary, ‘normal’ aging is associated with increased vascular stiffness of large elastic arteries, impaired vascular endothelial function, increased intima-media wall thickness and peripheral vasoconstriction (e.g. reduced blood flow to active vascular beds at rest and during exercise). Compared with sedentary groups, adults who are habitually active demonstrate smaller or no age associated increases in large elastic artery stiffness, reductions in vascular endothelial function and increases in intima-media thickness [40]. A mechanism by which this may be achieved is through enhanced endothelial function and lower adrenergic activity at rest, thus resulting in greater redistributions in blood flow during exercise. In chronic heart failure, it has been documented that there is a severe limitation in exercise tolerance [118], and in contrast to normal subjects, heart failure patients do not develop major redistributions of blood flow from non-exercising beds to exercising muscle. Exercise training has been shown to enhance blood flow capacity and vasodilatory behavior of the vasculature in aging, diabetes and heart failure.

Whether these adaptations involve changes in the responsiveness of both trained and non-trained vascular beds, relating to greater redistributions in blood flow has yet to be determined.

A.8: Conclusion

In summary of this review, the vasculature is divided among specific domains serving the purpose supplying adequate blood flow to the metabolically active tissue. The elastic arteries are low resistance pathways which transfer the blood ejected from the ventricle under high pressure towards the periphery. The conduit vessels transport the blood to a particular region in the periphery and respond predominately to hemodynamic forces (e.g. shear stress) by releasing endothelial derived relaxing factors (NO^- , PGI-2, EDHF) which act on the adjacent smooth muscle cells [22]. The arterioles feed the vascular beds of a tissue and have a high degree of basal tone. Along with the arterioles, the venous system responds predominately to neuro-humoral stimulation (e.g. NE, Ach, ANG-II) and serves as a major contributor of blood flow distribution.

There are a number of techniques currently being used in clinical screening and research to assess vascular physiology and it is important to examine all aspects of the vessel. In order to appreciate the techniques in assessing vascular physiology one must apply them to a model. Macro-vascular and micro-circulatory function, as well as regional blood flow can be assessed in a limb in response to various physiological stimuli (**Figure A.28**). Vascular reactivity observed in response to an endothelial mediated (e.g. cuff ischemia) and autonomic stimuli (e.g. cold pressor test) can serve as the physiological operating range from which blood flow is distributed. Theoretically, a higher vascular operating range (e.g. balanced vasodilatory and vasoconstrictive

responsiveness in a particular vessel and bed) may translate into higher blood flow capacity and physical functionality in the subject.

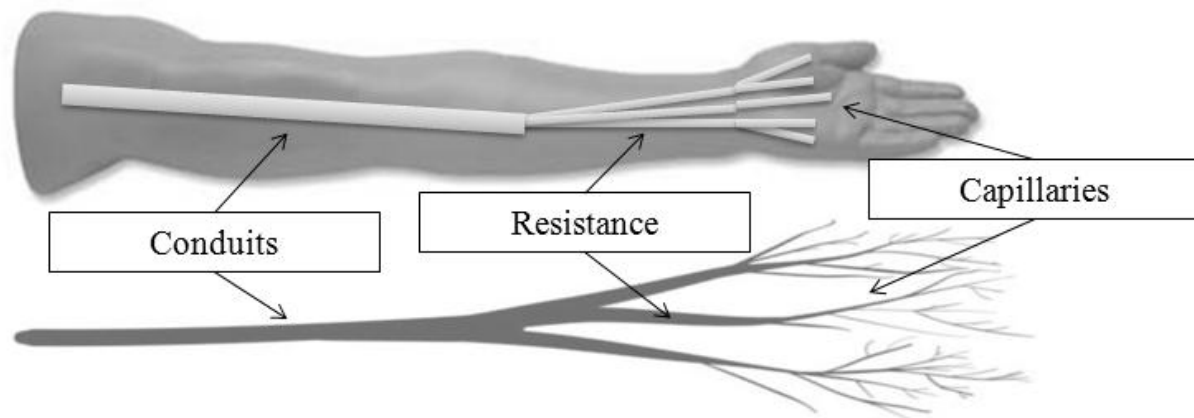


Figure A.28: Depicts s specific vascular domains which work in synch to distribute blood flow to the tissue.

