The Pennsylvania State University

The Graduate School

College of Medicine

BLOOD FLOW REGULATION DURING EXERCISE

IN PERIPHERAL ARTERIAL DISEASE

A Dissertation in

Neuroscience

by

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Submitted in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

August 2017
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Peripheral arterial disease (PAD) affects 8-10 million Americans and greatly increases risk of cardiovascular mortality. PAD is a form of atherosclerosis that affects medium to large arteries in the legs and causes pain with walking. Previous studies reveal that blood pressure (BP) responses to exercise are augmented in PAD patients. However, how blood flow in the heart and legs are regulated during exercise in PAD is unknown. The studies comprising this dissertation investigated BP and blood flow responses to exercise in PAD and how leg vascular interventions affect those responses. In the first study, 12 PAD patients and 15 healthy subjects performed dynamic plantar flexion exercise and isometric handgrip exercise while BP and coronary blood velocity (CBV) were measured. BP and skeletal muscle oxygen saturation (SmO₂) in the legs were measured during treadmill walking in 8 PAD patients and 8 healthy subjects (study 2). For the third study, BP and CBV responses to plantar flexion exercise were measured before and 1 month following a leg vascular intervention in 17 PAD patients. Coronary blood velocity responses to plantar flexion exercise (PAD: Δ 2.4 ± 1.2, healthy: Δ 6.0 ± 1.6 cm/s, $P = 0.039$) and to isometric handgrip exercise (PAD: Δ 8.3 ± 4.2, healthy: Δ 16.9 ± 3.6, $P = 0.033$) were attenuated in PAD patients. The fall in SmO₂ in response to treadmill walking was greater in PAD (healthy: 15 ± 12 vs. PAD: 49 ± 5 %, $P < 0.001$). Leg revascularization decreased the mean BP response (pre-intervention: 15 ± 4 vs. post-intervention: 7 ± 3 mmHg, $P = 0.013$) and increased the CBV response (pre-intervention: -1 ± 2 vs. post-intervention: 4 ± 1 vs. cm/s, $P = 0.038$) to plantar flexion exercise in PAD. Collectively, data from these studies suggest that exercise hyperemia is impaired in PAD patients compared to healthy subjects. Furthermore, the origin of the impaired coronary hyperemia and exaggerated exercise BP in PAD may be related to leg ischemia.
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LIST OF ABBREVIATIONS

Ankle-brachial index (ABI)
Blood pressure (BP)
Body mass index (BMI)
Claudication onset time (COT)
Coronary blood velocity (CBV)
Electrocardiogram (EKG)
Exercise pressor reflex (EPR)
Heart rate (HR)
Left anterior descending coronary artery (LAD)
Low-density lipoprotein (LDL)
Near-infrared spectroscopy (NIRS)
Oxygen (O$_2$)
Peak walking time (PWT)
Peripheral arterial disease (PAD)
Skeletal muscle oxygen saturation (SmO$_2$)
ACKNOWLEDGEMENTS

I want to thank my mentor Dr. Lawrence Sinoway for funding and inspiring these studies and for encouraging my love of clinical science and for teaching me to think outside the box. I acknowledge Dr. Matthew Muller for overseeing my daily progress in lab and teaching me about study design, statistical analysis, and manuscript and grant writing. I acknowledge Dr. Gail Thomas and Dr. Marc Kaufman for their mentoring and advice on all my projects. I thank Carter Luck and Dr. Dani Kim for being my colleagues in lab and assisting with data collection and analysis. I thank Michael Herr for technical support. I thank Cheryl Blaha and Aimee Cauffman for nursing support for my experiments, scheduling subjects, and answering my clinical questions. I want to thank Kris Brandt for backing up my ultrasound data. I acknowledge Dr. Zhaohui Gao and Dr. Jian Cui for teaching me technical skills such as ultrasonography and microneurography that were valuable for my training. I thank Kris Gray, Jen Stoner, Lynn Dawson, Kathy Simon, and Kathy Shuey for administrative support.
Chapter 1

Introduction

Peripheral arterial disease (PAD) is a chronic and progressive vascular disease that is estimated to affect 8-10 million Americans, although many individuals are asymptomatic (51). PAD costs the United States Medicare system $3.9 billion per year (60). The prevalence of PAD increases with age and it is estimated that 29% of people over 70 years have PAD (59). Therefore, as people continue to live longer lives and the “baby boomer” generation ages, cases of PAD are likely to increase.

PAD is a form of atherosclerosis that occurs in medium to large arteries in the lower extremities. Although the main risks directly associated with PAD are gangrene and amputation (93), PAD also increases overall cardiovascular risk. In this regard, PAD patients are 5 times more likely to die of cardiovascular causes in 10 years compared to healthy individuals (27). Myocardial infarction accounts for half of all deaths in PAD (93). However, the reason for the high cardiovascular risk in PAD is not well understood.

Studies in PAD reveal an exaggerated blood pressure (BP) response to leg exercise (4, 5, 77, 87, 106). The purpose of this exaggerated BP response to exercise is poorly understood. It is hypothesized that the higher BP is a result of narrowed arteries and needed to effectively perfuse organs (92). However, a higher BP is also damaging to organs such as the heart and the brain and increases cardiovascular mortality (70, 73). Exercise training, is often recommended for PAD, given its therapeutic effects (44). However, little is known about the physiological adaptations to exercise in PAD. In particular, it is unclear whether the exaggerated BP response to exercise in PAD is necessary to maintain perfusion to skeletal and myocardial muscle in PAD. Alternatively,
exercise hyperemia, the increase in blood flow with exercise, may be impaired in PAD patients regardless of the augmented BP response.

The studies comprising this dissertation were designed to evaluate the regulation of BP and blood flow during exercise in PAD patients. More specifically, I hypothesized that coronary blood flow responses to exercise were impaired in PAD patients compared to healthy subjects (study 1). I tested whether walking elicited altered hemodynamic responses in PAD patients (study 2). Third, I assessed whether peripheral revascularization procedures affected systemic cardiovascular responses to exercise in PAD (study 3).

Specific Aims and Hypotheses

Specific Aim 1: To determine whether coronary hyperemia during exercise is impaired in patients with peripheral arterial disease (PAD).

Hypothesis 1A: Despite the greater increase in myocardial metabolism, coronary hyperemia during plantar flexion exercise will be impaired in PAD patients compared to healthy subjects of similar age, sex, and body mass index (BMI).

Hypothesis 1B: BP and coronary blood flow responses to isometric handgrip exercise will be similar in PAD patients and healthy subjects since they are exercising a non-ischemic limb.

Aim 2: To investigate BP and leg skeletal muscle oxygenation during treadmill walking exercise in PAD patients and age-, sex-, and BMI-matched healthy subjects.

Hypothesis 2A: PAD patients will have a greater rise in BP and heart rate during treadmill walking compared to healthy subjects.
Hypothesis 2B: The decrease in leg skeletal muscle oxygen saturation will be greater and occur faster in PAD patients compared to healthy subjects.

Aim 3: To investigate the effects of peripheral revascularization on BP and coronary blood flow responses to exercise in PAD.

Hypothesis 3A: The augmented BP response to plantar flexion exercise observed in PAD will be attenuated one month following peripheral revascularization.

Hypothesis 3B: The impaired coronary exercise hyperemia in PAD will be unchanged one month following peripheral revascularization since especially if cardiac metabolism (BP and heart rate) decreases.
Chapter 2 Literature Review

This chapter will address the literature relevant to the topics in this dissertation including 1) peripheral arterial disease, 2) the exercise pressor reflex, 3) coronary blood flow regulation, and 4) leg muscle oxygen extraction.

Background of Peripheral Arterial Disease

Atherosclerosis Pathogenesis

Peripheral arterial disease (PAD) is an atherosclerotic disease caused by progressive narrowing of the lower extremity conduit vasculature due to development of an intraluminal thrombus in the medium to large arteries of the leg (37). The development of atherosclerosis is a chronic inflammatory process that involves interactions between modified lipids, monocyte-derived macrophages, T cells, and the arterial wall (50). Endothelial dysfunction is a precursor to atherosclerosis (121). Low-density lipoproteins (LDL) migrate through the damaged endothelial cell layer into the intima of the artery where they are oxidized. Oxidation of LDL triggers the migration of monocytes to the intima where they are differentiated into macrophages (98). Macrophages bind oxidized LDL molecules through scavenger receptors and form “foam cells,” which are macrophages that contain large amounts of cholesterol (124). Foam cells interact with T-cells and produce cytokines that interact with the vessel wall. This triggers migration of smooth muscle cells from the media to the intima of the arterial wall where they secrete extracellular matrix proteins that form a fibrous cap over the foam cells. The capped foam cells form plaques that protrude into the arterial lumen causing obstruction of flow. These plaques can also rupture causing thrombosis which can lead to adverse cardiovascular events such as myocardial infarction.
and cerebral ischemia (109). While hypercholesterolemia is sufficient to drive the atherosclerosis process alone (50), other risk factors for PAD (age, hypertension, diabetes, and smoking) may fuel the inflammatory process and increase the susceptibility of the arterial wall (37).

The development of atherosclerosis occurs in arteries exposed to nonlaminar flow and oscillating shear stress which both promote oxidative and proinflammatory states in the arterial wall (29). This may partially explain why coronary artery disease is much more common than PAD and why PAD lesions occur at bifurcations in arteries. Why some patients develop atherosclerosis in the legs rather than the coronary or carotid arteries remains unclear. While all atherosclerotic diseases have the same risk factors, the association between smoking and PAD is stronger than the association between smoking and coronary and carotid artery disease (93). Furthermore, the severity of PAD increases with the number of cigarettes smoked (39) and smokers develop PAD about 10 years earlier than non-smokers (59). Long-term smoke exposure generates oxidative stress that leads to greater lipid oxidation as well as changes in markers of endothelial dysfunction and platelet hyperaggregation, which contribute to the development of atherosclerosis (125). However, atherosclerosis is a systemic disease and 86% of patients with PAD patients have evidence of symptomatic coronary or carotid atherosclerosis (59) making it difficult to decipher whether smoking contributes specifically to PAD rather than to atherosclerosis in general.

**Symptoms and Diagnosis of Peripheral Arterial Disease**

The hallmark symptom of PAD is ischemic leg pain that occurs with walking and is relieved by rest, termed “intermittent claudication” (92). Other typical symptoms of PAD are leg numbness or weakness, hair loss, skin atrophy, and sores on the legs, feet, or toes. Due to the non-specific nature of the symptoms, PAD is underdiagnosed in the primary care setting and patients
with PAD are often not treated as aggressively as patients with other cardiovascular diseases (59). However, patients with PAD have the same relative risk of cardiovascular death compared to patients with coronary or cerebrovascular disease (52). Myocardial infarction accounts for over half of all deaths in PAD and 75% of patients with PAD die from cardiovascular causes (93). Furthermore, PAD patients are about 5 times more likely to die of cardiovascular disease in 10 years compared to healthy individuals (27), which makes proper diagnosis and treatment crucial.

