ABSTRACT

Calf Muscle Hemoglobin Oxygen Saturation Characteristics
in Healthy and Clinical Populations

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Peripheral artery disease (PAD), a manifestation of systemic atherosclerosis, is characterized by atherosclerotic blockages of the arteries supplying blood to the legs. The occlusion results in decreased blood flow during exercise which alters calf muscle hemoglobin oxygen saturation (StO_2) limiting functional capacity. Sarcopenia is the age-associated loss of muscle mass and function. This study examined changes in calf muscle StO_2 across the lifespan in younger and older healthy individuals. The goal was to differentiate between changes seen from ageing and from PAD and associate common sarcopenia tests, 4-meter walk speed and handgrip strength, with StO_2 kinetics. Subjects completed the same tests, Gardner Treadmill test and 6-minute walk test, commonly performed with PAD patients. The results show that ageing, regardless of health, lowers and slows StO_2 kinetics during and after exercise. They also support findings from previous studies that PAD further negatively affects StO_2 kinetics beyond that of ageing.

Calf Muscle Hemoglobin Oxygen Saturation Characteristics in Healthy and Clinical Populations

by

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A Thesis

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DEDICATION

To my parents, Stu and Teresa and my fiancée, Jaime

CHAPTER ONE

Introduction

Motivation

Peripheral artery disease (PAD) is a circulatory disorder characterized by the narrowing of peripheral arteries in the legs. The most common cause of PAD is atherosclerosis, and it manifests significantly in people over 50 (Fowkes et al., 2013). The occlusion results in decreased blood flow during exercise which can create an oxygen deficit during exercise. This deficit can cause intermittent claudication, walking induced muscle pain relieved by rest, limiting the functional capacity in patients with PAD (McDermott, 2015). When compared to age matched controls, limits in functional capacity have been demonstrated through reductions in peak exercise performance of approximately 50% (Hiatt, Nawaz, Regensteriner, & Hossack, 1988). In an effort to maintain functional capacity, exercise programs have been a cost-effective and commonly used treatment in patients with mild to moderate PAD (Bermingham et al., 2013). Results from multiple trials showing exercise improving outcome measures such as pain-free walking distance, 6-minute walking distance, claudication onset time, and peak walking time has led to supervised exercise being a Class IA recommendation by the American College of Cardiology and the American Heart Association for initial treatment of PAD and intermittent claudication (Gardner, Parker, & Montgomery, 2016).

One of the common theories on mechanisms behind the functional gains from exercise is the improvements to calf muscle hemoglobin oxygen saturation (StO_2)

kinetics. StO_2 is the measure of the ratio of oxygenated hemoglobin concentration to the total hemoglobin concentration. This is measured via near-infrared spectroscopy (NIRS) as oxygenated and deoxygenated hemoglobin have different absorbance spectra within the near-infrared region. NIRS devices can measure these differences and report the amount of hemoglobin carrying oxygen as a percentage, otherwise known as StO_2. Muscle StO_2 reflects the relative contribution of oxygen delivery and oxygen use in local muscle. Current research has shown PAD to contribute to slowed StO_2 kinetics in calf muscle through treadmill exercise testing. This is illustrated through reports of larger StO_2 decreases within the first minute of exercise, quicker times to minimum StO_2, and longer recovery times compared to controls (Gardner et al., 2008; Mesquita et al., 2013; Mohler, Gwen, Gregory, Hao, & Britton, 2006). Three recent studies measuring changes from exercise programs in patients with PAD have used calf muscle StO_2 kinetics as one of their parameters (Baker et al., 2017; Beckitt, Day, Morgan, & Lamont, 2012; Gardner, Parker, Montgomery, & Blevins, 2014). The results have been mixed, but have generally found at least mild improvements in StO_2 kinetics in relation to functional improvements. The largest of the three studies found improvements in time to minimum StO_2 and recovery half time (Gardner et al., 2014). As a result, credence has been given to the theory that improvement in microcirculation could be a mechanism behind the functional improvements from exercise.

Another seemingly unrelated condition which presents in otherwise healthy individuals as they age is sarcopenia. Sarcopenia is the age-associated loss of muscle mass and muscle function (Landi et al., 2013). It is characterized by a decrease in the number of excitable motor units and muscle fiber atrophy (Vandervoort 2002). The loss

of motor units is not linear across all aging and seems to increase in most individuals around 60-70 years of age (Powers et al., 2013). Overall this leads to approximately a 1.4% muscle mass lost per year over 65 (Frontera et al., 2000) and up to a 40% decline in muscle cross sectional area by 80 (Lexell et al., 1988). While not immediately obvious, the potential link between sarcopenia and PAD lies in their underlying pathology. The increase in the loss of motor units and atrophy associated with sarcopenia occurs in the age range an increase in the prevalence PAD is seen. The amount of non-contractile tissue proportional to cross sectional area increases (Vandervoort, 2002) similarly to that seen in PAD. Sarcopenia is associated with similar functional limitations as PAD such as poor endurance, physical inactivity, slow gait speed, and decreased mobility (Roberts et al., 2011; Rockwood, 2005). Finally, recent research around the topic suggests that similar to PAD, sarcopenia is the result of reduced blood flow to working skeletal muscle (Arango-Lopera, Arroyo, Gutierrez-Robledo, Perez-Zepeda, & Cesari, 2013; Bowen, Schuler, & Adams, 2015; Ryall, Schertzer, & Lynch, 2008). Theoretically, this could lead to similar results in StO_2 kinetics seen in patients with PAD during exercise as reduced blood flow would reduce the amount of oxygen delivered to the muscle. However, it is difficult to compare current data surrounding sarcopenia to that of PAD because measures of limitations from sarcopenia rely on different tests. The four-meter walking speed and handgrip strength are the common tests associated with sarcopenia (Cruz-Jentoft et al., 2010), but not used in patients with PAD. Likewise, the Gardner Treadmill protocol is used for patients with PAD, but not those with sarcopenia.

While the effects of ageing on exercise capacity overall has been well researched, there is limited data examining the changes in StO_2 kinetics via near-infrared

spectroscopy throughout ageing (Bhambhani, 2004). Despite the similar pathology, many of these changes in StO_2 kinetics have largely been documented strictly in patients already presenting with PAD. There is a lack of data examining StO_2 kinetics in healthy young and old individuals. Thus, relatively little is known about the effects of aging in healthy individuals on StO_2 kinetics. Collecting data on calf muscle StO_2 kinetics in healthy younger and older individuals could reveal differences caused by age and potential mechanisms underlying sarcopenia. The limited work surrounding this topic has largely been done at exercise levels higher than those capable by patients with PAD and using a variety of testing methods (DeLorey, Paterson, & Kowalchuk, 2007). Thus, collecting data from younger and older subjects performing the same methods used to test patient with PAD would allow for comparisons between the data. The benefits of having these data are that it could allow for greater differentiation between the effects of intermittent claudication on functional capacity in patients with PAD versus potential changes due to aging. It is important to know the effects of ischemia and intermittent claudication on exercise in these patients beyond which is caused by sarcopenia. It could increase the understanding behind the mechanisms of the limited function associated with PAD and allow for guidance on future research to develop better exercise guidelines for improvements.

Objectives

Objective One

The primary objective of this study is to determine changes in calf muscle StO_2 across the lifespan in younger and older healthy individuals. The goal is to measure this

characteristic while performing a treadmill walking test and 6-minute walking distance test commonly used in patients with PAD. The main data points used for this comparison will be minimum StO_2 values during each test and the time it takes for StO_2 to return to baseline, recovery time, after completion. This will allow insight into natural changes in StO_2 as people age due to sarcopenia and not caused by PAD.

Objective Two

The second objective is to use the treadmill data from the primary objective for comparison with similar data from patients with PAD to determine more direct effects of claudication while walking. Minimum StO_2 and recovery time during and after treadmill testing will be compared between patients with PAD and the older healthy controls. The comparison of these data could allow insight into mechanisms of StO_2 alterations from claudication and the overall understanding of PAD. Long term, this could prove useful to help with the diagnosis of PAD and exercise planning for those with PAD.

Objective Three

The third objective is to determine the participants' amount of force exerted when performing a handgrip strength test and their four-meter gait speed to associate with the calf muscle StO_2. Handgrip strength and gait speed are common measurements for determining sarcopenia (Cruz-Jentoft et al., 2010). Relating the changes in StO_2 to the functional measures of sarcopenia could reveal if an association exists. This would provide further evidence to the nature of the relationship between sarcopenia and oxygen delivery and utilization.

CHAPTER TWO

Review of Literature

Background

Peripheral artery disease (PAD) is a very broad term which can include all noncoronary arterial diseases. However, in academic literature PAD has been accepted to usually mean arterial occlusive disease because of arteriosclerosis (Cooke & Chen, 2015). Of note, though, is that many other disorders can cause PAD, such as dysfunction to the intrinsic pathobiology of the vessel wall, thromboangiitis obliterans, and fibromuscular dysplasia, but arteriosclerosis is the most common (Cooke & Chen, 2015; Criqui & Aboyans, 2015; Hiatt, Armstrong, Larson, & Brass, 2015; McDermott, 2015). Under this definition, PAD is the partial or complete obstruction of one or more peripheral arteries resulting in insufficient blood supply to the legs (Criqui & Aboyans, 2015; Hiatt et al., 2015; McDermott, 2015). Once severe enough, the blood supply can no longer meet the oxygen demands of the muscle which can lead to intermittent claudication (IC) and critical limb ischemia (CLI) (Criqui & Aboyans, 2015; Lau, 2011; McDermott, 2015). IC is walking induced muscle pain which is relieved by rest while ischemia is inadequate blood supply to part of the body. For patients with PAD, ischemia refers to the musculature of the lower extremity and particularly their calves.

Risk factors behind PAD have been well research and are well known with smoking, hypertension, dyslipidemia, and diabetes as the consensus top four causes (Criqui & Aboyans, 2015; Fowkes et al., 2013; Joosten, 2012; Lau, 2011). Smoking

carries its own special risk as active smoking is 2 to 3 times more strongly associated with PAD than coronary heart disease and data have shown an elevated risk even after 20 years since cessation (Joosten, 2012). Some studies estimate an increased risk of almost 4 times from current smoking versus nonsmoking (Criqui & Aboyans, 2015). Joosten et al. (2012) collected data from 537 separate cases of PAD and found population attributable factors of 44% for smoking, 41% hypertension, 17% for dyslipidemia and 14% for type II diabetes. As noted by Criqui and Aboyans (2015) in their review, hypertension consistently comes in second right behind smoking as the most prevalent risk factor. However, while ranking as a lower risk factor, diabetes may cause the most severe progressions of PAD. One study comparing diabetic PAD patients to other PAD patients found the diabetic PAD patients were 5 times more likely to have an amputation and had slightly more than 3 times the odds of mortality compared to other PAD patients (Jude, 2001).

The complex pathophysiology behind PAD leads to more than just arteriosclerosis and is associated with several other musculoskeletal dysfunctions. Muscle apoptosis and atrophy, increased fiber type switching, altered myosin heavy-chain expression, and muscle fiber denervation are common changes seen in patients with PAD (Hiatt et al., 2015). Biopsies from patients with PAD have shown approximately 4% of gastrocnemius cells are apoptotic (Mitchell, 2007). Other manifestations include smaller muscle area and higher fat content in the calf muscle along with an association of impaired peripheral nerve function in those with severe PAD (McDermott, 2015).

Importance

Awareness of PAD is important as recent estimates show PAD affecting over 200 million people worldwide, including 8 million men and women in the United States (Fowkes et al., 2013). Rates are generally similar between men and women and begin to rise significantly with age in both around 50-60 years old (Allison et al., 2007; Fowkes et al., 2013). African American males appear to be the most at risk compared to their counterparts with a prevalence more than double non-Hispanic whites, Hispanics, American Indians, and Asian Americans. Similar trends can be found amongst females, although Hispanic females have a similar prevalence to African American females (Allison et al., 2007). By age 70, prevalence is near or greater than 10% for all ethnicities and increases to almost 20% for all ethnicities by age 80 (Allison et al., 2007). PAD has become a global problem as both high income countries and low-income countries are experiencing increasing rates. In the last decade the number of individuals with PAD has increased 28.7% in low income countries and 13.1% in high income countries (Fowkes et al., 2013). PAD is proving to be a growing issue worldwide as new data have shown it affects men and women of all ethnicities in all countries.

