A Dissertation

entitled

Passive Leg Movement and NO-Mediated Vascular Function: The Impact of Obstructive Sleep Apnea (OSA)

by

Zakaria A. Alyousif

Submitted to the Graduate Faculty as partial fulfillment of

the requirements for the Doctor of Philosophy Degree in Exercise Science

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May 2020

An Abstract of

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Abstract: Passive leg movement (PLM) has been gaining popularity as a simple, reliable method for assessing endothelial function for a variety of chronic conditions including obstructive sleep apnea (OSA). Previous research has demonstrated that PLM leads to a significant increase in femoral artery blood flow (FBF), although studies suggest that the increase in FBF during PLM is mediated by the bioavailability of nitric oxide (NO). Meanwhile, research is growing on the use of antioxidants to attenuate endothelial dysfunction; for example, prior research has investigated the use of intravenous injection of vitamin C to demonstrate the therapeutic benefits of antioxidant supplements for endothelial function in OSA patients. However, the effect of vitamin C administered orally on the FBF response to PLM has not been previously examined. **Purpose**: The two aims of the study included in this dissertation were (1) to investigate whether the PLM approach

is an acceptable diagnostic tool to distinguish between healthy and OSA patients, and (2) to examine the effectiveness of oral vitamin C in terms of attenuating endothelial dysfunction in OSA. It was hypothesized that PLM would be a useful diagnostic tool to distinguish between healthy and OSA. In addition, it was hypothesized oral vitamin C would improve the FBF response in OSA with vitamin C compared to OSA without vitamin C. **Methods**: Subjects included two groups: healthy and OSA. Thirteen healthy

male adult subjects aged 18-55 years were included as well as 13 male subjects with OSA. PLM was accomplished using an isokinetic (Biodex) machine, which allowed the lower leg to move through 90° range of motion at 30 cycles/min. Femoral artery blood velocities (FBVs) were measured continuously at baseline (60 s) and during PLM (120 s) using Doppler ultrasound. FBF was calculated using the arterial diameter measured at baseline. Each subject consumed a 1000mg of oral vitamin C and, after 2 hrs of rest, subjects returned to the isokinetic machine and completed a second PLM trial. **Results**: The repeated measures ANOVA revealed a significant difference between OSA and healthy groups in FBF (healthy, 162.1 ml/min, OSA, 76.6ml/min; $p < 0.05$). However, the peak FBF responses were not different ($p>0.05$) between Pre-vitamin C (76.6 ml/min) and Post-VC (79.9 ml/min) in OSA patients. **Conclusion**: PLM has shown to be a good diagnostic tool to distinguish between healthy and OSA patients. However, vitamin C did not make a difference in either OSA or healthy groups. These findings challenge the hypothesis that the vitamin C would result in an improved FABF response during PLM. It is possible that vitamin C could still be effective if different administration methods, higher dosages, or more severe conditions were used. Despite these findings, research in the areas of alternative treatment methods for OSA that are less invasive and more time-efficient remains important as the incidence of OSA continues to increase and as adherence to current treatments remains inconsistent among many sufferers.

This dissertation is dedicated to my wife, who always encouraged me to do what made me happy. Without her support, I would not be the man I am today

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List of Abbreviations

BH4.....Tetrahydrobiopterin

Vit C.....Vitamin C

CPAP.....Continuous Positive Airway Pressure

FMD.....Flow Mediated Dilation

HEAL.....Healthy Control Group

PLM .....Passive Leg Movement

NO.....Nitric Oxide

eNOS.....Endothelial Nitric Oxide Synthase

FABF.....Femoral Arterial Blood Flow

FABV.....Femoral Artery Antegrade Blood Velocity

FAD.....Femoral Arterial Diameter

FRBV .....Femoral Artery Retrograde Blood Velocity

MFABV .....Mean Blood Velocity

HR.....Heart rate

CO.....Cardiac Output

SBP .....Systolic Blood Pressure

DBP.....Diastolic Blood Pressure

MAP.....Mean Arterial Pressure

TPR .....Total Peripheral Resistance

OSA.....Obstructive Sleep Apnea

Chapter 1

Introduction

1.1 General Background

Obstructive sleep apnea (OSA) is a sleep disorder characterized by repetitive episodes of either partial or complete upper airway collapse, leading to hypoxemia and disturbed sleep (Garvey et al., 2015). OSA is an important public health problem that affects an estimated 15 million adults in the United States, or approximately 5% the adult population. The majority of whom are male and obese; however, the exact number of individuals who suffer from OSA is difficult to determine since many cases go unreported and untreated (Drager et al., 2007; Gozal & Kheirandish-Gozal, 2008; Ryan, Taylor, & McNicholas, 2008; Somers et al., 2008), with the prevalence in the adult population has been estimated to be as high as 75% (Punjabi, 2008).

In addition to being associated with males and obesity, there has been a steady increase in the literature indicating that OSA is also associated with an increase in the risk for cardiovascular disease (CVD). Untreated obstructive sleep apnea can lead to or exacerbate several cardiovascular diseases such as, hypertension, atherosclerosis, and coronary artery disorders that then contribute to life-threatening diseases such as myocardial ischemia, heart failure, and stroke (Dopp, 2007; Gozal & Kheirandish-Gozal,

2008; Koehler & Schafer, 1996; Somers et al., 2008). However, because of the comorbidity factors linking obesity and OSA (i.e. approximately 80% of those individuals with OSA also are also considered obese), the cause and effect relationship is considerably more complicated than it first appears (Gozal & Kheirandish-Gozal, 2008). However, the treatment of OSA may represent a novel approach that may reduce the risk of a cardiovascular death significantly.

The underlying mechanism(s) whereby OSA may contribute to cardiovascular disease has not been fully elucidated however, several potential pathways have been identified. One possible mechanism may involve OSA as an initiating event for vascular endothelial dysfunction through diverse pathways such as hypoxemia, the production of reactive oxygen species (ROS), and activation of the sympathetic nervous system (Budhiraja et al., 2007). Since endothelial cells from the innermost lining of all blood vessels, these cells are directly exposed to changes in mechanical stress (i.e. sudden changes in shear stress associated with rapid changes in blood flow) and to changes in neurohumoral stimuli that are released into the bloodstream as a result of various stresses (Laughlin & Korzik, 2001). Thus, endothelial cells are also exposed to turbulent blood flow, pro-inflammatory factors, pro-coagulation factors and harmful chemicals that may lead to endothelial damage and dysfunction over a period of time. Compelling findings from the results of several large cross-sectional as well as longitudinal investigations strongly suggests that OSA may play a significant role in the development of endothelial dysfunction, which may lead to CVD.