During exercise, it is important for blood to be adequately distributed to contracting skeletal muscle [175]. Blood flow must match the metabolic demand of tissue in order for activity to be sustained. Sympathetic vasoconstriction of most major arteries occurs during exercise in order to increase driving pressure [31], with a subsequent increase in tissue perfusion. Remensnyder et al. (1962) termed a phenomenon known as functional sympatholysis, in which local metabolites (e.g. H^+ , Adenosine, P_i^-) produced in contracting skeletal muscle appear to override the sensitivity of vascular endothelium and smooth muscle to catecholamines, resulting in dilation and increases in blood flow to contracting skeletal muscle, while reducing flow to less active areas (e.g. mesentery, non-working skeletal muscle) [112].

In response to exercise and training, vascular adaptations include increases in maximal blood flow capacity, which are dependent on alterations in vasodilatory and vasoconstrictive properties of large arteries, arterioles and capacitance vessels. The relative contributions of these adaptations and distribution throughout the vascular tree are not uniform. Depending on the mode intensity, duration of training and region exercised, the mechanisms can be activated to differing degrees in order to achieve homeostasis and adequate perfusion. Exercise-induced changes in vasodilatory behavior include increases in EDD of large conduits and arterioles. The signals for these adaptations stem from muscular contraction-induced increases in shear as well as increases in tissue metabolism. Changes in vasoconstrictor responsiveness include alterations in adrenergic reactivity. Available evidence indicates that vasoactive constriction can be decreased in an exercised limb or region. The redistribution of flow is critical as it relates to exercise tolerance, and functional capacity (VO_2 peak) especially in situations which the vasculature is compromised (e.g. cardiovascular disease).

In consideration of functional limitation in individuals with disease, it is estimated that 45 million people in the United States suffer from disabilities related to cardiovascular disease, which can be defined as a difficulty performing activities of daily living and job work [3]. With advancing age and disease, our ability to function is greatly limited by poor perfusion defined by an inability shunt blood to areas of high demand. This not only includes blood flow to skeletal muscle, but also redistributions within the brain, gastro-intestinal tract, kidneys, liver, lungs, etc. It is very important as researchers and clinicians to continue our view of studying all areas of the vasculature in its relation to blood flow distribution, defined by the balance between vasodilation and vasoconstriction.

Appendix B: IRB Approval

Application for Approval of Projects Which Use Human Subjects

This application is used for projects/studies that cannot be reviewed through the exemption process.



Institutional Review Board
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Baton Rouge, LA 70803
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irb@lsu.edu
lsu.edu/irb

-- Applicant, Please fill out the application in its entirety and include two copies of the completed application as well as parts A-E, listed below. Once the application is completed, please submit to the IRB Office for review and please allow ample time for the application to be reviewed. Expedited reviews usually takes 2 weeks. Carefully completed applications should be submitted 3 weeks before a meeting to ensure a prompt decision.

-- A Complete Application Includes All of the Following:

(A) Two copies of this completed form and two copies of part B thru E.

(B) A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1&2)

(C) Copies of all instruments to be used.

*If this proposal is part of a grant proposal, include a copy of the proposal and all recruitment material.

(D) The consent form that you will use in the study (see part 3 for more information.)

(E) Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing or handling data, unless already on file with the IRB. Training link: (<http://phrp.nihtaining.com/users/login.php>.)

(F) IRB Security of Data Agreement: (<http://www.lsu.edu/irb/IRB%20Security%20of%20Data.pdf>)

1) Principal Investigator*: Michael A. Welsch

Rank Associate Prof

*PI **must be** an LSU Faculty Member

Dept: Kinesiology

Ph:

578-9143

E-mail:

mwelsch@lsu.edu

2) Co Investigator(s): please include department, rank, phone and e-mail for each

Denzil Moraes, MD, F.A.C.C., Louisiana Cardiology Associates
David Thomas, MD, PhD, F.A.C.P., Assistant Professor Tulane Univ. School of Medicine
Dwayne Labatut, N.P., Louisiana Cardiology Associates
Daniel Credeur, BS, Research Assistant, Louisiana State University

3) Project Title:

Plasma Nitrite-Reserve and Vascular Function before and after handgrip training in patients with Heart Failure

4) Proposal Start Date: 02/01/2011

5) Proposed Duration Months: 12 months

6) Number of Subjects Requested: 40

7) LSU Proposal #: 2914

8) Funding Sought From: Internal

ASSURANCE OF PRINCIPAL INVESTIGATOR named above

I accept personal responsibility for the conduct of this study (including ensuring compliance of co-investigators/co-workers) in accordance with the documents submitted herewith and the following guidelines for human subject protection: The Belmont Report, LSU's Assurance (FWA00003892) with OHRP and 45 CFR 46 (available from <http://www.lsu.edu/irb>). I also understand that copies of all consent forms **must be maintained at LSU for three years after the completion of the project**. If I leave LSU before that time, the consent forms should be preserved in the Departmental Office.

