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Post-Exercise and Post-Recovery Blood Lactate in Peripheral Arterial Disease

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Post-Exercise and Post-Recovery Blood Lactate in Peripheral Arterial Disease
Post-Exercise and Post-Recovery Blood Lactate in Peripheral Arterial Disease

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Kinesiology

by

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ABSTRACT

The purpose of this study was to observe how the presence of peripheral arterial disease affects the level of post-exercise blood lactate and post-recovery blood lactate during and following the six-minute walk test (6MWT). The 6MWT was administered to 49 participants (33 classified as Non-PAD and 16 classified as PAD) over the age of 50 with no uncontrolled cardiovascular or metabolic diseases or a cardiovascular event in the previous 6 months. Results demonstrated that no significant statistical difference exists between the presence of PAD and resting blood lactate ($F = 0.86, p = .36$), post-exercise blood lactate ($F= 0.48, p = .49$), or post-recovery blood lactate ($F = 1.62, p = .21$). Although the data failed to demonstrate statistical significant difference between the presence of PAD and these variables, this could be due, in part, to the relatively mild arterial occlusion seen in the population used in this study. Perhaps more severe cases of PAD would yield significant differences between the two groups following the 6MWT.
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CHAPTER ONE

INTRODUCTION

Peripheral Arterial Disease (PAD) is a subset of cardiovascular disease affecting 12-20% of adults over the age of 65 in the United States (Roger et al., 2012). PAD is the systemic build-up of plaque in the femoral artery of the leg(s) which leads to decreased blood flow to the affected limb. It often goes unnoticed until symptoms of intermittent claudication (IC) are felt and the individual’s functional capacity is decreased significantly. PAD is typically found in adults over the age of 65 and symptoms can range from very mild to life threatening. When compared to healthy controls, PAD patients have many physiological adaptations and abnormalities due to different manifestations of the disease. These abnormalities can be seen as changes in peripheral blood flow, skeletal muscle metabolic myopathies, rate of oxygen desaturation, oxygen uptake kinetics, rate of metabolic intermediate build-up, and a surplus of certain enzymes during exercise (Bhat, Hiatt, Hoppel, & Brass, 1999).

The prominent change with PAD is decreased blood flow to the limb with the occluded vessel. This results in ischemia which implies an insufficient supply of oxygen, glucose, and other important nutrients to the working muscle (Hlavova, Linhart, Prerovský, Ganz, & Fronek, 1965). The other important impact of decreased blood flow is that there is less blood to clear away the harmful metabolic waste of the working skeletal muscle. This causes an uncharacteristically rapid build-up of different substrates in the muscle and in the blood which can debilitate the muscle’s ability to keep working (Robbins et al., 2011). The extent of vessel occlusion determines the extent to which blood flow is diminished. This is easily measured by the Ankle Brachial Index (ABI) and this number is indicative of PAD severity (e.g. the lower the ABI, the greater the decrease in blood flow to the limb) (McDermott et al., 1999).
The other physiological changes with PAD frequently go unnoticed because they occur at the cellular level. The skeletal muscles in the affected limbs of PAD patients often have many different metabolic abnormalities when compared to individuals without PAD. These irregularities include atypical muscle mitochondrial enzyme expression (Lundgren, Dahllof, Schersten, & Bylund-Fellenius, 1988), abnormal accumulation of oxidative metabolism intermediates (i.e. lactate) (Hiatt, Wolfel, Regensteiner, & Brass, 1992), and slowed kinetic response to exercise (Kemp et al., 1993). These changes in the skeletal muscles of patients with PAD are the cause of the more traditional symptoms that are generally associated with the disease.

Symptoms of PAD are dependent on the extent of vessel involvement and collateral circulation to the specific area. Traditional symptoms with PAD include IC, decreased functional capacity, decreased exercise capacity, and an overall sedentary lifestyle (Hiatt, Regensteiner, Wolfel, Carry, & Brass, 1996). Intermittent claudication, or ischemia-induced leg pain, is the most noticeable of all symptoms but, like the others, it is the result of the metabolic dysfunction of the skeletal muscle as well as the ischemia due to the occluded femoral artery. PAD is often associated with a decreased quality of life (Liles, Kallen, Petersen, & Bush, 2006) and increased risk of depression (Arseven, Guralnik, O’Brien, Liu, & McDermott, 2001) due to the functional restrictions caused by the metabolic changes and abnormalities.

When evaluating exercise performance in patients with PAD, maximal walking time and peak VO$_2$ can be used to define maximal performance. Oxygen consumption is an objective performance measure that is used to define functional capacity in all individuals. In healthy individuals, a plateau in VO$_2$ signifies maximal exercise. However, in individuals who have impaired exercise performance due to cardiovascular disease, peak VO$_2$ can be used because it
defines the highest VO\(_2\) achieved during exercise, regardless of whether a plateau was reached or not. Because of this, peak VO\(_2\) has been shown to be more closely correlated than VO\(_2\) max to the degree of functional impairment in individuals with cardiovascular disease. In individuals both with and without PAD, peak VO\(_2\) is correlated with maximal walking time (Hiatt, Nawaz, Regensteiner, & Hossack, 1988). However, skeletal muscle ischemia limits exercise performance in PAD patients and maximal heart rate, VO\(_2\), and the respiratory exchange ratio in this population are lower than age-matched healthy individuals (Eldridge & Hossack, 1987). These observations would suggest that, during the 6MWT, performance variables such as heart rate would not be elevated to the same degree in PAD participants when compared to non-PAD participants due to skeletal muscle ischemic limitations.

A common mode to measure functional capacity in this population is the 6MWT. The 6MWT was developed for clinical settings by the American Thoracic Society Pulmonary Function Standards Committee and was used originally for patients with asthma but has since been adopted as a measure of functional ability in many diseased populations (Enright, 2003). It has become a staple in the cardiovascular community, particularly for patients with PAD. Basic standards for completing this test require an individual to walk as far as they can in the allotted six minutes. The primary outcome variable is total distance covered (Enright & Sherrill, 1998). This test is a commonly accepted and often required measure of functional ability in individuals with diagnosed PAD and is used to measure both the extent of functional loss as well as the extent of functional improvements from PAD treatment and exercise therapy. Typically, individuals with PAD exhibit significantly lower scores than healthy individuals in the 6MWT due to traditional PAD symptoms and restraints such as IC, decreased functional capacity, and decreased exercise capacity. A normal distance for healthy adults over the age of 50 to score
during the 6MWT is between 450-700 yards (Chetta et al., 2006). A typical distance for adults with PAD is between 250-400 yards (Regensteiner et al., 1993). This lower score is also associated with the lower functional and exercise capacities observed in individuals affected by PAD (Gardner, Katzel, Sorkin, & Goldberg, 1998).

Hemoglobin saturation refers to the degree to which hemoglobin is loaded with oxygen molecules. Each hemoglobin molecule has the capacity to bind with up to four oxygen molecules and carry them to the working muscle. If all four oxygen molecules are attached to the hemoglobin, the hemoglobin is fully saturated (Bauer, Regensteiner, Brass, & Hiatt, 1999). Measuring hemoglobin saturation is a safety tool often utilized to ensure that an individual is supplying an adequate amount of oxygen to their working muscles. Hemoglobin saturation can be measured easily and non-invasively by a pulse oximeter. In a healthy individual, a hemoglobin saturation of <90% is a contraindication to continue exercising. However, because this study included a diseased population group, this value was placed at a more conservative <92% cut off. In accordance with these extra precautions, hemoglobin saturation values were recorded to ensure that the participants maintained a healthy and sufficient amount of oxygen to their muscles throughout the test.

Statement of Problem

It is known that individuals with peripheral arterial disease have elevated lactate levels at rest and after exercise (Barker, Green, Green, & Walker, 2004). However, these measures have not been taken during the 6MWT to determine if these abnormalities occur in this short time span and to what degree they occur, if they do. The rate of lactate clearing following the 6MWT is also unknown in this population. This thesis sought to determine the level of post-exercise
blood lactate and the level of post-recovery blood lactate in individuals with PAD when compared with a control population during the 6MWT.

**Purpose**

The purpose of this study was to observe how the presence of peripheral arterial disease affects the level of post-exercise blood lactate and post-recovery level during and following the 6MWT. Blood lactate levels were measured before, immediately following the 6MWT, and post-recovery in order to define differences between PAD and Non-PAD populations.

**Hypotheses**

**Null:**

1. There will be no difference in resting blood lactate levels between PAD patients and healthy controls
2. There will be no difference in post-exercise blood lactate levels between PAD patients and healthy controls during the 6MWT
3. There will be no difference in post-recovery blood lactate levels between PAD patients and healthy controls post the 6MWT

**Research:**

1. PAD patients will have a higher resting blood lactate level when compared to healthy controls
2. PAD patients will have a higher level of blood lactate post-exercise during the 6MWT when compared to healthy controls
3. PAD patients will have a higher level of blood lactate post-recovery following the 6MWT when compared to healthy controls

**Operational Definitions**

*Ischemia* is defined as the restriction of blood to a tissue which infers a shortage of oxygen and other nutrients necessary for tissue functioning (Hlavova et al., 1965).

*Intermittent Claudication* is defined as severe leg pain during exertion. This pain is generally felt in the calf muscles and is described as severe cramping or tightness. This pain slowly builds with exercise until severe enough that the individual has to call an end to all physical activity. The pain subsides at rest. Intermittent claudication is associated with a 50% reduction in walking performance and is the primary external cause of decreased functional capacity in patients with Peripheral Arterial Disease (Hou et al., 2002).

The *Ankle-Brachial Index* is a noninvasive test used to define the presence and severity of PAD. ABI compares the blood pressure in the ankle to the blood pressure in the arm. A low ABI can indicate narrowing or blocking of the arteries in the legs (McDermott et al., 1999). Below is the general classification of blockage severities:

*Healthy Adults* = 0.91-1.30

*Blockage* = 0.40-0.90

*Severe Blockage* = less than 0.40

In accordance, *Peripheral Arterial Disease* was defined by an ABI of less than or equal to 0.90 when being classified as PAD positive.
The Six Minute Walk Test is a walking test to determine fitness level in unhealthy populations. The American Thoracic Society has determined that this test is safe, easy to administer, well tolerated, and reflects the daily activities of the target population. Primarily, this test measures overall distance covered by an individual in six minutes (Enright, 2003). This test is an often used measure in the PAD population because of the disease-induced limitations and intermittent claudication experienced by patients.

Post-exercise lactate in this study was determined by subtracting resting blood lactate from blood lactate taken immediately following the 6MWT.

Post-recovery lactate was determined by subtracting post-exercise blood lactate from the blood lactate value taken following 20 minutes of rest after the 6MWT.

Functional capacity is a term used to describe an individual’s ability to perform normal daily tasks. This does not include exercise capacity; it refers strictly to activities of daily living.

Exercise capacity is a term used to describe an individual’s ability to exercise at any intensity for a prescribed period of time.

Assumptions

1. Based on an overview of the literature, it is assumed that lactate will accumulate in the blood during the 6MWT in both PAD patients and healthy controls.

2. It is assumed that during the 6MWT, participants are giving 100% effort and performing the test to volitional exhaustion or the conclusion of six minutes.