Unfortunately, these estimates are also likely to be very conservative and on the low side. Only in the last two decades has research begun to establish that most patients with PAD do not show classic symptoms of IC which was a main parameter used to diagnose PAD (McDermott, 2015). The first study to examine this was in 1985 when Crique et al. performed a noninvasive vascular study with 575 men and women and found 65 to have signs consistent with PAD, but only 6 (9.2%) had classic symptoms of IC (M. Criqui, Fronek, Barrett-Connor, & Gabriel, 1985). Shortly following that study

was another which identified 144 participants with an ankle-brachial index, ABI, less than 0.90, but only 15% of those patients had classic symptoms of IC and 35% reported no exertional leg symptoms at all (Fowkes et al., 1991). Since then, the ABI has become the accepted initial noninvasive choice to screen for PAD. The ABI measures the systolic ankle pressure in the lower extremity and the systolic brachial pressure in the arm and divides the former by the later. A ratio of ≤0.90 is diagnostic of PAD. A drop ≥0.15 in one year is also significant regardless of starting point. An ABI of <0.90 has a 95% sensitivity and 99% specificity for diagnostic purposes (Lau, 2011; Sibley, 2017). More recent studies have continued to show only a small percentage (10-30%) of PAD presenting classic IC symptoms and 70-90% having atypical leg symptoms or being asymptomatic (Lau, 2011). Patients with atypical leg pain or who are asymptomatic are the most likely causes of continued underdiagnoses and undertreatment of PAD even with the ABI in place (Lau, 2011; McDermott, 2015; Sibley, 2017). This underdiagnoses due to lack of symptoms likely occurs because many patients at risk for PAD lead sedentary lifestyles, so they never exercise to the point where IC occurs and thus remain unaware that anything is wrong. There are also several cases reported in retrospect by patients who experienced IC while walking, so slowed their walking pace until they did not experience symptoms. After a while, the slower walking pace becomes their normal walking pace and they forget they ever experienced pain while walking and don't report it during routine health checkups.

The underdiagnoses is particularly troublesome because as PAD becomes more severe, the more prominent other risk factors become. After accounting for other factors, decreasing the vessel radius by 50% leads to a 16-fold reduction in blood flow (Sibley,

2017). This puts the patient at risk for other cardiovascular events. The 5 year rate of nonfatal cardiovascular events, including MI and stroke, for patients with symptomatic PAD is approximately 20% and the 5 year mortality is 15-30% (Lau, 2011). The Cardiovascular Heart Study found an ABI <0.90 to have increased the prevalence of myocardial infarction 2.5x, angina 1.9x, congestive heart failure 3.3x, stroke 3.1x, and transient ischemic attack 2.3x (Newman et al., 1993). These increased risks of cardiovascular events and mortality underscore the need for early detection and treatment of patients with PAD even if they are asymptomatic.

Treatments

Pharmacological treatment of PAD continues to have limited success. Recommendations are still made to use medications when indicated to reduce common risk factors that can lead to PAD, such as statins for diabetes and anti-hypertensives for hypertension, but data is mixed on their effectiveness and very limited in the studies showing modest success (Bonaca & Creager, 2015). Anticoagulants and their ability to reduce acute limb events have been the focus of current research. At a 35 month follow up of The Warfarin Antiplatelet Vascular Evaluation Trial, which randomized 2161 patients with PAD to warfarin or antiplatelet therapy, there was no difference in peripheral severe ischemia between the groups, but a significant increase in life-threatening bleeding with warfarin compared to the antiplatelet group (Anand, 2007). The only two drugs currently approved by the FDA for treatment of PAD in the United States are pentoxifylline and cilostazol (Stevens et al., 2012). Pentoxifylline has been shown to have no to very small effect over a placebo at improving measures related to PAD through several meta-analyses (Bonaca & Creager, 2015; Stevens et al., 2012).

Cilostazol however has shown some promise with three separate meta-analyses finding improvements of 50-67% in pain-free walking distance, translating to an increase of 31.1-42.1 meters before initial claudication (Pande, Hiatt, Zhang, Hittel, & Creager, 2010; Robless, Mikhailidis, & Stansby, 2008; Thompson, Simet, Forbes, & Zhang, 2002). Both of these drugs target the limitations in vasodilation during exercise seen in patients with PAD. They are both vasodilators while cilostazol is also a blood thinner and pentoxifylline is also an anti-inflammatory. Neither drug targets the underlying arteriosclerosis behind most cases of PAD. Another drug, Naftidrofuryl Oxalate, has shown moderate success in treatment of PAD by increasing maximal walking distance by 60% and pain-free walking distance by 49%, but is not currently approved by the FDA for use in the United States (Stevens et al., 2012). Naftidrofuryl Oxalate is also a vasodilator. Several angiogenic and cell therapies have shown preclinical promise which has not translated into clinical results and neither therapy has any FDA approval after two decades of research (Cooke & Losordo, 2015).

Revascularization is a necessary treatment once PAD has progressed to severe enough levels. This treatment is typically considered in patients with PAD who have any of the following three clinical presentations: lifestyle-altering claudication no longer responsive to conservative therapy, critical limb ischemia, or acute limb ischemia (Thukkani & Kinlay, 2015). Surgical procedures, endarterectomy and bypass, used to be the only form of revascularization. Endarterectomy is the direct removal of obstructive plaque from an arterial segment. While largely replaced with endovascular interventions now, femoral endarterectomy remains a common procedure in patients with PAD (Vartanian & Conte, 2015). Surgical bypass can be performed at multiple locations on

patients with PAD depending on the level of the lesion. Regardless of the level, surgical bypass has the best patency rates of any revascularization method with the expected 5-year patency rates for aortofemoral bypass at 80-95%, iliofemoral bypass 80-90%, femorofemoral bypass 55-85%, and axillobifemoral bypass 50-75% (Vartanian & Conte, 2015). Surgical interventions are well documented and known for providing immediate relief to patients with PAD. However, due to the risks inherently involved in undergoing surgery, the procedures are almost exclusively used in patients with severe PAD.

Largely replacing the surgical procedures are newer endovascular treatments including bare-metal stents, drug-eluting stents, and balloon angioplasty (Thukkani & Kinlay, 2015). Patency rates vary widely depending on the level of the lesion and the method used, but overall initial patency is significantly improved and well over 50% in many studies (Thukkani & Kinlay, 2015). The Bypass versus Angioplasty in Severe Ischemia of the Leg (BASIL) is the only clinical trial so far to have randomized patients with CLI to either surgery or balloon angioplasty. Twelve and thirty-six month follow-ups showed equivalent rates of amputation-free survival, but it should be noted that the angioplasty group had significantly lower patency (Adam, 2005). A new trial, the Best Endovascular versus Best Surgical Therapy for Critical Limb Ischemia (BEST-CLI) is just beginning in the U.S. and Canada. The BEST-CLI is the first randomized controlled trial to compare endovascular therapy to open surgery bypass in North America (Menard et al., 2016). This trial will hopefully help provide more in-depth analysis into functional improvements, quality of life, and costs differences between the methods clarifying the role each treatment strategy has within the PAD population. Also, although already widely used elsewhere, the FDA recently approved drug-coated balloons for use in the

U.S. (Thukkani & Kinlay, 2015). Several clinical studies are currently underway involving this newer procedure and more data are needed to compare its efficacy to previously established methods. Overall endovascular procedures have largely replaced many open surgeries due to their initial success and decreased invasiveness (Thukkani & Kinlay, 2015), but they are not without their drawbacks.

Patency rates tend to drop significantly while fracture rates rise within the first couple of year post endovascular procedure. Patency rates have been reported as low as 26.5% in two-year follow-ups on stents and high stent fracture rates (31%) have been associated with drug-eluting stents (Thukkani & Kinlay, 2015). These problems have led to extremely high revascularization rates. A database review of 7,452 patients with PAD who had undergone angioplasty or stenting found approximately 23% had a repeat endovascular procedure by the two year post index date (Meyers J, 2016). Compounding cost for repeat procedures has become a significant issue. In the U.S., mean cost by a two-year follow up were $206,015 for patients who only received angioplasty and $76,834 for stent only patients (Meyers J, 2016). Even the newer drug-coated balloons have not appeared to solve this problem with high revascularization rate increases of 50% between 12 and 24 months (Micari et al., 2013). Unfortunately, once lower extremity amputation becomes the only option for patients with CLI costs increase even more. A simulation model using data from the Centers for Medicare and Medicaid Services reported lower extremity amputations at 73,000 annually with an estimate of lifetime direct healthcare cost at $640,849 per patient (Palli et al., 2016). The high repeat revascularization rates and costs associated with many endovascular procedures have led researchers to look for alternative treatments.

Exercise

Due to the limited success of pharmacological treatments and barriers to endovascular and surgical procedures, exercise has become one of the most popular topics of research surrounding PAD. The principal disability of IC is limited walking ability and exercise performance. Patients with PAD have approximately a 50% reduction in peak exercise performance when compared to an age-matched group (Hiatt, Nawaz, Regensteiner, & Hossack, 1988). Supervised exercise programs have shown tremendous promise in increasing main outcome measures such as pain-free walking distance (PFWD), 6-minute walking distance (6MWD), claudication onset time (COT), and peak walking time (PWT) (Fakhry et al., 2015; Gardner, Parker, & Montgomery, 2016; Murphy et al., 2015; Parmenter, Dieberg, Phipps, & Smart, 2015). Results from supervised exercise have shown 50%-200% improvement in maximal treadmill walking performance (McDermott et al., 2013). This has led supervised exercise to be considered the gold standard for initial, noninvasive treatment of PAD and IC and a Class IA recommendation by the American College of Cardiology and the American Heart Association (Gardner et al., 2016). In two large clinical trials, the ERASE Trial and CLEVER study, supervised exercise has shown similar or greater improvements in primary outcome measures compared with endovascular revascularization (Fakhry et al., 2015; Murphy et al., 2015). At a 6-month follow-up in the CLEVER trial while both stenting and supervised exercise showed significant improvements over current optimal medical care guidelines, PWT was significantly higher for the exercise group than stent group (Murphy et al., 2015). The ERASE trial showed that a combination of revascularization and exercise is most likely the best intervention for most patients with

PAD. The combination group had greater improvements across almost all measures at a one-year follow-up, although it should be noted the exercise only group also showed significant improvements (Fakhry et al., 2015).

Due to the success of supervised exercise, but lack of access for many patients with PAD, new research has investigated home-based exercise programs. In 2011, Gardner et al. completed a prospective, randomized, controlled clinical trial directly comparing a home-based program to a supervised exercise program and a usual care control group. The study found improvements in COT and PWT for both home and supervised exercise, but no significant difference between the two groups meaning that home-based exercise may be as effective as supervised exercise (Gardner, Parker, Montgomery, Scott, & Blevins, 2011). Another randomized trial split 194 patients with PAD into a home-based exercise group and control group and found after 6 months the home-based exercise improved their max treadmill walking time and 6MWD by 53.5 meters (a 50-meter improvement is considered large and significant) as primary outcomes (McDermott et al., 2013). It should be noted, though, that almost three quarters (72.2%) of the participants in the McDermott study had only mild PAD and no classic symptoms of IC, so it is hard to generalize these results to patients with more severe PAD. Both of these studies also had surprisingly high adherence rates of 82.5% and 84% respectively suggesting that patients with PAD may be more willing to participate in home-based exercise programs if given the opportunity. Due to most other research on home-based exercise only coming from Level B evidence in nonrandomized trials, unsupervised home-based exercise currently only has a Class IIb recommendation by American College of Cardiology and the American Heart Association (Gardner et al., 2011). This

is also likely due to the one other randomized trial on home-based exercise showing no difference between control and treatment groups, but all participants had diabetes along with PAD (Collins et al., 2011). More research is needed to understand why this one trial was not successful.

Limits to Exercise

Despite its success, exercise as a treatment for PAD still has many limits. Even though exercise has repeatedly shown to improve physical aspects of PAD and IC, multiple studies show little to no improvement in quality of life (QoL) as self-reported by the participants (Collins et al., 2011; Fakhry et al., 2015; Murphy et al., 2015; Parmenter et al., 2015). Opposite to exercise, endovascular revascularization procedures show consistent and significant improvements in QoL of patients who undergo treatment (Fakhry et al., 2015; Murphy et al., 2015). It is still unclear as to why the physical improvements from exercise do not correlate to improvements in QoL as do revascularization procedures. It may be because patients undergoing revascularization procedures tend to have later stages of PAD compared to those in exercise protocols. Thus, they were more limited prior to treatment allowing more room for potential significant improvements to their QoL. Perhaps it is time to take a more qualitative approach to studying patients with PAD to help understand why QoL improvements are not showing up for exercise. Another major limitation to exercise is access to programs for patients with PAD. Only a few programs exist across the nation, mostly in research centers, allowing feasible access to a very small portion of patients to regularly attend (Gardner et al., 2011). Supervised exercise is also not currently reimbursed by any health policies further limiting access. Surprisingly, there is also no set protocol for exercise

rehab programs. Testing standards have been set with the Gardner Graded Maximal Treadmill Test, but nothing formal has been established to follow for exercise protocol. Most studies on supervised exercise used walking until a certain degree of claudication pain. Home-based exercise is even less well established with some studies using time, while others use distance or steps taken as guidelines for exercise protocol. Different routines need to be tested and well documented to establish set protocols for future studies to use.

Arguably an even larger problem surrounding exercise is our lack of understanding of the mechanisms exercise alters or improves. There is consensus in the literature that exercise has many benefits for patients with PAD, but there is no clear understanding as to why. It is known that patients with PAD have significant abnormalities in endothelium-dependent vasodilation during exercise (Liao et al., 1991). This is a main cause of decreased blood flow to the lower extremities during exercise. Thus, patients with PAD experience ischemia of their calves when metabolic demand exceeds the available oxygen supply during exercise followed by muscle reperfusion during rest when blood supply increases enough to meet oxygen requirements again (McDermott, 2015). This ischemia-reperfusion cycle produces reactive oxygen species that damage the muscle fibers, impair mitochondrial function and promote apoptosis (Pipinos et al., 2006; Pipinos et al., 2008; Weiss et al., 2013). Exercise training meanwhile is known to improve flow-mediated dilation (Brendle, Joseph, Corretti, Gardner, & Katzel, 2001). This means that exercise training allows for greater blood flow to the lower extremities which can help decrease the deficit between O_2 supply and demand. Thus, the ischemia-reperfusion cycle should theoretically not be as strong. This

is just one of many mechanisms proposed around limb ischemia and exercise (Hiatt et al., 2015; McDermott, 2015). However, while improvements in blood flow are significant, they do not increase to levels seen in healthy adults. It appears many of the causes of leg claudication and impaired walking capacity center around an imbalance of O_2 supply and demand. Understanding the exact cause of claudication during exercise may help reveal the mechanisms underlying the benefits of exercise which are currently largely unknown.