Signature of PI

Date 01-20-2011

ASSURANCE OF STUDENT/PROJECT COORDINATOR named above. If multiple Co-Investigators, please create a "signature page" for all Co-Investigators to sign. Attach the "signature page" to the application.

I agree to adhere to the terms of this document and am familiar with the documents referenced above.

Signature of Co-PI (s)

Date 01-21-2011

IRB# 2914 LSU Proposal #

☐ Full

☒ Expedited

☐ Human Subjects Training

☐ Complete Application

Study Approved By:
Dr. Robert C. Mathews, Chairman
Institutional Review Board
Louisiana State University
203 B-1 David Boyd Hall
225-578-8692 | www.lsu.edu/irb
Approval Expires: 02-22-11

Application for Approval of Projects Which Use Human Subjects

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1) Principal Investigator*: Michael A. Welsch

Rank Associate Prof

*PI must be an LSU Faculty Member

Dept: Kinesiology

Ph: 578-9143

E-mail: mwelsch@lsu.edu

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4) Proposal Start Date: 02/01/2011

5) Proposed Duration Months: 12 months

6) Number of Subjects Requested: 40

7) LSU Proposal #:

8) Funding Sought From: Internal

IRB# 2011-LSU Proposal #

☐ Full

☒ Expedited

☐ Human Subjects Training

☐ Complete Application

ASSURANCE OF PRINCIPAL INVESTIGATOR named above

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Signature of PI

Date 01-20-2011

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I agree to adhere to the terms of this document and am familiar with the documents referenced above.

Signature of Co-PI (s)

Date 01-20-2011

Study Approved By: Dr. Robert C. Mathews, Chairman
Institutional Review Board
Louisiana State University
203 B-1 David Boyd Hall
225-578-8692 | www.lsu.edu/irb
Approval Expires: 02-20-11

Application for Approval of Projects Which Use Human Subjects

This application is used for projects/studies that cannot be reviewed through the exemption process.



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4) Proposal Start Date: 02/01/2011

5) Proposed Duration Months: 12 months

6) Number of Subjects Requested: 40

7) LSU Proposal #:

8) Funding Sought From: Internal

IRB# 2514 LSU Proposal #

☐ Full

☒ Expedited

☐ Human Subjects Training

☐ Complete Application

ASSURANCE OF PRINCIPAL INVESTIGATOR named above

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Signature of PI

Date 01-20-2011

ASSURANCE OF STUDENT/PROJECT COORDINATOR named above. If multiple Co-Investigators, please create a "signature page" for all Co-Investigators to sign. Attach the "signature page" to the application.

I agree to adhere to the terms of this document and am familiar with the documents referenced above.

Signature of Co-PI (s)

Date

1/25/11

Study Approved By: Dr. Robert C. Mathews, Chairman
Institutional Review Board
Louisiana State University
203 B-1 David Boyd Hall
225-578-8692 | www.lsu.edu/irb
Approval Expires: 02-22-11

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6) Number of Subjects Requested: 40

7) LSU Proposal #:

8) Funding Sought From: Internal

IRB# 2914 LSU Proposal #

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☐ Complete Application

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Date 1/20/2011

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Approval Expires: 02-20-11

Part 1: A. Is a HIPAA Agreement Needed?

Are you obtaining any health information **from a health care provider** that contains any of the identifiers listed below?

A. Names

B. Address: street address, city, county, precinct, ZIP code, and their equivalent geocodes. Exception for Zip codes: the initial three digits of the ZIP Code may be used, if according to current publicly available data from the Bureau of the Census: (1) The geographic unit formed by combining all ZIP codes with the same three initial digits contains more than 20,000 people; and (2) the initial three digits of a ZIP code for all such geographic units containing 20,000 or fewer people is changed to '000'. (Note: The 17 currently restricted 3-digit ZIP codes to be replaced with '000' include: 036, 059, 063, 102, 203, 556, 692, 790, 921, 830, 831, 878, 879, 884, 890, and 893.)