3. It is assumed that participant’s blood lactate will not be elevated due to any other cause apart from walking.
4. It is assumed that all participants will be able to perform and complete the 6MWT due to the self-selected intensity.

Limitations

There are several limitations that presented themselves in this study. First, the severity of the artery occlusion in PAD patients was a limitation due to varying severities playing a role in overall post-exercise and post-recovery blood lactate. Second, the inability to control for an entirely healthy population versus a strictly PAD population was a limitation because almost all participants had other comorbidities that could have affected results. Third, all of the different medications being taken by each individual for different conditions and diseases was a limitation because the physiological effects of these medications was not considered. And last, inability to force individuals into high intensity exercise from a more low-to-moderate state was a limitation because it changed the 6MWT from a maximal test to a timed, moderate-intensity test and this, in turn, could have affected the overall rate and amount of post-exercise and post-recovery blood lactate.
CHAPTER TWO

REVIEW OF LITERATURE

Peripheral Arterial Disease (PAD) is systemic atherosclerosis that typically occurs in the lower extremity arteries. Having PAD puts an individual at an increased risk of stroke, myocardial infarction, and cardiovascular death when compared to healthy individuals (Liles et al., 2006). It is estimated that between 10-12 million people in the United States have PAD, however this number may be underestimated due to those individuals that remain undiagnosed because they are currently asymptomatic. There are many treatment options for individuals with PAD including pharmacotherapy, risk factor reduction, and exercise interventions. These treatments, when used effectively, can decrease the severity of the atherosclerosis and increase the patient’s overall quality of life. It is estimated that approximately 70% of individuals diagnosed with PAD remain stable or improve if proper medical advice and treatments are utilized (Liles et al., 2006).

Generally, atherosclerosis, including that manifested in the periphery, is a dangerous inflammatory disorder that may be initiated by several lifestyle factors such as diabetes, smoking, hypertension, and dyslipidemia. One of the most important factors responsible for atherosclerosis is low density lipoprotein (LDL) levels in the blood. LDLs enter the artery wall from the plasma. Once the plasma level of LDLs exceeds the threshold, LDLs enter the sub-endothelial space faster than they can be removed and accumulate. These accumulated LDLs then become oxidized and stimulate endothelial cells to express a protein, monocyte chemotactic protein-1 (MCP-1), that causes a release of monocytes from the blood into the artery wall. The modified LDLs then convert monocytes into macrophages and then undergo an unregulated uptake of oxidized LDLs until they rupture, causing fatty streaks on the endothelium. Macrophages express
several cytokines that activate endothelial cells to express adhesion molecules. These proteins bind the plasma monocytes to the endothelium and then are attracted into the artery wall by MCP-1. The entry of LDLs into the endothelium begins a cycle that commences atherosclerosis and can also lead to its progression (Deedwania et al., 2006). In patients with PAD, this plaque buildup occurs in the femoral artery and is known to cause many issues including inhibited blood flow, ischemia, claudication, and other pathophysiological adaptations in the skeletal muscles and cells.

PAD is associated with two primary consequences: the first is a significant decrease in quality of life and overall well-being due to intermittent ischemic pain of the lower limbs. This is typically exemplified in an overall sedentary lifestyle and possible depression. The second consequence is a significantly higher likelihood of cardiovascular morbidity and mortality (Olin & Sealove, 2010). This risk of cardiovascular mortality has been found to be similar between diagnosed PAD patients experiencing claudication pain and patients that are asymptomatic.

Symptoms of PAD are dependent on the extent of vessel involvement and collateral circulation to the specific area. The primary symptom of PAD is claudication which is an impairment in an individual’s walking ability due to pain, discomfort, tiredness, cramping, or aching in the legs. Claudication symptoms generally grow more severe as exertion and/or duration increases. This leg pain can range from intermittent claudication during exertion to claudication at rest and even to gangrene (Oka, Szuba, Giacomini, & Cooke, 2003). Patients with PAD have significant functional impairments and physical restrictions due to claudication symptoms.

Quality of life (QoL) is significantly associated with PAD severity. PAD affects the individual’s perceived QoL in both physical and psychosocial functioning. Decreases in physical
functioning can lead to depression, loneliness, emotional distress, and social role limitations (Liles et al., 2006). An individual’s psychosocial functioning decreases as a result of the knowledge that they are living with a chronic disease, the label of being a “PAD patient,” and the perceived loss of control over their body and life (Liles et al., 2006). In patients with PAD versus patients with cardiovascular disease (CVD), Health Related Quality of Life (HRQoL) was found to decrease similarly despite the specific disease diagnosis. Both populations also experienced a decrease in physical function although PAD patient’s losses were attributed to leg-related symptoms while CVD patient’s losses were attributed to cardiac symptoms (Regensteiner et al., 2008).

**Risk Factors for PAD**

There are several discrepancies in the literature about which demographics are at a higher risk for being diagnosed with PAD. The Rotterdam study sought to determine if a relationship existed between age and PAD prevalence. They sampled a group of 7715 subjects over 55 years old living in Rotterdam, the Netherlands. Of these participants, 19.1% were classified as having peripheral arterial disease. The Rotterdam study found that the prevalence of PAD and intermittent claudication symptoms increases drastically with age. Men who were 55 years old were at 6.6% risk for developing PAD but when looking men who were 85 years old, there was a sharp increase to 52.0%. Women in these corresponding age groups saw increases in PAD risk grow from 9.5% to 59.6% (Meijer et al., 1998). This data showed that the risk for developing PAD increased exponentially with age.

Several studies have also been conducted to determine if gender contributes to an individual’s risk to develop PAD. These results are inconsistent between studies. The Rotterdam study showed that the prevalence of PAD was higher among women than men (20.5% vs 16.0%)
but the presence of intermittent claudication in individuals with PAD was higher among men when compared to women (8.7% vs 4.9%) (Meijer et al., 1998). Conversely, a study done with 403 patients showed that the prevalence of PAD in women was 15.9% compared with a higher 17.4% in men. They also reported that physical functioning in patients with PAD was significantly lower in women when compared with men (Collins, Suarez-Almazor, Bush, & Peterson, 2006). Physical functioning differences between genders could be seen when evaluating individual’s ability to perform daily tasks, functional ability, and overall physical ability; this discrepancy between genders could be attributed to the increased claudication symptoms experienced by women (Meijer et al., 1998). Other researchers looked at the effect of gender on PAD patients’ overall quality of life. Oka et al. (2003) compared the perception of PAD between genders and respective quality of life. Results from this study showed that PAD has a much greater negative impact on women than men in regards to health related quality of life (HRQoL), physical functioning, and emotional distress. Similar results were also found by McDermott et al. (2003). She associated women’s greater decrease in physical functioning with the poorer leg strength generally found in women with PAD when compared to men with PAD. This decreased physical functioning in women plays a role in their overall perception of their quality of life and therefore, greater functional decreases in women could be the reason that women express lower HRQoL scores. The irregularity in the results when examining gender as a risk factor for developing PAD shows that gender is not a strong risk factor for the development of PAD and therefore prevention and treatment methods for this disease should be focused on both genders equally.
Comorbidities

Research has found that individuals with PAD are at an increased risk of cardiovascular morbidity and mortality compared to individuals without PAD (Meijer et al., 1998). This increased risk is seen, in part, because of the high degree of overlap between the pathophysiology of peripheral arterial disease and cardiovascular disease. Schainfield (2001) found that CVD can be detected in as many as 90% of individuals with present intermittent claudication. In addition, diabetes and impaired glucose tolerance have a high correlation with the development and progression of PAD. Among a group of 7715 individuals with known PAD, approximately 10% had also been diagnosed with diabetes mellitus (Meijer, 1998). Because of the high association with atherosclerotic diseases and cholesterol levels, specifically elevated LDLs, lipid abnormalities are also a very prevalent comorbidity with PAD. In individuals with PAD who are also hypertensive, the risk of claudication can increase 2-3-fold (Schainfield, 2001). Meijer and colleagues reported that up to 33% of patients with PAD were also hypertensive (1998). Other studies have identified increased fibrinogen and blood viscosity as important risk factors in the development of PAD (Schainfield, 2001). Robeer and colleagues (1998) examined 460 PAD patients and determined the prevalence of cancer, pulmonary disease, and history of coronary revascularization. They found that the percent of individuals with both conditions were 16.0%, 32.5%, and 35.7% respectively. These findings show that the presence of PAD in individuals is often coupled with the presence of other comorbidities and medical complications and needs to be treated in conjunction with these other conditions.

Femoral Vessel Occlusion

The femoral artery and its branches supply blood flow to the entire leg. When individuals age, there is a decrease in leg blood flow and femoral artery vascular conductance when
compared with younger adults; this decrease is seen even in trained individuals (Proctor et al., 1998). Results from a study examining these age-related, hemodynamic changes in trained older men found that the fit older men had a 25% decrease in leg blood flow at different exercise intensities when compared to trained younger men (Proctor et al., 1998). The study also looked at leg vascular conductance which is calculated as limb blood flow divided by the mean arterial pressure (Proctor, Le, & Ridout, 2005). Vascular conductance was shown to be, on average, 26-30% lower in older versus younger men. Lastly, Proctor et al. (1998) examined oxygen saturation in the femoral artery and found that hemoglobin saturation is lower in healthy older men versus the healthy younger men. These studies have shown that vascular conductance, blood flow, and oxygen saturation all decrease with age in a healthy population. In a diseased population, these numbers decrease even more drastically. With vessel occlusion, blood flow decreases to a greater degree along with vessel conductance, compliance, and oxygen kinetics. Blood flow in individuals with claudication symptoms was found to decrease by almost 50% when compared to healthy controls during exercise (Pernow, Saltin, Wahren, Cronestrand, & Ekestrom, 1975). This decrease in blood flow as a result of femoral vessel occlusion has significant implications on skeletal muscle contractility as well as metabolite production in individuals with peripheral arterial disease.

**Blood Flow**

Blood flow is known to be decreased in individuals with atherosclerotic vessels. Hlavova, Linhart, Prerovsky, Ganz, and Fronek (1965) compared differences in leg blood flow at rest, during exercise, and after exercise in PAD patients compared to healthy controls. They found that resting blood flow did not significantly differ amongst the groups; however, blood flow during exercise was significantly lower in the PAD patients versus the control subjects. Pernow
et al. (1975) found that common resting femoral artery blood flow was 0.53 liters/min in healthy adults compared to 0.29 liters/min in adults with claudication symptoms. When exercising, blood flow in the normal adults increased by 2.23 liters/min while the claudication group’s blood flow only increased by 1.11 liters/min. This suggests that in individuals with severe femoral vessel occlusion, such as in PAD patients, blood flow during exercise is dramatically compromised which can cause many functional complications which inhibit performance and functional ability.

Dilation of larger peripheral arteries has been shown to occur in response to increased blood flow through that vessel in a healthy adult; this phenomena is called flow–mediated dilation. When blood flow is increased in a vessel, an increase in sheer stress occurs which triggers the release of nitric oxide (NO), a potent vasodilator. This vasodilation of the vessel provides a greater area for the blood to travel. However, in atherosclerotic vessels, arterial compliance is significantly decreased and the body’s flow–mediated dilation response is impaired (Cox et al., 1989). This is a functional mechanism that can partially explain why an individual with atherosclerotic vessels does not have the proper blood flow response to exercise. This data can also be used to understand why an individual with PAD’s ABI decreases with exercise.