PAD is diagnosed by the ankle-brachial index (ABI). ABI is calculated for each leg as each ankle systolic BP divided by the highest arm systolic blood pressure (BP) (93). In healthy people there is no difference between leg and arm blood pressure and ABI is close to 1. In PAD, ankle blood pressure is lower than brachial blood pressure and an ABI < 0.9 is indicative of PAD. Low ABIs (< 0.9) are predictive of greater risk of cardiovascular events and all-cause mortality (103). However, high ABIs (> 1.40 or incompressible) are also associated with a high risk of mortality as they may indicate calcification of the arteries (103). The severity of PAD can also be qualitatively classified into Fontaine stages (I = asymptomatic, IIa = mild claudication, IIb = moderate to severe claudication, III = ischemic rest pain, IV = ulceration or gangrene) or Rutherford categories (0 = asymptomatic, 1 = mild claudication, 2 = moderate claudication, 3 = severe claudication, 4 = ischemic rest pain, 5 = minor tissue loss, 6 = major tissue loss) (93). The most severe form of PAD is termed “critical limb ischemia” which is defined by resting leg pain and ABI < 0.5; critical limb ischemia is associated with very high morbidity and mortality. To further emphasize, 20% of patients with critical limb ischemia die within one year of diagnosis and 40% have a leg amputated within 6 months of onset if vascular reconstruction fails (93).
Treatment of Peripheral Arterial Disease

Treatment of PAD is aimed at relieving symptoms and reducing overall cardiovascular risk. The first line of treatment for PAD is typically medical management, which consists of lifestyle changes (smoking cessation and exercise), antiplatelet agents to prevent blood clots, statins to lower cholesterol, and various hypertensive medications to control blood pressure (78). While medical management slows disease progression and reduces cardiovascular risk it rarely relieves symptoms. However, cilostazol, a phosphodiesterase III inhibitor, has been shown to improve pain in PAD perhaps through its combined effects on anti-coagulation and vasodilation (57, 101). Patients with more severe occlusion and symptoms may receive surgery such as angioplasty, stenting, or lower extremity bypass to restore limb blood flow. While surgery improves ABI, it does not always improve walking distance or symptoms (1, 95, 100).

Exercise training has the most promise for increasing functional capacity and decreasing cardiovascular risk in PAD but the mechanisms of improvement are incompletely understood (46). Most exercise training programs for PAD involve walking but sometimes other exercise modalities like cycling and lower extremity strength exercises are also utilized (97). Also, much of the research on exercise training in PAD combines exercise with other treatments such as revascularization surgery and medications. Exercise training has many physiological benefits in PAD such as increasing endothelial function, whole body maximal oxygen consumption, and oxygen utilization in the leg muscles (83, 97, 116). For functional status, exercise training improves total walking distance and claudication onset time in, and overall quality of life in PAD (54, 97). Exercise training has been shown to decrease mortality in several disease populations including PAD. PAD patients with intermittent claudication who engage in moderate intensity exercise at least on a weekly basis have a lower mortality rate in 5 years than sedentary PAD patients (44).
There are several problems in implementing exercise programs for PAD patients. First, there are very few exercise programs for PAD and these are underutilized because of the lack of medicare reimbursement for such programs despite their proven efficacy (56). If patients have comorbid coronary or heart disease, a modified cardiac rehabilitation can be prescribed. However, recruitment is difficult for research-based exercise programs for PAD that are free or compensated; this is partly attributed to transportation issues. In general, PAD patients poorly adhere to at home exercise programs, likely because of pain with exertion (56). More recently, remotely monitoring steps for at-home exercise programs has shown many of the same functional and physiological improvements as the in facility programs with higher adherance and lower attrition (45). Yet, it remains unclear whether exercise training is completely safe for PAD (given their cardiovascular risk) and what modality, frequency, and intensity of training are optimal.

The Exercise Pressor Reflex

The cardiovascular response to exercise is mediated by central command and the exercise pressor reflex (EPR) (65). Central command is a “top-down” mechanism in which neurons in the motor cortex and thalamus elicit the cardiovascular response to exercise through connections in the medulla and spinal cord neurons (36). The EPR describes a reflex originating in the contracting skeletal muscle that triggers the hemodynamic response to exercise (25). There is evidence to support that both mechanisms contribute to the cardiovascular and respiratory responses to exercise and the two pathways are not mutually exclusive (65).

Evidence for the EPR was first reported by Alam and Smirk in 1937 (2). Alam and Smirk noted that the BP increase from contracting skeletal muscle in humans remained if blood was trapped in the muscle; this is now referred to as the muscle metaboreflex. The EPR is carried from the muscle to the spinal cord via group III and IV muscle afferent fibers; these have thinly
or unmyelinated axons and free nerve endings (81). Group III muscle afferent fibers are predominantly stimulated by mechanical stimuli and group IV muscle afferent fibers are more responsive to metabolic stimuli (66). Some muscle afferents respond to both mechanical and metabolic stimuli (67). There are two major components of the EPR, the muscle mechanoreflex and the metaboreflex. The muscle mechanoreflex is evoked by actively or passively contracting the muscle; this occurs rapidly within the first 30 seconds of muscle contraction (10). The muscle metaboreflex is evoked by a build-up of chemical metabolites in the muscle. This process takes longer to evoke but can last for several minutes following muscle contraction if the metabolites are trapped in the muscle (94).

The type of exercise and the intensity dictate the cardiovascular response that the EPR evokes. Dynamic exercise, such as walking and plantar flexion, increases cardiac output and HR but increases in mean BP are minimal- at least in healthy humans (72). Static exercise, such as weight lifting or isometric handgrip, evokes a large rise in BP and a small change in cardiac output (72).

**Blood Pressure in Peripheral Arterial Disease**

An exaggerated EPR has been observed in several disease states including hypertension, heart failure, and PAD (4, 85, 114). An exaggerated BP response to exercise is predictive of cardiovascular events and all-cause mortality in PAD (30) and future cardiovascular events in healthy humans (70, 73, 75, 122). About half of PAD patients are hypertensive (24), which makes it difficult to discern whether abnormal BP responses to exercise are unique to PAD or a trait of hypertension.

PAD patients have an exaggerated BP response to dynamic leg exercise; which does not evoke dramatic changes in BP in healthy humans. However, the exaggerated BP response in PAD
seems to be unique to leg exercise (4, 5, 77, 87, 106). Hemodynamic responses to arm exercise (isometric handgrip) are similar in PAD patients and healthy subjects (6, 107). These data suggest that the exaggerated BP response to leg exercise in PAD is due to a change in the skeletal muscle afferents in the leg that sensitize the EPR, rather than a change in central command. Studies have also postulated that specifically muscle mechanoreceptor sensitivity is augmented in PAD since the exaggerated BP response to exercise is rapid onset (within 20 seconds) and occurs during low-intensity exercise (53, 87).

**Coronary Blood Flow Regulation**

The coronary blood vessels play a vital role in maintaining oxygen (O₂) and substrate delivery to the myocardium. In healthy humans, physiological stress increases myocardial O₂ demand, which is compensated for by an increase in coronary blood flow since the myocardium cannot significantly increase O₂ extraction (34).

There are two main methods to measure coronary blood velocity (CBV) in humans. The gold-standard technique for measuring coronary blood flow in humans is Doppler guide-wire which is performed by feeding a catheter from the femoral artery to the heart; this measurement aids in angiography or angioplasty (32). Doppler guide-wire can accurately measure velocity and diameter of coronary vessels. However, Doppler guide-wire is invasive, requires an interventional cardiologist to perform and sedation for the patients. Doppler guide-wire also carries several including vessel rupture, thrombosis, and hemorrhage at the arterial access location. Transthoracic Doppler echocardiography is a non-invasive technique to measure CBV in humans. Transthoracic Doppler echocardiography is performed by placing a 7S Doppler probe on the chest, between and below the ribs. A skilled ultrasound technician or student can perform this
technique. Transthoracic Doppler echocardiography is utilized in this dissertation to measure velocity in the left anterior descending coronary artery (LAD) during exercise.

The problem with using transthoracic Doppler echocardiography is that the technique is not sensitive enough to accurately measure diameter of the LAD. Without a measure of diameter or area of the vessel, blood flow cannot be calculated. However, measuring changes in CBV may be indicative of coronary blood flow responses. One study found that LAD velocity changes 10 times more than diameter in response to adenosine infusion (52). In addition, adenosine-induced changes in LAD velocity are correlated to changes in flow measured by Doppler guide-wire (69, 102). CBV responses to cold pressor test, a physiological stressor, are also correlated to coronary blood flow responses (52). Furthermore, CBV responses to pharmacological stimuli are predictive of adverse cardiovascular events in healthy subjects and patients with coronary artery disease (55, 104, 105, 112, 113). Therefore, evidence suggests changes in CBV measured by Doppler are largely indicative of changes in coronary blood flow. However, we cannot be certain that changes in CBV to exercise precisely track changes in coronary blood flow as this has not been verified and this represents a limitation of the studies in this dissertation.

**Coronary Blood Flow in Peripheral Arterial Disease**

Despite the high cardiovascular risk in PAD, there have been few studies to investigate coronary blood flow in PAD. One study by Pellegrino, et al. (99) found that PAD patients have decreased coronary flow reserve (the ratio of coronary blood flow at stress to coronary blood flow at rest). This study also found that coronary flow reserve was correlated to flow-mediated dilation of the brachial artery (99). These data suggest two things: 1) the ability of the coronary blood vessels to dilate is impaired in PAD patients and 2) this may be related to global endothelial dysfunction. Yet, this study only tested maximal coronary vasodilation in PAD to
pharmacological stimuli. Prior to my recent publication (107), no studies had examined coronary blood flow responses to physiological stressors or exercise in PAD.

Investigating coronary blood flow responses to exercise in PAD is imperative because of the high risk of myocardial infarction (93). While the mechanisms contributing to mortality in PAD are unknown, an augmented rise in myocardial metabolism coupled with impaired O₂ delivery may lead to myocardial ischemia. Several studies support that PAD patients have an exaggerated BP response to leg exercise (4, 5, 77, 87, 106), that increases myocardial O₂ demand (34). Furthermore, an augmented BP response to exercise predicts mortality in healthy subjects (75, 122) and in patients with PAD (30). Impaired O₂ delivery to the myocardium during stress may link augmented BP (myocardial O₂ demand) in PAD to high cardiovascular risk.

One problem with measuring CBV in PAD is that it is not a clear phenotype. Since atherosclerosis is a systemic disease, approximately 50% of PAD patients also have coronary artery disease (12). Even PAD patients who do not have clinically significant coronary atherosclerosis (>80% vessel occlusion or angina) likely have pre-clinical manifestations of coronary artery disease such as endothelial dysfunction (109). Study 1 in my dissertation excluded patients with symptomatic coronary artery disease but we can not be certain that patients did not have subclinical atherosclerosis that might confound our data. If PAD patients have co-morbid coronary disease, this could decrease their CBV more, especially if they have not had any coronary revascularization procedures.

PAD patients without coronary artery disease have the same cardiovascular mortality rates as PAD patients without coronary artery disease (26). This suggests that something inherent to PAD can limit blood flow to the heart and brain causing cardiovascular insults. There are some data to suggest that peripheral artery changes can impede coronary flow. A study in an animal model of PAD found that mechanically occluding the femoral artery in swine reduced adenosine diphosphate and bradykinin induced vasodilation in the LAD (3). These animals had no systemic
atherosclerosis. Therefore, data suggest that occluding blood flow in the leg may have an effect on blood flow in the heart in the absence of systemic disease. This concept will be explored in this dissertation.

**Leg Oxygen Extraction in Peripheral Arterial Disease**

During exercise, the metabolic demand of the exercising muscle increases, which is met by increases in blood flow to the exercising skeletal muscle via local vasodilator factors (110). The skeletal muscle also increases its extraction of oxygen from the blood up to 500% of resting levels (20). There is an ongoing debate on whether impairments in blood flow or muscle metabolism contributes more to PAD symptoms. While PAD primarily affects large artery blood flow, limited blood flow does not fully explain claudication pain (47) and vascular bypass surgery that restores flow, does not always improve walking ability (100). Furthermore, it is unclear whether exercise hyperemia is impaired in the legs in PAD. PAD patients may actually experience compensatory vasodilation to maintain flow around thrombi as supported by clinical findings (31) and studies of ischemic exercise in healthy humans (38). Joyner and Casey (22) developed a purely mechanical model of arterial obstruction by inflating an intra-arterial balloon catheter in the brachial artery in healthy humans at rest and during rhythmic handgrip exercise. They observed enhanced brachial artery vasodilation when the balloon was inflated during exercise to maintain flow (22). However, this compensatory vasodilation is dependent on adenosine and nitric oxide, two mechanisms that are impaired in PAD (13, 18, 61). Whether blood flow limitations during exercise impair oxygen delivery to skeletal muscle in PAD remains unclear.