C. Dates related to individuals

i. Birth date

ii. Admission date

iii. Discharge date

iv. Date of death

v. And all ages over 89 and all elements of dates (including year) indicative of such ago. Such ages and elements may be aggregated into a single category of age 90 or older.

D. Telephone numbers;

E. Fax numbers;

F. Electronic mail addresses;

G. Social security numbers;

H. Medical record numbers; (including prescription numbers and clinical trial numbers)

I. Health plan beneficiary numbers;

J. Account numbers;

K. Certificate/license numbers;

L. Vehicle identifiers and serial numbers including license plate numbers;

M. Device identifiers and serial numbers;

N. Web Universal Resource Locators (URLs);

O. Internet Protocol (IP) address numbers;

P. Biometric identifiers, including finger and voice prints;

Q. Full face photographic images and any comparable images; and

R. Any other unique identifying number, characteristic, or code; except a code used alone or in combination with other information to identify an individual who is the subject of the information.

- ☐ **YES** Your study falls under the HIPAA (Health Information Privacy and Accountability Act) and you must obtain either a limited data set use agreement or a HIPAA authorization agreement from the health care provider. This agreement must be submitted with your IRB protocol.
- ☒ **NO** You do not need a HIPAA agreement.

B. Are pregnant women specifically excluded from participation on the consent form?

- ☐ **YES** Skip to Part C.
- ☒ **NO** You need to document the following:

- ☐ 1. Is the purpose of the activity to meet the health needs of the mother and -
- ☐ a. Fetus will be placed at risk only to minimum to meet mothers needs.
 - ☐ b. Fetus risk is minimal.
- ☐ 2. Have mother and father given informed consent including potential affects on the fetus?
- ☐ 3. Father's consent to be omitted when:
- ☐ a. Purpose of activity is to meet health needs of the mother
 - ☐ b. His identity can not be ascertained
 - ☐ c. He is not reasonably available
 - ☐ d. Pregnancy is from rape

Continue on the next page

C. Are any of your participants incarcerated?

☐ **YES** - You must document the following information:

- ☐ 1. Is the study minimal risk? (it must be)
- ☐ 2. Research fits one of the allowed categories below
 - ☐ Causes or effects of incarceration
 - ☐ Study of prisons or prisoners
 - ☐ Conditions affecting prisoners as a class
 - ☐ Practices that may improve health or well-being of subjects
- ☐ 3. Are the risks commensurate with risks accepted by non-prisoners?
 - ☐ Selection of subjects is fair - controls random
 - ☐ Language is understandable
 - ☐ Study does not affect parole
 - ☐ If necessary, follow up care will be provided

☒ **NO**

D. Are children involved?

☐ **YES** - You need both parental consent form and a child assent form

- If the study has greater than minimal risk and no direct benefits, then you must show that the
- ☐ risk is only a minor increase above minimal, and it involves experiences that are commensurate with ordinary medical, psychological, social or educational situations

☒ **NO**

Part 2: Project Abstract - Provide a brief abstract of the project

☒ I have attached a project abstract to this application

Part 3: Research Protocol

A. Describe study procedures

Describe study procedures with emphasis on those procedures affecting subjects and safety measures. Also provide script for telephone surveys.

☒ I have attached a description of my study procedures to this application

B. Answer each of the following questions

1. Specify sites of data collection

Louisiana Cardiology Associates, 7777 Hennessey Blvd., Suite 1000, Baton Rouge, 70808

2. If surgical or invasive procedures are used, give name, address, and telephone number of supervising physician and the qualifications of the person(s) performing the procedures. Comparable information when qualified participation is required or appropriate.

Denzil Moraes, MD, F.A.C.C., Louisiana Cardiology Associates
David Thomas, MD, PhD, F.A.C.P., Assistant Professor Tulane Univ. School of Medicine
Dwayne Labatut, N.P., Louisiana Cardiology Associates

3. Provide the names, dosage, and actions of any drugs or other materials administered to the subjects and the qualifications of the person(s) administering the drugs.