It is clearly evident that OSA is a very serious health issue that, when left untreated, increases the potential threat of a lethal cardiovascular event for an OSA

patient. Currently, continuous positive airway pressure therapy (CPAP) is the most common and currently, the most effective treatment for OSA, but patients do not necessarily accept, tolerate or comply with CPAP treatment and thus, many factors influencing the efficacy and longer-term benefits of CPAP (Catcheside, 2010). However, the results of recent studies suggest with improved understanding of the role of nitric oxide (NO) and endothelial dysfunction may lead to the development of alternative treatments for OSA that may have therapeutic benefits for those individuals with both OSA and CVD. For example, vitamin C is a potent anti-oxidant that can reduce oxidative stress and may prove beneficial in reducing or slowing the progression of endothelial dysfunction in patients with OSA (Grebe et al., 2006; Wray et al., 2012).

Because endothelial dysfunction has been linked to cardiovascular disease, the non-invasive assessment of endothelial function (or dysfunction) is important in both clinical and research settings, in particular as a measure of the effectiveness of OSA treatments (Celermajer, 2008). Endothelial dysfunction has been measured in both the clinical and research setting using the non-invasive flow-mediated dilation (FMD) approach whereby the magnitude of the blood response in an artery is measured immediately following a brief period of blood flow occlusion (Celermajer et al., 1992). It is generally held that the magnitude of the blood flow response following the release of the cuff and the sudden increase in arterial shear stress represents endothelial cell function which has been attributed to NO bioavailability. While the results of recent studies have questioned the role of NO in the traditional FMD response (Parker et al., 2011; Pyke & Tschakovsky, 2005; Wray et al., 2013), there is a plethora of data indicating the FMD response is endothelial dependent and therefore, a good measure of

endothelial cell "health". Given the established relationship between endothelial function and the risk for CV, it is not surprising that studies have evaluated endothelial function in patient with OSA, including the functional assessment of vascular responses by assessing changes in blood flow in response to FMD technique (Budhiraja et al., 2007). The challenge with the FMD technique is that it requires a very skilled sonographer to perform the test since it requires the continuous measurement of the arterial walls and it requires prolonged occlusion of the artery (i.e., typically 5 min) which can be quite uncomfortable due to the occlusion pressure being above the individual's systolic blood pressure.

The results of several recent investigations have shown that blood flow in the femoral artery increases significantly, albeit transiently, at the onset of passive leg movement (PLM), which can trigger additional central hemodynamics changes in heart rate, stroke volume and cardiac output (Wray et al., 2005; Trinity et al., 2010; Trinity et al., 2012; Mortensen et al., 2012; Groot et al., 2015). Furthermore, it appears as the PLM technique can be used to assess NO-mediated endothelial function (Wray et al., 2005; Trinity et al., 2010; Trinity et al., 2012). Thus, the PLM technique may prove to be a very useful approach for evaluating vascular disease associated with OSA and to monitor the response to various treatments. This approach is easily adapted in the clinical or research setting since blood velocity (flow) is measured during limb movement with less emphasis on the borders of the vascular wall (which is more difficult) and does not require occlusion of the limb and therefore, is appreciably more comfortable for the subject or patient. However, we are not currently aware of any studies that have

examined the use of the PLM technique as a diagnostic tool for assessing endothelial function or dysfunction in patients diagnosed with OSA.

1.2 Statement of the Problem

It is clearly evident that OSA is a serious health issue that, when left untreated, a patient with OSA is at a higher risk for suffering from a cardiovascular event or death. Currently, continuous positive airway pressure (CPAP) is the most common and effective treatment, but patients do not necessarily accept, tolerate or comply with this treatment. While CPAP may be a highly efficacious therapy for treating OSA patients, many factors influence CPAP use both in the short- and long-term users. A better understanding of the role of systemic inflammation and oxidative stress may lead to the development of alternative treatments that may prevent CVD resulting from OSA or may improve the effectiveness of current treatments for OSA. This may be an important first step in establishing, through the use of long-term prospective studies, that an effective treatment for sleep apnea may decrease the risk of cardiovascular events in terms of either primary or secondary prevention.

1.3 Statement of Purpose

The purposes of this study were to (i) to investigate whether the passive leg movement (PLM) technique could be used to distinguish differences in endothelial function between young, healthy individuals compared to individuals known to have OSA, and (ii) to examine the effectiveness of oral administration of vitamin C on endothelial dysfunction in patients with OSA compared to healthy idividuals. More

specifically, this dissertation investigates the application of the PLM technique as a novel, non-invasive approach to measuring the vascular endothelial response in both healthy individuals and individuals with obstructive sleep apnea.

In this study, endothelial function will be determined using a non-invasive approach whereby femoral artery blood flow will be measured using standard Doppler ultrasound techniques during passive movement of the lower limb through a range of motion of approximately 90° at the knee. This investigation will examine the blood flow response in four conditions; subjects with OSA, subjects with OSA following administration of a vitamin C regimen, healthy subjects, and healthy subjects following administration of a vitamin C regimen. The responses following PLM will be compared before and following post ingestion of 1000 mg of vitamin C (i.e. within group) as well as comparing the healthy and OSA patients (i.e. between groups).

The hypotheses are as follows:

1. Passive leg movement paradigm will be a technical approach to distinguish between healthy and patients with OSA.
2. Vitamin C administration will attenuate endothelial dysfunction in patients with OSA.