The Role of Hemoglobin Oxygen Saturation (StO_2) in Exercise

Muscle StO_2 reflects the relative contributions of oxygen delivery and oxygen use in local muscle. Thus, understanding the differences in dynamics of the StO_2 response between rest and exercise could provide valuable understanding into the causes of exercise limitations in patients with PAD. Current research has built a base knowledge of the StO_2 response in patients with PAD using treadmill exercise testing. This has allowed for insight into how StO_2 changes, but not why it changes. Below outlines the current knowledge of StO_2 changes during one exercise session.

Preliminary data show that calf muscle StO_2 decreases more in patients with IC during exercise than in controls (Comerota, Throm, Kelly, & Jaff, 2003; Kemp et al., 2001; Kooijman, Hopman, Colier, Adam van der Vliet, & Oesebrg, 1997; Mesquita et al., 2013; Mohler, Gwen, Gregory, Hao, & Britton, 2006). The degree of oxygen desaturation is also related with the severity of the disease and claudication (Komiyama, Onozuka, Miyata, & Shigematsu, 2002; Manfredini et al., 2009). This decrease can be as much as a 72% from resting levels and generally occurs within the first minute of exercise (Afaq et al., 2007; Afaq et al., 2008; Gardner et al., 2008). There is mild variability across data in the time it takes to reach minimal StO_2 ranging from as short as

3.4 minutes to as long as 7.65 minutes, a difference of 4.25 minutes (Gardner et al., 2015; Gardner et al., 2008). However, all times from the 8 studies which reported on this measure were within this range and the majority under 5 minutes regardless of the subset population of patients with PAD studied (Afaq et al., 2007; Afaq et al., 2008; Bauer, Brass, & Hiatt, 2004; Gardner, Parker, Montgomery, & Blevins, 2014; Gardner et al., 2012; Gardner et al., 2015; Gardner et al., 2008; Watanabe et al., 2004). In general, patients with PAD regardless of compounding variables such as diabetes, hypercholesterolemia, or type of leg pain will reach minimal StO_2 within 5 minutes of beginning exercise. Several studies have also shown the sooner the patient reaches this minimum StO_2, the sooner they will reach initial claudication pain and absolute claudication pain (Afaq et al., 2007; Afaq et al., 2008). This has led to a strong association between StO_2 and COT and PWT (Gardner et al., 2009; Gardner et al., 2008). While factors such as smoking (Afaq et al., 2007), hypercholesterolemia (Afaq et al., 2008), and diabetes (Mohler et al., 2006) do not appear to affect the timing of StO_2 measures, they do cause significantly lower levels overall of StO_2 at all time points during exercise. Interestingly, minimum StO_2 and time to minimum StO_2 does not correlate to ABI at rest or post exercise (Khurana et al., 2012). Even though ABI and StO_2 are both used to asses PAD, minimum StO_2 and time to minimum StO_2 more accurately reflect the severity of PAD because ABI is a macrovascular measure which is an imprecise measure of claudication (Khurana et al., 2012). This may explain why a correlation does not appear.

These same data sets have also shown a longer recovery time in patients with PAD (Comerota et al., 2003; Gardner et al., 2008; Kemp et al., 2001; Kooijman et al.,

1997; McCully, Halber, & Posner, 1994; Mesquita et al., 2013; Mohler et al., 2006). This could be because of the association between relative blood flow and StO_2 levels in the extremities of patients with PAD (Mesquita et al., 2013) as the limited blood flow delays StO_2 recovery. Half recovery and full recovery time for calf StO_2 were similar across many of the studies. Times ranged from 129-174 seconds for half recovery and 225-311 seconds for full recovery time (Gardner et al., 2014; Gardner et al., 2009; Gardner et al., 2015; Gardner et al., 2008). Similar to time to minimum StO_2 during exercise, regardless of factors such as type or severity of leg pain, recovery time is roughly the same. Patients were also able to increase to 79%-82% maximal saturation after completion of exercise (Gardner et al., 2015; Gardner et al., 2008). One study has discussed qualitative patterns of StO_2 response to exercise in patients with PAD (Bauer et al., 2004). The study involved 6 PAD subjects and 6 healthy controls who completed three 6-minute treadmill sessions at low (60% of measured peak exercise work rate), medium (80%), and high (100%) constant work rates. The authors concluded the initial StO_2 response had similar qualitative patterns between both groups at all work rates. Both groups showed an initial increase in StO_2 followed by a sharp decline which plateaued below baseline after the start of exercise. Three healthy and one PAD patient had no drop below baseline during the low work rate. Although qualitatively similar, interestingly, StO_2 desaturation was quicker in control subjects. Control subjects plateaued below baseline within 40 seconds of starting exercise while patients with PAD did not reach this point until approximately 100 seconds in to exercise (Bauer et al., 2004). The magnitudes of the decrease were similar to other studies as patients with PAD had greater desaturation overall compared to the healthy controls.

Three recent studies have been completed examining the impact exercise programs have on StO_2 kinetics (Baker et al., 2017; Beckitt, Day, Morgan, & Lamont, 2012; Gardner et al., 2014). In the two which reported on resting StO_2 (Baker et al., 2017; Beckitt et al., 2012), no changes were noted before or after the exercise programs. This is not surprising because resting blood flow is not usually altered or different at rest between patients with PAD and healthy controls. Results differed on the effect of exercise programs on oxygen desaturation. One study (Gardner et al., 2014) found the time to minimum StO_2 to be significantly increased after home-based and supervised exercise programs while another (Beckitt et al., 2012) found no reduction in oxygen desaturation kinetics during exercise. Two of the three (Beckitt et al., 2012; Gardner et al., 2014) showed improvements in recovery half time of hemoglobin/myoglobin desaturation compared to controls while the other (Baker et al., 2017) reported no improvement in recovery half time. All three studies used an exercise program spanning three months and had at least one group completing supervised exercise, so other factors would need to be examined to try to explain the differences in results for recovery half time. Testing differences could explain the results as two used Gardner protocol to take measurements (Baker et al., 2017; Gardner et al., 2014) while the other used their own treadmill protocol (Beckitt et al., 2012). The different protocols tested patients at different intensities, so it is possible that the improvements seen manifests only at certain exercise intensities. Also, while they all used some form of NIRS to collect data, the study which did not find significant decreases in recovery half time had customized their sensors (Baker et al., 2017). The largest of the three trials involving 180 patients with PAD and using home and supervised programs showed the potential benefits of exercise

in improving microcirculation to the lower extremities during exercise by means of improved time to minimum calf StO_2 and recovery half time (Gardner et al., 2014).

From these studies, several common themes appeared: 1) PAD likely causes a rapid decline in StO_2 within the first minute of exercise before the desaturation rate slows (Afaq et al., 2007; Afaq et al., 2008; Mohler et al., 2006), 2) the faster a patient reaches minimal calf muscle StO_2 the sooner claudication begins and the shorter the max walking distance (Afaq et al., 2007; Afaq et al., 2008; Gardner et al., 2009; Gardner et al., 2008), 3) StO_2 measures are able to recover within minutes of stopping exercise (Afaq et al., 2007; Afaq et al., 2008; Gardner et al., 2014; Gardner et al., 2009; Gardner et al., 2015; Gardner et al., 2008), 4) external factors such as diabetes, smoking, and hypercholesterolemia further lower StO_2 levels (Afaq et al., 2007; Afaq et al., 2008; Mohler et al., 2006).

While all this is good information to know, it is only a starting point in trying to understand the mechanisms behind improvements in oxygen supply and demand during exercise for patients with PAD. All these data have been collected from patients with PAD, but it remains unclear how to apply this knowledge to help solve the problem. Now that the data is documented and available, the question remains on how it can help improve treatment of patients with PAD. This is largely due to minimal concurrent research on StO_2 measures in healthy subjects. Of the 20 articles reviewed, only 9 used healthy control groups with the most recent study being in 2013 (Bauer, Brass, Barstow, & Hiatt, 2007; Bauer et al., 2004; Comerota et al., 2003; Kemp et al., 2001; Kooijman et al., 1997; Manfredini et al., 2009; McCully et al., 1994; Mesquita et al., 2013; Mohler et al., 2006). Of these 9 studies, 3 had healthy control groups of less than 10 participants

(Bauer et al., 2007; Bauer et al., 2004; Kemp et al., 2001). Putting this into perspective, data have been collected on only 183 healthy control subjects compared to 1,128 patients with varying severities of PAD when it comes to StO_2 measures. The literature has been documenting patterns and changes in StO_2 in patients with PAD, but not documenting anything to compare it to and draw conclusions from. This becomes especially difficult due to the older nature of the studies that used healthy controls. The Gardner Graded Treadmill Protocol has become the standard for exercising testing and was used in 11 of the 20 studies reviewed (Afaq et al., 2007; Afaq et al., 2008; Bauer et al., 2004; Beckitt et al., 2012; Comerota et al., 2003; Gardner et al., 2009; Gardner et al., 2012; Gardner et al., 2015; Gardner et al., 2008; Khurana et al., 2012; McCully et al., 1994). However, only 4 of the 9 studies using healthy controls used this protocol (Bauer et al., 2004; Comerota et al., 2003; McCully et al., 1994; Mesquita et al., 2013). The others either used their own treadmill protocol (Kooijman et al., 1997; Manfredini et al., 2009), did not state the protocol they used (Mohler et al., 2006), or tested plantarflexion max voluntary contraction rather than treadmill exercise testing (Bauer et al., 2007; Kemp et al., 2001). This makes it difficult to generalize any of their results to the rest of the subjects who were tested using the Gardner Graded Treadmill Protocol. One study not previously mentioned because it only reported intraclass correlation constants and not data directly on oxygen saturation suggested adding yet another test into the fold to determine capabilities of patients with PAD (Pedersen, Baekgaard, & Quistorff, 2015). The authors compared a plantarflexion test to a treadmill protocol and suggested it could be used for those with limited functionality. However, the study only had 9 patients with PAD, two healthy controls, and did not use Gardner Graded Treadmill Protocol making it hard to

draw comparisons to the most well-known methods. Now that there is an understanding of the pattern of StO_2 during exercise in patients with PAD, research should focus on exploring how StO_2 kinetics differ from healthy subjects under the same conditions. Then, it may become clearer not only that exercise can improve claudication symptoms in patients with PAD, but also help to understand why it does.

Some research has been completed looking at StO_2 kinetics in healthy individuals, but not using the same tests making them poor comparisons. A few interesting notes can be taken from a select few studies looking at StO_2 in healthy populations though. One study measured calf StO_2 kinetics in 6 healthy females following a plantarflexion protocol similar to ones used in various PAD research (Quaresima et al., 2001). They found that StO_2 reached its minimal value within the first minute of exercise and was followed by StO_2 increases and partial recovery before the end of the exercise (Quaresima et al., 2001). Another study found this same pattern of partial re-saturation in 7 untrained males during running on a treadmill (Quaresima, Komiyama, & Ferrari, 2002). These show the ability in healthy individuals to partially recover while still completing exercise, which is not seen in patients with PAD. In patients with PAD, oxygen desaturation continues even after the initial drop during exercising testing and they usually do not show any signs of recovery until the complete cessation of exercise. Recently, a small study containing 7 subjects found less StO_2 desaturation during high pace running on a treadmill than slow pace running (Steimers et al., 2016). This is opposite to common thought as high intensity exercise generally correlates to greater oxygen deficits and thus greater StO_2 desaturation. The results can be explained by differences in mechanical efficiency. The authors concluded that the slower pace led to a

lower stride frequency, longer stride time, and increased contact which could have increased the metabolic cost of running at the slower pace compared to faster pace (Steimers et al., 2016). This could translate into a wide range of results when testing healthy individuals using PAD tests and protocols. The Gardner Protocol maintains a steady 2 mph walking pace throughout the entire test which could prove awkward to maintain for those whose normal walking speed is faster. Thus, it is plausible to see unexpected results in healthy individuals if their mechanics must change to accommodate walking at 2 mph. These studies in healthy individuals can be used to raise potential questions that need answering within PAD research, but it must be noted they all had very small sample sizes and none looked at the data qualitatively

Using Near-Infrared Spectroscopy (NIRS) to Measure StO_2

Near-infrared spectroscopy is the process of projecting near-infrared light (650nm-1000nm) into tissue to measure relative changes to oxy and deoxy-hemoglobin based on the amount of light reflected back to a detector (Jones, Chiesa, Chaturvedi, & Hughes, 2016). The technology has long been considered a versatile, non-invasive and easily applied method to provide measures of tissue oxygenation (Boushel et al., 2001). 2014 marked the 20th year the non-invasive technology, functional NIRS, has been in use to measure hemoglobin concentrations (Boas, Elwell, Ferrari, & Taga, 2014; Scholkmann et al., 2014). The technology is well researched and has been covered multiple times in several reviews throughout the years. More recent reviews have summed data from early reviews and covered advances in the technology (Boas et al., 2014; Jones et al., 2016; Scholkmann et al., 2014). NIRS has been validated as a tool for evaluating StO_2 (Bhambhani, 2004). As such, it has been used with various conditions such as primary

valvular insufficiency (Yamaki et al., 2013), critically ill patients (Mesquida, Gruartmoner, & Espinal, 2013), hemodialysis (De Blasi et al., 2009), PAD (Vardi & Nini, 2008), and heart failure (Fu et al., 2013). In healthy populations, NIRS has been used primarily to examine physiology changes during athletic performances such as aerobic exercise (Bhambhani, 2004) and resistance exercise (Pereira, Gomes, & Bhambhani, 2007), and wearing compression garments (Coza, Dunn, Anderson, & Nigg, 2012). In a systematic review of using NIRS to evaluate PAD, no adverse events were reported and the authors noted that while no gold standard exists for the method, the technique is promising in its ability to reflect tissue dynamics (Vardi & Nini, 2008). One study reported a 88% sensitivity and 81% specificity for determining PAD using NIRS and a full recovery cut-off of 165 seconds (Comerota et al., 2003).