Microcirculation, or capillary density, has also been the target of several studies when investigating the decreased functional capacity in PAD patients. The proposed relationship between capillary density and walking capacity represents the amount of blood that is reaching the working muscles. Robbins et al. (2011) examined this phenomena and found that capillary density was 18% lower in PAD patients when compared to healthy controls. This decrease in capillary density was also correlated to an individual’s functional impairment as shown by a lower peak VO₂, peak walking time, and claudication onset time when compared to healthy
controls. These findings suggest that adaptations in microcirculation may play a role in the decreased functional capacity associated with PAD.

**Ankle-Brachial Index**

The Ankle-Brachial Index (ABI) is the primary method used to diagnose PAD as well as determine the severity of the vessel occlusion and ischemia. ABI is a non-invasive screening tool that can easily identify the presence of PAD. ABI measurement is a number expressing the systolic blood pressure in the ankle over the blood pressure in the brachial artery. If there is a large discrepancy in the blood pressures, that is representative of femoral vessel occlusion and ischemia in the affected limb. PAD is typically defined as an ABI of less than or equal to 0.90 (McDermott et al., 1999). This shows that blood flow in the lower limbs compared to the upper limbs is drastically altered by an occluded vessel. The ABI has a sensitivity of 79% to 95% and a specificity of 95% to 100% (Olin et al., 2010). An ABI of less than or equal to 0.90 is considered PAD even though leg symptoms of claudication might not always be present. A high ABI has been shown to be correlated with a loss in functional ability, a decreased walking distance, a slower walking speed, and an increased prevalence of claudication pain (McDermott et al., 1999). Typically, an ABI of 0.80-0.90 is considered relatively mild PAD; an ABI of 0.50-0.70 represents more severe PAD and individuals with these values typically have claudication symptoms and significant decreased functional capacity (Ouriel, 2001).

**Intermittent Claudication**

Approximately one third of individuals with PAD exhibit traditional claudication symptoms which are characterized by pain in one or both legs upon exertion, primarily in the calves, that is not relieved until the activity is stopped (Hiatt, 2004). Claudication symptoms progress in severity as time goes on; 5% of individuals with claudication symptoms undergo an
amputation of the claudicating limb within five years. Even in individuals without traditional claudication symptoms, atypical leg pain remains common and can affect ambulatory activity and an individual’s overall quality of life (McDermott et al., 1999). Claudication symptoms are the primary inhibitor of physical activity in patients with PAD and the presence of claudication is directly correlated to the individual’s functional capacity. Exercise induces claudication symptoms acutely but can chronically improve them by inspiring the formation of collateral vessels, improving microcirculation and endothelial function, attenuating inflammatory responses, and improving walking economy (Stewart, Hiatt, Regensteiner, & Hirsch, 2002). Regensteiner and colleagues (1996) performed a 24-week walking exercise intervention study for individuals with PAD and claudication symptoms. They found that with the implementation of the exercise program, claudication severity was decreased by 29%. Claudication symptoms are often determined by using a claudication scale as a guide for describing claudication pain (Appendix 1). Claudication pain is a detrimental symptom of PAD and drastically decreases an individual’s functional capacity and ability to perform physical activity.

Post-Exercise Lactate

Lactate is a byproduct of glycolysis formed through the reduction of pyruvate. The conversion of pyruvate to lactate is driven by the enzyme lactate dehydrogenase (LDH). When enough oxygen is provided to the working muscle, pyruvate is oxidized via pyruvate dehydrogenase (PDH) and converted to acetyl-CoA to be used in the Citrus Acid Cycle. If PDH is inhibited, as in the anaerobic milieu, pyruvate will be converted to lactate. Thus, in a hypoxic state, lactate accumulates quickly (McNelis et al., 2001). It is important to note, however, that lactic acid is constantly being produced in the body, not just at extreme exercise levels. At resting states, a basal level of about 0.8-1.2 mM/minute of lactate is constantly circling in the
blood (Mazzeo, Brooks, Schoeller, & Budinger, 1986; Hultman & Sahlin, 1980). The body maintains this basal level by balancing the entry and removal of lactate from the blood; this is called lactate turnover rate. Therefore, when lactate levels rise above basal values, it means that entry is exceeding removal (Billat, 1996). Many studies have shown that lactate turnover rates are higher in trained vs. untrained individuals (Hurley et al., 1984; Seip, Snead, Pierce, Stein, & Weltman, 1991) and in young adults vs. older adults (Strandell, 1964). With decreased blood flow both to and from the working muscle, as with PAD, lactate accumulation can occur at a much higher rate and rises above basal level more quickly.

Adenosine triphosphate (ATP) is the energy source needed to initiate muscle contraction. This is produced through different metabolic processes throughout the body. Primarily, ATP production occurs when a glucose molecule is broken down through a process called glycolysis. Glycolysis is the catabolism of glucose from its normal form to two pyruvate molecules via many different steps that are driven by different enzymes. This process does not require oxygen but results in a net four ATP molecules. Once pyruvate is formed, it then travels into the mitochondria of the skeletal muscle where it is converted to Acetyl CoA and then enters the Citrus Acid Cycle where many more ATP molecules are produced. However, when oxygen is limited by respiration or ischemia, the process for the production of ATP is altered.

There are two primary types of exercise based on the two different metabolic states that an individual is in: these are anaerobic metabolism and aerobic metabolism. Aerobic metabolism typically occurs during submaximal work or long endurance activities where oxygen is available and able to be delivered to the working muscle as needed. As previously described, when oxygen is available, aerobic metabolism can occur by means of glycolysis with an end product of pyruvate which is converted to Acetyl CoA and used in the Citrus Acid Cycle to produce energy.
Alternately, anaerobic metabolism typically occurs in sprints or in maximal work where oxygen is not available to be delivered to the working muscle at the amount that is required to sustain work. When there is a lack of oxygen delivered to the working muscle, other mechanisms are relied on to provide energy to the body such as anaerobic glycolysis or the use of high energy phosphates like ATP or creatine phosphate (CP). During anaerobic glycolysis, pyruvate is formed and then converted to lactic acid via lactate dehydrogenase (Guyton & Hall, 2000). This lactic acid production is due to the fact that activation of glycolysis is occurring more rapidly than the activation of oxidative phosphorylation. This results in an elevated net lactic acid production. Elevated post-exercise lactate levels can be attributed to increases in power rather than an insufficient oxygen supply because lactate accumulates as a result of the increase in the activation of glycolysis (Billat, 1996). Lactate production is believed to be due to a “mass-action effect” of high amounts of pyruvate production exceeding its rate of oxidation (Heighenhauser & Parolin, 1999). The conversion to lactic acid prevents severe acidosis in the cell that would occur from the excess of pyruvate and hydrogen atoms that are the end products of anaerobic glycolysis. With the build up of either of these two substrates, the glycolytic process would halt and the formation of ATP would be ceased. However, when in excess, these two end products interact with each other to form lactic acid. This allows for the body to keep functioning during a hypoxic state longer than it would be able to without this process. When oxygen is introduced back into the system after an anaerobic state, lactate is reconverted to pyruvic acid where it can then be converted to acetyl coA and enter the Citrus Acid Cycle to efficiently produce ATP (Guyton & Hall, 2000). This is an important factor in individuals with decreased blood flow, as seen in PAD, because there is an inadequate supply of oxygen to meet the demand of the working muscle and the ensuing metabolic processes are often altered and pyruvate and
hydrogen atoms interact to form lactic acid. This occurs at a higher rate when an inadequate supply of oxygen forces the muscle into anaerobic metabolism in order to maintain the muscle’s ability to contract.

In general, lactate levels are elevated during exercise. This can be attributed to many different factors. Contracting skeletal muscle stimulates glycogenolysis and lactate production. Also, during exercise, there are hormone-induced increases in glycogenolysis and glycolysis which contribute to lactate production and therefore accumulation. Lastly, with exercise there is a shunting of blood from lactate-removing, gluconeogenic tissues to lactate-producing glycolytic tissues. This redistribution of blood flow causes blood lactate to rise during exercise (Brooks, Fahey, & White, 1996). Depending on the exercise intensity, lactate begins accumulating above resting levels within 15 seconds-5 minutes or as heart rate exceeds 120 beats/minute in healthy adults (Margaria, Oliva, Prampero, & Ceretelli, 1969). It is generally recognized that at work rates exceeding 70% of VO$_2$max, blood lactate levels systemically increase (Tanaka & Matsuura, 1984). At rest, pyruvate production matches pyruvate oxidation and so lactate is stable. However, beginning in exercise around 60% of VO$_2$max, pyruvate production begins to exceed pyruvate oxidation and some pyruvate is converted to lactic acid via LDH (Heighenhauser & Parolin, 1999). Increasing blood lactate levels indicate that lactate production exceeds lactate catabolism. Several factors contribute to blood lactate levels in an individual such as age, training level, and mitochondrial deficiencies and diseases (i.e. PAD). These findings suggest that an increased level of blood lactate above basal level could be expected during short duration, high intensity activities, such as the six-minute walk test (6MWT), that require near-maximal effort.

Blood lactate levels represent overall lactate accumulation versus overall lactate production. However, this blood lactate measurement is proportional to the net lactate
accumulation in the whole body. Blood lactate measurements approximate the equilibrium between lactate production rate and elimination rate as determined by many different physiological modulators such as glycolysis kinetics, the activity of LDH, and mitochondrial respiration. Blood lactate levels compared with skeletal muscle lactate levels have been found to be different in humans. Lactate concentration in the blood is lower than in the working muscle. This is because the process of lactate transport from the muscle into the blood is a relatively slow process. This process is thought to be slow to protect the blood from acidosis (Sachs & Sachs, 1937). The increase in blood lactate as exercise progresses occurs due to the transport of lactate from the muscle. Skeletal muscle lactate accumulates with repeated contractions and then is released into the blood. Jorfeldt, Julin-Dannfelt, and Karlsson (1978) found that the release of lactate from working skeletal muscle increased linearly with the amount of muscle lactate content produced during a cycling protocol. This shows that blood lactate can be used as a measure of overall lactate accumulation in an exercising individual due to its correlation to the amount of lactate actually produced in the working muscle.

In PAD patients, oxidative metabolism kinetics during exercise are altered. In healthy adults, the skeletal muscles rapidly use oxygen as exercise begins and these increases in oxygen utilization by the muscle can be measured as changes in oxygen consumption (Barker et al., 2004). By using nuclear magnetic resonance spectroscopy, Pipinos et al. (2000) further showed that metabolic kinetics are slowed at the muscular level in PAD patients. Slowed oxygen kinetics with PAD implies that the working muscle is unable to utilize oxygen efficiently. At rest, this is not a big factor for individuals with PAD. However, with exercise, oxygen demand increases but there is a decreased oxygen delivery to the working muscle due to vessel occlusion and a decreased ability of the muscle to utilize the oxygen that is supplied. If there was an inadequate
supply of oxygen to a working muscle with intact muscle oxygen utilization, there would be a rapid hemoglobin desaturation rate. However, if oxygen was being delivered at an adequate amount to a muscle with impaired muscle oxygen utilization, then at the onset of exercise there would be a delayed hemoglobin desaturation rate. When looking at the onset of exercise in PAD patients, hemoglobin desaturation is slowed when compared with healthy controls which shows that there is a defect in the utilization of oxygen in the muscle. This shows that PAD patients have impaired oxidative metabolic kinetics (Brass, Hiatt, & Green, 2004).