Growing evidence suggests that changes in skeletal muscle metabolism distal to obstruction contribute to the pathophysiology of claudication (15). Muscle biopsies in PAD
reveal reduced Nicotinamide adenine dinucleotide dehydrogenase and ubiquinol-cytochrome c oxidoreductase activity in mitochondria which may decrease electron transport chain activity (16). These studies also reveal accumulation of acylcarnitines and lactate suggesting a shift to anaerobic metabolism (58). Together these data imply that oxidative metabolism is reduced in skeletal muscle in PAD (17). Since limitations in large artery blood flow do not fully explain claudication in PAD, mechanisms to measure blood flow in the microvasculature and skeletal muscle oxygen extraction (SmO₂) in PAD are imperative.

Some studies have utilized magnetic resonance imaging to examine blood flow and oxygenation in leg muscles in PAD (71, 76). The problems with magnetic resonance imaging are that it is very expensive, there are size constraints (not all subjects can fit in the scanner), claustrophobic patients can not participate, and even slight movement can greatly damage the signal, which limits exercise studies. In addition, functional magnetic resonance imaging was designed for use in the brain and programs to image skeletal muscle are difficult to find and use. Near-infrared spectroscopy (NIRS) has emerged as a more feasible way to measure muscle oxygenation. NIRS devices are relatively inexpensive, portable, and non-invasive (84). The devices are worn outside the skin and light penetrates to the skeletal muscle. The NIRS signal measures absorption of light by the heme groups and is altered by oxygen (84). The signal is derived from oxygen bound to hemoglobin and myoglobin in the microvasculature (8). With NIRS, we are able to measure local tissue oxygenation. NIRS data can suggest the balance between local oxygen delivery (blood flow) and oxygen extraction in the skeletal muscle but cannot decipher between the two unless blood flow is not changing. NIRS-measured oxygen saturation has been shown to decline with exercise (11). Changes in NIRS-measured SmO₂ correlates to decreased oxygen saturation measured with direct measurements of venous and arterial O₂ saturation via venous catheters (123).
Previous studies using NIRS on the calf muscles in PAD found that SmO₂ decreases faster and to a greater extent in PAD patients compared to age-matched healthy controls during treadmill walking (8, 9, 35, 68, 79, 82). While the drop in leg SmO₂ in PAD is usually attributed to decreased delivery of oxygen-rich blood to the muscle, the higher BP response to exercise may actually improve flow and impaired oxygen extraction in the muscle may account for the drop in leg SmO₂. Surprisingly, no studies have examined the link between changes in SmO₂ and BP during exercise in PAD. We will address this gap in Study 2 (Chapter 4) of this dissertation.
Chapter 3

Coronary Exercise Hyperemia is Impaired in Patients with Peripheral Arterial Disease


Introduction

Peripheral arterial disease (PAD) is an atherosclerotic vascular disease that affects over eight million people in the United States and 202 million worldwide (40, 51). PAD is characterized by progressive narrowing of the lower extremity arterial vasculature and ischemic leg pain that occurs with walking, termed “intermittent claudication” (93). Whether or not patients experience claudication, a diagnosis of PAD heightens overall cardiovascular risk. Patients with PAD have a five times greater incidence of cardiovascular mortality in 10 years compared to healthy individuals of similar age (27). Myocardial infarction accounts for 60% of all deaths in PAD (93) and it is suspected that impaired coronary vasomotor control contributes to these outcomes. A recent study found that occluding the femoral arteries in swine blunts coronary vasodilation (3). However, very few studies have evaluated coronary blood flow responses in patients with PAD (99).

The coronary blood vessels play a vital role in maintaining oxygen (O$_2$) and substrate delivery to the myocardium. In healthy humans, physiological stress increases myocardial O$_2$ demand, which is compensated by an increase in coronary blood flow since the myocardium cannot significantly increase O$_2$ extraction (34). Changes in peak coronary blood velocity (CBV) reflect changes in coronary blood flow (69, 102). Furthermore, CBV responses to pharmacological stimuli are predictive of adverse cardiovascular events in healthy subjects and
patients with coronary artery disease (55, 104, 105, 112, 113). PAD patients also have attenuated coronary vasodilation to pharmacological stimuli (99), but whether coronary hyperemia is impaired in these patients during physiological stress is unknown.

Previous studies show that patients with PAD have augmented blood pressure (BP) responses to low-intensity exercise (4, 5, 77, 87), which is prognostic of cardiovascular events and all-cause mortality (30). While the mechanisms contributing to mortality are unknown, an augmented rise in myocardial metabolism coupled with impaired O₂ delivery may lead to myocardial ischemia. Therefore, the main purpose of this study was to investigate coronary blood velocity (CBV) responses to plantar flexion exercise in patients with PAD. A subset of subjects also performed isometric handgrip exercise to assess coronary exercise hyperemia without exercising an ischemic leg. We hypothesized that PAD patients have attenuated coronary exercise hyperemia despite an augmented rise in myocardial O₂ demand.

Methods

Subjects and Design

These laboratory studies used an independent-subjects design whereby physiological parameters were compared between groups (PAD patients, healthy control subjects). Twelve PAD patients (65 ± 2 yr, 7 men) and 15 healthy control subjects (64 ± 2 yr, 9 men) were enrolled. The sample size was determined after the first four subjects in each group had completed testing. Specifically, we determined that if the true difference in the mean ΔCBV/Δrate pressure product (RPP = HR x SBP; an index of myocardial O₂ demand) from baseline to plantar flexion exercise at 2.0 kg was 5.6 a.u. with a standard deviation of 4.0 a.u. then we would need to study 12 subjects in each group to reject the null hypothesis with 90% power and α = 0.05. In order to
account for attrition and potential missing data, we enrolled 12 PAD patients and 15 healthy subjects. Subsequently, for the handgrip experiments we determined after the first three subjects that if the true difference in the mean $\Delta \text{CBV}/\Delta \text{RPP}$ from baseline to peak handgrip was 5.27 a.u. with a standard deviation of 2.5 a.u. then we would need to study 6 subjects in each group to reject the null hypothesis with 90% power and $\alpha = 0.05$. We enrolled 7 PAD patients and 8 healthy controls to account for potential missing data.

PAD patients were recruited from the Penn State Hershey Medical Center vascular outpatient clinic lists and from our database of subjects who had previously participated in our studies. We made an effort to match PAD patients to healthy subjects from our database (first by sex, then by age, then BMI). All PAD patients had an ankle-brachial index (ABI) < 0.9 and were classified as Fontaine stage II. All PAD patients in the current study did not have clinically active coronary artery disease, aortic stenosis, or prior myocardial infarction based on medical history, symptoms (no angina with exertion), EKG and resting echocardiogram interpreted by a cardiologist (U. Leuenberger). More specifically, all PAD patients had normal ejection fraction (55-70%) and left ventricle wall thickness (< 1.1 cm) as well as no wall motion abnormalities. Patients with diabetes or were not excluded from the study; two PAD patients were diabetic and one PAD patient was pre-diabetic. Most PAD patients (9/12) had undergone at least one leg vascular intervention prior to enrollment but remained symptomatic with ABI < 0.9. These procedures included femoral-popliteal bypass (N=4), peripheral stents (N=3), angioplasty without stenting (N=3), aortic-bifemoral bypass (N=1), and popliteal endarterectomy (N=1). PAD patients were on several medications including antiplatelet medications (N=8), statins (N=8), aspirin (N=8), ACE inhibitors (N=7), hydrochlorothiazide (N=6), fish oil supplements (N=5), amlodipine (N=4), selective β1 blockers (N=4), proton pump inhibitors (N=3), Angiotensin II receptor blockers (N=2), cilostazol (N=3), and metformin (N=1). PAD patients withheld cardiovascular medications for at least 12 hours prior to the plantar flexion exercise trials but stayed on
medications for the handgrip trials. Two PAD patients were active smokers and we did not ask that they refrain from smoking before the study. Healthy subjects were normotensive, non-obese, and non-smokers who were not on any medications and had no chronic illnesses. Healthy subjects were recreationally active but not competitive athletes. All subjects refrained from caffeine, alcohol, and vigorous exercise for 24h prior to the study.

**Experimental Protocol**

All study protocols were approved in advance by the Institutional Review Board of Penn State Hershey and conformed to the Declaration of Helsinki. All subjects provided written and informed consent. The study protocols were performed in the supine position in a thermoneutral laboratory (20-21°C). First, ABIs were assessed at rest in all subjects. Subjects were then instrumented with a three-lead EKG (Cardiocap/5, GE Healthcare), a finger BP cuff (Finometer, FMS), and pneumotrace to monitor respiratory activity; these variables were continuously collected at 200 Hz by PowerLab (ADInstruments) and analyzed offline. Resting BPs were obtained in triplicate by automated oscillometry of the right brachial artery (Phillips Sure Signs VS3) after 15 min of quiet rest and these values were used to verify the Finometer values as previously described (90). A transthoracic echocardiogram (GE Vivid 7) was performed at rest in the supine posture to determine left ventricular size and function, mitral inflow at the mitral leaflet tips, and Doppler velocities at the septal mitral annulus (table 1). Peak diastolic CBV in the left anterior descending artery (LAD) was obtained from the adjusted apical four-chamber view using a 7S probe (all images acquired by Z. Gao). The specific procedures for measuring CBV in LAD have been previously described by our laboratory and the reproducibility within subjects has been verified (41-43, 86). Subjects also completed the walking impairment questionnaire (WIQ), a pen and paper test to evaluate walking performance that correlates to peak walking time.
and absolute claudication distance in PAD (111). The WIQ contains 14 questions and each response is weighted based on the difficulty of the task; scores range from 0 to 100 with lower scores indicating worse walking ability (greater impairment).

To test our hypothesis, subjects performed plantar flexion exercise by contracting the calf muscles of a single leg 30 times per minute with increasing amounts of resistance attached to the device. The resistance started at 0.5 kg and increased by 0.5 kg every minute until 7 kg, fatigue, significant pain, or inability to maintain the cadence occurred as previously performed in our lab (33, 87, 88). Ratings of perceived exertion and pain were obtained each minute using the Borg scales (14). PAD patients performed the exercise with their most symptomatic limb. In a subsequent study on a separate day, a subset of subjects who were willing to participate in another experiment (N=7 PAD, N=8 healthy) performed isometric handgrip exercise at 40% maximal voluntary contraction until fatigue by squeezing a handgrip dynamometer using their right forearm muscles. This stressor was chosen because it raises sympathetic nervous system activity, HR, and BP without evoking ischemic leg pain (80).

**Data Collection and Statistical Analysis**

All variables were measured continuously and analyzed offline. An average of the last 20 s of each minute is presented. Rate pressure product (RPP, the product of HR and systolic BP) was used as an index of myocardial O₂ demand. CBV was used as an index of myocardial O₂ supply. Our primary outcome variable was the ΔCBV to ΔRPP ratio from baseline to exercise, which was calculated as ΔCBV/ΔRPP x 1000. This ratio has been used previously to quantify coronary hyperemia normalized to cardiac metabolism; higher numbers indicate greater myocardial O₂ supply relative to demand (89, 91, 108, 119). Statistical analysis was performed using SPSS 22. Mann-Whitney U tests were used to compare anthropometric and non-parametric
data between groups. To analyze responses to plantar flexion exercise, two group (PAD, healthy) by 6 time point (baseline, exercise at 0.5 kg, 1.0 kg, 1.5 kg, 2.0 kg, fatigue) repeated measures ANOVA were conducted on the raw physiological variables (Figure 3-1). For significant interactions, post-hoc Tukey-Kramer tests were employed. Changes (Δ) from baseline to plantar flexion exercise at 2.0 kg and from baseline to peak handgrip exercise were compared between groups using Student’s t-tests for independent samples. Cohen’s d (mean 1 - mean 2/ combined standard deviation) was calculated on primary outcomes to determine effect size. We also conducted planned correlations between WIQ scores, ABIs, and physiological responses to exercise. All data are shown as mean ± standard error mean (SEM) unless otherwise stated. Significance was set at P < 0.05 for all tests.