Despite its widespread use, NIRS is not without its limitations. It cannot differentiate between the contributions of hemoglobin and myoglobin since the absorbency of near-infrared light of the two chromophores overlap (Bhambhani, 2004). Thus, there will always be some error when using NIRS to calculate StO_2 since myoglobin is bound to contribute at some level, but is often thought to be negligible (Wolf et al., 2007). This is not without contention, though, as many studies have reported very high contributions from myoglobin (Jones et al., 2016). Skin pigmentation can also alter NIRS readings and may require applying a physiological calibration to allow inter-subject comparisons (Jones et al., 2016). This is because light in the near-infrared range is also absorbed by melanin in the skin and cytochrome. Adipose tissue also changes the absorbency measurements of NIRS (Bhambhani, 2004; Jones et al., 2016). Thicker adipose tissue leads to greater scattering of the near-infrared light and thus less light

reaching active muscle. This makes the measurement site and important decision for accurate readings. It has been suggested to use the gastrocnemius for NIRS measurements of lower limbs because the subcutaneous adipose tissue rarely exceeds 1-cm at this location (Jones et al., 2016). Finally, the heterogeneity of blood flow in muscle makes consistent probe placement vital. A study mapped hemoglobin oxygen saturation at 22 locations in 30 legs of 15 healthy subjects and found highly significant differences comparing proximal and distal regions and medial and lateral regions (Wolf et al., 2007). Readings can vary widely in healthy subjects from minor changes in placements. However, these difference have been found to be less pronounced in patients with PAD (Wolf et al., 2003). Many of these limitations can now be accounted for and largely removed from error with modern equations such as the light propagation model and Beer-Lambert law and strong study methodology controlling for sensor placement and accounting for adipose thickness.

Conclusion

Research for PAD has come a long way over the years with new drugs and treatment methods continuously being tested. For now, exercise remains the most cost effective treatment for patients with mild to moderate PAD (Bermingham et al., 2013). As demonstrated throughout this review, research has proven exercise to be effective in improving symptoms of PAD and IC. Now research must begin to discover the mechanisms behind the benefits of exercise, much like is already being done with drug therapy research. A good place to start is with StO_2 measurements during treadmill testing with healthy populations. Comparing these data to data from patients with PAD could help form better exercise protocols as there is currently no established protocol.

Much of the data on patients with PAD is already documented and now needs a healthy comparison to help form and guide the next step in PAD exercise research.

CHAPTER THREE

Methodology

Subjects

Thirty-one young, middle-aged, and elderly male and female participants were recruited from the Central Texas area. The participants were recruited via word of mouth and generally considered healthy overall by the study administrators and recruiters. After being informed of the nature of the study and allowed the opportunity to read consent form, all participants provided written consent. The full consent form can be found in Appendix A. Screening was then performed via a Healthy History Questionnaire which was reviewed by the study's administrators for eligibility. The Health History Questionnaire can be found in Appendix B. The study was approved by the Baylor University Institutional Review Board (IRB #1081326). Data from these participants were sorted into younger and older samples for analysis and used for all objectives. Data from participants under 40 years old were classified as younger and data from participants aged 40 or above were considered older.

Additionally, 32 subjects with peripheral artery disease, PAD, were recruited, screened, and tested via means outlined in IRB 160390 under the direction of Dr. William Bohannon and Dr. Panagiotis Koutakis at Baylor Scott and White Medical Center in Temple, Texas. These participants only completed the treadmill protocol and did not complete a 6-minute walking distance, 4-meter walking speed, or handgrip strength test. Data from these participants were only used for analysis in the second objective

comparing treadmill testing results. Calf muscle StO_2 data were received for only some of the subjects from the administrators for use within the current study. Subjects tested under this IRB had both legs monitored during the treadmill test and data were collected for both legs. Data from the worst leg of each subject were kept by the original study's administrators and only unused data from the better leg of each subject with PAD was sent to be used for analysis within this study. Additionally, information about the current stage of PAD or condition of the leg was not included in the received data.

Inclusion and Exclusion Criteria

All volunteers were screened and only included those with the following characteristics: 1) 19 to 70 years of age; 2) no documented cardiovascular or metabolic disease; 3) no signs or symptoms of latent heart disease; and 4) no signs of musculoskeletal disease that can limit the participant's ability to walk. Inclusion criteria for subjects with PAD can be found under IRB 160390 through Baylor Scott and White Medical Center in Temple, Texas.

After screening, volunteers were excluded and not allowed to participate if they reported or exhibited any of the following: 1) known cardiovascular or metabolic disease; 2) signs or symptoms of cardiometabolic disease; 3) any musculoskeletal condition or disorder that limited their ability to walk; and 4) women who are pregnant or breast feeding. Exclusion criteria for subjects with PAD can be found under IRB 160390 through Baylor Scott and White Medical Center in Temple, Texas. No subjects were fully excluded from this study. Individual data points which were excluded during analysis will be covered in the results.

Equipment

Similar to other studies examining oxygen saturation in tissue, a NIRS device was used for data collection. The Moxy Monitor (MM) (Fortiori Design LLC, Hutchinson, MN) is a relatively new NIRS monitoring device release in 2013 (Figure 3.1). Measuring only 2.40"x1.72"x0.82" and weighing 40 grams, the MM is designed to be portable for use outside a traditional lab setting. Subsequently, several studies have already used the device to collect data on subjects competing in their natural environments such as trail running (Born, Stoggl, Swaren, & Bjorklund, 2017), rock climbing (Balas et al., 2016; Kodejška, Michailov, & Baláš, 2016), and skiing (Nyback et al., 2017). The MM uses four separate LEDs as light sources, covering wavelengths from 630-850 nm, which it sends into the tissue at one location. The intensity of the light is then recorded at two separate detectors 12.5 and 25 mm from the emitter. Since light does not travel straight through tissue and scatters in all directions, Moxy has developed a proprietary technique to calibrate the monitor based on a mathematical model of light propagation through tissue (Fortiori Design, 2018b). This algorithm combines a tissue light propagation model and the Beer-Lambert law to determine the light absorbed at various wavelengths and measure the ratio of the oxyhemoglobin concentration to the total hemoglobin concentration (Fortiori Design, 2018b). Each device computes these calculations onboard and reports it as the percentage of hemoglobin that is carrying oxygen. This percentage is more commonly known and referred to as muscle oxygen saturation, StO_2. The use of a digital filter to reject pulsating signals, four wavelengths, and two detectors also allow the MM to measure StO_2 levels in capillaries rather than arteries (Fortiori Design, 2018b). This is significant because measuring oxygenation levels in arteries

would not reveal differences as arterial blood oxygenation remains constant even during exercise, similar to the use of a pulse oximeter on a finger. Oxygenation levels must be measured in capillaries or veins to more accurately reflect oxygen delivery and use.

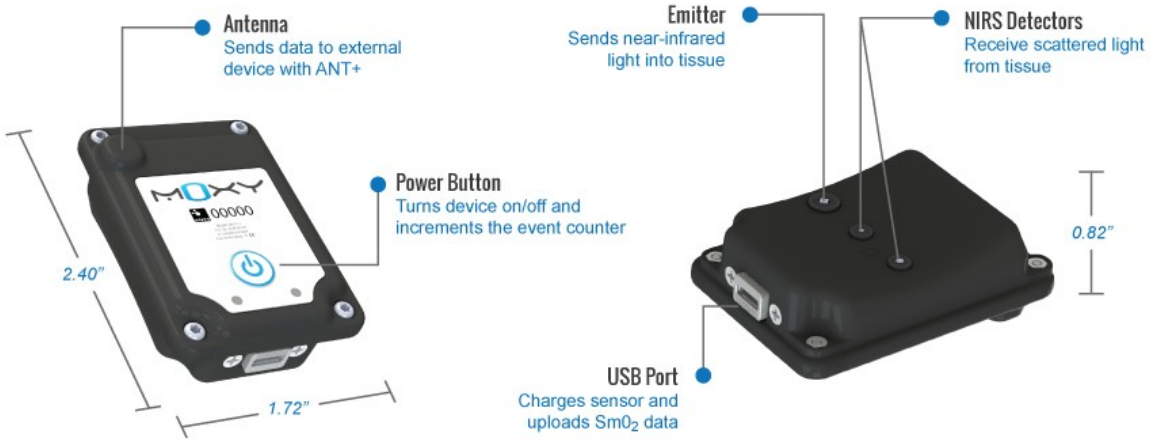

Figure 3.1. Specifications of the Moxy Monitor (Fortiori Design, 2018a)

Since its release in 2013, the MM has been employed across a variety of studies. Most importantly, the validity and reliability of the device were recently tested during incremental cycling exercise (Crum, O'Connor, Van Loo, Valckx, & Stannard, 2017). A strong correlation between trials was observed in all participants through Spearman's Order-Rank Coefficient (0.834-0.980) and Intraclass Correlation (0.773-0.922). The results showed a moderate to high reliability at low to moderate intensities, but less so as at higher intensities (Crum et al., 2017). The reliability of the MM for the tests involved in this study could therefore be considered high as all testing was performed at low intensities. However, these data were taken from cyclists, whereas this study used walking for all tests, so the transferability may be limited. In a study using a more similar method, the MM proved to be a more accurate measure of running intensity in

hilly terrain than heart rate (Born et al., 2017). The device could provide a tissue-saturation index which reflected alterations in VO_2 quicker and more accurately than heart rate throughout a trail run. The device has been used in trials examining ischemic preconditioning which involves muscles reaching similar ischemic conditions present in PAD (Zinner, Born, & Sperlich, 2017). This study used the device to record the minimum and maximum StO_2 during three sets of five-minute blood flow restrictions as well as the reperfusion time. Most importantly, the MM has recently been used in a study measuring calf muscle oxygen extraction during plantarflexion in patients with PAD (Luck et al., 2017). The device was attached to the medial gastrocnemius of each participant and used to record StO_2 levels during fatiguing plantarflexion exercise. The authors found greater reductions in oxygen saturation in the patients with PAD compared to age-matched healthy subjects along with the StO_2 drop preceding the rise in blood pressure (Luck et al., 2017). The MM has proven to be a reliable NIRS monitor in a variety of situations with a specialization for use during activity, including those similar to conditions outlined in this study.

Data Collection

Once volunteers provided written consent (Appendix A) and were deemed eligible from the HHQ (Appendix B), they were enrolled as participants in the study. Each participant was asked to wear comfortable clothing for mild exercise tasks which also provided access to their calves. A MM was attached to equivalent locations on both calves of the participant. The MMs were secured via Cover-Roll tape strips designed for use with a MM purchased through Fortiori Design LLC. Powerflex tape was wrapped around the device and participant's calf to further secure the device and limit outside light

interference. Once attached, each MM was turned on and connected to a Samsung Galaxy Tablet (Samsung, Seoul, South Korea) for data tracking throughout the study. After a secure connection was established, the participant sat quietly for 5 minutes to establish a baseline StO_2.

Following establishment of a baseline, the participant was asked to perform a 4-meter gait speed test. The participant lined up with their toes behind a mark on the floor and upon verbal command walked their normal walking pace through a second mark on the floor 6 meters away. The time it took to cover the first 4 meters was recorded. The participant was then asked to line up at the second and the test was repeated walking back to their chair. The fasted time was kept and recorded in cm/sec as the participant's normal walking speed on the Functional Test Report Form. A blank copy of this reporting form can be found in Appendix C. Once seated again, health related quality of life was assessed via a Quality of Life Questionnaire (SF-36 Health Survey). The SF-36 assesses eight health domains: physical function, limitation due to physical health, limitation due to emotional problems, energy, mental health, bodily pain, general health, and social function (Myers, 2007). Extensive examination of SF-36 has been done in several healthy and clinical populations across all ages of life. Internal consistency and clinical validity have consistently been high, satisfying rigorous psychometric criteria in distinguishing between distinct populations (Garratt, Ruta, Abdalla, Buckingham, & Russell, 1993; Jenkinson, Coulter, & Wright, 1993). The full survey can be found in Appendix B.