Chapter 2

Literature Review

2.1 Introduction

Sleep apnea is a sleep disorder characterized by repetitive episodes of either partial or complete upper airway collapse, leading to hypoxemia and disturbed sleep. Sleep apnea is an important public health problem that is known to affect approximately 5% the adult population, but an accurate estimate of the exact number of individuals who may experience at least some form of sleep apnea is difficult to determine since many sleep disturbances go undiagnosed (Drager et al., 2007; Ryan, Taylor, & McNicholas, 2008). There two forms of sleep apnea that are more common, central sleep apnea (CSA) and obstructive sleep apnea (OSA) with a third form that is considered very rare and that is complex sleep apnea, a combination of the other central and obstructed sleep apnea.

Central sleep apnea characterized by repetitive cessations of breathing caused by a loss of the ventilatory drive arising from a dysfunction in the areas of the brain that control breathing patterns. However, the mechanisms underlying the periodic cessations of breathing are not well understood at the current time (Somers et al., 2008). It has been shown that the underlying neurological causes associated with CSA resulting in malfunctioning signals that control breathing are typically associated with heart failure

and stroke in many of the patients that have been diagnosed with CSA (Somers et al., 2008).

Although similar to CSA in that it is a condition that affects breathing during sleep, obstructive sleep apnea (OSA) differs in that it is characterized by repetitive interruptions in sleep that are caused by some form of physical obstruction of the airway. Moreover, OSA comprises the majority of sleep apnea cases as it is estimated to affect more than 15 million adults in the U.S., the majority of whom are male and obese (Somers et al., 2008). Unlike CSA which may be idiopathic in its origin, OSA is directly linked to a physical cause, such as a collapsed or an obstructed airway.

In general, the extent that a person experiences sleep apnea is determined and is diagnosed by using the apnea–hypopnea index (AHI), which simply quantifies the number of apnea and hypopnea events per hour during sleep. An obstructive apnea pause is defined as a cessation of breathing of ≥10 seconds with an ongoing ventilatory effort. Obstructive hypopnea is defined as a decrease in breathing but does not result in a complete cessation of respiration (Somers et al., 2008). An AHI of less than five (5) events is considered normal whereas more than thirty (30) events in an hour is considered severe; between these extremes is often reported as being mild-to-moderate OSA (Zhang &Yi Si, 2012).

2.2 Pathophysiology of Obstructive Sleep Apnea

The pathophysiology of OSA may vary considerably from one individual to another resulting in the diagnoses, reporting and treatment of OSA a challenge to the healthcare professionals that treat sleep disorders. Important variables such as the upper

airway anatomy, the ability of the upper airway dilator muscles to respond to respiratory challenge during sleep, the tendency to wake from increased carbon dioxide (CO_2) accumulation during sleep, and the stability of the respiratory control system (Eckert et al., 2008). As a result, patients may respond to different treatment approaches based on the underlying factor leading to the sleep apnea.

Because of the wide range of potential mechanisms contributing to OSA, they have been further classified to two main areas: (i) the mechanisms that influence upper airway patency and (ii) ventilatory control instability during sleep. Although problems with maintenance of upper airway patency lead primarily in obstructive sleep apnea, ventilatory control instability can lead to either central or obstructive sleep apnea (White, 2005).

The upper airway is composed of muscles and soft tissue, but has relatively little bony or rigid support. Therefore, if dilating forces of upper airway is not potent enough there are anatomic and physiologic influences that tend to collapse the airway which must be offset to keep the airway open. The two primary forces tending to collapse the airway are the intraluminal negative pressure generated by the diaphragm during each inspiration phase and the extraluminal tissue pressure which is the pressure that resulting from tissue and bony structures surrounding the airway (White, 2005). The anatomy of the airway can also affect pharyngeal patency. In normal healthy individuals, when the respiratory muscles completely relaxed, the airway generally remains open and requires a pressure difference of -5 cm H_2O to collapse. The pressure in the soft tissue surrounding the airway is not sufficiently positive to overcome the elastance of the airway wall. In patients with OSA, this is not the case. With the respiratory muscles relaxed, the airway

collapses and positive pressure is required to keep the airway open (Eckert et al., 2008). As a result, the soft tissue pressure must be positive and high enough to overcome the elastance of the airway wall to prevent the wall from collapsing. Furthermore, several factors may also affect the size of the lumen of the airway including fat deposition, physical structures that impede airflow through the airway (i.e. tonsils and/or adenoids), and excessive airway secretions (White, 2005).

In addition to upper airway patency, ventilatory control instability during sleep also play an important role as an underlying factor that can lead to sleep apnea. According to Hudgel and colleagues (1987) and Badr and coworkers (1995), ventilatory control instability can contribute to OSA through two possible mechanisms. First, the muscles of the upper airway are quite responsive to the respiratory system with their activity increasing or decreasing substantially depending on respiratory drive. Therefore, if respiratory drive is unstable, then the responsiveness of the pharyngeal musculature will also be highly variable. This may increase the possibility of the upper airway collapsing because of the respiratory cycling. Second, it also has been demonstrated that airway collapse is common during a central sleep apnea. Thus, if respiratory drive is eliminated, the upper airway muscles are turned off as well and the pharynx may collapse resulting in the periodic cessation of breathing.

2.3 Risk Factors & Complications of Obstructive Sleep Apnea

In addition to being associated with males and obesity, OSA is associated with cardiovascular problems. Untreated OSA can lead to or exacerbate several cardiopulmonary conditions such as hypoxemia, hypertension, and arrhythmia, which

then contribute to life-threatening diseases such as heart failure, stroke, and myocardial ischemia. However, because of the comorbidity factors of obesity and OSA, with about 80% of OSA also being obese, the cause–effect relationship is complicated (Dopp, 2007; Gozal & Kheirandish-Gozal, 2008; Koehler & Schafer, 1996; Somers et al., 2008). Unfortunately, OSA like many other chronic diseases, individuals with OSA can be asymptomatic for long periods of time before the onset of any signs or symptoms of the underlying disease become apparent. Of course, this results in a delayed diagnosis and treatment for the disease, which by this point, the disease has already made considerable progression and damage to the body.