Results

Table 3-1 shows anthropometric measurements and resting hemodynamics in PAD patients and healthy control subjects. Age, height, weight, diastolic BP, and mean BP were similar between groups. While BMI was not statistically different between groups, BMI tended to be higher in PAD patients compared to healthy subjects. Systolic BP was higher in PAD patients compared to healthy subjects. The median WIQ score in PAD was 51 (range: 16 to 100). All healthy subjects scored 100 (i.e. no impairment) on the WIQ. There were no significant correlations between WIQ scores, ABIs, and physiological responses to exercise. More specifically, the CBV response to plantar flexion did not correlate to worst ABI (R = -0.093, P = 0.785) or to WIQ scores (R = -0.060, P = 0.860).
### Table 3-1. Baseline anthropometric measurements and resting hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>PAD (N = 12)</th>
<th>Healthy (N = 15)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male / female</td>
<td>7 / 5</td>
<td>9 / 6</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>65 ± 2</td>
<td>64 ± 2</td>
<td>0.940</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.67 ± 0.03</td>
<td>1.72 ± 0.02</td>
<td>0.104</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.7 ± 3.6</td>
<td>75.2 ± 3.3</td>
<td>0.481</td>
</tr>
<tr>
<td>Body Mass Index, kg/m²</td>
<td>28.1 ± 1.2</td>
<td>25.2 ± 0.7</td>
<td>0.072</td>
</tr>
<tr>
<td>ABI exercising leg</td>
<td>0.58 ± .03 *</td>
<td>1.09 ± .03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ABI non-exercising leg</td>
<td>0.72 ± .06 *</td>
<td>1.07 ± .03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pack years</td>
<td>29 ± 8 *</td>
<td>0 ± 0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>143 ± 7 *</td>
<td>123 ± 3</td>
<td>0.014</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>74 ± 2</td>
<td>76 ± 2</td>
<td>0.351</td>
</tr>
<tr>
<td>Mean BP, mmHg</td>
<td>96 ± 3</td>
<td>90 ± 2</td>
<td>0.194</td>
</tr>
<tr>
<td>Heart Rate, beats/min</td>
<td>63 ± 3 *</td>
<td>55 ± 2</td>
<td>0.020</td>
</tr>
<tr>
<td>S', cm/s</td>
<td>9.1 ± 0.7</td>
<td>8.5 ± 0.5</td>
<td>0.497</td>
</tr>
<tr>
<td>e', cm/s</td>
<td>8.7 ± 0.6</td>
<td>9.8 ± 0.6</td>
<td>0.209</td>
</tr>
<tr>
<td>a', cm/s</td>
<td>10.2 ± 0.8</td>
<td>10.0 ± 0.7</td>
<td>0.877</td>
</tr>
<tr>
<td>E, cm/s</td>
<td>86.1 ± 4.3 *</td>
<td>72.7 ± 3.3</td>
<td>0.024</td>
</tr>
<tr>
<td>A, cm/s</td>
<td>90.4 ± 7.7 *</td>
<td>68.8 ± 1.6</td>
<td>0.021</td>
</tr>
<tr>
<td>E-to-A ratio</td>
<td>1.00 ± 0.05</td>
<td>1.06 ± 0.05</td>
<td>0.383</td>
</tr>
<tr>
<td>E to e'</td>
<td>10.7 ± 0.9 *</td>
<td>7.6 ± 0.4</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. ABI, ankle-brachial index; BP, blood pressure; S’, systolic mitral annulus velocity; e’, early diastolic mitral annulus velocity; a’, late diastolic mitral annulus velocity; E, early diastolic mitral inflow; A, late diastolic mitral inflow. * P < 0.05 compared to healthy control subjects.

### Table 3-2. Perceived Exertion, Pain, and Exercise Duration

<table>
<thead>
<tr>
<th></th>
<th>PAD (N = 13)</th>
<th>Healthy (N = 14)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dynamic Plantar Flexion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last workload completed (kg)</td>
<td>4.0 ± 0.5 *</td>
<td>7.0 ± 0.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RPE at end of exercise (a.u., 6 – 20)</td>
<td>14 ± 1</td>
<td>16 ± 1</td>
<td>0.212</td>
</tr>
<tr>
<td>Pain at end of exercise (a.u., 0 – 10)</td>
<td>4 ± 1 *</td>
<td>0 ± 0</td>
<td>0.011</td>
</tr>
</tbody>
</table>

**Isometric Handgrip**

<p>| | | | |</p>
<table>
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<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to fatigue (seconds)</td>
<td>204 ± 15 *</td>
<td>280 ± 24</td>
<td>0.010</td>
</tr>
<tr>
<td>MVC forearm (kg)</td>
<td>34 ± 3</td>
<td>37 ± 2</td>
<td>0.252</td>
</tr>
<tr>
<td>RPE at fatigue (a.u., 6 – 20)</td>
<td>19 ± 1</td>
<td>19 ± 0</td>
<td>0.694</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. RPE, rating of perceived exertion; MVC, maximum voluntary contraction. * P < 0.05 compared to healthy control subjects.
During plantar flexion exercise, PAD patients experienced more pain and stopped at a lower workload compared to healthy subjects; yet ratings of perceived exertion were similar between groups during the last stage of exercise (table 3-2).

All PAD patients completed the 2.0kg workload (4 minutes) therefore statistical comparisons were made up to this time point. Significant group x time interactions were found for systolic BP and HR, which were higher in PAD compared to healthy subjects at all analyzed time points (Figure 3-1). Yet, CBV over time was not different between groups (Figure 3-1). The changes from baseline to exercise at 2.0 kg in systolic BP (PAD: Δ 18 ± 2, healthy: Δ 12 ± 2 mmHg, \( P = 0.020 \)) and HR (PAD: Δ 9 ± 1, healthy: Δ 3 ± 1 beats/min, \( P = 0.001 \)) were higher in PAD. However, the change in mean BP from baseline to exercise at 2.0kg was not different between groups (PAD: Δ 12 ± 2, healthy: Δ 8 ± 2 mmHg, \( P = 0.089 \)). The change in CBV (ΔCBV) from baseline to exercise at 2.0kg was attenuated in PAD vs. healthy subjects (Δ 2.4 ± 1.2 vs. Δ 6.0 ± 1.6 cm/s, \( P = 0.039 \), Cohen’s \( d = 0.7 \)). In addition, ΔCBV/ΔRPP (slope of the line from baseline to exercise

![Figure 3-1. Systolic blood pressure (BP), heart rate, and coronary blood velocity responses to plantar flexion exercise in patients with peripheral arterial disease (PAD) and healthy control subjects (N = 12 in each group). The fatigue time point represents the last workload performed by each PAD patient and the corresponding workload of each matched healthy subject. *P < 0.05 between groups.](image)
at 2.0 kg) was lower in PAD vs. healthy subjects (Δ 1.0 ± 0.6 vs. Δ 7.3 ± 2.9 a.u., \( P = 0.013 \), Cohen’s \( d = 0.9 \), figure 3-2A).

In response to isometric handgrip exercise, maximum voluntary contraction of the forearm and ratings of perceived exertion at fatigue were similar between groups, although healthy subjects exercised about one minute longer (Table 3-2). There were no group differences in mean BP (PAD: Δ 35 ± 3, healthy: Δ 30 ± 4 mmHg, \( P = 0.223 \)), HR (PAD: Δ 14 ± 2, healthy: Δ 14 ± 3 beats/min, \( P = 0.701 \)), or RPP (PAD: Δ 5450 ± 487, healthy: Δ 4307 ± 408 beats/min, \( P = 0.093 \)) responses to handgrip exercise.

Despite comparable increases in myocardial \( O_2 \) demand, ΔCBV from baseline to peak handgrip was attenuated in PAD vs. healthy subjects (Δ 8.3 ± 4.2 vs. Δ 16.9 ± 3.6, \( P = 0.033 \), Cohen’s \( d = 1.2 \)). The ΔCBV/ΔRPP ratio from baseline to peak grip was also attenuated in PAD vs. healthy subjects (Δ 1.7 ± 0.9 vs. Δ 4.5 ± 1.0, \( P = 0.030 \), Cohen’s \( d = 1.1 \), Figure 3-2B).

**Figure 3-2** A) Change in coronary blood velocity / rate pressure product from baseline to plantar flexion exercise at 2.0 kg in peripheral arterial disease patients (PAD, \( N = 12 \), solid lines) and healthy control subjects (\( N = 14 \), dashed lines). B) Change in coronary blood velocity / rate pressure product from baseline to peak fatiguing isometric handgrip exercise (40% maximal voluntary contraction) in PAD (\( N = 7 \), solid black lines) and healthy subjects (\( N = 8 \), gray dashed lines). Gray arrows represent individual responses and black arrows show group averages. A steeper slope indicates better myocardial \( O_2 \) supply for a given amount of increased metabolic demand.
Discussion

Summary and Main Findings

The purpose of this study was to investigate the effects of leg revascularization on the cardiovascular response to exercise in PAD. This study produced two novel findings. First, the BP and HR responses to exercise in PAD were attenuated following leg revascularization. Second, the coronary exercise hyperemia in PAD was improved one month after leg revascularization when exercising at the same workload with a similar myocardial oxygen demand. Together these data suggest that leg revascularization improves cardiovascular reflex control of blood flow in PAD.

Effect of Revascularization on the Exercise Pressor Reflex

Previous studies from our laboratory and others demonstrate that the BP response to plantar flexion exercise is augmented in PAD patients compared to healthy subjects (77, 87, 107). This heightened exercise pressor reflex in PAD may be caused by increased sympathetic nervous system activation to exercise or by sensitized skeletal muscle afferents (81). We have previously shown that reducing oxidative stress with vitamin C or prostanoids with the cyclooxygenase inhibitor ketorolac attenuates the BP response to exercise in PAD (87, 88). The current study adds to this literature by showing that restoring large artery blood flow, via leg revascularization, decreases the heightened BP and HR responses to exercise in PAD. In addition, the patients are able to perform plantar flexion longer (about 2 minutes on average) following revascularization. These data suggest that ischemia in the leg contributes to the heightened BP and HR responses to exercise in PAD.
Effect of Leg Revascularization on Coronary Exercise Hyperemia

Our recent study found that the CBV response to leg exercise is attenuated in PAD (107). Based on this previous study, we speculated that impaired coronary exercise hyperemia in PAD is attributed to increased sympathetic vasoconstriction in the coronary arteries or global endothelial dysfunction. Therefore, in the current study, we hypothesized that leg revascularization would not alter the CBV response to leg exercise in PAD because the coronary vasculature was not treated as part of the subjects’ clinical interventions during the timeframe of the study. Contrary to our hypothesis, we observed a significant change in CBV responses to plantar flexion exercise in 6/8 PAD patients following leg revascularization. At the pre revascularization visit, 4/8 subjects had a paradoxical decrease in CBV in response to exercise (ie. their hearts were getting less blood flow and oxygen during exercise compared to at rest). In all four of these subjects, their CBV response increased in response to exercise at their post revascularization visit. Despite this beneficial effect of peripheral revascularization, the CBV response in PAD post revascularization is still attenuated compared to healthy subjects’ CBV response using a similar exercise protocol (107).