The treadmill test was then explained to the participant. The test followed the Gardner Graded Treadmill Protocol (Gardner, Skinner, Cantwell, & Smith, 1991). The

test consisted of walking at 2-mph on the treadmill starting at 0% grade and every two minutes increasing the grade 2%. Since being established in 1991, the Gardner Graded Treadmill Protocol has been a widely used and accepted standard for exercise testing in patients with PAD (Afaq et al., 2007; Afaq et al., 2008; Bauer et al., 2004; Beckitt et al., 2012; Comerota, et al., 2003; Khurana et al., 2012; McCully et al., 1994). After the participant acknowledged understanding, they stood up and stepped onto an X7i Interactive Incline Trainer Treadmill model NTL14215.2 (NordicTrack, Logan, Utah). The test began as soon as the participant stepped on the treadmill. The Gardner protocol was followed until the participant either felt discomfort or the 10-minute point was reached. All healthy participants completed the full 10 minutes with no complaints of discomfort. After the treadmill test the participant returned to sitting and sat quietly resting for 15 minutes.

At the end of the rest period, the participant performed a handgrip strength test. The dominant hand of the participant was used. The participant held their arm straight out in 90-degree shoulder flexion and squeezed a handgrip dynamometer (Sammons Preston Rolyan, Bolingbrook, IL) for three seconds (Roberts et al. 2011). The maximum force registered on the dynamometer over the three seconds was recorded. After a short rest, the test was performed a second time. The highest value between the two tests was recorded on the Functional Test Report Form (Appendix C).

Once the handgrip strength was recorded, the six-minute walking test was explained to the participant. The test was performed in the hallway outside the lab where 100 feet had been marked on each end. The participant walked back and forth at their normal walking pace for six minutes and every 100 feet were marked. The participants

were given cues of time remaining every two minutes and a five second countdown at the end. At the conclusion of the test, the participant remained standing in place and the distance between them and the last mark was measured and added to the total distance. The total distance in feet was recorded on the Functional Test Report Form (Appendix C). The participant then returned to sitting and rested for ten minutes with the MMs still collecting data. After the ten minutes, the MMs were removed and the testing was concluded.

Data Extraction

Data were downloaded and cleared off each MM after every participant. The data were stored in separate excel documents for each leg. After the data was graphed, each leg was analyzed individually for every participant. The baseline StO_2, maximum treadmill StO_2 value, minimum treadmill StO_2 value, treadmill recovery time, maximum six-minute walk StO_2 value, minimum six-minute walk StO_2 value, and six-minute recovery time were all manually extracted from the graphs and confirmed with the raw data sets. The recovery times were calculated from the time the participant finished the test and sat down until StO_2 returned to baseline. If the participant ended the test with values higher than baseline then a recovery time of zero was recorded. These data points were then compiled into a separate excel document also containing the handgrip strength, four-meter walking speed, and six-minute walking distance from the Functional Test Report Form (Appendix C) for each participant.

Data Analysis

Datum for each leg of a participant was considered an independent point for analysis. Data distribution for each group was checked for normality and the Kolmogorov-Smirnov test was done to determine if the data were parametric or non-parametric. If a sample size was less than ten, a non-parametric test was run regardless of data distribution. Comparison of minimum treadmill value and treadmill recovery time between the three groups (PAD, young healthy, and older healthy) was done by the one-way analysis of variance (ANOVA) followed by Tukey's HSD test for post-hoc analysis or by the Kruskal Wallis Test, as appropriate. Further, differences between young healthy and older healthy subjects for maximum treadmill value, maximum 6-minute walk value, minimum 6-minute walk value, 6-minute walk recovery time, handgrip strength, 4 m walk speed, and 6-minute walk distance were analyzed by means of the independent *t* test or Mann-Whitney U test, as appropriate. Data were analyzed using SPSS version 24 (SPSS Inc., Chicago, IL). The level of significance was set at 0.05.

CHAPTER FOUR

Results

Data from a total of 19 PAD limbs (32 subjects) and 60 healthy limbs (31 subjects) were included in final analyses. Healthy limbs included 47 limbs of young subjects, and 13 limbs from older subjects (29 female limbs and 31 male limbs). Total limb count for subjects with PAD does not match the number of subjects because only partial data was received from the administrators of the IRB from which their data were collected. Three limbs were excluded from final analyses. One limb from a younger subject was excluded because of sensor malfunctions resulting in missing data during the tests. One limb from an older subject was excluded because of a history and current signs of vascular disease. The other limb had no history and showed no signs or symptoms of vascular disease so was included in the healthy older sample. One limb from data received on subjects with PAD was excluded because it fell above the third quartile plus 1.5 times the interquartile range which was the used definition of statistical outlier within this study. The average age for the older subjects was 58.15 (11.92) years and the average age for the younger subjects was 24.70 (5.23) years. Subject demographics are listed in Table 4.1.

There was a significant difference in minimum treadmill value between groups ($p<0.0001$) (Figure 4.1). Post-hoc analysis revealed that all groups differed significantly for minimum treadmill value (Young vs Older $p=0.01$, 95% CI (2.45,21.14); Young vs PAD $p<0.0001$, 95% CI (24.31,40.53); Older vs PAD $p<0.0001$, 95% CI (9.89,31.36)). There was also a significant difference between groups for treadmill recovery time

($p<0.0001$) (Figure 4.2). Table 4.2 displays the descriptive statistics for the variables of minimum treadmill value and treadmill recovery time for the three groups.

There were no significant differences in baseline value, maximum treadmill value, maximum 6MW value, minimum 6MW value, handgrip strength, and 6MW distance between young and older subjects. However, 6MW recovery time was significantly shorter in young vs older subjects ($p=0.004$) (Figure 4.3), and younger subjects had a significantly faster 4m walk speed than older subjects ($p=0.016$) (Figure 4.4). Table 4.3 lists the descriptive statistics for the remaining variables assessed for young and older healthy subjects.

Table 4.1. Subject Demographics

Group	Healthy Young	Healthy Older	PAD
Age, years	24.7 ± 5.2	58.2 ± 11.9	61.6 ± 5.2
Male (%)	11 (45.83)	5 (71.43)	30 (93.75)
Female (%)	13 (54.17)	2 (28.57)	2 (6.25)
Risk factors (%)			
Hypertension	0 (0)	2 (28.57)	28 (87.5)
Diabetes mellitus	0 (0)	0 (0)	12 (37.5)

Table 4.2. Descriptive statistics for minimum treadmill value and treadmill recovery time

Variable	Group	Mean	Std. Error
Min. Treadmill Value (%)	Young (n=47)	45.24	1.96
	Older (n=13)	33.31	3.55
	PAD (n=19)	12.56	2.49
Treadmill Recovery (min)	Young	0.132	0.018
	Older	0.626	0.179
	PAD	2.587	0.483

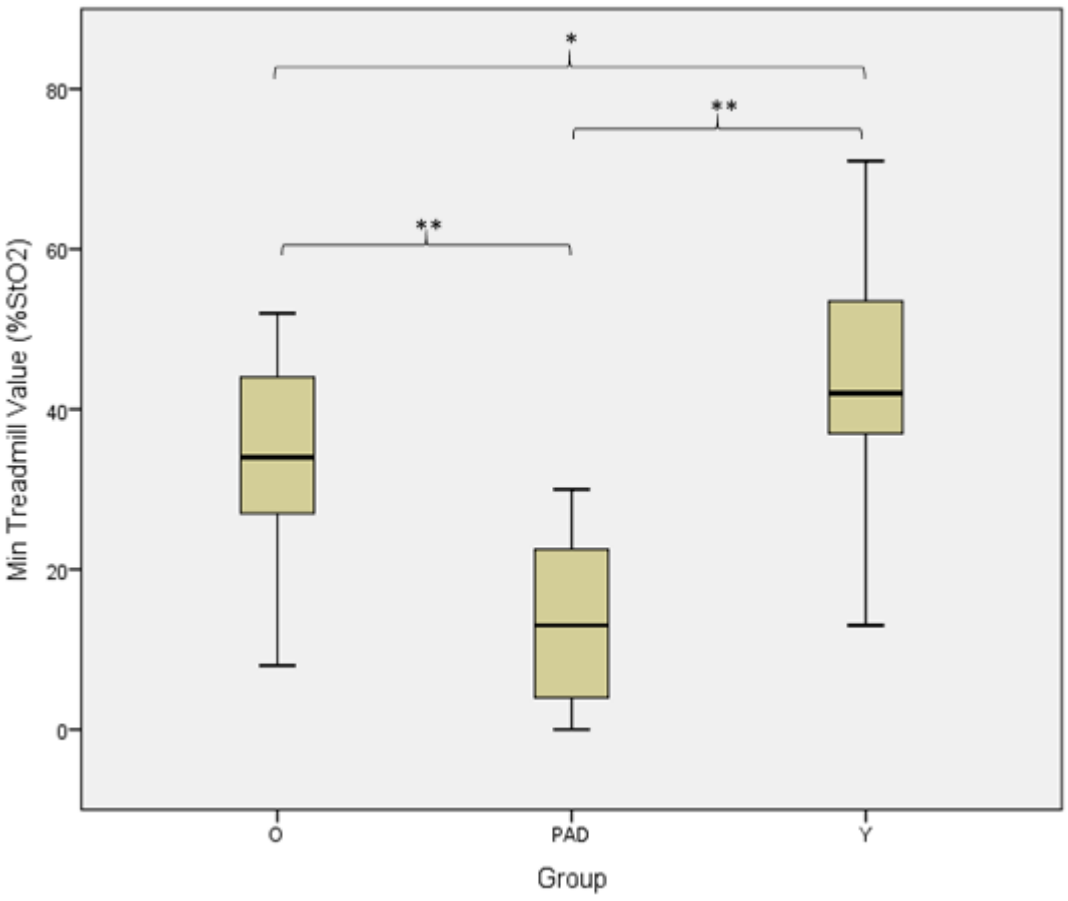

Figure 4.1. Minimum treadmill value between groups based on one-way ANOVA followed by Tukey's HSD post-hoc test. O=older, Y=young, PAD=patients with peripheral artery disease. * p=0.01 ** p<0.0001

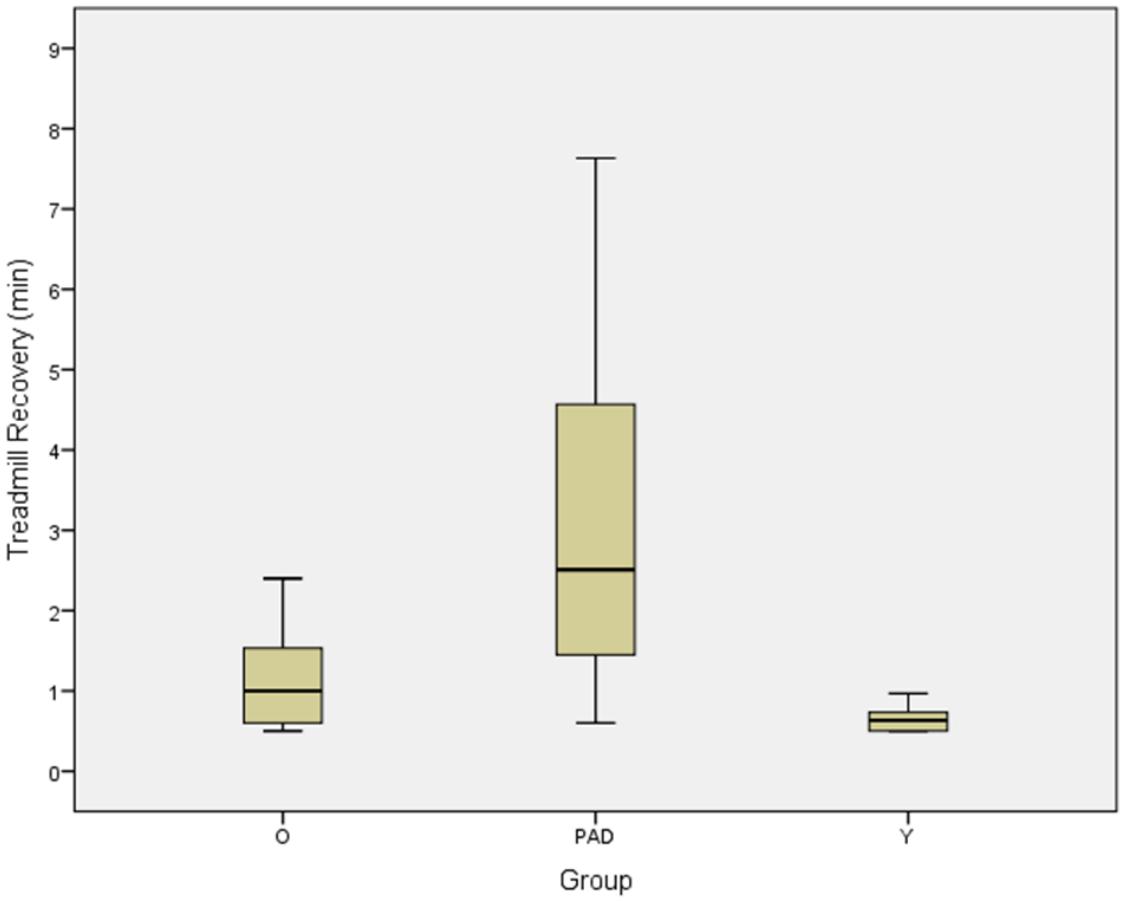

Figure 4.2. Treadmill recovery time based on Kruskal Wallis Test. O=older, Y=young, PAD=patients with peripheral artery disease. $p<0.05$ between groups