OSA is also associated with cardiac arrhythmias. Arrhythmias are reportedly more frequent in persons with OSA and increase with the number and severity of apneic episodes (Koehler & Schafer, 1996). Nocturnal arrhythmias have been shown to occur in up to 50% of patients that are also suffering from OSA (Somers et al., 2008). Prolonged apnea and hypoxemia in OSA elicits what is known as the diving reflex, which is defined as an increase in cardiac vagal activation, with simultaneous sympathetic activation to the peripheral blood vessels, including muscle, the renal system, and the splanchnic bed, but for some reason, the cerebral vasculature appears to be unaffected.

The detrimental health effects of OSA can also lead to life threatening events. Heart failure is one common condition experienced by people with OSA. The most direct mechanism by which long-standing OSA might induce left ventricular systolic dysfunction is by rising BP. Hypertension is the most common risk factor for left ventricular hypertrophy and heart failure. Nocturnal oxygen desaturation is an independent predictor of impaired ventricular relaxation during diastole (Somers et al.,

2008). OSA could potentially contribute to the progression of heart failure through several pathological mechanisms: 1) by eliciting greater sympathetic outflow to the heart, kidney, and resistance vessels during wakefulness and sleep; 2) by increasing left ventricular afterload both acutely and chronically; 3) by inducing hypoxia and secondary increases in right ventricular afterload; and 4) by increasing the risk of myocardial infarction (Somers et al., 2008). However, obesity again is a confounding factor. Increased BMI, an important predisposing factor for OSA, also was associated with greater risk of developing heart failure.

OSA can also lead directly or indirectly to increased risk of stroke. The evidence for sleep apnea as a risk factor for primary ischemic stroke is mostly inferential and derives from evidence implicating sleep apnea in hypertension and heart disease, both of which are risk factors for stroke. Mechanisms that have been implicated in any increased risk of stroke in OSA include BP swings, reduction in cerebral blood flow, altered cerebral autoregulation, impaired endothelial function, accelerated atherogenesis, and prothrombotic and proinflammatory states (Somers et al., 2008).

OSA can contribute to myocardial ischemia and infarction. Severe intermittent hypoxemia, acidosis, increased BP, and sympathetic vasoconstriction, in conjunction with simultaneous changes in intrathoracic and cardiac transmural pressures, all argue compellingly for obstructive apneas as a potential trigger for cardiac ischemia. In the longer term, the cardiac and vascular disease mechanisms, including endothelial dysfunction and systemic inflammation, may promote structural coronary artery damage (Somers et al., 2008).

2.4 Effects of Obstructive Sleep Apnea

Preceding these diseases is systemic inflammation, endothelial dysfunction, oxidative stress, thrombosis, intrathoracic pressure changes, and insulin resistance. Essentially, the stress on systems caused by frequently interrupted breathing can lead to long-term dysfunction, particularly in the cardiovascular system.

2.4.1 Obstructive Sleep Apnea & Hypertension

OSA is a recognized cause of secondary hypertension, which is high blood pressure that is caused by another medical condition (Dopp et al., 2007). Different percentages of OSA patients with hypertension have been reported, with the lowest reporting that 50% of OSA patients are hypertensive (Somers et al., 2008) while others have reported as high as 83% (Logan et al., 2001). Additionally, an estimated 30% of hypertensive patients have OSA (Somers et al., 2008). In most normal individuals, as well as untreated and treated hypertensive patients, blood pressure slowly decreases by about 20% while sleeping and increases to normal daytime levels upon awakening (Littler et al., 1975). Conversely, patients with sleep apnea and others who snore heavily have high blood pressure throughout the night (Dopp, 2007).

Several studies have found that OSA is linked to hypertension. According to Ruttanaumpawan and coworkers (2009), OSA increases the risk for developing hypertension. Moreover, Budhiraja and colleagues (2007) reported that OSA is associated cardiovascular diseases such as hypertension and coronary artery disease. Suffering from a sleep related breathing disorder is even a risk factor for poor blood pressure control in younger hypertensive patients $\leq$ 50 years of age (Grote et al., 2000).

Multiple factors contribute to the prevalence of hypertension in people with OSA. In general, apneas lead to several hemodynamic changes: (1) wide range fluctuations in intrathoracic pressure to as low as -90 cm H_2O; (2) gradual decreases in arterial blood oxygen and increased carbon dioxide levels, which stimulates peripheral chemoreceptors (Shepard, 1985); (3) decreased stroke volume; (4) increased afterload; and (5) a boost of sympathetic activity to vascular smooth muscle, leading to vasoconstriction in some vascular beds (Hedner, 1988). With normal breathing resumption, the normalization of heart rate, right ventricular preload, and left ventricular afterload probably contributes to a surge in cardiac output. When the larger blood volume enters the constricted peripheral blood vessels, an acute increase in blood pressure results.

Intermittent hypoxemia is thought to be the most important prohypertensive factor. Although the mechanisms underlying OSA-related hypertension are not fully understood, the current concept suggests that the sympathetic nervous system and the renin-angiotensin system alter vascular function and structure, resulting in blood pressure elevation. The repetitive intermittent hypoxemia triggers sympathetic nervous system activities which lead to an increase in systolic and diastolic pressure that keeps mean blood pressure levels elevated at night. In many OSA patients, the blood pressure remains elevated during the daytime even with normal breathing (Dopp, 2007).

In particular, OSA has been shown to be correlated with pulmonary artery hypertension. The most likely primary mechanism for any OSA-related pulmonary arterial hypertension is hypoxemia, which is known to reflexively induce an acute increase in pulmonary arterial pressure (Somers et al., 2008). However, there is debate as to whether OSA can be considered a primary cause of sustained pulmonary arterial

hypertension (Somers et al., 2008). Two confounding factors that may contribute to daytime hypoxemia are severe obesity (obesity-hypoventilation syndrome) and chronic obstructive pulmonary disorder in association with OSA (the so-called overlap syndrome) (Somers et al., 2008).

The problem with some research showing an association between OSA and hypertension is they do not adjust for other risk factors (Logan et al., 2001). Studies that have adjusted for factors such as BMI have found mixed results. For example, a longitudinal study of 709 participants observed an association between OSA and of hypertension that was independent of other known risk factors, including BMI after four years of follow-up (Peppard et al., 2000). However, another longitudinal study by O'Connor et al. (2009) found that after adjusting for BMI, OSA was not a significant independent risk factor for hypertension. Moreover, Cano-Pumarega and associates (2011) found no association between OSA and systemic hypertension in the middle-aged population after adjusting for confounding factors including age, sex, body mass index, neck circumference, fitness level, and consumption of alcohol, tobacco, and coffee.