In patients with PAD, post-exercise lactate is elevated higher over normal values at rest and at very low workloads. The ischemia associated with PAD plays a role in this metabolite build up, however, there are other physiological mechanisms behind this heightened lactate accumulation in circulation. Barker et al. (2004) showed that regulation of PDH is altered in PAD patients. This suggests that pyruvate was being moved from complete oxidation towards lactate production. PAD patients are also considered to have an acquired metabolic defect due to the relatively normal blood flow at rest followed by oxygen-supply discrepancies during exercise which is generally followed by maintained post-exercise hyperemia. This ischemia-reperfusion injury leads to increases in oxidative stress (Neumann et al., 1990). The exaggerated post-exercise blood lactate levels in PAD patients can be attributed to this oxidative stress as well as metabolic pathophysiology and decreases in blood flow.

**Post-Recovery Lactate**

Clearing lactate is an important component of lactate accumulation; if lactate is not cleared out of the blood, levels become dangerous and negatively feed back and inhibit muscle contraction. Because of this, lactate clearance is a very important mechanism. Sometimes, blood lactate accumulates as a result of overproduction but it is also increased as a result of poor lactate
clearance. Lactate clearance is an important factor in predicting mortality; prolonged lactate clearance is associated with an increased mortality rate in post-surgery patients (McNelis et al., 2001). Blood flow is a significant factor in lactate clearance implying that there could be drastic impairments in lactate clearance rates in individuals with decreased blood flow due to occluded vessels.

At rest, lactate is removed from the blood by the liver, kidneys, and the heart. It is oxidized in these organs or used in the gluconeogenic pathway for glucose production (Hultman & Sahlin, 1980). During exercise, blood lactate is transported from the blood back into the working muscle where it is used as a substrate for oxidative metabolism (Hultman & Sahlin, 1980). This contributes to the lactate turnover rate. Mazzeo et al. (1986) studied blood lactate disposal rates and found that the average disposal rate was 123.4 mg/kg/hour at rest. In different cardiovascular disease populations, the rate of lactate clearance is reduced due to the alterations in both blood flow and oxygen kinetics. Subsequently, the pathophysiology of cardiovascular disease and its subsets often alters the oxidative muscle metabolism of the exercising peripheral skeletal muscles which contributes to both the increased production and the inhibited clearance of lactate in the blood (Engelen et al., 2000).

**Hemoglobin Saturation**

Hemoglobin saturation is beneficial in understanding the role of blood flow and how that affects skeletal muscle metabolism with the slowed kinetics evident in PAD patients. Hemoglobin saturation can be measured non-invasively by using pulse oximetry technology. This lightweight device slips over an individual’s finger and determines pulse and percentage of hemoglobin saturation. It works by shining light through a cuvette filled with a lysed red blood cell solution. The concentration of hemoglobin is determined by measuring the incident and
transmitted light intensity through the known dimensions of the cuvette (Tremper, 1989). By placing the fingertip, which contains a pulsating capillary bed, between two-wavelength light source and a detector, it is able to determine hemoglobin saturation. In a study testing the reliability of pulse oximeters, Yelderman and New (1983) found that there was a correlation coefficient of 0.98 between pulse oximeter results and a carbon dioxide-oximeter and saturation valves. They did find however that pulse oximeters become less reliable when adequate finger pulsation is lost in situations such as hypothermia, hypotension, or severe vasoconstriction. They concluded that pulse oximeters can accurately measure arterial hemoglobin oxygen saturation between 70-100%. Another variable found to possibly alter the reliability of pulse oximeters is motion. Motion can disturb the processing of light absorbance and alter the readings. Barker and Shah (1997) evaluated the effect of motion on the accuracy of pulse oximeter readings. They found that motion does play a key role in their accuracy and needs to be controlled. When intending to use pulse oximetry during the 6MWT, it is important to limit hand movement during time of readings to decrease the chance of skewed results.

**Six-Minute Walk Test**

The Six-Minute Walk Test (6MWT) is a test originally developed for asthma patients by the American Thoracic Society (Enright, 2003). Its original intent was to measure maximal distance covered by an asthmatic individual in the allotted six minutes. Since then, the 6MWT has been utilized in many different populations as a measure of functional fitness due to the safety, the reproducibility, and the ease of administration of the test (Enright, 2003). The cardiovascular society is one of the main fields outside the thoracic society to use the 6MWT as a measure of functional fitness within cardiac rehab facilities.
The primary variable measured in the 6MWT is the overall distance covered by the individual. However, there are many secondary measures that can be taken as well. These include measures of fatigue or dyspnea, or in the case of the cardiovascular society, angina or claudication. Arterial oxygen saturation taken with a pulse oximeter is also a common measure recorded during the 6MWT. Blood samples can also be used to measure the build up of different enzymes and metabolites in the blood (Enright, 2003). The 6MWT evaluates the whole-body and integrated responses of the cardiovascular, pulmonary, and muscular systems in addition to measuring the functional capacity of an individual that translates into their ability to perform activities of daily living. Gardner et al. (1998) examined the relationship between activities of daily living and different ambulatory measurements in PAD patients with intermittent claudication symptoms. Energy expenditure of physical activity was determined and compared to 6MWT distances and other ambulatory measures. It was found that a low ability to perform daily activities was correlated strongly with a decrease in walking ability as measured in the 6MWT. This evidence shows that the 6MWT can represent an individual with PAD’s functional capacity.

Interpreting results of the 6MWT can be difficult when observing a specific diseased population. Reference equations and distances covered by healthy adults exist and can serve as a measuring stick to determine loss in functional capacity in unhealthy adults. Enright and Sherrill (1998) looked at almost 300 healthy adults to determine average distance covered for men and for women. The median distance walked was 576 meters for men and 494 meters for women. Chetta et al. (2006) found that mean distance for both genders was 614 meters. When looking at non-healthy individuals, mean distance covered is drastically lowered, particularly in individuals with cardiovascular disease. Bittner et al. (1993) measured patients with coronary obstructive pulmonary disease (COPD) and found that average distance covered by participants was 374
meters during the six minutes. Carter et al. (2003) also used the 6MWT in COPD patients and found that average distance walked for men was 416 meters and for women was 367 meters. These findings show that the 6MWT is an acceptable measure of functional capacity for patients with cardiovascular disease. They also show that the presence of cardiovascular disease decreases the overall distance covered during the 6MWT. Because of this, the 6MWT is the most common ambulatory measure used for individuals with PAD.

The 6MWT is a staple in the PAD community for testing current functional ability as well as declines over time. This test was adopted for these ischemic patients because walking performance depends heavily on aerobic metabolism and the ability of said metabolism to keep up with the energy requirements of the working muscle (Green & Askew, 2001). When PAD patients perform the 6MWT, intermittent claudication (IC) symptoms generally appear. In some patients, IC gets so severe that they have to take a break or cannot complete the test. For this reason, time to onset of claudication is generally measured and recorded. These factors can be compared as pain free walking versus non-pain free walking. The claudication scale is a scale from 0-4 that expresses the severity of claudication symptoms being felt at a specific moment in time. A score of “0” is indicative of no claudication pain while a score of “4” is indicative of extremely severe pain to the point of immobilization. Upon rest, this pain generally subsides within several minutes and the individual can continue walking.

Faucheur et al. (2008), observed that the average distance covered by 24 PAD patients was 184 meters with a range of 144-246 meters. Interesting to note in this study, 50% of participants had to stop at least once during the test before completing the test. Other studies have concluded that walking performance is 40-50% less in PAD patients compared to healthy controls (Regensteiner et al., 1993). Average time to onset of claudication in PAD patients is
generally between 2-4 minutes (Askew, Green, Hou, & Walker, 2002; Hiatt et al., 1988; Womack, Sieminski, Katzel, Yataco, & Gardner, 1997).

**Borg Scale**

Borg’s scale is a measure for assessing an individual’s rating of perceived exertion (RPE). The borg scale is based on numerical values that represent the individual’s current exertion level to determine exercise intensity. The scale ranges from 6-20 with “6” representing very easy work and “20” representing the absolute maximum for how hard an individual could work (Karavatas & Tavakol, 2005). The scale is often used at different points throughout an exercise testing protocol to determine the perceived exertion level of the subject. The RPE scale should not be confused with the claudication scale that measures pain; the RPE scale is a subjective tool measuring perceived exertion and is a subjective measure of exercise intensity. Karavatas and Tavakol (2005) examined the correlation between the RPE scale and heart rate. They found that the correlation between the two values was .58 which was significantly different from zero. Males were found to have a slightly higher correlation (0.60) than females (0.56). The borg scale is often used in different submaximal and maximal exercise tests to track how hard an individual is working. In the 6MWT, it can be used to track exertion as well as be used to determine what exertion levels are most often correlated to the onset of claudication.

**Summary**

Peripheral arterial disease causes decreases in blood flow and metabolic oxidative abnormalities that become more apparent with exertion. This pathophysiology leads to hemodynamic changes such as the rate of blood lactate accumulation and clearance during exercise. These variables will be observed in patients with PAD during the 6MWT and compared to healthy controls.
CHAPTER THREE

METHODOLOGY

The purpose of this study was to measure how the presence of peripheral arterial disease affects resting, post-exercise, and post-recovery blood lactate during and following the 6MWT.

Participants

A total of 49 individuals (21 males and 28 females) age ≥ 50 years, both with and without peripheral arterial disease voluntarily participated in this study (Table 1). Sixteen participants with an ABI ≤ 0.90 made up the experimental group while 33 participants with an ABI > 0.90 made up the control group. Volunteers were recruited from the northwest Arkansas and northeast Oklahoma areas. Health history was used to identify any immediate risks that may have been exacerbated with exercise (Appendix A). Participants could not have uncontrolled cardiovascular disease, diabetes, or hypertension in order to be approved to participate in this study. If the participant was under current instruction by their doctor to not participate in physical activity, they were excluded from the study. Other inclusion criteria included age and Montreal Cognitive Assessment (MoCA) score; all participants were required to be over the age of 50 years old and to have scored ≥ 26 on the MoCA.

Study Design

This study was a cross sectional design consisting of one testing day that required no more than three hours per participant.

Pre-Testing Procedures

Participation was voluntary and a signed informed consent provided clearance and indicated participant understanding of study protocols and risk factors associated with participation (Appendix B). The Montreal Cognitive Assessment was given to all participants to
ensure that they could ethically sign the informed consent. The protocol was approved by the Institutional Review Board at the University of Arkansas, Fayetteville (Appendix C). Height and weight of each participant was measured using a Detecto Physician’s Scale (Webb City, MO). A health history questionnaire was used to assess and identify any immediate risks that may be intensified with physical activity. Some of these risks included dyspnea, ischemia, heart palpitations, and drastic blood pressure changes.