Table 4.3. Descriptive statistics for the remaining variables assessed for young and older healthy subjects

Variable	Group	Mean	Std. Error
Max Treadmill Value (%)	Young (n=47)	73.84	1.39
	Older (n=13)	74.00	3.38
Max 6MW Value (%)	Young	79.00	1.17
	Older	79.25	2.39
Min 6MW Value (%)	Young	57.47	2.03
	Older	52.08	4.45
6MW Recovery (min)	Young	0.088	0.019
	Older	0.325	0.140
Handgrip (lbs)	Young	103.93	5.07
	Older	91.67	6.23
4m Walk Speed (cm/sec)	Young	126.78	3.16
	Older	107.99	5.91
6MW Distance (feet)	Young	1487.13	26.11
	Older	1430.58	32.96

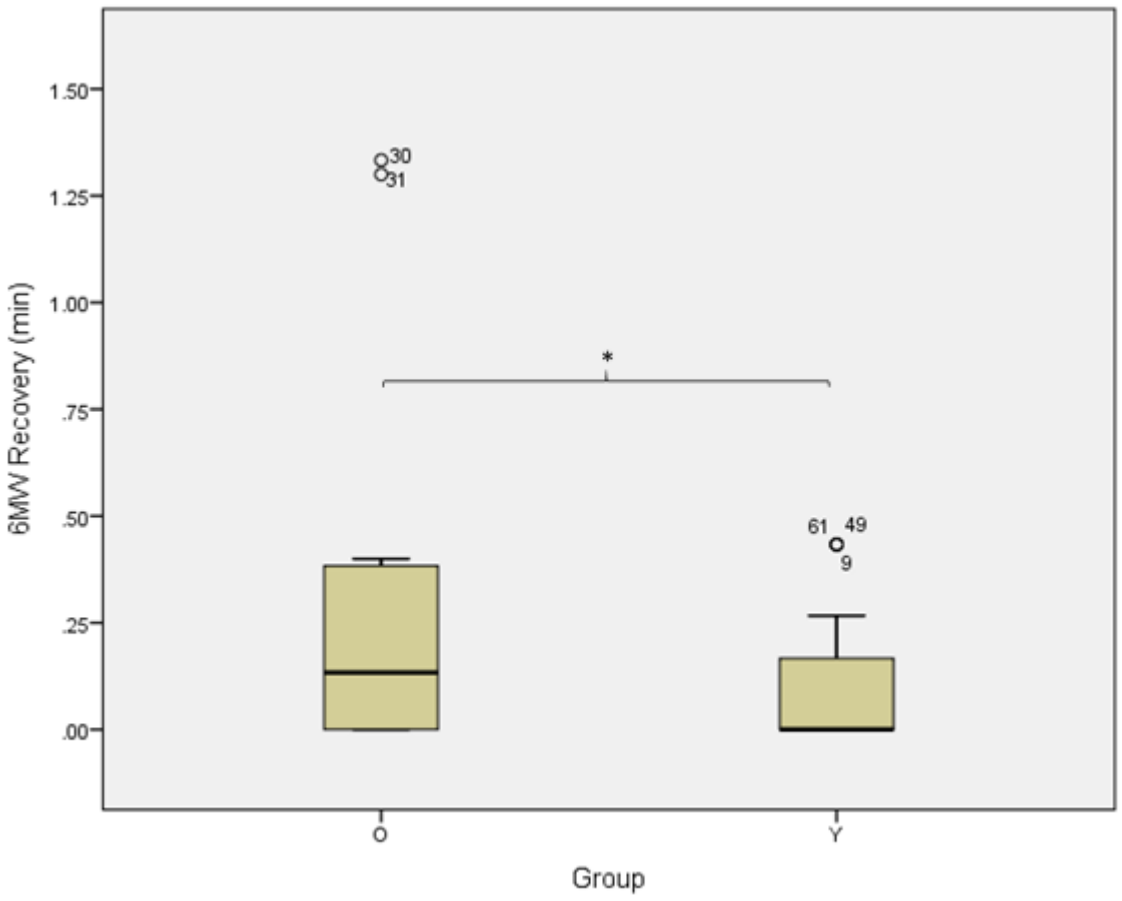

Figure 4.3. Six-minute walk recovery time based on Mann-Whitney U test. O=older, Y=young. *p<0.05

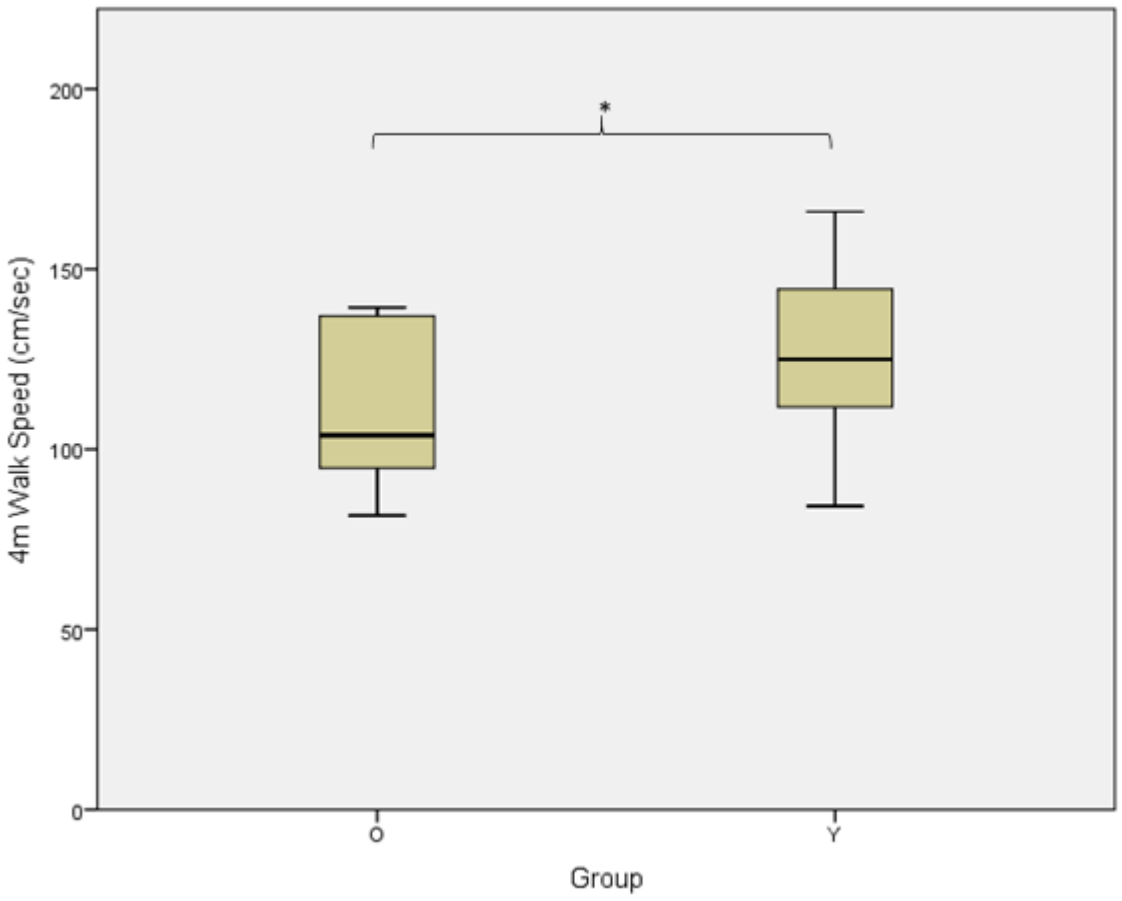

Figure 4.4. Four-meter walking speed based on independent samples t-test. O=older, Y=young. *p<0.05

CHAPTER FIVE

Discussion

This study was designed to compare StO_2 kinetics across three different samples: young healthy, older healthy, and patients with peripheral arterial disease (PAD). The same treadmill test was done across all groups to allow direct comparisons. The data show significant differences in minimum treadmill value, MTV, between all groups with lower values in the older and PAD subjects. Complete treadmill recovery time was also significantly longer in the presence of increased age and PAD. Similar to the treadmill recovery, for the young and older samples which completed further testing, 6MW recovery time was significantly shorter in young versus older subjects. However, the minimum 6MW value and the 6MW distance were not significantly different between the two groups. The baseline and maximum oxygen saturation values before and during either test, treadmill or 6MW, were also not significantly different between the groups. The younger subjects also showed a significantly faster four-meter walking speed than the older subjects.

We found that calf muscle StO_2 decreases more in patients with PAD than healthy controls, young and older. The average MTV for PAD was 12.6% compared to 45.2% and 33.3% for young and old respectively. These data showing differences in MTV support several previous findings of lower MTV in patients with PAD (Comerota et al., 2003; Kemp et al., 2001; Kooijman et al., 1997; Mesquita et al., 2013; Mohler et al., 2006). These findings could be explained by the relative exercise intensity of the

treadmill test based on individual subjects. The treadmill test is constant regardless of conditioning level, so the relative intensity of the test would be different for every individual. The occlusion and reduced blood flow from PAD (McDermott, 2015) which reduces functional capacity (Hiatt et al., 1988) means that patients with PAD may be working at a higher relative intensity compared to healthy subjects completing the same test. Findings by Kemp et al. (2001) showed that working at higher intensities, 75% vs. 50%, resulted in lower NIRS signals. Thus, if the intensity of the test is higher for patients with PAD compared to healthy controls, their StO_2 should reach lower levels. As there was no difference in baseline between the young and older samples and the MTV was significantly lower for older versus young, the decline in StO_2 during exercise was greater in older populations. The same concept likely explains the significant difference between the young and older subjects for MTV in this study, but heart rate or any other measure of exercise intensity for each individual was not measured making it impossible to draw firm conclusions.

The MTV was obtained within the first minute of treadmill testing for nearly all healthy subjects in this study. This occurred during a pattern of initial spike and sharp decline before rebounding and leveling off with the onset of exercise in healthy subjects. This data from healthy subjects is mildly comparable to studies reporting on time to MTV in patients with PAD. The majority of those studies found the patients to reach minimum StO_2 within five minutes of exercise initiation (Afaq et al., 2007; Afaq et al., 2008; Bauer et al., 2004; Gardner et al., 2012; Gardner et al., 2008; Watanabe et al., 2004). Subsequently, the faster a patient reaches minimum StO_2, the sooner the COT and absolute claudication pain (Afaq et al., 2007; Afaq et al., 2008) leading to strong

associations between StO_2 and COT and PWT (Gardner et al., 2009; Gardner et al., 2008). However, unlike data from patients with PAD, our data from healthy young and older subjects does not support associations between time to minimum StO_2 and peak walking time over the course of this treadmill test. All subjects completed the full 10-minute treadmill protocol with no complaints or stopping regardless of time to minimum StO_2. This is likely due to the design of the treadmill protocol used and relative intensity previously mentioned. When compared qualitatively, StO_2 levels in patients with PAD continue to drop throughout the treadmill test as the incline is raised every two minutes, but this pattern was not seen in most healthy individuals throughout this study. Healthy individuals, regardless of age group, tended to show an initial spike, decline and rebound before leveling off for the remainder of the test. Another study which examined the data qualitatively found a similar pattern (Bauer et al., 2004). This suggests that while a 2% grade increase is enough to increase intensity for patients with PAD resulting in lower StO_2 levels, it may not be enough to increase the relative intensity for most healthy individuals. Thus, it appears that at low enough levels of intensity, age does not hamper the body's ability to maintain StO_2 levels in healthy individuals over the course of a 10-minute treadmill protocol.

Similar to the MTV, the slowed StO_2 kinetics during post-exercise recovery support most previous findings. Complete StO_2 recovery times following the treadmill test were significantly different between the three groups. Several studies have shown longer recovery times in patients with PAD compared to healthy controls (Comerota et al., 2003; Gardner et al., 2008; Kemp et al., 2001; Kooijman et al., 1997; McCully et al., 1994; Mesquita et al., 2013; Mohler et al., 2006). The average recovery time for patients

with PAD in this study was 2.59 minutes which is slightly quicker than those reported in other studies ranging from 3.75-5.18 minutes (Gardner et al., 2014; Gardner et al., 2009; Gardner et al., 2015; Gardner et al., 2008). Since data were not received regarding the condition of each leg within the PAD sample, the severity of PAD was not taken into account during analysis for this study which could explain the recovery time difference. This could also explain why such variability is seen in the data from PAD subjects compared to the young and older samples as the limbs could a been from wide range of classifications. The slowed StO_2 recovery time could be because of the association between relative blood flow and StO_2 levels in the extremities of patients with PAD (Mesquita et al., 2013). Limited blood flow and supply delays the delivery of oxygen in the blood stream slowing StO_2 recovery. Both healthy groups had full recovery times of less than one-minute post treadmill test. Eleven subjects in the healthy young group had no recovery time in at least one leg post treadmill test because they finished with StO_2 values above baseline. The healthy subjects who did not need recovery time following the treadmill test appear to have fully recovered while still completing the test. While not measured in this study, it's possible this could due to the intensity being low enough that blood flow was not altered in a way to limit the muscular supply and always remained sufficient to maintain the needed muscle oxygen levels. Any oxygen deficiency was corrected while still testing in these subjects.