2.4.2 Obstructive Sleep Apnea & Endothelial Function-Dysfunction

One known early biomarker of cardiovascular disease resulting from OSA, as well as several other factors, is the inability of blood vessels to regulate vascular tone (i.e. the ability to vasodilate and/or vasoconstrict in response to a specific stimulus). Since the blood vessels of the body are lined with endothelial cells, this change in vascular responsiveness is known as endothelial dysfunction and has been associated with poor vascular health and in general, a poor prognosis unless immediate interventions are undertaken by the patient.

Endothelial cell dysfunction has been identified as one of the earliest identifiable biomarkers demonstrating the presence of underlying pathophysiology for cardiovascular disease (Furchgott, 1983). The vascular endothelium is an important regulatory organ that is involved in maintaining cardiovascular homeostasis and implicated in developing several cardiovascular disorders. Normally, the endothelium maintains the following functions in the vascular tissue: tone of the underlying smooth muscle, provide a non-adhesive luminal surface, cellular homeostasis, cellular proliferation, and inflammatory and immune mechanisms (Rubanyi, 1993). Endothelial cells regulate the tone of the vascular smooth muscles by responding to both vasodilatory and vasoconstricting factors that are released by the endothelial cells or other tissues involved in the regulation of vascular tone.

However, injury of the endothelium can lead to endothelial dysfunction, which is characterized by an imbalance in the production of vasoactive hormones, increased adherence of inflammatory mediators to endothelial cells and hypercoagulability (Budhiraja et al., 2007). When left undiagnosed, endothelial dysfunction progress undetected for many years causing considerable damage to the endothelium thereby contributing to very serious cardiovascular disorders (Budhiraja et al., 2007). While many factors may contribute to the onset of endothelial dysfunction, the progression of disease will lead to a lower production endothelium-dependent vasodilators, of which nitric oxide (NO) may be the most important (Widlansky et al., 2003).

The finding of several studies have now linked OSA to endothelial dysfunction suggesting that there may be direct causal relationship between OSA and the progression of cardiovascular disease (Ip et al., 2004; Nieto et al., 2004). Mary and colleagues (2003)

measured the vascular endothelial function in patients with OSA and found that most men with moderate to severe OSA have endothelial dysfunction, and, furthermore, that treatment with CPAP could reverse the abnormalities. Similarly, it was reported that OSA is associated with endothelial dysfunction, although these associations were lower after other cardiovascular risk factors, particularly after adjusting for differences in the body mass index (Nieto et al., 2004). However, the association observed was statistically significant even after adjustment for body mass index and other risk factors. Nieto and colleagues (2004) also reported that the associations were appreciably stronger among individuals who were younger than 80 years and among those who were hypertensive.

2.4.3 Oxidative Stress & Endothelial Function-Dysfunction

In order for the endothelium to function optimally, there must be a balance between pro-oxidants and anti-oxidants conditions. Oxidative stress occurs when the level of pro-oxidants outweighs that of anti-oxidants, which is known to occur in atherosclerotic populations. The reverse of this, having greater anti-oxidative forces than pro-oxidative forces, can also be problematic, as research has shown that young, healthy individuals who ingest antioxidants display a decreased vasodilatory effect compared to no antioxidant supplementation, further supporting the evidence that a certain level of pro-oxidative forces is necessary for ideal functioning (Donato et al., 2010; Wray et al., 2012)

Oxidative stress is an imbalance between antioxidant defenses and the production of reactive oxygen species (ROS), also known as free radicals (Betteridge, 2000). ROS are highly chemically reactive molecules that react with nucleic acids, lipids, and proteins and obstruct cellular metabolism, resulting in cell injury (Lavie, 2003). When two

radicals react with each other, they produce a non-radical product. When a radical molecule reacts with a non-radical molecule, the product of this reaction is a new radical, thus circulating radical chain reactions. When electrons are transferred from one molecule to another, reduction and oxidation reactions occur. Since ROS are by-products of normal oxygen metabolism and are usually created during normal cellular respiration, anti-oxidant systems are responsible for eliminating these excess free radicals. When ROS production exceeds the antioxidant mechanism's capacity to eliminate the ROS, oxidative stress and damage to cells and tissues occur (Lavie, 2003).

This process of imbalance contributes to many untreated cardiovascular diseases. Free radical leakage from mitochondria during cellular respiration is the main source of ROS generation, and it is estimated that about 5% of the oxygen consumed is transformed into ROS under normal conditions (Lavie, 2003). During hypoxia, as PO_2 decreases, ROS production increases due to excessive mitochondrial reduction (Dureanteau et al., 1998; Sanders et al., 1993). Therefore, the excessive ROS that is produced during hypoxia will lead to injury to the surrounding cellular components.

Lavie (2003) reported that exposure of leukocytes to hypoxia leads to increased production of ROS that have far reaching implications to cardiovascular function. In addition, increased ROS production by OSA monocytes under basal conditions will probably contribute to a constant increase in oxidative metabolism and oxidative stress in OSA patients (Lavie, 2003). Similarly, Schulz et al., (2002) found a significant increase in ROS production in patients with OSA. Conversely, results from Schulz and Mahmoudi (2000) demonstrated that the application of CPAP therapy lead to a decrease of ROS production in patients with OSA, suggesting that maintaining airflow and

preventing the intermittent cessations in breathing attenuated the production of ROS. Furthermore, antioxidants such as ascorbate and allopurinol improve OSA, especially vasoreactivity (El Solh et al., 2006; Grebe et al., 2006), which suggests that oxidative stress may contributes significantly to endothelial dysfunction which then may exacerbated by the OSA condition.

2.4.4 Shear Stress & Endothelial Cell Function-Dysfunction

Hemodynamic forces that are sensed by the vascular wall include circumferential stress and shear stress. Circumferential stress is associated with pulse pressure and is applied to each layer within the vessel wall with cyclic changes during the cardiac cycle, while shear stress is the tugging or viscous force each layer of blood applies to the next, affecting only the endothelium.