N/A

4. Detail all the physical, psychological, and social risks to which the subjects may be exposed.

The discomforts and risks of a blood test are the pain of a needle going into the vein, possible bruising and the remote risk of infection.
The 6-min walk test is recommended as a safe, useful, simple, noninvasive alternative for assessing physical activity in patients with heart failure. The 6-min walk test provides prognostic information about the heart failure patient and appears to be sensitive to interventions such as exercise training.
There are no known risks associated with squeezing a handgrip dynamometer. Dr. Welsch has conducted similar studies on younger and older subjects, with and without disease without a single adverse response.
There is no known risk from the use of ultrasound to measure the size of the brachial artery blood vessel. Inflation of the blood pressure cuff on the arm, during the ultrasound test, may cause some temporary discomfort and pain. If the discomfort is too severe, we may terminate the procedure immediately by asking the technician to stop the test. Temporary numbness and tingling in the hand may occur during cuff occlusion and during the cuff occlusion and during the cold pressor test.

5. What steps will be taken to minimize risks to subjects?

Any risks associated with this study will be minimized as a result of proper evaluation, education, and treatment, careful exercise prescription, and appropriate assessment of exercise responses. There will also be well-trained personnel capable of monitoring exercise equipment present for each testing and training visit. The investigators have extensive experience in the study of vascular function in a wide range of patients and disease severity.

6. Describe the recruitment pool (community, institution, group) and the criteria used to select and exclude subjects.

The participants for this study will be recruited from Louisiana Cardiology Associates, and the Baton Rouge community. Eligible patients include: (a) men and women; at least 18 years of age with a diagnosis of heart failure, (b) individuals with evidence of systolic or diastolic dysfunction, (c) individuals on optimal therapy as determined by the patient's physician, and (d) not pregnant. Eligible participants for the age-matched control group include those who do not have a diagnosis of heart failure or any other chronic degenerative disease. Individuals who have an acute illness, or are less than 3 months post-hospitalization, or those who are medically unstable, and those who are pregnant will be excluded from participation.

Those individuals who have no contraindications will be asked to volunteer for the study.

7. List any vulnerable population whose members are included in this project (e.g., children under the age of 18; mentally impaired persons; pregnant women; prisoners; the aged).

Eligible patients include men and women; at least 18 years of age with a diagnosis of heart failure.

8. Describe the process through which informed consent will be obtained. (Informed consent usually requires an oral explanation, discussion, and opportunity for questions before seeking consent form signature.)

Those individuals who have no contraindications will be asked to volunteer for the study. All participants will receive a comprehensive explanation of the proposed study, its benefits, inherent risks and expected commitments with regard to time. Following the explanation of the proposed study, all patients will be allowed a period of questioning. Individuals who are willing to participate in the study will be required to read and sign the informed consent document during the first visit and prior to any experiments.

9. (A) Is this study anonymous or confidential? (Anonymous means that the identity of the subjects is never linked to the data, directly or indirectly through a code system.)

(B) If a confidential study, detail how the privacy of subjects and security of their data will be protected.

This study will be a confidential study. The identity of the subjects will only be known through a study code which will remain with the PI of the study and locked in a safe and secure area. The identity of the subjects will not be revealed at any time following completion of the study.

Part 4: Consent Form (Including Assent Form and Parental Permission Form if minors are involved)

▷ **Please note:** The consent form must be written in non-technical language which can be understood by the subjects. It should be free of any exculpatory language through which the participant is made to waive, or appears to be made to waive any legal rights, including

▷ For example consent forms and a complete checklist of required items, please refer to our website, www.lsu.edu/irb. Remember, **IRB contact information must be included** on the consent form!

▷ To waive signed consent, **IRB must be provided with the consent script** that will present the informed consent information to human subjects regarding the study/research. Also, note that waiving signed consent required full IRM approval, which may delay approval of your study.

I am requesting waiver of signed Informed Consent because:

☐ (a) Having a participant sign the consent form would create the **principal risk** of participating in the study.

or that

☐ (b) The research presents **no more than minimal risk** of harm to subjects and involves no procedures for which having signed consent is normally required.

Expedited review usually takes 2 weeks. See our website for information about meeting dates. Carefully completed applications should be submitted 3 weeks before a meeting to ensure a prompt decision.

Institutional Review Board
Dr. Robert Mathews, Chair
131 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 22.578.6792
irb@lsu.edu
lsu.edu/irb

Print Form

Vita

Daniel Paul Credeur is a resident of Louisiana, originally from the city of Thibodaux. Daniel received his Bachelor of Science in kinesiology from Louisiana State University, with a concentration in fitness studies, in the summer of 2007. Daniel continued his graduate work as a doctoral student from the fall of 2007 to the present in kinesiology, with a concentration in exercise physiology. Daniel defended his graduate work July 13, 2011 and will graduate with a doctoral degree in kinesiology in December, 2011.