Interestingly, unlike the findings from MTV in this study, there was no significant difference in minimum 6MW StO_2 value between young and old. The 6MW test allows the participant to walk at their normal self-selected walking pace which could explain the lack of difference in minimum StO_2 value. Since pace is not set, older participants could

walk at slower paces if desired. Data from the four-meter walking speed test supports this notion as the young group had a significantly faster walking pace than the older (126.78 cm/sec vs. 107.99 cm/sec). However, the average distance covered between each group was not significantly different during the 6MW test with only a 57-foot difference. This suggests that for the 6MW test participants from both groups should have been walking roughly the same pace, so a slower pace for the old group may not explain the results. Based on observations during the 6MW test, it appeared the older subjects cared more about the distance they covered and didn't want to be considered "slow." This observation was based on repeated questions from the participants about their pace and where they matched with others their age and the younger sample. No information was given to them on their standing and they were instructed to continue at their normal self-selected walking pace. This may explain why the difference in walking speed was significant for the four-meter walking speed, but did not appear during the 6MW test. The four-meter walking speed test is designed to test normal walking pace over a short distance that does not allow the participants time to think about and change their pace, so might be more indicative of the true differences in walking speed between the groups. The older group in this study also could have been at an above average fitness level. Four of the seven participants in the older sample described themselves as having above average fitness on the HHQ and five of the seven listed their overall health as very good or better on the SF-36. It appears that each participant selected a pace that did not lead to oxygen deficits while walking in this study. This suggests that when given an option, individuals will walk at a pace which does not lead to significant StO_2 alterations,

resulting in minimal StO_2 changes appearing during self-selected exercise as a person ages.

Despite no difference in minimum 6MW value, after completion of the test, full StO_2 recovery time was significantly shorter for the young group. Healthy young subjects only required an average of 5.28 seconds to return to baseline following the test. Eighteen of the twenty-four subjects in the healthy young group had a recovery time of zero in at least one leg. The older group had a significantly slower average recovery time of 19.5 seconds, with only three subjects having at least one leg with no recovery time. The ability to recover while still completing exercise has previously been shown in healthy subjects using a plantarflexion protocol (Quaresima et al., 2001). Another study found a similar pattern of re-saturation during running in healthy young individuals (Quaresima et al., 2002). The ability to recover does not appear in any data for patients with PAD and this study shows that ageing may impair this ability as well. Young subjects showed a remarkable ability to recover and maintain oxygen levels when walking at a self-selected pace. Thus, it appears that ageing could affect an individual's ability to recover while still completing a task even when healthy. This study did not have the means to examine a possible cause for this delay, but like those with PAD, it could be the result of altered blood flow in older subjects.

There was not a significant difference between max treadmill or max 6MW StO_2 values between the young and old. Few studies have reported on maximum StO_2 values during exercise or testing as minimum values are generally the focus. However, Comerota et al. (2003) found peak exercise StO_2 to be significantly lower in patients with PAD compared to controls. This suggests that while PAD may impact peak oxygenation

levels, ageing alone may not. In our study, the peak treadmill value and peak 6MW value generally occurred at the beginning of testing in initial response to the onset of exercise. Thus, when putting these data together, it is possible that age alone does not have a significant impact on the body's initial response to beginning exercise, while PAD may hamper the body's initial reaction. A possible explanation for this could be alterations in relative blood flow due to PAD at the onset of exercise, but the topic warrants further investigation. While not previously considered important, peak StO_2 values may be worth more examination as it appears PAD may significantly alter the measure earlier during exercise than the minimum StO_2 levels not seen until later in exercise. Examining peak StO_2 may lead to more insight into the potential pathology of restrictions caused by PAD. As expected, there was no difference in baseline StO_2 between the younger and older groups. This is because leg blood flow at rest is not altered between most populations, including those with PAD (Comerota et al., 2003; Kooijman et al., 1997). Ageing does not alter resting mechanics of blood flow and oxygen delivery in a way that affects baseline StO_2.

The results from the handgrip strength and gait speed as markers of sarcopenia contrast each other within this study. While the older group had a significantly slower four-meter walking speed, there was no significant difference in handgrip strength between them and the young group. As handgrip strength and gait speed are both common functional measures of sarcopenia (Cruz-Jentoft et al., 2010), the contrasting results make it difficult to determine if calf muscle StO_2 kinetics have a use when determining sarcopenia. This makes it difficult to explore associations between calf muscle StO_2 kinetics and these tests in relation to sarcopenia. The differing results may

be explained by the average age of the participants in the older group as it was only 58.15 years. The loss of motor units is not linear across all ageing and seems to increase in most individuals around 60-70 years of age (Power et al., 2013). It is possible that within this study, the participants had not begun to experience significant muscle mass loss from sarcopenia. However, four of the seven older participants were over 65 years old placing them well within the expected range of significantly decreased muscle mass loss. Three of the four participants over 65, though, self-described their physical fitness as above average or excellent and all reported engaging in physical fitness a minimum of 3-4 times/week, including two reporting exercise 5 or more times/week. As routine exercise has been shown to have profound effects at preventing age related muscle mass loss (Powers et al., 2013; Makanae & Fujita, 2015; Wall et al., 2013), it is likely that the oldest participants in this study were at decreased risk of significant losses even considering their age. Thus, sarcopenia may not have been a significant factor within the older sample in this study. As such, more controlled studies are needed with subjects clearly demonstrating signs of sarcopenia to determine if an association exists with StO_2 kinetics.

Limitations

There were multiple limitations to this study. The small sample size of healthy older subjects resulted in lower than optimal power calculations. This is largely due to difficulty in finding older individuals with no history of cardiometabolic disease or current musculoskeletal condition in a limited time frame. Each leg was counted individually to help increase the power of the study. While the sensors were always placed on the medial gastrocnemius on both calves of each participant, the exact

placement was not directly measured on each participant. Research has shown regional differences in the calf of StO_2 measurements using NIRS based on sensor placement (Wolf et al., 2007). This is likely due to the heterogeneity of blood flow in the muscle (Jones et al., 2016). These findings suggest that the study could have benefited from a stronger design using a measured position to maintain more consistent inter-subject sensor placement.

Conclusion

This study shows that ageing, regardless of health, lowers and slows StO_2 kinetics during and after exercise. These alterations may even present in older persons in the absence of sarcopenia. It also supports findings from several previous studies that the presence of PAD further negatively affects StO_2 kinetics beyond that of ageing. Healthy young subjects have a strong ability to recover during exercise when the intensity is low enough. Healthy older subjects have also shown a milder form of this ability when a task is performed at a self-selected pace. Future research should focus on angiogenesis as a method to help increase blood flow to lower extremities. Increased blood flow could lead to major improvements in the lowered and slowed StO_2 kinetics found in older subjects and patients with PAD. The associations between ageing, sarcopenia, and StO_2 kinetics also warrant further investigation as this study indicates alterations in StO_2 may occur from ageing even without sarcopenia present. It is likely other factors beyond the loss in muscle mass and function contribute to the age-related alterations in calf muscle StO_2 kinetics. The Moxy Monitor is a good instrument to explore more underlying causes behind StO_2 alterations from ageing and to see if improvements occur in StO_2 kinetics from angiogenesis as it can be used in practical environments outside a lab.

APPENDICES

APPENDIX A

Consent Form

Baylor University
Health Human Performance and Recreation

Consent Form for Research

PROTOCOL TITLE: **Calf muscle hemoglobin oxygen saturation characteristics in healthy participants**

PRINCIPAL INVESTIGATOR: **Panagiotis Koutakis, Ph.D.**

SUPPORTED BY: **Baylor University**

Introduction

Please read this form carefully. The purpose of this form is to provide you with important information about taking part in a research study. If any of the statements or words in this form are unclear, please let us know. We would be happy to answer any questions. You have the right to discuss this study with another person, including those not a part of the research team, before making your decision regarding participation in the study.

Taking part in this research study is your decision. If you decide to participate, we ask that you sign this form. We will provide you a copy of the signed form.

The person in charge of this study is **Panagiotis Koutakis**. We will refer to this person as the "researcher" throughout this form.

Why is this study being done?

The purpose of this study is to determine changes in tissue oxygen saturation across the life-span and associate these changes with parameters of sarcopenia. Secondary objectives are to determine differential changes in tissue oxygen saturation with smoking status, exercise activity and quality of life.

We are asking you to participate in this study because you are an apparently-healthy, man or woman between 19 and 70 years of age. You have never been diagnosed with cardiovascular or metabolic disease and you do not have any musculoskeletal disease that can limit your ability to walk.

About **60** participants will take part in this research study at Baylor University.

How long will I participate in this research study?

We expect that you will be in this research study for **1.5 hours**. During this time, we will ask you to make **a one-time** visit to Marrs McLean Gym in rooms 233.11. If you are selected for the reliability/validity sub-analysis study, we will ask you to make 5 visits in order to complete the study.

What will happen if I participate in this research study?

Upon entry to the study you will perform the following tasks:

1) You will be asked to complete the Health History Questionnaire.
2) You will wear comfortable clothes and athletic shoes. The researcher will strap a small portable device on your calf muscles that measures the amount of oxygen delivered to the muscle.
3) You will be asked to perform the 4m-gait speed test.
4) You will be asked to complete the Short-Form 36 questionnaire.
5) You will be asked to complete a graded treadmill test. The test consists of starting at 2mph-0% grade and every two minutes a 2% increase in the grade occurs. The test will stop when you walk for 10 consecutive minutes or if you feel any discomfort that will prohibit you from finishing the test.
6) You will rest for 15 minutes and then you will perform the hand grip strength.
7) You will be asked to perform the six-minute walking test. The test consists of walking in a 100ft hallway at your regular walking pace for six minutes.
8) You will rest for 10 minutes, we will remove the portable device and you will have completed the study.

If you agree to participate in this study, we will ask you to sign the consent form before we do any study procedures.

What are the risks of taking part in this research study?

No foreseeable risks: To the best of our knowledge, taking part in this study will not hurt you.

Evaluation of your leg function

Evaluation of leg function poses minimal risks similar to those produced by activities of daily living such as walking. The treadmill test requires a low level of exercise which may cause some leg discomfort without placing undue stress on your heart and lungs. The leg discomfort you may experience will be the same that you may experience when walking or climbing the stairs, however, if you do not want to continue the evaluation, you may stop at any time.

Wearable infrared spectroscopy device risks

There are no reasonable/foreseeable physical risks when using the wearable infrared spectroscopy device. You may feel some discomfort if the device is strapped too tight on your calf muscle. If that is the case, the strap will be adjusted accordingly to alleviate the discomfort.

Risks of Completing Tasks

You may get tired during the tasks. You can rest at any time.

Questionnaire/Survey Risks
You may be uncomfortable with some of the questions and topics we will ask about. You do not have to answer any questions that make you feel uncomfortable.

Loss of Confidentiality
A risk of participating in this study is the possibility of a loss of confidentiality. Loss of confidentiality includes having your personal information shared with someone who is not on the study team and was not supposed to see or know about your information. The researcher plans to protect your confidentiality. Their plans for keeping your information private are described later in this consent form.

Are there any benefits from being in this research study?
For participation, you will receive an individualized and confidential report of their calf muscle oxygenation tests.

What alternatives are available?
You may choose to not participate in this research study.

Storing Study Information for Future Use
We would like to store your study information for future research related to calf muscle oxygenation. We will label all your study information with a code instead of your name. The key to the code connects your name to your study information. The researcher will keep the code in a password-protected computer/locked file.

How Will You Keep My Study Records Confidential?
We will keep the records of this study confidential. Participant names and contact information will be retained separately from hard copy data and from electronic data bases. Hard copy records of participant information will be maintained in a locked filing cabinet that is separate from the filing cabinet containing hard copy data records. We propose to retain identifying information and all data until all human participant data has been collected, analyzed and assimilated into an electronic data set that includes only a numeric identifier for each participant.

Study Participation and Early Withdrawal
Participating in this study is your choice. You are free to not take part or to withdraw at any time for any reason. Regardless of your decision, there will be no penalty or loss of benefit to which you were previously entitled. If you decide to withdraw from this study, the information that you have already provided will be kept confidential. You cannot withdraw information collected prior to your withdrawal.

If you are a student, you may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or your grades at Baylor University. You will not be offered or receive any special consideration if you take part in this research study.
If you are a Baylor faculty or employee, you may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your job status at Baylor University. You will not be offered or receive any special consideration if you take part in this research study.

Will I get paid for taking part in this research study?

You will not be paid for taking part in this study.

What will it cost me to take part in this research study?

There are no costs to you for taking part in this research study.

What happens if I am injured as a result of participating in this research study?

If you become ill or injured as a result of your participation in the study, you should seek medical treatment from your doctor or treatment center of choice. You should promptly tell the researcher about any illness or injury.

There are no plans for Baylor University to pay you or give you other compensation for your injury or illness. You do not give up any of your legal rights to seek compensation by signing this form.

What if I have any questions or concerns about this research study?

You can call us with any concerns or questions about the research. Please contact Panagiotis Koutakis at 254-710-4010 weekdays between the hours of 8:00 AM and 5:00 PM.