As the suspension of red blood cells in plasma flows within a blood vessel, parabolic flow is created where the red blood cells travelling closest to the vessel wall flow at a considerably slower rate than those red blood cells towards the center of the lumen. This results in multiple layers of red blood cells flowing past each other at different velocities due to frictional forces, referred to as shear rate. Since the blood vessel walls form the boundary for the flowing blood, this produces a mechanical force or shear stress that acts on the endothelial cells lining the walls of the blood vessel (Fåhraeus & Lindqvist, 1993). When there is an increase in blood velocity (i.e., an increase in blood flow) through the blood vessel, there is also an increase in shear stress that initiates a cascade of reactions ultimately resulting in the release of NO from the endothelial cells. The release of NO causes the blood vessels to vasodilate to reduce the shear stress acting on the vascular walls (Joannides et al., 1995).

Shear stress has both a magnitude and a direction (Lipowsky et al., 1995). Its magnitude is directly associated with blood flow and the viscosity of blood and is inversely proportional to its radius. As a result, smaller blood vessels with high flow rates experience a greater shear stress compared to larger diameter vessels with lower flow rates. Because blood flow rate through the arterial and venous systems are not similar, the exposure of arteries and veins to shear stress is also quite heterogeneous. Normally, arterial shear stress is within the range of 10-70 dynes/cm^2, while normal venous shear stress falls within 1-6 dynes/cm^2 (Lipowsky et al., 1995; Malek, Alper, & Izumo, 1999).

Importantly, shear stress within the normal arterial range (10-70 dynes/cm^2) stimulates the production of NO (Ballermann, Dardik, Eng, & Liu, 1998; Ross, 1993; Sumpio, Riley, & Dardik, 2002; Walpola et al., 1995), all while impairing the proliferation of smooth muscle cells and the development of atherogenesis, thrombosis, leukocyte adhesion, and vascular remodeling (Ross, 1993).

Shear stress has been shown to promote vascular health, as evidence indicates that when endothelial and vascular smooth muscle cells experience normal shear stress, they exhibit low rates of proliferation (Cooke et al., 1991). The endothelium responds to shear stress by promoting a complex series of reactions that induce biochemical responses, like vasodilation, to occur in the vasculature. Acute shear stress is associated with short-term increases in blood flow (Davies et al., 1992). When exposed to frequent elevations in shear stress, vascular remodeling occurs, increasing the thickness of the vascular wall and the lumen of the vessel in order to return shear stress back to normal and inhibit the proliferation of vascular smooth muscle cells and leukocyte adhesion and migration

(Frangos, Eskin, McIntire, & Ives, 1985; Helmlinger, Berk, & Nerem, 1995; Malek, Jackman, Rosenberg, & Izumo, 1994; Nagel et al., 1994; Rubanyi, Romero, & Vanhoutte, 1986). Additionally, shear stress promotes endothelial-derived production of NO (Cooke et al., 1991), and it appears to be crucial for vasodilation that occurs as a result of PLM (Trinity et al., 2012), which is a method for assessing NO-mediated vascular function.

2.4.5 Role of Nitric Oxide in Endothelial Function-Dysfunction

NO is derived in the endothelium from L-arginine, and it is catalyzed by the enzyme endothelial NO synthase (eNOS) (Vallance, 2001). This reaction can only occur, however, if the protein cofactor tetrahydrobiopterin (BH4) is present in the endothelial cell. Once NO is produced, it must diffuse to the vascular smooth muscle cell and activate NO-sensitive guanylyl cyclase, signaling a cascade of events that induces vascular smooth muscle cell relaxation.

Tetrahydrobiopterin is a component of all NOS isoforms, and decreased BH4 has been implicated in disease states including hypertension, and atherosclerosis. In these disease states, NO production is inhibited, leading to vascular dysfunction, despite normal or even elevated levels of eNOS (Li, Wallerath, & Forstermann, 2002). Decreased NO production could be due to eNOS uncoupling, in which eNOS induces superoxide radical formation instead of NO, possibly because of the reduced content or absence of BH4 (Förstermann & Münzel, 2006; Schmidt & Alp, 2007). Superoxide is a NO scavenger, and when NO and superoxide react with each other, peroxynitrite is formed, which can uncouple eNOS either directly or by reducing BH4. Vitamins C and

E, as well as folic acids and statin drugs are able to increase BH4 levels (Förstermann & Münzel, 2006; Moens & Kass, 2007; Schmidt & Alp, 2007).

It has been shown that in conduit arteries, NO is the primary endothelium-dependent vasodilator. NO provides several benefits to the vasculature in addition to its role regarding the regulation of vascular tone through the induction of vascular smooth muscle cell relaxation. By reducing the accumulation of adhesion molecules, inflammation, vascular smooth muscle cell migration, and platelet aggregation (Anderson, 2003), endothelial dysfunction may be attenuated thereby affording greater protection from the development of cardiovascular disease.

NO serves a regulatory function in most tissues, and the balance between the production and reduction of NO determines its bioavailability (Williams, Wheatcroft, Shah, & Kearney, 2002). It is the most potent vascular relaxing factor and an intracellular signaling molecule (Lavie, 2003). Therefore, NO is involved in the various causes of obstructive sleep apnea (OSA), whether developmental, infectious, medicinal, hormonal, metabolic, or other, as well as in its immediate mechanism, the collapse of the upper airway, and in its complications, both short and long term (Haight et al., 2003). Limited availability of oxygen significantly lowers NO output which will impact airflow significantly. The results of studies by Schulz et al., (2000) and Ip et al., (2000) both demonstrated that NO levels increased after CPAP treatment, which supports the hypothesis that the reduced endothelium-dependent vasodilation in OSA patients resulted from a decrease in the bioavailability of NO.