**Resting Measures**

Ankle-brachial index (ABI) was taken in each individual at rest before the commencement of the 6MWT. A Portable Doppler with 8-10 MHz probe was utilized to measure the systolic blood pressure of the brachial artery in the arms and the dorsalis pedis of the ankles. To take the measure, the participant lie supine as the pressure cuffs were placed with the bottom of the cuff approximately 2-3 cm above the cubital fossa on the arms and malleolus at the ankle. The participant remained supine for 10 minutes before the measure was taken. First, the brachial pulse was palpated to determine its precise location to obtain an audible pulse. Once the location was confirmed, transmission gel was applied over the pulse site. The tip of the Doppler probe was placed at a 45° angle pointed in the direction of the participant’s head until an audible pulse signal was obtained and the pressure cuff was then inflated. The cuff was inflated 20-30 mmHg above the point where the pulse was no longer audible. From this point, the pressure cuff was deflated at a rate of 2-3 mmHg per second while observing the manometer and reading where the first pulse signal was heard and that value was recorded as the systolic value. Once the systolic value was found, the pulse site was cleaned and the procedure was repeated on the other arm. In the circumstance that a pressure needed to be repeated, at least one minute was allowed between readings. BP was then taken using the same technique in the legs; however in the legs,
blood pressure was taken approximately 2-3 cm proximal to the malleolus at the ankle. After the pulse was palpated and an audible pulse was confirmed, transmission gel was applied and the pressure was taken and recorded for each leg. The higher brachial systolic pressure was used to calculate the ABI for both legs. One measure was taken in each arm and in each ankle.

Prior to the initiation of the 6MWT, resting lactate was measured by the standard finger-prick method. The Accutrend Lactate (Indianapolis, IN) was utilized in the participant’s non-dominant hand. Accutrend Lactate analyzer uses light impulses from an LED to measure the color produced on the lactate test strip during the reaction and then compares this to a baseline value to easily determine lactate levels. Each participant had their own individual lancet to ensure safety and prevent contamination. Capillary blood samples were taken from the side of the fingertip. Three different lactate measures were taken: 1) at rest, 2) at the immediate conclusion of the 6MWT, and 3) 20 minutes following the cessation of exercise. To take lactate measurements, the administrator first put on gloves and cleaned the pricking site with an alcohol swab. The lancet was then inserted into the fingertip and the first drop of blood was wiped away. The finger was then massaged until a large drop of blood rested on the top of the wound. This was then squeezed onto the lactate strip which was then analyzed with the Accutrend lactate to determine the lactate level at that particular time point.

Also, prior to the initiation of the 6MWT, resting hemoglobin saturation was assessed using the Nonin SportStat Pulse Oximeter (Plymouth, MN). This was done quickly and non-invasively by placing the device on the fingertip of the participant’s dominant hand to determine saturation levels. This also measured the participant’s resting heart rate. Hemoglobin saturation was also measured every minute throughout the 6MWT to ensure that saturation levels did not
fall below 92%. If levels dropped below this mark for two consecutive minutes, the test was ceased and the individual was asked to rest until levels read at least 92%.

**6MWT**

The 6MWT took place on a hard, flat surface. The course was 50 yards long marked by cones to signify where the participant should turn and return in the opposite direction. Participants were instructed to walk as far as possible in the allotted six minutes. Encouragement was given in moderation to ensure that the individual was pushing themselves as far as they could before resting or stopping. Only one trial was performed per participant.

Two scales were utilized at every 50-yard increment during the 6MWT. The Borg’s Scale of rating of perceived exertion (RPE) was used to determine how hard the individual felt that they were working (Appendix D). They were asked to put their overall exertion into one number on the Borg’s scale and that number was recorded for each lap. A score of “6” on the RPE scale was described as the exertion required to sit on a couch while a score of “20” was described as the exertion required to work as hard as possible before immediately before failure. To determine claudication symptoms during the 6MWT, the claudication scale was used (Appendix E). Individuals ranked their overall claudication pain every 50 yards and onset of claudication was marked in all participants. A “0” on the claudication scale was described as no claudication pain at all. A “4” was described as unbearable claudication pain that required an individual to stop exercise altogether.

Immediately following the 6MWT, HR and hemoglobin saturation were read from the pulse oximeter and blood lactate was taken again from the participant’s same hand. The participant was then asked to lie down for 20 minutes and lactate was taken for a third time at the conclusion of the 20 minute recovery period.
Variables

The dependent variables in this study were 1) total distance covered in the 6MWT and 2) blood lactate levels. Within blood lactate levels, three specific time points were measured: a) blood lactate at rest, b) blood lactate at the immediate conclusion of the 6MWT, and c) blood lactate at 20 minutes post 6MWT. The independent variable assessed in this study was the presence of PAD as defined by an individual’s ABI.

Statistical Analysis

Statistical significance was set at the $\alpha \leq .05$ level and all results are reported as means $\pm$ standard deviations. SPSS statistical software (Armonk, New York) was utilized to analyze all data. A repeated-measures analysis of variance was conducted to compare the effect of having PAD on lactate concentrations at rest, post 6MWT, and 20 minutes post 6MWT.

The 6MWT total distance, rating of perceived exertion scale, claudication scale, hemoglobin saturation, and heart rate were measured in each participant at rest and immediately following the 6MWT in order to determine intensity. A repeated measures analysis of variance was conducted to determine intensity between the two groups.
CHAPTER FOUR

RESULTS

Upon analyzing blood lactate levels at all three time intervals, the data did not demonstrate a significant statistical difference between the presence of peripheral arterial disease and level of blood lactate. Results indicated that PAD, as classified by an individual’s ABI, did not show lead to a significantly different amount of blood lactate in an individual at rest, immediately following the 6MWT, or after a 20-minute rest period. Blood lactate concentrations taken at these three time intervals appeared to follow the same pattern and values were not statistically different between groups.

Demographics

When evaluating the demographic variables between groups, no significant differences were found besides ABI ($F[1, 47] = 48.72, p = <.0001$) (Figure 1) and resting HR ($F[1, 47] = 4.03, p = .05$). Individuals in the PAD group had ABI values that were, on average, 20% less than individuals in the healthy control group. Height ($F[1, 47] = 2.31, p = .14$), weight ($F[1, 47] = 0.00, p = .95$), age ($F[1, 47] = 1.39, p = .24$), MoCA score ($F[1, 47] = 3.23, p = .08$), resting hemoglobin saturation ($F[1, 47] = 3.83, p = .57$), and resting lactate ($F[1, 47] = 0.82, p = .37$) did not differ between groups indicating that the groups were similar and comparable (Table 1).

Classification of Groups

Ankle Brachial Index values were taken at rest on each individual and were used to classify an individual as PAD or Non-PAD using the 0.90 cut off. Out of 49 total participants, 33 were classified as Non-PAD with an average ABI of $1.03 \pm 0.09$ and 16 were classified as PAD with an average ABI of $0.83 \pm 0.09$ (Figure 1).
Hypothesis One

The primary purpose of this study was to observe how the presence of peripheral arterial disease affects resting blood lactate and the rate of post-exercise blood lactate and post-recovery blood lactate during and following the 6MWT. The first hypothesis stated that PAD patients would have a higher resting blood lactate level when compared to healthy controls. A repeated-measures ANOVA statistical analysis was performed on each of the tested dependent variables to determine the differences between blood lactate values at each time period (Figure 2). At rest, there was not a significant difference between blood lactate in Non-PAD (2.93 ± 0.83 mmol/kg) versus PAD (2.72 ± 0.58 mmol/kg). The repeated-measures ANOVA showed that there was not a significant difference between groups ($F[1, 47] = 0.86, p = .36$) (Table 2). These results did not support the hypothesis because resting blood lactate levels were not significantly different between groups.

Hypothesis Two

The second hypothesis in this study asserted that PAD patients would have a higher amount of blood lactate post-exercise (lactate taken immediately following 6MWT – resting lactate) during the 6MWT when compared to healthy controls (Figure 2). Immediately following the 6MWT, there was not a significant difference between blood lactate values in the Non-PAD (1.35 ± 1.73 mmol/kg) vs the PAD group (1.03 ± 1.08 mmol/kg) ($F[1, 47] = 0.48, p = .49$) (Table 2). These data do not support the hypothesis as there was no significant difference between post-exercise blood lactate between PAD and Non-PAD groups.

Hypothesis Three

The third hypothesis in this study indicated that PAD patients would have a slower rate of blood lactate clearing post-recovery (lactate taken 20 minutes post 6MWT – post-exercise
lactate) following the 6MWT when compared to healthy controls (Figure 2). Following 20 minutes of rest after the 6MWT, there was not a significant difference between the Non-PAD (-1.33 ±1.29 mmol/kg) vs. the PAD (-0.87 ± 0.94 mmol/kg) in blood lactate post-recovery ($F[1, 47] = 1.62, p = 0.21$) (Table 2). The PAD group had a post-recovery blood lactate of approximately 35% lower than the Non-PAD group; however, this value was not found to be significant by the repeated measures ANOVA statistical analysis. This hypothesis was not supported by the data because post-recovery blood lactate between groups was not significantly different.

**Summary of Repeated-Measures ANOVA**

Specifically, these results suggest that blood lactate levels at rest, post-exercise (following 6MWT), and post-recovery (20 minutes following 6MWT) do not significantly differ with the reduced circulation and blood flow seen in individuals with PAD compared to individuals without PAD (Table 2, Figure 2). The repeated-measures ANOVA also demonstrates that there was no group by time interaction between relative blood lactate concentration changes at the time points observed between groups (Table 4).

**Standardization of Intensity**

During the 6MWT, a self-selected pace allowed for possible differences in intensity variables such as overall distance covered, rating of perceived exertion (RPE) values, claudication score (CS) values, hemoglobin saturation (HS) values, and changes in heart rate (HR). A repeated-measures ANOVA statistical analysis was performed on each of these values to check for differences in intensity achieved between groups (Table 3).

**6MWT distance.** The mean distance traveled during the 6MWT for each group was found and compared. The Non-PAD group (606.24 ± 118.88 yards) did not walk significantly
further than the PAD group (596.06 ± 104.98 yards) (Figure 3). The repeated-measures ANOVA showed that the presence of PAD did not impact overall distance traveled ($F[1, 47] = 0.08, p = .77$).

**Rating of perceived exertion.** The change in perceived exertion from rest to the conclusion of the 6MWT was determined for each individual and the mean for each group was found and compared. The Non-PAD group had a mean change of 7.76 ± 2.25 while the PAD group had a mean change of 7.25 ± 2.02 (Figure 4). The ANOVA showed that the change in perceived exertion following the 6MWT was independent of disease classification ($F[1, 47] = 0.58, p = .45$).

**Claudication scale.** The change in the claudication pain scale from rest to the conclusion of the 6MWT was also determined for each participant and then the mean for each group was determined. When comparing these means, it was found that the Non-PAD group (0.30 ± 0.77) did not differ significantly from the PAD group (0.38 ± 0.72) (Figure 5). The repeated-measures ANOVA showed that the change in the claudication pain scale from rest-to-conclusion of the 6MWT was not dependent on the presence of PAD ($F[1, 47] = 0.10, p = .76$).