There are a few possible mechanisms that may underlie this change in coronary physiology that occurs one month following leg revascularization. First, revascularization may have decreased sympathetic nervous system activity. While we did not directly measure sympathetic activity in this study, less sympathetic tone during exercise may have decreased BP and coronary vasoconstrictor responses to exercise in PAD. Second, there may have been alterations in the timing and amplitude of the arterial pulse wave reflection following revascularization, which could alter CBV. Peripheral arterial obstruction causes premature arterial pulse wave reflection in PAD and augments afterload (aortic blood pressure) reducing coronary perfusion (126). Removing the arterial obstruction may delay pulse wave reflection and improve coronary perfusion. This hypothesis is supported by one study that found a decrease in
augmentation index in PAD patients three months following a peripheral stent placement (63). Therefore, revascularization could delay the arrival of the reflected pulse wave and normalize CBV responses in PAD. Third, there is evidence of improved endothelial-dependent vasodilation in the arm in PAD patients following leg revascularization (19, 62, 120). Furthermore, endothelial-dependent vasodilation in the arm is correlated to coronary vasodilation in PAD (99). Therefore, if flow-mediated dilation is improved in the arm following leg revascularization as the literature suggests (19, 62, 120), it is likely that endothelial-dependent vasodilation in the coronary arteries is improved as well although this has not been investigated directly. In summary, these data suggest that leg revascularization may improve endothelial function in PAD in multiple vascular beds, not just the leg although the mechanism of physiological improvement remains unclear. Finally, it is also possible that post revascularization the PAD patients exercised more, which could have profound physiological effects (56). We do not have data on how much our study subjects walked pre- or post-revascularization and the literature does not support that PAD patients walk significantly more following leg revascularization even if their pain decreases (1, 95). However, if PAD patients did walk more following their procedures, this could certainly improve their cardiovascular health and responses to exercise.

Limitations

The main limitations of this study are typical of human physiology experiments in clinical populations. Subjects underwent different types of interventions including femoral to popliteal bypass (N = 1), angioplasty with stent placed (N = 9), and angioplasty without stent placed (N = 7). While our study was not powered to investigate whether one type of peripheral vascular intervention affected the cardiovascular response to exercise more, there doesn’t appear to be a difference between the treatment groups (ie. the one subject who underwent bypass had a
similar change in BP and CBV responses to exercise compared to the patients who underwent angioplasty). The subjects were on several medications during the study visits that may have affected their BP responses such as β1 blockers, α1-blockers, amlodipine, and ACE inhibitors. All subjects were also on platelet inhibitors such as aspirin and clopidogrel and one subject was taking the vasodilator cilastazol; these medications may increase blood flow. All patients took their usual medications during both study visits and we did not have patients withhold certain medications for safety and ethical reasons. Yet, the effect of medications on physiological responses is probably minimized since patients were taking the same medications at both study visits and the study is a within-subjects design (all patients were their own control). However, clopidogrel was added to 6 subjects’ medication lists following revascularization since it is prescribed specifically following stent implantation. Our study was not randomized and we did not have a control group or control treatment such as sham surgery or catheterization. In addition, the PAD patients in this study had co-morbid conditions including coronary artery disease and diabetes. Since some PAD patients also had coronary artery disease, this may have affected their CBV responses but every subject served as his or her own control and no procedure was performed on their coronary arteries between visits. Finally, we studied cardiovascular responses to exercise in PAD patients one month following their procedure and we do not know if the changes we observed occurred earlier than one month or remained long-term.

**Conclusion**

Leg revascularization decreased the BP and HR response to exercise and improved coronary exercise hyperemia in PAD. Further studies are need to investigate possible mechanisms that lead to widespread changes in the endothelium and central blood pressure that may improve cardiovascular reflexes in PAD.
Chapter 4

Blood Pressure and leg Deoxygenation are Augmented During Treadmill Walking in Patients with Peripheral Arterial Disease

Introduction

Peripheral arterial disease (PAD) is a large vessel, atherosclerotic vascular disease that is estimated to affect approximately 200 million people worldwide (40). The classic symptom of PAD is leg pain during walking that improves with rest, termed “intermittent claudication.” Patients with PAD also have an exaggerated blood pressure (BP) response to treadmill walking (4, 5), which may be related to increased risk of cardiovascular incidents (30, 75, 122). Furthermore, PAD patients are advised to walk as part of their treatment (74) even though the physiological mechanisms that lead to pain and the exaggerated BP response to walking in PAD are incompletely understood. Claudication pain is not fully explained by decreased large artery blood flow (47) and vascular bypass surgery that restores flow, does not always improve walking ability (100).

Growing evidence suggests that changes in the microvasculature and skeletal muscle metabolism of oxygen in the leg contribute to claudication in PAD (15). We can measure changes in skeletal muscle oxygen saturation (SmO₂) in the leg non-invasively during walking using near-infrared spectroscopy (NIRS). Several studies suggest that the fall in leg SmO₂ during walking is greater and occurs faster in PAD patients compared to healthy people (8, 9). Since understanding the mechanisms that contribute to morbidity and mortality in PAD is imperative for improving treatment, we investigated SmO₂ and BP responses during graded treadmill walking in PAD patients and healthy control subjects. We hypothesized that PAD patients would
have an exaggerated increase in BP and decrease in leg SmO$_2$ during treadmill walking compared to healthy subjects.

**Methods**

**Subjects and Design**

These studies used a repeated measures design in which physiological parameters were measured at baseline and during treadmill walking. Group (PAD, control) was a between subject factor and time (baseline, walking) was a within-subjects factor. Eight patients with PAD (age 66 ± 3, 1 female) and 8 healthy control subjects (age 65 ± 2, 1 female) participated in this study. The sample size needed was calculated after the first 7 subjects in each group had completed the protocol. We determined that if the true difference in the mean change in systolic BP from baseline to peak walking time (PWT) was 26 mmHg with a standard deviation of 14 mmHg then we would need to study 7 subjects in each group to reject the null hypothesis with 90% power and $\alpha = 0.05$. Since we already had the next subject in each group enrolled, we studied 8 PAD patients and 8 healthy subjects.

PAD patients were recruited from the Penn State Hershey Medical Center vascular outpatient clinic lists and from our database of subjects who had previously participated in our studies. We made an effort to match PAD patients to healthy subjects from our database (first by sex, then by age, then by BMI). All PAD patients had an ankle-brachial index (ABI) < 0.9 and were classified as Fontaine stage II. All PAD patients were hypertensive and had a history of cigarette smoking (1/8 subjects was currently smoking). Two out of eight subjects had coronary artery disease, 1/8 had carotid artery disease, 1/8 had obstructive sleep apnea, 1/8 had chronic obstructive pulmonary disease, 1/8 was diabetic. The healthy subjects did not have any chronic
medical conditions. Healthy subjects were taking multivitamins (2/8), Vitamin D (2/8), proton-pump inhibitors (2/8), flax seed oil (1/8), and vitamin B-12 (1/8). PAD patients were taking statins (8/8), platelet inhibitors (7/8), ACE inhibitors (4/8), Ang II antagonists (2/8), vasodilators (2/8), calcium channel blockers (2/8), fish oil (2/8), vitamin D (2/8), hydrochlorothiazide (1/8), anti-diabetic medications (1/8), and multivitamins (1/8).

Protocol

This experiment was approved by the Institutional review Board of the Pennsylvania State University College of Medicine and all procedures conformed to guidelines stated in the Declaration of Helsinki. All procedures were verbally explained, and informed consent was obtained from all participants prior to the study. The study protocols were performed in a thermoneutral laboratory (20-21°C). First, ankle-brachial indices (ABIs) were assessed at rest in all subjects. Subjects were then instrumented with a three-lead electrocardiogram (EKG, Cardiocap/5, GE Healthcare) and a separate twelve-lead EKG (SensorMedics, Milan, Italy) for safety purposes. Blood pressure was measured with an auscultatory blood pressure (BP) cuff (SunTech Medical, Morrisville, NC, USA). The SunTech Tango BP system uses an auscultatory method aided by EKG R-wave gating. The automated ausculometric transducer determines the systolic BP and diastolic BP. This BP system has been verified to accurately track systolic BP and diastolic BP during treadmill stress tests when compared to a brachial artery indwelling catheter measurements (21).

$\text{SmO}_2$ was monitored noninvasively on the medial gastrocnemius in both legs using continuous wave NIRS (Moxy muscle oxygen monitor, Fortiori Design LLC, Hutchinson, MN, USA). The NIRS device uses a Monte Carlo model to trace the propagation of photons through a tissue with an adipose tissue thickness up to $\sim$12.0 mm. The mathematical model treats a medium
(tissue) as four layers (epidermis, dermis, adipose, and muscle) by which the adipose tissue thickness is unknown. The subcutaneous adipose tissue thickness measurements are made to ensure that our NIRS photon path is reaching the correct depth to infiltrate skeletal muscle. The approximate depth of penetration is equal to half the distance between the NIRS light source and its detector (23, 28). Therefore, the NIRS device used in the present study using one emitter optode at 0 mm and two detector optodes at 12.5 mm and 25 mm has a maximum penetration depth of ~ 12.5 mm. We used ultrasound imaging to measure from the epidermis to the superficial aponeurosis of the medial gastrocnemius. An average of three manually selected points are used to account for the length between the emitter optode furthest detector optode at 25 mm.

Following instrumentation subjects remained seated for 3 minutes of baseline rest then stepped onto the treadmill (Trackmaster, Full Vision Inc., Newton, KS, USA) and remained standing for 1 minute before the treadmill was started. All subjects walked until maximal discomfort following the Gardner protocol which is a walking protocol developed for PAD patients (48). Briefly, subjects walked at 2.0 miles/hour for the entire duration. The grade began at 0% and increased 2% every 2 minutes; each increase in the grade is considered a new stage. Subjects kept walking until maximal discomfort or 22 minutes (11 stages) was reached. Subjects rated their discomfort on a scale from 0 to 4 every minute (0 = no discomfort, 1 = onset of discomfort, 2 = moderate discomfort, 3 = severe discomfort, 4 = maximal discomfort). When subjects first rated their discomfort as 1, this was used to calculate claudication onset time (COT). The time subjects said their discomfort was 4 was used to calculate peak walking time (PWT); this is also when the treadmill was lowered, slowed, and then stopped. HR was recorded from the EKG every minute and BP measurements were taken every 2 minutes, 1 minute into each walking stage.
Data Collection and Statistical Analysis

BP and HR data were recorded at the end of each stage on paper data sheets and in PowerLab (ADInstruments) and analyzed offline. EKG data were recorded continuously through PowerLab. All NIRS data were collected continuously and recorded electronically at 2 Hz and transmitted wirelessly via ANT+ to a laptop for offline analysis (PeriPedal v2.4.8, Napoleon, IN, USA). NIRS data were analyzed in 20-second bins and the last 20 seconds of each stage is reported. The NIRS devices were placed on both legs and data are reported as most affected and least affected leg in PAD; which is based on symptoms. Controls’ legs are matched to their PAD match. Stage 2 (4% incline, 4 minutes of walking) was the last stage that all PAD patients completed; therefore statistical comparisons were only made at baseline, stages 1 and 2, COT, and PWT. Each healthy subject was time matched for COT and PWT to their PAD patient match.

Statistics were performed using IBM SPSS 24.0. To analyze physiological responses to treadmill walking between groups, 2 group (PAD, healthy) by 6 time comparisons (sitting, standing, stage 1, stage 2, COT, PWT) repeated measures ANOVA were conducted on the raw physiological variables (Figure 4-1). For significant interactions, post-hoc Student’s t-tests for independent samples with modified Holm Adjustments were performed. Changes from baseline to PWT were calculated and compared between groups using Student’s t-tests for independent samples. Data are presented as mean ± standard deviation unless otherwise noted.