2.4.6 Systemic Inflammation & Endothelial Function-Dysfunction

Cross-sectional and prospective studies have implicated OSA as an important causal factor in the development of cardiovascular disease (Peker et al. 2016). The mechanisms underlying the association between OSA and cardiovascular disease are not fully understood. Multiple causal factors leading to vessel wall damage and development of atherosclerotic plaques have been proposed, including reflex sympathetic activation and consequent increases in blood pressure, endothelial dysfunction and systemic inflammation (Ip et al., 2004). Inflammation plays a central role in the initiation and progress of atherosclerosis and has been shown to be involved at the onset of adverse clinical vascular events when activated cells within an atherosclerotic plaque secrete proteases that degrade the fibrous cap, leading to rupture of the plaque and thrombus formation (Tedgui et al., 2006).

The effect OSA has on systemic inflammation is important because systemic inflammation has been known to contribute to pulmonary hypertension. Endothelial cells play a major role in inflammation and immune reactions, and subsequently inflammatory cytokines cause endothelial dysfunction. Endothelial dysfunction is a hallmark of pulmonary hypertension (Mathew, 2010). The prevalence of pulmonary hypertension in patients with systemic inflammatory diseases is well documented (Mathew, 2010). Systemic inflammation, sympathetic activation, pressor surges, and oxidative stress all contribute to the development of endothelial dysfunction (Somers et al., 2008). Two major, and to some extent overlapping, mechanisms have been proposed to explain the morbid consequences of OSA, namely increased generation and propagation of reactive

oxygen species and initiation and amplification of inflammatory processes (Gozal & Kheirandish-Gozal, 2008).

2.4.7 Assessment of Endothelial Function/Dysfunction

The assessment of endothelial function is important in both clinical and research settings and may prove to be a very useful measure of the effectiveness of OSA treatments. Two methods for assessing endothelial function is flow-mediated dilation (FMD) and passive leg movement (PLM)

In both clinical as well as research settings the ability of the blood vessel wall to vasodilate in response to a sudden increase in blood velocity acting on the endothelial cells (i.e., an increase in shear stress) can be measured using FMD. Endothelial function is commonly examined by FMD following blood flow occlusion. It has been demonstrated that as shear stress increases, eNOS catalyzes the reaction from L-arginine to L-citrulline and NO is activated. Once NO is produced, it diffuses to the vascular smooth muscles surrounding the blood vessels where a series of reactions are initiated ultimately resulting in the release of calcium (Ca^{2+}) from the smooth muscle cells causing relaxation (i.e. vasodilation).

The results of previous studies have demonstrated that the production of NO from this reaction leads to the increase in arterial diameter or FMD response and that the magnitude of the response is proportional to the bioavailability of NO (Dakak et al. 1998; Joannides et al. 1995; Pohl and de Wit 1999). Therefore, the prognostic value of measuring the endothelial response to a sudden increase in shear stress in the early detection of vascular disease has been shown to be a good, non-invasive measure of endothelial or vascular health. Given that CVD resulting from OSA is an insidious

disease that develops over time without the appearance of any signs or symptoms, the identification of early biomarkers for CVD is essential to the prevention of this disease because it allows for the assessment of risks and early intervention thereby preventing the deadly disease from progressing.

In the past, other tests like intracoronary infusions have been used to assess cardiovascular function and risk; however, given the invasiveness of the assessments, these tests are impractical for widespread clinical application and not possible in most research settings. As mentioned above, the FMD technique has been used successfully and routinely to assess endothelial function in both clinical as well as research settings. Since the FMD technique is a non-invasive procedure, it is highly preferred over previous methods, but the reliability or specificity of the technique is controversial due to the precise, complex measurements that are required for an accurate assessment of vascular function (Rossman et al., 2016). For this reason, a new approach that utilizes PLM has recently been proposed as an early diagnostic tool for identifying endothelial function or dysfunction (Trinity et al., 2012; Mortensen et al., 2012).

While both FMD and PLM techniques are non-invasive procedures, PLM is preferred over the FMD approach for a number of reasons. First, the FMD technique requires the continuous recording of changes in vessel diameter, a skill that requires considerable experience and specialized analytical software. The PLM approach is gaining favor because the method measures changes in blood flow in the femoral artery which does not vasodilate appreciably making this a more stable measurement. This means that the precise measurements of the arterial diameter are not required continuously throughout the entire PLM protocol (Rossman et al., 2016). Second, the

PLM technique has no known discomforts whereas the FMD techniques requires the use of a vascular occlusion cuff that may cause subjects considerable discomfort during the cuff occlusion period. Finally, another benefit of PLM is that it is applied in the lower limb, which is at a greater predisposition to develop vascular disease in humans because of the change in activity patterns and regional changes in blood flow as one ages (Groot et al., 2016). By testing PLM in the lower limbs, not in the brachial artery like the FMD approach, PLM may serve as an even earlier indicator of the onset of vascular disease and, thus, may lead to earlier interventions and improved overall mortality rates. These factors make the PLM test more accessible and could make it very practical for clinical use as well as research settings.

Indeed, the results of preliminary research have shown that using PLM to assess endothelial function is very useful in identifying endothelial dysfunction. In 2016, a study by Rossman et al. (2016) compared the results obtained using FMD with those measured using PLM and demonstrated that the results were very similar. In addition, Research shows that in healthy individuals, PLM leads to increases in limb blood flow without increases in skeletal muscle metabolism (Groot et al., 2015). According to Mortensen and coworkers (2012), PLM is associated with a ~3-fold increase in leg blood flow, but the underlying mechanisms remains unknown. It has also been shown that following passive movement in healthy individuals, blood flow increases, which can trigger additional central hemodynamic changes, including an increase in heart rate and cardiac output (Groot et al., 2015).

However, for populations with cardiovascular disease, PLM does not increase limb blood flow, making the PLM technique an indicator of vascular health similar to the

more traditional FMD test (Rossman et al., 2016). Trinity et al. (2015) and Groot et al. (2015) used PLM to examine the impact of age on vascular function and found that age is associated with reduction in PLM-induced limb blood flow in the elderly. Furthermore, Mortensen et al. (2012) found PLM did not increase leg blood flow in individuals with peripheral arterial disease.

It has been suggested that PLM leads to an increase limb blood flow by increasing shear stress and by passively stretching the muscle tissue, both of which can stimulate NO formation (Cheng et al., 2009). Boushel and coworkers (2002) has also suggested that the blood flow response to PLM is mediated by an increase in NO acting on the vascular endothelium. Similarly, Hellsten and colleagues (2008) found that PLM enhanced limb blood flow and increased endothelial cell proliferation. Thus, the PLM technique may prove to be very useful in diagnosing endothelial dysfunction in patients with OSA and for monitoring the response to various therapeutic treatments.