**Hemoglobin saturation.** Hemoglobin saturation was taken at every minute during the 6MWT but changes for each participant were recorded by subtracting the value at rest from the value at the conclusion of the 6MWT. Means were found for each group and then compared to identify differences between groups. The Non-PAD group exhibited a mean change of -1.24 ± 2.57 while the PAD group exhibited a mean change of -1.38 ± 2.90 (Figure 6). The repeated-measures ANOVA again showed that the change in hemoglobin saturation during the 6MWT was not different between groups ($F[1, 47] = 0.03, p = .87$).
**Heart rate.** Lastly, resting heart rate was subtracted from the individual’s heart rate at the conclusion of the 6MWT. The mean change in heart rate was calculated for each group and compared between groups to identify any differences between change in HR during the 6MWT. Mean change in HR for the Non-PAD group was 48.9 ± 19.6 bpm which was not significantly different from the mean change of 44.9 ± 10.3 bpm in the PAD group (Figure 7). The repeated-measures ANOVA demonstrated that the change in HR from rest to the conclusion of the 6MWT was not significant between groups ($F[1, 33] = 0.39, p = .53$).

**Summary of Repeated-Measures ANOVA**

Specifically, these results suggest that the exercise intensity achieved was not different between groups. The repeated-measures ANOVA also demonstrates that there was no group by time interaction between relative changes in claudication score, hemoglobin saturation, and rating of perceived exertion (Table 5) or ankle-brachial index and heart rate (Table 6) during the 6MWT.
CHAPTER FIVE
DISCUSSION

The primary purpose of this thesis was to investigate differences in resting blood lactate, post-exercise blood lactate, and post-recovery blood lactate between groups. Based on the results found during this study, the data do not indicate that there is a significant difference between these examined variables. Several factors could be responsible for the inability of the data collected throughout this study to demonstrate significance between groups in blood lactate values.

First, the inclusion criteria did not exclude individuals for any medication they were taking. It also did not exclude individuals with controlled cardiovascular disease, controlled diabetes, cancer, and musculoskeletal injuries. All but one participant in this study had at least one medical condition ranging from high blood pressure to cancer. Medications taken by participants, as disclosed in the health history questionnaire, include but are not limited to medications intended for depression, osteoporosis, allergies, thyroid disorders, arthritis, cholesterol, diabetes, blood pressure, and long term medications taken for post-cancer and post-cardiovascular event individuals. Several patients also regularly took anti-inflammatory drugs and pain relievers for chronic joint pain or joint relief after replacement. All of these medications play different roles in normal body functioning and can therefore cause or prevent certain functions of the body to occur. They can also affect different aspects of the cardiovascular system such as circulation, blood pressure, arterial compliance, and blood viscosity. Musculoskeletal injuries were also disclosed by each participant in this study and varied from individual to individual. These injuries included but were not limited to hip replacements, knee replacements, bunionectomy, ankle edema, and obesity-related functional impairments. The fact
that individuals with these different medicinal and functional issues were not excluded from participation led to a very diverse population of participants with varying diseases, functional limitations, and medications all of which had differing effects on walking ability and cardiovascular function as well as perhaps even having different metabolic implications. For example, some participants with joint-replacements complained of joint pain throughout the walk and had a harder time completing the walk due to this pain. Others were unable to push past a low-intensity speed without aggravating a replaced joint or other injury. In addition, without a detailed analysis of all medications taken by participants, it is impossible to know all of the potential effects that these medications could have had on the variables analyzed in this study.

Second, because the 6MWT has a self-selected pace, individuals were all working at different intensities and to different points of exhaustion. While results did not show a significant difference between groups in any of the “intensity” variables (RPE, HR, distance covered), they did show that overall, individuals worked at a low-to-moderate intensity rather than a maximal intensity. In both groups, the average post-exercise RPE was 13.59 ± 2.17 which is classified by the RPE scale as “somewhat hard“ work. Based on self-report via the RPE scale, this shows that individuals struggled to achieve maximal effort in the allotted six-minute time frame. Intensity in older adults is defined by ACSM on a scale from 6-20 of physical exertion. They define a 12 or a 13 on this scale as moderate-intensity exercise which produces a noticeable increase in HR and breathing and a 16 or an 17 as vigorous-intensity exercise producing a large increase in HR and breathing (Thompson, Gordon, & Pescatello, 2010). In this study, post-exercise heart rate for both groups following the 6MWT was 121.91 ± 16.18 bpm. For their average age (64.82 years), based on target heart rates, they were performing work at approximately 75% of their HR max which is defined as a moderate intensity. This again shows that individuals were not at maximal
effort during the six-minute test (Tanaka, Monahan, & Seals, 2001). Perhaps a walk-to-exhaustion test that allowed each individual to achieve maximal exertion would yield significant results rather than a timed test where most individuals did not make it past a moderate intensity.

However, despite participants not achieving high intensity during the 6MWT, Brass et al. (2004) showed that lactate accumulates in the muscles of individuals with PAD at rest and at low-to-moderate workloads. He suggest that this elevated lactate in muscle is due to pyruvate being shunted from total oxidation towards lactate production because of a dramatically different metabolic milieu seen in PAD individuals versus healthy controls. Brass et al. found that lactate can build up in the muscle at rest and at low workloads; however, in contrast, when looking at lactate in the blood, the present study did not find a significantly higher amount of lactate at rest and low intensities in individuals with PAD.

Because the 6MWT is only six minutes, perhaps blood lactate differences would be more apparent and statistically significant during longer exercise bouts where higher intensities are achieved. Urhausen and Kindermann (1992) examined blood lactate concentrations at different exercise intensities and found that, in healthy adults, blood lactate only increased at high intensities near an individual’s anaerobic threshold. It is commonly taught that higher intensity exercise leads to a greater lactate production than lower intensity exercises (McArdle, Katch, & Katch, 2010). Because the 6MWT is technically a “maximal” test, it can be inferred that it would be a high intensity exercise resulting in large amounts of blood lactate build-up. However, according to our results, most participants only performed at a low-to-moderate intensity even when instructed to walk at maximal pace. Therefore, because high intensities were not achieved during the 6MWT, blood lactate levels would not be expected to be elevated drastically higher than resting levels.
Third, because severity of PAD was not considered, nor was the presence of claudication symptoms, it could be possible that mild-to-moderately blocked femoral arteries in individuals (with no claudication pain) do not exhibit excessive blood lactate accumulation or prolonged clearance time whereas individuals with severely blocked arteries (with present claudication pain) would exhibit a stronger difference with blood lactate abnormalities compared to age-matched controls. Because there is a correlation between the severity of vessel occlusion, abnormal metabolic milieu in the muscles of individuals with PAD, and exercise impairment, it is known that with less-severe occlusion, there would be less-severe substrate build-up (Brass, Hiatt, & Green, 2004). It is known that with more occluded vessels, less blood flow is able to travel to working muscle. This decreased blood flow and consequential ischemia is directly responsible for metabolic abnormalities and substrate build-up in healthy individuals (Robbins et al., 2011 & Hiatt et al., 1992). It can be asserted that in an individual with occluded blood flow to one or both legs that the extent to which the vessel is occluded would play a part in the extent to which metabolic abnormalities are present and therefore the abnormalities in substrate build-up (Robbins et al., 2011, Hiatt et al., 1992, & McDermott et al., 1999). Because the mean ABI for participants in the PAD group was 0.83 ± 0.09, most participants in this study fell in the “mild blockage” category for PAD implying that they did not have extremely occluded vessels with drastic reductions in blood flow. Because most of the PAD group did have such mild PAD and close-to-normal blood flow and there was no statistical difference between groups in resting, post-exercise, and post-recovery blood lactate, perhaps a more drastic experimental group is needed to see if these variables become significant with more occluded vessels and more inhibited blood flow.
Last, this study classified participants as PAD based on either limb scoring an ABI of less than or equal to 0.90. Because of this classification, only 3 of the 49 participants enrolled in this study exhibited vessel occlusion in both limbs. All other PAD subjects had only one limb with an ABI $\leq 0.90$. In studies that have used muscle biopsies to determine enzyme and substrate differences with PAD, the contralateral “healthy” limb is often used as its own control for the “diseased” limb with vessel occlusion. It has been found that the activity, as well as expression, of mitochondrial enzymes is increased only in the limb with PAD and these changes are not observed in the healthy limb (Jansson, Johansson, Sylven, & Kaijser, 1988). Because blood lactate is representative of lactate in the entire body and not just the affected limb, analysis of lactate from a muscle biopsy would be a much more effective and accurate way to determine differences in substrate build-up with PAD.

In regards to 6MWT distance, interestingly, this study did not show a significantly different distance covered between groups as often found in other research. Chetta et al. (2006) found that healthy adults typically walk between 450-700 yards during the 6MWT. Conversely, Regensteiner et al. (1993) found that PAD individuals walk an average of 250-400 yards. However, in this study, there was no significant difference between groups and the average distance for Non-PAD (606.24 ± 118.88) and PAD (596.06 ±104.98) fell comfortably into Chetta et al.’s range for healthy classification (450-700 yards).

Although results for the study failed to demonstrate a significant difference between the presence of PAD in an individual and blood lactate at rest, post-exercise, and post-recovery, it is important to note that peripheral arterial disease still impacts blood flow to working muscle and may still have an effect on these variables under different exercise or functional conditions. McDermott et al. (2004) showed that lower ABI values were associated with annual decline in
6MWT time. They also showed that individuals with PAD had greater annual 6MWT time decreases than individuals without PAD. Regensteiner et al. (1993) showed that individuals with PAD and intermittent claudication had an approximate 50% reduction in walking performance when compared to healthy individuals of the same age. Blood flow is often singled out as the main cause of this reduction in functional ability and exercise capacity. Therefore, because a large portion of the experimental population in this study did not have drastically reduced blood flow (as inferred from their ABI), perhaps these results would be more significant if the population had more drastic occlusion and thereby a significant decrease in blood flow that would lead to the previously observed functional impairment.

With vessel occlusion, blood flow decreases to a greater degree along with vessel conductance, compliance, and oxygen kinetics than in healthy individuals. Blood flow in individuals with claudication symptoms has been found to decrease by almost 50% when compared to healthy controls during exercise (Pernow et al., 1975). This decrease in blood flow as a result of femoral vessel occlusion has significant implications on skeletal muscle contractility as well as metabolite production in individuals with peripheral arterial disease. Brass et al. (2004) showed that lactate accumulates in the muscles of individuals with PAD at rest and at low-to-moderate workloads. This shows that the ischemic nature of blood flow in individuals with PAD can lead to a more-than-normal blood lactate accumulation at all work loads.