Results

Table 4-1 shows anthropometric measurements and resting hemodynamics in PAD patients and healthy control subjects. Age, height, weight, BMI, resting diastolic BP and HR did not differ between groups ($P > 0.05$). Resting systolic BP was higher in PAD patients compared
to healthy subjects. ABIs were lower in PAD patients. Pack years (packs smoked per day x number of years smoked) were higher in PAD patients compared to healthy subjects and only one healthy subject had a smoking history.

Table 4-1. Demographic Data and Resting Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>PAD (N = 8)</th>
<th>Healthy (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>7/1</td>
<td>7/1</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>66 ± 8</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>174.9 ± 10.7</td>
<td>176.5 ± 7.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.6 ± 13.4</td>
<td>83.9 ± 14.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.1 ± 4.4</td>
<td>26.8 ± 3.1</td>
</tr>
<tr>
<td>ABI right</td>
<td>0.7 ± 0.1 *</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>ABI left</td>
<td>0.8 ± 0.2 *</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Pack years</td>
<td>31 ± 20 *</td>
<td>3 ± 8</td>
</tr>
<tr>
<td>Smoking history/current smokers</td>
<td>8/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>137 ± 17*</td>
<td>117 ± 12</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>78 ± 8</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>65 ± 9</td>
<td>68 ± 10</td>
</tr>
<tr>
<td>Adipose Tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>6.20 ± 2.20</td>
<td>5.65 ± 1.45</td>
</tr>
</tbody>
</table>

Peripheral arterial disease (PAD); body-mass index (BMI); ankle-brachial Index (ABI); blood pressure (BP). Data are shown as mean ± standard deviation. * P < 0.05 compared to healthy subjects.

Significant group x time interactions were observed in all measured variables during treadmill walking (Figure 4-1). PAD patients had a greater rise in systolic BP and diastolic BP in response to treadmill walking compared to healthy subjects. Systolic BP increased over time in both groups but was higher in PAD patients compared to healthy subjects during standing (P < 0.001), stage 1 (P = 0.023), COT (P = 0.014), and PWT (P = 0.001, Figure 4-1). The change in systolic BP from seated to PWT was greater in PAD (healthy: 23 ± 9 vs. PAD: 44 ± 19 mmHg, P = 0.007). While diastolic BP fell slightly in healthy subjects, it increased slightly in PAD patients over time. Diastolic BP was higher in PAD patients at stage 2 (P = 0.006), COT (P = 0.089), and PWT (P < 0.001, Figure 4-1). The change in mean BP from seated to PWT was greater in PAD
(healthy: 2 ± 6 vs. PAD: 23 ± 5 mmHg, \( P < 0.001 \)). HR increased over time in all subjects but HR was higher in PAD patients at PWT \( (P = 0.011, \text{Figure 4-1}) \). The change in RPP from seated to PWT was exaggerated in PAD (healthy: 6596 ± 1580 vs. PAD: 12820 ± 3470 mmHg * beats/min, \( P < 0.001 \), Figure 2).

Leg SmO\(_2\) data is shown in Figure 4-1 for the most affected leg. In the most affected leg, PAD patients had a sharp fall in leg SMO\(_2\) following the onset of walking while SMO\(_2\) fell less and remained relatively stable in healthy subjects. SMO\(_2\) in the most affected leg was lower in PAD patients at stage 2 \( (P = 0.050) \), COT \( (P < 0.001) \), and PWT \( (P < 0.001) \).

The change in SMO\(_2\) (PWT-seated) was greater in PAD (healthy: 15 ± 12 vs. PAD: 49 ± 5 %, \( P < 0.001 \)) in the most affected leg.

**Figure 4-1.** Effects of treadmill walking test on systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and local oxygen saturation (SmO\(_2\)) in the medial gastrocnemius muscle in PAD patients (\( N = 8 \), filled circles) and healthy subjects (\( N = 8 \), open diamonds). All subjects underwent a graded treadmill walking test designed to evoke symptoms of claudication in PAD. The claudication onset time (COT) and peak walking time (PWT) time points represent the initial and maximum claudication symptoms reported by each PAD patient. Data are presented as M ± SD, *\( P < 0.05 \) between groups.
affected leg and in the least affected leg (healthy: 12 ± 11 vs. PAD: 32 ± 18%, \( P = 0.003 \)).

**Discussion**

**Summary and Main Findings**

The main findings of this study are that PAD patients have an exaggerated increase in BP and HR as well as a greater drop in leg SmO\(_2\) during treadmill walking compared to healthy subjects. It is important to note that these exaggerated responses in PAD occurred before the onset of pain (claudication onset time). To our knowledge, this is the first study to simultaneously measure BP and SmO\(_2\) during treadmill walking in PAD patients and healthy subjects.

**Implications of the in Exaggerated Exercise Pressor Reflex in PAD**

Patients with PAD have a five times greater incidence of cardiovascular mortality in 10 years compared to healthy individuals of similar age (27). Our data show that systolic BP and HR responses to treadmill walking are exaggerated in PAD, which may help explain the heightened cardiovascular risk. Data from large epidemiological studies suggest that a greater rise in systolic BP during a maximal or submaximal treadmill exercise in healthy subjects is associated with a greater incidence of an adverse cardiovascular events (70, 73, 75, 122). Furthermore, one study...
found that in PAD patients who had an increase in systolic BP greater than 55mmHg (post-treadmill walking minus baseline) had a greater incidence of cardiovascular and all-cause mortality compared to PAD patients who had systolic BP increase of less than 55mmHg (30).

Myocardial infarction is the leading cause of death in PAD (93). This may be caused by decreased oxygen delivery to the heart during exercise. Our recent study found that coronary blood flow responses to supine plantar flexion exercise are impaired in PAD despite a greater rise in myocardial oxygen demand (RPP) (107). In the current study, PAD patients had higher RPP responses to exercise (Figure 4-2), which increases myocardial oxygen demand. However, measuring coronary blood flow non-invasively during upright exercise is extremely difficult, so we were not able to collect this data. We can only speculate that coronary blood flow may also be impaired in PAD patients during treadmill walking. Since physical activity often triggers myocardial infarction (115) and walking is a part of treatment for PAD, it is important to understand the physiology during walking in PAD and potential adverse effects.

While healthy subjects had no change or a slight decrease in diastolic BP during our treadmill walking protocol, PAD patients had a slight increase in diastolic BP. This may be caused by increased vascular stiffness or altered sensitivity of muscle afferents in PAD (117). Regardless of the mechanism, a rise in diastolic BP during dynamic exercise is also associated with increased risk of adverse cardiovascular events. Data from 3045 healthy participants in the Framingham Heart study suggests that a higher diastolic BP (above 80th percentile) during a treadmill exercise test predicted cardiovascular events (75). These data suggest, that the counterintuitive increase in diastolic BP during slow-paced treadmill walking in PAD may relate to cardiovascular morbidity and mortality.

Other studies have measured BP during treadmill walking in PAD. Bakke, et al. (5) measured beat-to-beat BP during treadmill walking at a range of speed and distances in PAD patients and healthy subjects. However, BP in this study was measured by finger
plethysmography (Finometer) which has not been validated during treadmill or walking exercise. Although the arm was held with a sling, in our experience slight movement can greatly alter finometer recordings. In addition, each subject walked at a different speed (1-4.5 km/hr), which makes between subjects comparisons complicated. Ritti-Dias, et al. (106) also measured BP during walking in PAD. However, this study did not include control subjects and BP was measured infrequently and the methods are not described in detail (106). In one study by Baccelli, et al. (4), BP was measured continuously via a radial artery catheter during short bouts of treadmill walking at a 10° slope and variable speeds. These studies all support that PAD patients have a greater increase in systolic BP during treadmill walking and a rise in diastolic BP as well (4), which is consistent with our findings. However, our finding that HR at PWT was exaggerated in PAD patients conflicts with previous findings that the HR response to treadmill walking is similar between PAD patients and healthy subjects (4).

**Importance of NIRS Findings During Walking**

In the present study, we also used NIRS to track oxygen saturation in calf muscle along with BP and HR during treadmill walking. The major finding of this study is a drop in SmO₂ is coincident with the augmented rise in BP during treadmill walking in PAD. In particular, both the fall in SmO₂ and the rise in BP occur before the onset of symptomatic leg pain suggesting there is a desaturation threshold in the leg muscle that contributes to the exercise pressor response. This suggests that a mismatch in oxygen delivery and/or utilization in the muscle during walking contribute to the augmented exercise pressor response in PAD. Interestingly, there is no change in SmO₂ or BP in healthy participants who walked longer and at greater inclines than matched PAD patients. This could be explained in part by the normal cardiovascular system and circulation’s ability to supply adequate oxygenation to the exercising muscles during walking by increasing
blood flow and oxygen extraction by the muscle. In contrast, PAD patients are less able to increase blood flow to the legs due to impaired endothelial-mediated vasodilation (13, 18, 61). PAD patients also have impaired oxygen metabolism in the muscle (17). Therefore triggering the exercise pressor reflex during slow walking may be necessary in PAD patients to send more oxygenated blood through an occluded vessel to deliver oxygen to the exercising muscle. However, the exaggerated pressor response during treadmill walking did not restore leg SmO₂.

**Limitations**

Because both blood flow (O₂ delivery) to skeletal muscle and O₂ extraction by skeletal muscle increase greatly during exercise (20, 110), the NIRS signal (O₂ saturation in the capillaries of muscle tissue) is a mixture of the two. There is evidence that both blood flow and skeletal muscle O₂ extraction are impaired in PAD (13, 17, 18, 61) and it is impossible to determine which mechanism contributes more to the drop in SmO₂ in PAD observed in this study. In addition the PAD patients in this study were on several medications that may affect blood pressure such as Angiotensin converting enzyme inhibitors, Angiotensin II antagonists, vasodilators, and calcium channel blockers. However, despite these anti-hypertensive drugs, the BP response to exercise was still augmented in PAD.

**Overall Significance and Future Directions**

We found that BP, HR, and leg SmO₂ responses to treadmill walking are exaggerated in PAD before the onset of pain. Our findings may help explain hemodynamic adaptations to walking in PAD as well as heightened cardiovascular risk. Additional studies are needed to investigate the connection between SmO₂ and the exercise pressor reflex in PAD. Furthermore,
larger epidemiological studies are needed to show whether the drop in SmO$_2$ or the exaggerated exercise pressor reflex have prognostic value in PAD and to determine if exercise training or acute exercise increases cardiovascular risk in PAD.
Chapter 5

Peripheral Revascularization Decreases the Exercise Pressor Reflex and Increases Coronary Exercise Hyperemia in Peripheral Arterial Disease

Introduction

Peripheral arterial disease (PAD) is a form of atherosclerosis that affects the descending aorta and arteries in the lower limbs. It is estimated that 8 million people in US have PAD although many are asymptomatic and undiagnosed (51). PAD increases the overall disease burden of atherosclerosis and patients with PAD have greater cardiovascular risk than patients with coronary or carotid artery disease (93). The standard of care for PAD includes medical management, exercise therapy, and endovascular or surgical interventions. The goal of treating PAD is to decrease cardiovascular risk and ease symptoms (93). Many studies have investigated the effects of pharmacological, endovascular, and surgical interventions at improving symptoms and cardiovascular risk in PAD (96). Previous studies show that lower limb revascularization procedures (angioplasty, stenting, and bypass grafts) in PAD decrease the risk of cardiovascular events, amputation-free survival, and quality of life (49, 64). While some studies suggest that global endothelial function improves in PAD patients following revascularization (19, 62, 120), it is unknown whether hemodynamic or hyperemic responses to exercise are improved.