If you want to speak with someone **not** directly involved in this research study, you may contact the Baylor University IRB through the Office of the Vice Provost for Research at 254-710-1438. You can talk to them about:

- Your rights as a research subject
- Your concerns about the research
- A complaint about the research

Future Contact

We may like to contact you in the future either to follow-up to this study or to see if you are interested in other studies taking place at Baylor University.

Do you agree to let us contact you in the future?

_____YES _____NO _____INITIALS

Statement of Consent

I have read the information in this consent form including potential risks and benefits. I have been given the chance to ask questions. My questions have been answered to my satisfaction, and I agree to participate in the study.

_____ _____

Signature of Subject Date

Signature of Person Obtaining Consent:

I have explained the research to the subject and answered all his/her questions. I will give a copy of the signed consent form to the subject.

_____ _____

Signature of Person Obtaining Consent Date

APPENDIX B

Questionnaires

Health History Questionnaire

Please complete this form (2 pages front and back) as accurately as possible. The information you provide will be used to evaluate your health by the exercise physiologist who will see you in our facility.

All information you provide will be treated as privileged and confidential.

1. IDENTIFICATION & GENERAL INFORMATION

Name			Today's Date
			/ /
Age	**Date of Birth**	**Gender**	**e-mail (optional)**
	/ /19		

Please check the box that applies to you:

Race or Ethnic Background

☐ American Indian / Alaskan native ☐ Black, not of Hispanic origin ☐ White, not of Hispanic origin

☐ Asian ☐ Hispanic ☐ Other: _____

2. ILLNESS & MEDICAL HISTORY

Identify all of the conditions or diseases for which you have been diagnosed and/or treated and indicate if the condition is current.

Cardiovascular Conditions	Check if Applicable	Current? (Yes / No)	Cardiovascular Conditions (cont.)	Check if Applicable	Current? (Yes / No)
Angina			Heart Problem (other)		
Anemia (low iron)			High Blood Pressure (controlled)		
Coronary Disease			High Blood Pressure (uncontrolled)		
Disease of the Arteries			Peripheral Vascular Disease		
Enlarged Heart			Phlebitis or Emboli		
Heart Attack			Pulmonary Emboli		
Heart Murmur			Rheumatic Heart Disease		
Heart Rhythm Problem			Other (Please List):		
Heart Valve Problem			Other (Please List):		

Metabolic, Renal & Hepatic Conditions	Check if Applicable	Current? (Yes / No)	Metabolic, Renal & Hepatic Conditions (cont.)	Check if Applicable	Current? (Yes / No)
Diabetes (Type1)			Renal / Kidney Problems		
Diabetes (Type 2)			Thyroid Problems		
Gout			Other (Please List):		

Pulmonary Conditions	Check if Applicable	Current? (Yes / No)	Pulmonary Conditions (cont.)	Check if Applicable	Current? (Yes / No)
Allergies			Chronic Restrictive Pulmonary Disease		
Asthma			Emphysema		
Bronchitis (chronic)			Orthopnea		
Chronic Obstructive Pulmonary Disease			Other (Please List):		

Inflammatory, Immune & Hematological Conditions	Check if Applicable	Current? (Yes / No)	Inflammatory, Immune & Hematological Conditions	Check if Applicable	Current? (Yes / No)
Aids / HIV			Lupus		
Anemia (Type):			Osteoarthritis		
Blood Clotting Disorders			Rheumatoid Arthritis		
Chronic Fatigue Syndrome			Other (Please List):		
Fibromyalgia			Other (Please List):		

Other Medical Conditions	Check if Applicable	Current? (Yes / No)	Other Medical Conditions (cont.)	Check if Applicable	Current? (Yes / No)
Cancer (Type):			High Anxiety / Phobias		
Depression			Hysterectomy		
Eating Disorders (anorexia, bulimia)			Menstruation Problems		
Epilepsy			Sleeping Problems		
Gallstones / Gallbladder Disease			Stomach / Duodenal Ulcer		
Hearing Loss			Substance Abuse Problems		
Other Health Problems					
Please list any other health problems and/or illnesses that may influence your physical activity.					

Orthopedic Problems / Conditions	Check if Applicable	Current? (Yes / No)	Orthopedic Problems / Conditions (cont.)	Check if Applicable	Current? (Yes / No)
Ankle or Foot Problems			Low Back Pain		
Elbow Pain			Osteoporosis		
Hip Problems			Shoulder Pain		
Knee Problems			Wrist or Hand Pain		
Orthopedic Problems					
Please describe the orthopedic issue identified above and/or list any orthopedic problem(s) that may influence your physical activity.					

3. SYMPTOMS or SIGNS SUGGESTIVE of DISEASE

Do you presently have or recently had (Check if Applicable):

Yes	Description	Yes	Description
☐	Have you experienced unusual pain or discomfort in your chest, neck, jaw, arms, or other areas that may be due to heart problems?	☐	Do you suffer from swelling of the ankles (ankle edema)?
☐	Have you experienced unusual fatigue or shortness of breath at rest, during usual activities, or during mild-to-moderate exercise (e.g., climbing stairs, carrying groceries, brisk walking, cycling?	☐	Have you ever experienced an unusual and rapid throbbing or fluttering of the heart?
☐	Have you had any problems with dizziness or fainting?	☐	Have you ever experienced severe pain in your leg muscles during walking?
☐	When you stand up, or sometimes during the night while you are sleeping, do you have difficulty breathing?	☐	Has your doctor told you that you have a heart murmur?
☐	Have you ever experienced a seizure?	☐	Have you ever had unexpected weight loss of 10 lbs or more?

Have you ever had:	Check if Applicable	Date Diagnosed (M / Yr)
An abnormal chest x-ray?		
An abnormal electrocardiogram (ECG)?		
An exercise stress test?		
An abnormal exercise stress test?		

4. ADDITIONAL FAMILY HISTORY

Check all of the conditions or diseases for which ***any member of your immediate family, including grandparents,*** have been diagnosed and/or treated. Please provide their age and the date of occurrence or diagnosis if known.

Medical Condition	List Relative & Age at Diagnosis	Date Diagnosed (M / Yr)
High Blood Pressure before age 40		
High Cholesterol		
Obesity		
Diabetes		

Stroke under age 50		
Heart Attack under age 50		
Heart Operation		

5. PHYSICAL ACTIVITY

Please check the box that best describes you.

1. In general, compared to other persons your age, rate how physically fit you are:

Not at all fit	Slightly below average fitness	Average fitness	Slightly above average fitness	Extremely fit
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. Outside of your normal work, or daily responsibilities, how often do you engage in physical exercise?

☐ 5 or more times/week ☐ 3 - 4 times/week ☐ 1 - 2 times/week

☐ Less than 1 time/week ☐ Seldom or never

3. On average, how long do you exercise on each occasion?

☐ 10 - 20 min ☐ 20 - 30 min ☐ 30 - 40 min ☐ 40 - 50 min ☐ > 50 min

4. On a scale of 1 to 10 (1 being the lowest, 10 being the highest), how would you rate your exercise intensity ?

☐ Very Low (1 - 2) ☐ Low (3 - 4) ☐ Moderate (5 - 6) ☐ Mod-High (7 - 8) ☐ High (9 - 10)

5. How much strenuous physical work is required on your job?

☐ > 80% ☐ 60 – 80% ☐ 40 – 60% ☐ 20 – 40% ☐ None

7. STRESS INDEX

Rate how closely you agree with each of the following statements by filling in the blank preceding each statement with a number from **1 to 5** as indicated below.

1. In general, I seem to have many responsibilities but little authority.

Strongly disagree	Somewhat disagree	Undecided	Somewhat agree	Strongly agree
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. I rarely have enough time to do a good job, accomplish what I want or for family, social obligations or personal needs.

Strongly disagree	Somewhat disagree	Undecided	Somewhat agree	Strongly agree
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. Most of the time I have little control over my life at work, school, or home.

Strongly disagree	Somewhat disagree	Undecided	Somewhat agree	Strongly agree
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

4. On average, how many hours of sleep do you get in a 24-hour period?

☐ 3 to 4 ☐ 5 to 6 ☐ 7 to 8 ☐ 9 to 10 ☐ > 10

8. MEDICATIONS

Please indicate any medications, prescription or "over the counter" by providing the name and dosage:

Medication Type	Name of Medication	Dosage
Heart Medicine		
Blood Pressure Medicine		

4

Medication Type	Name of Medication	Dosage
Blood Cholesterol Medicine		
Insulin		
Other Medicine for Diabetes		
Thyroid Medicine		
Medicine for Breathing / Lungs		
Medicine for Weight Loss / Weight Control		
Hormones		
Birth Control Pills		
Painkiller Medicine		
Arthritis Medicine		
Medicine for Depression		
Medicine for Anxiety		
Medicine for Ulcers		
Allergy Medicine		
Other (please specify):		

Date: __/__/__ Participant ID:_____

QUALITY OF LIFE QUESTIONNAIRE (SF- 36 Health Survey)

This survey asks for your views about your health, how you feel and how well you are able to do your usual activities. Answer every question by checking the appropriate response. There are no right or wrong answers. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is: (Please check a box.)

- ☐ Excellent
- ☐ Very Good
- ☐ Good
- ☐ Fair
- ☐ Poor

2. Compared to one year ago, how would you rate your health in general now? (Please check a box.)

- ☐ Much better than one year ago
- ☐ Somewhat better now than one year ago
- ☐ About the same as one year ago
- ☐ Somewhat worse now than one year ago
- ☐ Much worse now than one year ago

3. The following questions are abour activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? (Please circle one number on each line.)

Activities	Yes, Limited A Lot	Yes, Limited A Little	Not Limited At All
(a) Vigorous, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
(b) Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	1	2	3
(c) Lifting or carrying groceries	1	2	3
(d) Climbing **several** flights of stairs	1	2	3
(e) Climbing **one** flight of stairs	1	2	3
(f) Bending, kneeling or stooping	1	2	3
(g) Walking **more than a mile**	1	2	3
(h) Walking **several blocks**	1	2	3
(i) Walking **one block**	1	2	3
(j) Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health? (Please circle one number on each line.)	**Yes**	**No**
(a) Cut down on the **amount of time** you spent on work or other activities	1	2
(b) Accomplished less than you would have liked	1	2
(c) Were **limited** in the kind of work or other activities	1	2
(d) Had **difficulty** performing the work or other activities (e.g.it took extra effort)	1	2

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?	Yes	No
(a) Cut down on the **amount of time** you spent on work or other activities	1	2
(b) Accomplished less than you would like	1	2
(c) Did your work or activities **less carefully than usual**	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
0	1	2	3	4

7. How much physical pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very Severe
0	1	2	3	4	5

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	Slightly	Moderately	Quite a bit	Extremely
0	1	2	3	4

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. (Please circle one number on each line.)

How much of the time during the Past 4 weeks….	All of the time	Most of the time	Some of the time	A little of the time	None of the time
(a) Did you feel full of life?	4	3	2	1	0
(b) Have you been very nervous?	4	3	2	1	0
(c) Have you felt so down in the dumps that nothing could cheer you up?	4	3	2	1	0
(d) Have you felt calm and peaceful?	4	3	2	1	0
(e) Did you have a lot of energy?	4	3	2	1	0
(f) Have you felt downhearted and depressed?	4	3	2	1	0
(g) Did you feel worn out?	4	3	2	1	0
(h) Have you been happy?	4	3	2	1	0
(i) Did you feel tired?	4	3	2	1	0

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	Never
0	1	2	3	4

11. How TRUE or FALSE is each of the following statements for you?

	Definitely True	Mostly true	Don't know	Mostly False	Definitely False
(a) I seem to get sick a little easier than other people	4	3	2	1	0
(b) I am as healthy as anybody I know	4	3	2	1	0
(c) I expect my health to get worse	4	3	2	1	0
(d) My health is excellent	4	3	2	1	0

APPENDIX C

Leg Function Report Form

Date:___/___/___ **Participant ID:_____**

Functional Tests Report Form

Six-minute walk test

Participants are asked to walk up and down a 100-foot hallway for six minutes. They are instructed to cover as much distance as possible using normal walking speed.

Distance covered in feet: _____

Notes:_____
_____
_____

Four-meter walking speed

a. Participants are asked to walk at their usual pace over a four-meter distance.
b. Participants are instructed to stand with both feet touching the starting line and to start walking after a verbal command.
c. Timing begins when the command is given, and the time in seconds needed to complete the entire distance is recorded.
d. The test can be repeated twice and the best time is used to calculate walking speed in cm/sec.

Walking speed in cm/sec: _____

Graded treadmill protocol

a. Treadmill speed is held constant at 2 mph
b. Treadmill grade begins at 0 percent and increases 2 percent every 2 minutes
c. The patient is asked about claudication symptoms at regular intervals (every 30 seconds)
d. Walking distances will be calculated from the time stamps reported in the report

Onset time of claudication symptoms: _____

Maximum walking time with claudication symptoms: _____

Notes:_____
_____
_____
_____

Please circle the stage completed:

Stage:	Speed (MPH):	Elevation (%):	METS:
1	2.0	0.0	2.5
2	2.0	2.0	3.1
3	2.0	4.0	3.4
4	2.0	6.0	4.2
5	2.0	8.0	4.7
6	2.0	10.0	5.3
7	2.0	12.0	5.8
8	2.0	14.0	6.4

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