Many studies concur that exercise blood flow is markedly decreased in individuals with PAD when compared to healthy individuals (Pernow et al., 1975). Decreased blood flow to a working muscle leads to reduced nutrient delivery, ischemia, and impaired metabolite clearance. These physiological changes are important because they often define functional capacity. As
individuals with PAD age, the energy requirements for most daily tasks exceeds that of their energy capacity due to these functional limitations. This impacts individuals as they try to perform simple tasks in every-day life and are unable to do so without taking a break or reaching exhaustion (Oka et al., 2003; Garg et al., 2006). Ischemia is also very important because with recurring ischemia, muscle and tissue damage can occur. All of this information in conjunction would infer that there would be a significant difference between femoral vessel occlusion, decreases in exercise blood flow, and blood lactate abnormalities between PAD and Non-PAD individuals. However, data between groups during the 6MWT was not found to be significantly different in this study.

**Conclusion**

Although no difference was observed between blood lactate levels between groups, it is important to note that physiologically, PAD elicits exercise metabolic abnormalities and therefore a relationship may still exist between these two variables at different intensities, durations, or exercise modes.

Future research in this area should include different functional tests than the 6MWT to determine if a walk-to-exhaustion or a timed, set-pace exercise protocol would elicit blood lactate differences between individuals with PAD and without PAD. Additional research could also control for PAD severity by breaking down the population into severe PAD, moderate PAD, mild PAD, and non-PAD groups rather than just PAD and non-PAD.

With the conclusion of this research project, it is important to continue the promotion of walking and functional training in aging individuals whether they have decreased circulation to their legs or not. This training can improve and prevent occluded vessels in aging adults as well
as improve vessel compliance and blood pressure regulation. It also allows an adult to maintain their functional capacity as they age.
Table 1

Demographic differences between height, weight, age, MoCA score, resting ABI, resting HS, resting HR, resting lactate, and 6MWT distance

<table>
<thead>
<tr>
<th></th>
<th>Non-PAD</th>
<th>PAD</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>166.80 ± 7.14</td>
<td>169.88 ± 8.05</td>
<td>2.31</td>
<td>.14</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.94± 21.36</td>
<td>78.13 ± 16.73</td>
<td>0.00</td>
<td>.95</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.67 ± 8.34</td>
<td>67.19 ± 9.75</td>
<td>1.39</td>
<td>.24</td>
</tr>
<tr>
<td>MoCA</td>
<td>28.09 ± 1.42</td>
<td>27.31 ± 1.08</td>
<td>3.23</td>
<td>.08</td>
</tr>
<tr>
<td>Resting ABI*</td>
<td>1.03 ± 0.09</td>
<td>0.83 ± 0.09</td>
<td>48.72</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Resting HS (%)</td>
<td>96.64 ± 1.65</td>
<td>97.50 ± 1.46</td>
<td>3.83</td>
<td>.57</td>
</tr>
<tr>
<td>Resting HR* (bpm)</td>
<td>76.09 ± 13.29</td>
<td>67.88 ± 10.74</td>
<td>4.03</td>
<td>.05</td>
</tr>
<tr>
<td>Resting Lactate (mmol/kg)</td>
<td>2.93 ± 0.83</td>
<td>2.71 ± 0.58</td>
<td>0.82</td>
<td>.37</td>
</tr>
<tr>
<td>6MWT Distance (yards)</td>
<td>605.97 ± 120.77</td>
<td>596.06 ± 104.98</td>
<td>0.08</td>
<td>.78</td>
</tr>
</tbody>
</table>

Note. MoCA = Montreal Cognitive Assessment, ABI = ankle-brachial index, HS = hemoglobin saturation, HR = heart rate, 6MWT = six-minute walk test. No statistical difference in measured factors between individuals with (PAD) and individuals without (Non-PAD) peripheral arterial disease. Values are means ± sd. *Indicates significant difference between Non-PAD and PAD scores, p < .05
Table 2

ANOVA analysis. Differences in blood lactate at rest, post-exercise, and post-recovery during the six-minute walk test (6MWT).

<table>
<thead>
<tr>
<th></th>
<th>Non-PAD</th>
<th>PAD</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting Lactate (mmol/L)</td>
<td>2.93 ± 0.83</td>
<td>2.72 ± 0.58</td>
<td>.86</td>
<td>.36</td>
</tr>
<tr>
<td>Post-Exercise (mmol/L)</td>
<td>1.35 ± 1.73</td>
<td>1.03 ± 1.08</td>
<td>.48</td>
<td>.49</td>
</tr>
<tr>
<td>Post-Recovery (mmol/L)</td>
<td>-1.33 ± 1.29</td>
<td>-0.87 ± 0.94</td>
<td>1.62</td>
<td>.21</td>
</tr>
</tbody>
</table>

*Note.* No statistical difference in measured factors between individuals with (PAD) and individuals without (Non-PAD) peripheral arterial disease. Values are means ± sd. *Indicates significant difference between Non-PAD and PAD scores, p < .05
Table 3

ANOVA analysis: Differences in 6MWT distance, RPE, CS, HS, and HR

<table>
<thead>
<tr>
<th></th>
<th>Non-PAD</th>
<th>PAD</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWT Distance (yards)</td>
<td>606.24 ± 118.88</td>
<td>596.06 ± 104.98</td>
<td>0.08</td>
<td>.77</td>
</tr>
<tr>
<td>Change in RPE</td>
<td>7.76 ± 2.25</td>
<td>7.25 ± 2.02</td>
<td>0.58</td>
<td>.45</td>
</tr>
<tr>
<td>Change in CS</td>
<td>0.30 ± 0.77</td>
<td>0.38 ± 0.72</td>
<td>0.10</td>
<td>.76</td>
</tr>
<tr>
<td>Change in HS (%)</td>
<td>-1.24 ± 2.57</td>
<td>-1.38 ± 2.90</td>
<td>0.03</td>
<td>.87</td>
</tr>
<tr>
<td>Change in HR (bpm)</td>
<td>48.88 ± 19.64</td>
<td>44.91 ± 10.25</td>
<td>0.39</td>
<td>.54</td>
</tr>
</tbody>
</table>

Note. 6MWT = six-minute walk test, RPE = rating of perceived exertion, CS = claudication scale, HS = hemoglobin saturation, HR = heart rate. No statistical difference in measured factors between individuals with (PAD) and individuals without (Non-PAD) peripheral arterial disease. Values are means ± sd. *Indicates significant difference between Non-PAD and PAD scores, p < .05
Table 4
Repeated measures ANOVA analysis. Group by time interaction between blood lactate at rest, post-exercise, and post-recovery during the six-minute walk test (6MWT).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>2</td>
<td>37.85</td>
<td>18.92</td>
<td>23.28</td>
<td>.00</td>
</tr>
<tr>
<td>Time * PAD</td>
<td>2</td>
<td>1.23</td>
<td>0.61</td>
<td>.75</td>
<td>.47</td>
</tr>
<tr>
<td>Error (Time)</td>
<td>94</td>
<td>76.40</td>
<td>0.81</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. No group by time interaction was found between the PAD group versus the Non-PAD group when looking at blood lactate values over time (n=49).
Table 5
Repeated measures ANOVA analysis. Group by time interaction between Claudication Scale, Hemoglobin saturation, and Rating of Perceived Exertion throughout the six-minute walk test (6MWT).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>6</td>
<td>3.65</td>
<td>0.608</td>
<td>4.67</td>
<td>0.00</td>
</tr>
<tr>
<td>HS</td>
<td>6</td>
<td>97.93</td>
<td>16.32</td>
<td>6.04</td>
<td>0.00</td>
</tr>
<tr>
<td>RPE</td>
<td>6</td>
<td>935.68</td>
<td>155.95</td>
<td>125.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Time * PAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>6</td>
<td>0.29</td>
<td>0.05</td>
<td>0.38</td>
<td>0.89</td>
</tr>
<tr>
<td>HS</td>
<td>6</td>
<td>11.99</td>
<td>2.00</td>
<td>0.74</td>
<td>0.62</td>
</tr>
<tr>
<td>RPE</td>
<td>6</td>
<td>3.28</td>
<td>0.55</td>
<td>0.44</td>
<td>0.85</td>
</tr>
<tr>
<td>Error (Time)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>174</td>
<td>22.64</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>174</td>
<td>469.89</td>
<td>2.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE</td>
<td>174</td>
<td>217.05</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* No group by time interaction was found between the PAD group versus the Non-PAD group when looking at CS (claudication score), HS (hemoglobin saturation), and RPE (rating of perceived exertion) throughout the 6MWT (n=49).
Table 6
Repeated measures ANOVA analysis. Group by time interaction for ankle-brachial index and heart rate at rest and post-exercise following the six-minute walk test (6MWT).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TimeABI</td>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.81</td>
<td>.38</td>
</tr>
<tr>
<td>TimeHR</td>
<td>1</td>
<td>33171.43</td>
<td>33171.43</td>
<td>220.66</td>
<td>.00</td>
</tr>
<tr>
<td>Time * PADABI</td>
<td>1</td>
<td>0.07</td>
<td>0.07</td>
<td>6.40</td>
<td>.02</td>
</tr>
<tr>
<td>Time * PADHR</td>
<td>1</td>
<td>59.32</td>
<td>59.32</td>
<td>0.40</td>
<td>.53</td>
</tr>
<tr>
<td>Error (TimeABI)</td>
<td>33</td>
<td>0.36</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error (TimeHR)</td>
<td>33</td>
<td>4960.77</td>
<td>150.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. No group by time interaction was found between the PAD group versus the Non-PAD group when looking at ABI (ankle-brachial index) and HR (heart rate) throughout the 6MWT (n=49).
Figure 1 *ABI distribution*

*Note.* ABI = ankle-brachial index. This figure illustrates the distribution of individuals with PAD ( ) and without PAD ( ) in terms of their ABI (*n* = 49).
Figure 2 Blood lactate at rest, post-exercise, and post-recovery during the six-minute walk test

Note. This figure illustrates there is no statistical difference in blood lactate levels at any stage between individuals with PAD (---) and individuals without PAD (--) \((n = 49)\).
Note. 6MWT = six-minute walk test. This figure illustrates there is no statistical difference in the overall distance covered between individuals with (PAD) peripheral arterial disease and individuals without (Non-PAD). ($n = 49$)
Figure 4 *RPE during the 6MWT*

*Note:* RPE = rating of perceived exertion, 6MWT = Six-Minute Walk Test. Figure illustrates there is no statistical difference in the changes in RPE between individuals with (PAD) peripheral arterial disease and individuals without (Non-PAD) (*n* = 49).
Figure 5  *CS score during the 6MWT*

*Note.* CS = Claudication Scale, 6MWT = six-minute walk test. This figure illustrates there is no statistical difference in the changes in the CS score between individuals with (PAD) peripheral arterial disease and individuals without (Non-PAD) (*n* = 49).
Figure 6 HS during the 6MWT

Note. HS = Hemoglobin saturation, 6MWT = six-minute walk test. HS is written in terms of a percent. This figure illustrates there is no statistical difference in the changes in HS between individuals with (PAD) peripheral arterial disease and individuals without (Non-PAD). \( n = 49 \)
Figure 7 HR during the 6MWT

Note. HR = heart rate, 6MWT = six-minute walk test. This figure illustrates there is no statistical difference in the changes in HR between individuals with (PAD) peripheral arterial disease and individuals without (Non-PAD). (n = 35)
REFERENCES


APPENDIX A

Health History Questionnaire
Please answer the following questions.  Today’s Date: ____________

Date of Birth ____________  What is your current age? ____________

(3) No diagnosis of unstable or unmanaged cardiovascular disease, hypertension;

Have you ever had any of the following conditions? Check yes or no. If yes, explain.
Heart Disease  □ Yes □ No
Heart Attack  □ Yes □ No
Angina (Chest Pain)  □ Yes □ No
Peripheral Artery Disease  □ Yes □ No
Stroke  □ Yes □ No
High Cholesterol (>220)  □ Yes □ No
High Blood Pressure (>140/90)  □ Yes □ No
Diabetes  □ Yes □ No
Rheumatic Fever  □ Yes □ No
Aneurysm  □ Yes □ No

No history of hospitalization within the past year that would prohibit physical activity

Have you ever had any of the following conditions? Check yes or no. If yes, explain.
Severe Illness (in the last year)  □ Yes □ No
Operations (in the last year)  □ Yes □ No
Broken bone/fracture (in the last year)  □ Yes □ No

History of a fall within the preceding one year
Have you fallen in the past 12 months?  □ Yes □ No  If yes, explain.
**Additional information**

**Have you experienced any of these symptoms? Check yes or no.**

If yes, explain.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain and/or discomfort in the chest, neck, jaw, or arms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortness of breath at rest or with mild exertion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankle edema (swelling)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid or irregular beating heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg pain, cramping, or tightness during exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart murmur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue or shortness of breath during the day</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do you smoke?  