PAD patients have an augmented blood pressure response to leg exercise that may be predictive of cardiovascular and all-cause mortality in PAD (30). The exaggerated blood pressure (BP) response to exercise, or exercise pressor reflex, in PAD appears to be triggered by leg ischemia since the exaggerated exercise pressor reflex in PAD is not elicited by upper body exercise (6, 107). Therefore, the exaggerated exercise pressor reflex in PAD is likely triggered by
leg ischemia and may serve to push blood flow through an obstructed vessel. Yet, it is unknown whether improving leg blood flow in PAD via revascularization attenuates the exaggerated exercise pressor reflex in PAD.

We recently found that PAD patients without coronary artery disease have attenuated coronary hyperemic responses to exercise, which may contribute to cardiovascular risk (107). It is unclear whether peripheral vascular interventions in PAD would alter the BP or coronary vascular responses to leg exercise. Therefore we investigated BP and coronary blood flow responses to leg exercise before and one month following leg revascularization. We hypothesized 1) that leg revascularization would reduce the BP response to plantar flexion exercise in PAD and 2) that leg revascularization would not alter the coronary blood flow response to this exercise paradigm.

Methods

Subjects and Design

These laboratory studies employed a repeated-measures within subjects design. We studied 17 patients with PAD (66 ± 2 yr, 11 men) before and one month following a leg revascularization procedure. PAD patients were recruited from the Penn State Hershey Medical Center vascular outpatient clinic lists and from our database of subjects who had previously participated in our studies.

One out of 17 of the PAD patients underwent vascular bypass surgery during the study; this patient had a right femoral to popliteal bypass graft. Sixteen of the PAD patients underwent angioplasty and 9/16 of these patients also had a stent placed. The stents were placed in iliac (8/16), femoral (8/16), popliteal (4/16), and tibial (2/16) arteries. Three out of the 16 patients who had a stent placed also underwent femoral endarterectomy during their procedure (2 common
femoral artery, 1 superficial femoral artery). Patients with other vascular diseases or diabetes or were not excluded from the study; 5 subjects had coronary artery disease, 14 were hypertensive, and 4 were diabetic.

At the study visits, subjects were on several medications and supplements including statins (N = 13), fenofibrate (N = 1), aspirin (N = 13), clopidogrel (N = 11), cilostazol (N = 1), angiotensin converting enzyme inhibitors (N = 10), amlodipine (N = 5), selective β1 blockers (N = 8), diuretics (N = 4), α1-blocker (N = 1), proton pump inhibitors (N = 4), and anti-diabetic medications (N = 5), anti-depressants (N = 3), levothyroxine (N = 2), bone health medications (N = 2), albuterol (N = 1), multivitamins (N = 6), vitamin D (N = 2), iron (N = 2), fish oil (N = 1), co-enzyme Q10 (N = 1), St. John’s wort (N = 1). Mostly, subjects were on the same medications at both study visits except 6 subjects added clopidogrel to their medications after their intervention; at the post-intervention visit all 17 subjects were taking clopidogrel.

**Experimental Protocol**

Each subject completed 2 identical laboratory visits: one approximately 24 hours before their leg revascularization procedure and one approximately 1 month after their procedure. All study protocols were approved in advance by the Institutional Review Board of Penn State Hershey and conformed to the Declaration of Helsinki. All subjects provided written and informed consent. The study protocols were performed in the supine position in a thermoneutral laboratory (20-21°C). At each visit ankle-brachial indices (ABIs) were measured first. Subjects were then instrumented with a three-lead EKG (Cardiocap/5, GE Healthcare), a finger BP cuff (Finometer, FMS), and pneumotrace to monitor respiratory activity; these variables were continuously collected at 200 Hz by PowerLab (ADInstruments) and analyzed offline. Resting BPs were obtained in triplicate by automated oscillometry of the right brachial artery (Phillips
Sure Signs VS3) after 15 min of quiet rest and these values were used to verify the Finometer values as previously described (90). Peak diastolic CBV in the left anterior descending artery (LAD) was obtained from the adjusted apical four-chamber view using a 7S probe (all images acquired by Z. Gao). The specific procedures for measuring CBV in LAD have been previously described by our laboratory and the reproducibility within subjects has been verified (41-43, 86).

To test our hypothesis, subjects performed plantar flexion exercise by contracting the calf muscles of a single leg 30 times per minute with increasing amounts of resistance attached to the device. Subjects exercised with their leg undergoing the revascularization procedure. If a leg revascularization procedure was to be performed on both legs, subjects exercised with their most symptomatic leg. The resistance started at 0.5 kg and increased by 0.5 kg every minute until 3.0 kg, fatigue, significant pain, or inability to maintain the cadence occurred. Ratings of perceived exertion and pain were obtained each minute using the Borg scales (14).

**Data Collection and Statistical Analysis**

All variables were measured continuously and analyzed offline. An average of the last 20s of each minute is presented. Statistical analysis was performed using SPSS 22. Wilcoxon signed-rank tests were used to compare non-parametric anthropometric data between groups. Changes (Δ) from baseline to plantar flexion exercise at each subjects’ maximal workload were compared between groups using paired Student’s t-tests. Each subjects’ maximal workload from the pre-intervention visit was also used for comparisons at the post-intervention visit (since most subjects exercised longer at the post-intervention visit). All data are shown as mean ± standard error mean (SEM) unless otherwise stated. Significance was set at $P < 0.05$ for all tests.
Results

Demographic, resting hemodynamics, and exercise tolerance data are shown in table 5-1. There were no changes in weight, BMI, or resting BP and HR data between study visits. ABI increased from the pre- to post-intervention visit in the leg that was revascularized. Fifteen out of 18 subjects had a history of smoking; 3 were current smokers and pack years ranged from 0 to 105 in our subjects. All subjects had symptomatic PAD with claudication, but not rest pain (Fontaine stage II). Subjects were able to exercise on average 2 minutes longer and reach a higher maximal workload (1.0 kg greater) at the 1 month post-intervention visit compared to the pre-intervention visit (Table 5-1). Subjects also had less pain and lower perceived exertion at their maximal workload or end of exercise at the post-intervention visit compared to the pre-intervention visit.
<table>
<thead>
<tr>
<th>PAD patients (N = 17)</th>
<th>Pre-Intervention</th>
<th>Post-Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>11/6</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>66 ± 2</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84 ± 5</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.5 ± 1.3</td>
<td>29.3 ± 1.2</td>
</tr>
<tr>
<td>ABI of leg revascularized</td>
<td>0.61 ± 0.03</td>
<td>0.84 ± 0.04 *</td>
</tr>
<tr>
<td>ABI of other leg</td>
<td>0.81 ± 0.05</td>
<td>0.82 ± 0.05</td>
</tr>
</tbody>
</table>

**Smoking**
- Pack years: 33 ± 8
- Smoking history/current smokers: 15/3

**Resting Hemodynamics**
- Systolic BP (mmHg): 140 ± 5
- Diastolic BP (mmHg): 75 ± 3
- Heart Rate (beats/min): 68 ± 3

**Exercise Tolerance**
- Maximal workload (kg): 2.0 (1.0 – 3.0)
- Pain at end (0-10): 5 (0 – 8)
- RPE at end (6-20): 14 (8 – 17)

Peripheral arterial disease (PAD); body-mass index (BMI); ankle-brachial Index (ABI); blood pressure (BP), Rating of perceived exertion (RPE). Data are shown as mean ± standard error or median (range) for non-parametric data. * P < 0.05 compared to pre-intervention visit.
Figure 5-1. Systolic blood pressure (BP), diastolic BP, and heart rate responses to plantar flexion exercise over time (baseline, 0.5 kg, 1.0 kg, 1.5 kg, maximum workload) and by treatment (pre revascularization and post revascularization) (N = 17). * P < 0.05 between treatments.
**Effect of Revascularization on the Exercise Pressor Reflex**

The HR and BP responses to exercise are displayed in figure 5-1. Significant interactions were observed for diastolic BP and HR. Although the systolic BP response to plantar flexion exercise tended to be lower following leg revascularization, the difference was not statistically significant (pre-intervention: 21 ± 5 vs. post-intervention: 15 ± 3 mmHg, $P = 0.058$). The mean BP response to plantar flexion exercise was attenuated by leg revascularization (pre-intervention: 15 ± 4 vs. post-intervention: 7 ± 3 mmHg, $P = 0.013$, Figure 5-2). Diastolic BP increased less in response to plantar flexion post-intervention: 14 ± 4 vs. pre-intervention: 4 ± 2 mmHg ($P = 0.014$). The HR response to plantar flexion was also attenuated following leg revascularization (pre-intervention: 9 ± 1 vs. post-intervention: 6 ± 1 beats/min, $P = 0.014$, Figure 5-2).

**Figure 5-2.** Mean blood pressure and heart rate responses (changes from baseline to maximum exercise) to single-leg plantar flexion exercise pre-revascularization (gray bars) and one month post revascularization (white bars) ($N = 17$).

**Effect of Revascularization on Coronary Blood Flow**

CBV was measured before and after revascularization in 9/17 subjects. One subject was excluded from analysis because his CBV measurements were more than 2 standard deviations above the mean. CBV responses to plantar flexion were greater following leg revascularization in
6/8 subjects (Figure 5-3). At the pre revascularization visit, subjects had no increase in CBV in response to plantar flexion exercise on average (baseline: 22.55 ± 3.14 vs. max: 22.17 ± 4.46 cm/s, \( P = 0.282 \)). Yet, at the post revascularization visit CBV increased with exercise (baseline: 19.98 ± 2.61 vs. max: 24.10 ± 2.94, \( P = 0.013 \)). The change in CBV with exercise was significantly greater at the post revascularization visit: 4.11 ± 1.57 vs. pre revascularization: -1.20 ± 2.09 cm/s (\( P = 0.038 \), Figure 5-3). The change in RPP with exercise was not significantly altered by revascularization (pre revascularization: 2796 ± 871 vs. post revascularization: 1766 ± 378 mmHg*beats/minute, \( P = 0.082 \), Figure 5-3). Although, the RPP response to plantar flexion was attenuated following revascularization in 5/8 subjects.

**Figure 5-3.** Coronary blood velocity and rate pressure product responses to single-leg plantar flexion exercise pre revascularization (closed circles) and one month post revascularization (open circles). Each circle represents the change from baseline to maximum exercise in one subject (N = 8).
Discussion

Summary and Main Findings

The purpose of this study was to investigate the effects of leg revascularization on the cardiovascular response to exercise in PAD. This study has two novel findings. First, the exaggerated BP and HR responses to exercise in PAD were attenuated following leg revascularization. Second, the attenuated coronary exercise hyperemia in PAD was improved 1 month after leg revascularization. Together these data suggest that leg revascularization alters cardiovascular reflex control of blood flow in PAD.

Effect of Revascularization on the Exercise Pressor Reflex

Previous studies from our laboratory and others demonstrate that the BP response to plantar flexion exercise is augmented in PAD patients compared to healthy subjects (77, 87, 107). This heightened exercise pressor reflex in PAD may be caused by greater activation of the sympathetic nervous system or sensitized skeletal muscle afferents (81). We have previously shown that reducing oxidative stress with vitamin C or prostanoids with cyclooxygenase attenuates the BP response to exercise in PAD (87, 88). The current study adds to this literature by showing that restoring large artery blood flow, via leg revascularization, decreases the heightened BP and HR responses to exercise in PAD. These data suggest that ischemia in the leg contributes to the heightened BP and HR responses to exercise in PAD.
Effect of Leg Revascularization on Coronary Exercise Hyperemia

Our recent study found that the CBV response to leg exercise is also attenuated in PAD (107). We hypothesized that impaired coronary exercise hyperemia in PAD is attributed to increased sympathetic vasoconstriction in the coronary arteries or global endothelial dysfunction. Therefore, in the current study, we hypothesized that leg revascularization would not alter the CBV response to leg exercise in PAD because the effects of revascularization should be localized to the leg and corresponding reflexes (such as the exercise pressor reflex). Contrary to our hypothesis, we saw a significant change in CBV responses to plantar flexion exercise in 6/8 PAD patients following leg revascularization. At the pre-intervention visit, 4/8 subjects had a paradoxical decrease in CBV in response to exercise (ie. their hearts were getting less blood flow and oxygen during exercise compared to at rest). In all 4 of these subjects, their CBV response increased in response to exercise at their post-intervention visit. Although, the coronary hyperemic response in PAD post-intervention is still attenuated compared to healthy subjects’ CBV response using a similar exercise protocol (107).