- Yes
- No
- Quit

(8) Please attach a list of all medication (prescription or over-the-counter) you are currently taking or use the form below.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Reason Prescribed</th>
<th>When do you take this medication?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>□ Morning □ Mid-Day □ Evening □ Bedtime</td>
</tr>
<tr>
<td></td>
<td></td>
<td>□ Morning □ Mid-Day □ Evening □ Bedtime</td>
</tr>
<tr>
<td></td>
<td></td>
<td>□ Morning □ Mid-Day □ Evening □ Bedtime</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>□ Morning □ Mid-Day □ Evening □ Bedtime</td>
</tr>
</tbody>
</table>
APPENDIX B

INFORMED CONSENT

Title: Peripheral arterial disease, balance, sensation, muscle activity, function, lactate levels, and ankle brachial index measures.

Investigator(s):
Jennifer Vincenzo  Michelle Gray  Rebecca Roderick
HPER  HPER  HPER
University of Arkansas  University of Arkansas  University of Arkansas
155 Stadium Drive-HPER 321Q 155 Stadium Drive-HPER 155 Stadium Drive – HPER
Fayetteville, AR 72701  Fayetteville, AR 72701  Fayetteville, AR 72701
479-575-2975  479-575-2975  479-575-2975
jvincenz@uark.edu  rgray@uark.edu  rroderic@uark.edu

Description: You are being asked to participate in a research study. The purpose of this research study is to determine the relationship between physical measurements (such as leg muscle activity, balance, sensation, and mobility), blood lactate levels, and peripheral arterial disease. You will complete a series of assessments in one day. It is expected these assessments will take less than three hours of your time. You will be asked questions to assess your thinking ability, your age, and medical history. Your height and weight will be taken on a scale. Your blood pressure in your arm and leg will be measured to determine how your blood flow is. Your sensation will be assessed for your feeling of pressure on the bottom of your feet. Your balance will be measured on a standing machine called the Biodex Balance System. Your muscle activity during all these tests will be measured by surface electrodes that are placed on your legs during the balance testing. You will also perform standing up, standing in place, and walking tests. During one of the walking tests, you will walk across a flat mat on the floor to assess your walking. There will be rest breaks as needed. There will be a trained clinician with you at all times. You may choose to stop the testing at any time if you feel that you have reached a level in which you can no longer continue. Four small blood samples will be taken from your fingertips at different time points during the 6 minute walk test. You may choose to not participate in this portion of the study. Any information you possess about your health status or previous experiences of unusual feelings with physical activity may affect the safety and value of your testing. You are responsible to fully disclose such information when requested to the testing staff. All testing will be designed by trained personnel and monitored throughout the course of the study.

Risks and Benefits: There is no compensation for participating in this study. There exists the possibility of certain changes occurring during the assessments. They include, but are not limited to, muscle or joint injury, possible dizziness and predisposition to injury, and falls. There is a risk of pain and contamination with the finger pric test. Trained personnel will be available and with you during testing at all times in the event an unforeseen instance may arise. The results obtained will assist in the assessment and prevention of falls and balance deficits in individuals with peripheral arterial disease.
**Voluntary Participation:** Permission for you to engage in the testing is voluntary. You are free to deny or withdraw from testing at any time, if you so desire.

**Confidentiality:** All information collected will be kept confidential to the extent allowed by law and University policy. Test results will be kept in a secure location (HPER 309). After initial contact with the primary investigator, a code number (e.g. 100,101, etc) will be assigned to you. All data collection sheets and electronic data files will only have the code number to identify you.

**Right to Withdraw:** You are free to refuse to participate and to withdraw at any time. Your decision to withdraw will bring no penalty to you.

**Questions:** If you have questions or concerns about this study, you may contact Jennifer Vincenzo or Dr. Michelle Gray at (479) 575-2975 or by e-mail at jvincenz@uark.edu. For questions or concerns about your rights as a research participant, please contact Ro Windwalker, the University’s IRB Coordinator, at (479) 575-2208 or by e-mail at irb@uark.edu.

**Informed Consent:** I, _______________________________ , have read the description of this program, including the purpose of the program, the procedures to be used, the potential risks and side effects, the confidentiality, as well as the option to withdraw from the program at any time. The investigator has explained each of these items to me.

My signature below indicates that I freely agree to participate in this study and that I have received a copy of this agreement from the investigator. I understand that participation in all activities related to this project is voluntary on behalf of all participants. The university makes no commitment to provide free medical care or payment for any unfavorable outcomes resulting from participation in this research.

Participant: _______________________________ 

Date: ________________

Witness: _______________________________ 

Date: ___________
APPENDIX C

September 28, 2012

MEMORANDUM

TO: Jennifer Vincenzo
    Rebecca Roderick
    Michelle Gray

FROM: Ro Windwalker
    IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 12-09-076

Protocol Title: Physical and Functional Assessment of Individuals with Peripheral Arterial Disease Compared to Individuals without Peripheral Arterial Disease

Review Type: ☒ EXPEDITED □ EXEMPT □ FULL IRB

Approved Project Period: Start Date: 09/28/2012 Expiration Date: 09/27/2013

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form Continuing Review for IRB Approved Projects, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (http://vpred.uark.edu/210.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 40 participants. If you wish to make any modifications in the approved protocol, including enrolling more than this number, you must seek approval prior to implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.
January 17, 2013

MEMORANDUM

TO: Jennifer Vincenzo
    Rebecca Roderick
    Michelle Gray

FROM: Ro Windwalker
      IRB Coordinator

RE: PROJECT MODIFICATION

IRB Protocol #: 12-09-076

Protocol Title: *Physical and Functional Assessment of Individuals with Peripheral Arterial Disease Compared to Individuals without Peripheral Arterial Disease*

Review Type: ☒ EXEMPT  ☒ EXPEDITED  ☐ FULL IRB

Approved Project Period: Start Date: 01/15/2013 Expiration Date: 09/27/2013

Your request to modify the referenced protocol has been approved by the IRB. **This protocol is currently approved for 80 total participants.** If you wish to make any further modifications in the approved protocol, including enrolling more than this number, you must seek approval prior to implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

Please note that this approval does not extend the Approved Project Period. Should you wish to extend your project beyond the current expiration date, you must submit a request for continuation using the UAF IRB form “Continuing Review for IRB Approved Projects.” The request should be sent to the IRB Coordinator, 210 Administration.

For protocols requiring FULL IRB review, please submit your request at least one month prior to the current expiration date. (High-risk protocols may require even more time for approval.) For protocols requiring an EXPEDITED or EXEMPT review, submit your request at least two weeks prior to the current expiration date. Failure to obtain approval for a continuation on or prior to the currently approved expiration date will result in termination of the protocol and you will be required to submit a new protocol to the IRB before continuing the project. Data collected past the protocol expiration date may need to be eliminated from the dataset should you wish to publish. Only data collected under a currently approved protocol can be certified by the IRB for any purpose.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.
April 10, 2013

MEMORANDUM

TO: Jennifer Vincenzo
   Rebecca Roderick
   Michelle Gray

FROM: Ro Windwalker
   IRB Coordinator

RE: PROJECT MODIFICATION

IRB Protocol #: 12-09-076

Protocol Title: Physical and Functional Assessment of Individuals with Peripheral Arterial Disease Compared to Individuals without Peripheral Arterial Disease

Review Type: ☒ EXEMPT   ☐ EXPEDITED   ☐ FULL IRB

Approved Project Period: Start Date: 04/08/2013 Expiration Date: 09/27/2013

Your request to modify the referenced protocol has been approved by the IRB. This protocol is currently approved for 80 total participants. If you wish to make any further modifications in the approved protocol, including enrolling more than this number, you must seek approval prior to implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

Please note that this approval does not extend the Approved Project Period. Should you wish to extend your project beyond the current expiration date, you must submit a request for continuation using the UAF IRB form “Continuing Review for IRB Approved Projects.” The request should be sent to the IRB Coordinator, 210 Administration.

For protocols requiring FULL IRB review, please submit your request at least one month prior to the current expiration date. (High-risk protocols may require even more time for approval.) For protocols requiring an EXPEDITED or EXEMPT review, submit your request at least two weeks prior to the current expiration date. Failure to obtain approval for a continuation on or prior to the currently approved expiration date will result in termination of the protocol and you will be required to submit a new protocol to the IRB before continuing the project. Data collected past the protocol expiration date may need to be eliminated from the dataset should you wish to publish. Only data collected under a currently approved protocol can be certified by the IRB for any purpose.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.
## APPENDIX D

Rate of Perceived Exertion

<table>
<thead>
<tr>
<th>Rate</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td><strong>NO EXERTION AT ALL</strong></td>
</tr>
<tr>
<td>7</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><strong>EXTREMELY LIGHT</strong></td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><strong>VERY LIGHT</strong></td>
</tr>
<tr>
<td>11</td>
<td><strong>LIGHT</strong></td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td><strong>SOMEWHAHT HARD</strong></td>
</tr>
<tr>
<td>14</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td><strong>HARD (HEAVY)</strong></td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td><strong>VERY HARD</strong></td>
</tr>
<tr>
<td>18</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td><strong>EXTREMELY HARD</strong></td>
</tr>
<tr>
<td>20</td>
<td><strong>MAXIMAL EXERTION</strong></td>
</tr>
</tbody>
</table>


### APPENDIX E

Claudication Scale

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No claudication symptoms or discomfort</td>
</tr>
<tr>
<td>1</td>
<td>Mild discomfort or pain</td>
</tr>
<tr>
<td>2</td>
<td>Moderate discomfort or pain</td>
</tr>
<tr>
<td>3</td>
<td>Intense pain from which you cannot be distracted</td>
</tr>
<tr>
<td>4</td>
<td>Unbearable pain—you must stop</td>
</tr>
</tbody>
</table>