There are a few possible mechanisms that may underlie this change in coronary physiology that occurs one month following revascularizing the leg. First, it is important to note that the HR response to exercise was also attenuated at the post-intervention visit. Because coronary blood flow occurs during diastole, at lower HRs there is more time for flow to occur which may increase CBV. Second, there may have been alterations in the timing and amplitude of the arterial-wave reflection following revascularization, which could alter CBV. Peripheral arterial obstruction causes premature arterial-wave reflection in PAD and augments afterload (aortic blood pressure) reducing coronary perfusion (126). Removing the arterial obstruction from the leg may decrease stiffness of the peripheral arteries. If the leg artery stretches more, reflected blood flow waves from the leg arrive at the heart later (during diastole rather than during systole).
This would decrease the augmented central systolic pressure observed in PAD and improve coronary perfusion. This hypothesis is supported by one study that found a decrease in augmentation index in PAD patients 3 months following a peripheral stent placement (63). Therefore, revascularization could decrease central blood pressure and normalize CBV responses in PAD.

Third, there is evidence of improvement in endothelial-dependent vasodilation in the arm in PAD patients following leg revascularization (19, 62, 120). Furthermore, endothelial-dependent vasodilation in the arm is correlated to coronary vasodilation in PAD (99). Therefore, if flow-mediated dilation is improved in the arm following leg revascularization as the literature suggests (19, 62, 120), it is likely that endothelial-dependent vasodilation in the coronary arteries is improved as well, although that has not been investigated. In summary, these data suggest that leg revascularization may improve endothelial function in PAD in several arteries, not just the leg, although the mechanism of physiological improvement remains unclear. Finally, it is also possible that post-intervention the PAD patients exercised more, which could have profound physiological effects (56). We do not have data on how much our study subjects walked pre- or post-intervention and the literature does not support that PAD patients walk significantly more following leg revascularization even if their pain decreases (1, 95). However, if PAD patients did walk more following their procedures, this could certainly improve their cardiovascular health and responses to exercise.

**Limitations**

The main limitations of this study are typical of human physiology experiments in clinical populations. Subjects underwent different types of interventions femoral to popliteal bypass (N = 1), angioplasty with stent placed (N = 9), angioplasty without stent placed (N = 7).
While our study was not powered to investigate whether one type of peripheral vascular intervention affected the cardiovascular response to exercise more, there does not appear to be a difference between the treatment groups (ie. the one subject who underwent bypass had a similar change in BP and CBV responses to exercise compared to the patients who underwent angioplasty). The subjects were on several medications during the study visits that may have affected their BP responses such as β1-blockers, α1-blockers, amlodipine, and angiotensin converting enzyme inhibitors. All subjects were also on platelet inhibitors such as aspirin and clopidogrel and 1 subject was taking the vasodilator cilastazol; these medications may increase blood flow. All patients took their usual medications during both study visits and we did not have patients withhold certain medications for safety and ethics reasons. Yet, the effect of medications on physiological responses is probably minimized since patients were taking the same medications at both study visits and the study is a within-subjects design (all patients were their own control). However, clopidogrel, an anticoagulant, was added to 6 subjects’ medication lists post-intervention since it is prescribed specifically following stent implantation. Our study was not randomized and we did not have a control group or control treatment such as sham surgery or catheterization. In addition, the PAD patients in this study had co-morbid conditions including coronary artery disease and diabetes. Since some PAD patients also had coronary artery disease, this may have affected their CBV responses; however, every subject served as his or her own control and no procedure was done to their coronary arteries between visits. Finally, we studied cardiovascular responses to exercise in PAD patients 1 month following their procedure and we do not know if the changes we observed occurred earlier than one month or remained long-term.
Conclusion

In conclusion, leg revascularization decreased the BP and HR response to exercise and improved coronary exercise hyperemia in PAD. More controlled studies are needed to minimize confounding variables and validate these effects. Further studies are need to investigate possible mechanisms that lead to widespread changes in the endothelium and central blood pressure that may alter cardiovascular reflexes in PAD.
Chapter 6
Conclusions and Future Directions

The studies comprising this dissertation provide several new findings that enhance current understanding on blood flow responses during exercise in patients with peripheral arterial disease (PAD). In the first study (Chapter 3), we found that coronary hyperemia to both leg and arm exercise was impaired in PAD patients without coronary artery disease. The main finding of the second study (Chapter 4) is that PAD patients have an exaggerated increase in blood pressure (BP) and heart rate (HR) as well as a greater drop in leg skeletal muscle oxygen saturation (SmO$_2$) during treadmill walking compared to healthy subjects. In the third study (Chapter 5), we determined that leg revascularization decreases the exaggerated BP response and increases the attenuated coronary blood velocity (CBV) response to leg exercise in PAD patients. More in depth conclusions for each individual study can be found in the Discussion sections of Chapters 3 through 5. Collectively, data from these studies suggest that exercise hyperemia is impaired in PAD patients compared to healthy subjects despite a higher BP response to leg exercise. Furthermore, the origin of the impaired coronary hyperemia and exaggerated exercise pressor reflex (EPR) in PAD may be related to leg ischemia.

This set of studies reveals that there are impaired physiological responses to exercise in PAD patients compared to healthy controls of similar age, sex, and BMI. However, many questions remain regarding 1) the mechanisms that alter the physiological responses to exercise in PAD and 2) how leg revascularization improves the systemic cardiovascular response to exercise in PAD patients.
First, it is unclear why the coronary hyperemic response to exercise is decreased in PAD patients. In the first study, we found that the increase in CBV to single-leg plantar flexion exercise was attenuated in PAD patients compared to healthy subjects. In the third study, CBV did not change, on average, in response to plantar flexion exercise in PAD patients before revascularization. The difference between the two study groups may be because the patients in third study had more severe PAD since they were preparing to undergo a leg vascular intervention. The third study also included PAD patients who had coronary artery disease since this study was a within-subjects design in which every subject served as their own control. Indeed, 2 out of 8 subjects who had CBV measurements before and after revascularization had coronary artery disease; one of these subjects had the biggest decrease in CBV in response to exercise and the other had the biggest increase in CBV in response to exercise before revascularization. Neither subject is an outlier and removing both subjects from the data set does not affect the mean change in CBV. Therefore, PAD itself likely contributes to a blunted CBV response to plantar flexion exercise.

However, the subjects in the third study did have a positive CBV response to exercise following revascularization even though nothing was done to alter their coronary disease between study visits. These data further support that PAD plays a role in limiting coronary exercise hyperemia. How PAD alters CBV responses to exercise remains unclear. It is possible that the coronary arteries are unable to dilate as much in PAD patients due to systemic endothelial dysfunction (13, 18, 61). Another proposed mechanism for impaired CBV responses in PAD is that an afferent reflex from the leg impedes coronary vasodilation. However, in the first experiment we found that CBV responses to handgrip exercise are also impaired in PAD. Since handgrip exercise does not cause leg ischemia or an exaggerated EPR in PAD, leg afferents may not be responsible for limiting CBV reactivity in PAD. It is also possible that an exaggerated sympathetic nervous system response to exercise in PAD impedes CBV. PAD patients might
have increased α-adrenergic receptor mediated coronary vasoconstriction to physiological stress, which impedes dilation (7). It is also possible that β-adrenergic receptor-mediated coronary vasodilation is impaired in PAD as it is in older men (108) and in patients with coronary disease (118). Investigating sympathetic nervous system responses to stress and adrenergic receptor sensitivity in the coronary arteries in PAD was something I planned to do earlier in graduate school since it naturally followed one of my prior studies (108). This study was not feasible due to lack of PAD patients willing to participate in that protocol. However, investigating the role of the sympathetic nervous system on coronary blood flow in PAD would be a potential future direction if subject recruitment becomes easier.

Regarding the greater decrease in SmO₂ in PAD that was observed in the second study of this dissertation, the mechanism also remains unclear. The NIRS signal tracks skeletal muscle oxygen saturation (SmO₂) in the capillaries of muscle tissue. Because both blood flow (O₂ delivery) to skeletal muscle and O₂ extraction by skeletal muscle from blood increase greatly during exercise (20, 110), the NIRS signal (O₂ saturation) does not allow us to differentiate between the two. The SmO₂ signal could decrease more in PAD because O₂ delivery (ie. blood flow) is decreased or because O₂ extraction is increased. It is impossible to determine which mechanism contributes more to the drop in SmO₂ in PAD observed in this study. There is evidence that both blood flow and skeletal muscle O₂ extraction are impaired in PAD (13, 17, 18, 61). Therefore, it is unlikely that skeletal muscle O₂ extraction is increased in PAD patients compared to healthy subjects. Yet, SmO₂ dropped quickly in PAD patients before the onset of pain suggesting that the patients were getting oxygen to their muscle and the SmO₂ signal may not have been entirely indicative of blood flow. Future studies using Doppler ultrasound or thermodilution to measure blood flow and femoral venous catheters to measure arterial-venous oxygen difference will be able to address whether decreased leg blood flow or increased oxygen extraction is contributing more to the greater drop in SmO₂ during exercise in PAD.
It is also unclear why leg revascularization improved the BP or CBV response to exercise in PAD. We hypothesized that leg revascularization would improve the EPR in PAD by decreasing ischemia in the leg and thereby reducing the sensitization of afferents that evoke the EPR (65). Yet, we did not expect CBV responses to change following leg revascularization. Contrary to our hypothesis leg revascularization also improved the CBV response to exercise in PAD. The increased CBV response to exercise may be due to systemic improvements in endothelial function. There is evidence that leg revascularization improves endothelial-mediated vasodilation in the arm in PAD (19, 62, 120). Therefore, it is possible that revascularization improves CBV responses to exercise by improving endothelial-mediated vasodilation in the coronary arteries. Improving blood flow in the leg may also alter the pattern of blood flow going back to the heart. Further studies are needed to investigate mechanisms of coronary blood flow changes following revascularization in PAD.

Finally, a major assumption of the studies in this dissertation is that CBV responses to exercise are important and the attenuated CBV response to exercise in PAD may relate to the high risk of cardiovascular events, particularly myocardial infarction, in PAD (93). There is evidence that decreased coronary flow reserve (ie. vasodilation to infusion of vasoactive drugs such as acetylcholine or adenosine) is predictive of cardiovascular events and mortality in healthy humans (55, 104, 113) and in patients with coronary disease (105, 112). We also know that a greater BP response to exercise is predictive of future cardiovascular disease and mortality (70, 73, 75, 122). Furthermore, a higher systolic BP response to slow walking may be predictive of cardiovascular events and mortality in PAD patients (30). However, it is unknown whether the CBV response to exercise in PAD has any prognostic value and future epidemiological studies are needed to address this.
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VITA

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Amanda received a B.A. in Behavioral Biology from Johns Hopkins University in Spring 2013. She matriculated into the Neuroscience Graduate Program at Penn State College of Medicine in Fall 2013. Throughout graduate school, Amanda has researched several topics including sex differences in vascular transduction and the effects of exercise on chemoreflex sensitivity. She was awarded a predoctoral fellowship from the American Heart Association to study coronary blood flow in patients with peripheral arterial disease; this research became the basis for her dissertation. Amanda also serves on the trainee advisory committee for the American Physiological Society. She currently has 12 publications, 6 as first author, with 3 additional publications in preparation.