2.5 Treatments for Obstructive Sleep Apnea

Treatments for sleep apnea include lifestyle changes and CPAP in most cases, and surgery in extreme cases. The appropriate treatment depends on the type and severity of the sleep apnea.

2.5.1 Lifestyle Changes

According to the National Heart, Lung, and Blood Institute (NHLBI, 2011), a division of the National Institute of Health (NIH), if a patient exhibits the signs and symptoms of mild sleep apnea, alterations in their activities of daily living or daily

activity patterns might be all the treatment that patient needs in order to resolve their symptoms. The suggested changes in lifestyle include but are not limited to such things as, avoiding alcohol and medicines that make the patient sleepy, losing weight if the person is overweight or obese, sleeping on the side instead of the back to help keep the airway open, keeping nasal passages open at night with nasal sprays or allergy medicines, and quitting smoking, if they are smokers. If caught early and in a mild state, these lifestyle changes can prevent worsening of the condition and the need for more serious treatments.

2.5.2 Surgical Interventions

For some individuals who suffer from obstructive sleep apnea, they will benefit by having surgery. The type of surgery and how well it works will depend on the cause of the sleep apnea. For example, surgeries that are performed in order to widen the airways and breathing passages are typically beneficial to some patients with OSA. The actual procedure typically involves either shrinking, stiffening, or removing excessive tissue in the mouth and throat areas or it may involve resetting the lower jaw into a position that improves airflow while sleeping. Surgery to shrink or stiffen excess tissue is done in a doctor's office or a hospital. Shrinking tissue may involve small shots or other treatments to the tissue and often requires multiple visits for the procedure to be successful in shrinking the excessive tissue. In order to stiffen the excess tissue, the surgeon makes a small incision in the tissue and inserts a piece of stiff plastic. Surgery to remove excess tissue is done in a hospital. Patient is given medicine to help sleeping during the surgery. After surgery, patient may have throat pain that lasts for 1 to 2 weeks (NLHBI, 2011).

The challenge that confronts the surgeon is determining which part of the upper airway is causing the obstruction to airflow. There are many possible sites, and conventional sleep testing does not identify the area the surgeon should modify. If the surgeon does not treat that site in the airway, or if there are multiple sites of obstruction, it is unlikely that the sleep apnea will diminish to a degree that eliminates the need for other treatment.

2.5.3 Continuous Positive Airway Pressure

The most common treatment for OSA is CPAP (Kribbs et al., 1993). The effect of CPAP on systemic inflammation in OSA has generated mixed results in the literature. One study found four weeks of CPAP treatment had no beneficial effect on blood markers of inflammation and adiponectin in patients with moderate–severe OSA (Kohler et al., 2008). However, four months of CPAP treatment for OSA significantly improved early signs of atherosclerosis in Luciano et al., (2007), supporting the concept that OSA is an independent risk factor for atherosclerosis. Studies on this topic are limited, so it remains to be seen if the duration of CPAP treatment or the different measures of systemic inflammation were the critical factors in the different findings.

Although some studies have cast doubt on the relationship between OSA and hypertension, some of the most convincing evidence is the fact that treating patients with OSA can lead to significant decreases in symptoms associated with the hypertensive condition. Evidence from clinical trials have demonstrated the link between OSA and hypertension by showing that there is reduction in blood pressure after the nasal CPAP treatment (Giles et al., 2006; Martínez-García et al., 2013). Likewise, the use of CPAP treatments in patients with moderate to severe OSA also significantly reduced arterial

blood pressure during the day and at night (Pepperell et al., 2001; Becker et al., 2003). Even more convincing evidence comes from a meta-analysis conducted by Iftikhar et al. (2014) that found CPAP treatment in patients with resistant hypertension and OSA reduced blood pressure after 24 hours. The pooled estimates from six studies show a favorable reduction of BP with CPAP treatment in patients with resistant hypertension and OSA. The effects sizes are larger than those previously reported in patients with OSA without resistant hypertension (Iftikhar et al., 2014).

While CPAP is generally considered to be the most effective and the current primary treatment for OSA, Ferguson et al. (1996) stated that long-term use of CPAP in patients with OSA is 50 to 80%, and less symptomatic patients are more likely to discontinue treatment. In addition, even among OSA patients who report the use of the CPAP treatment, Kribbs et al. (1993) reported that average usage is less than 50% of the night. Patients who are suffering from OSA sometimes discontinue CPAP therapy due to undesirable side effects include; anxiety, insomnia, and nasal discomfort. Therefore, alternative treatments that are safe, effective, and acceptable are needed.

2.5.4 Oral Appliance

Another treatment for OSA is an oral appliance mouthpiece, also known as a dental appliance. The oral appliance treatment approach is simple, reversible, and cost-effective treatment option for simple snoring and OSA. According to Ferguson and colleagues (1996), the use of an oral appliance is an effective treatment in some patients with mild to moderate OSA and has greater patient satisfaction than using CPAP. Similarly, Schmidt and associates (1995) recommended oral appliance therapy to be used for the treatment of mild OSA. Oral appliances are an appealing therapy because they are

easy to use, portable, more easily tolerated while sleeping and generally have no side effects (Ferguson, 2003). There are two main appliance groups in commonly used; (i) tongue repositioning device and (ii) a mandibular repositioning appliance (Ferguson, 2003). According to Schmidt and coworkers (1991), oral appliances are an effective treatment for the symptoms of snoring and can effectively treat OSA of moderate severity. Ferguson and associates (1996) reported that oral appliances have fewer side effects and greater patient satisfaction than nasal CPAP in mild-moderate OSA patients.

2.5.5 Supplements (Vitamin C, Antioxidant) & Obstructive Sleep Apnea

One alternative approach to prevent some of the consequences of oxidative stress and endothelial dysfunction in people with OSA is vitamin C. Vitamin C has been reported to improve endothelial function in many diseases, including diabetes mellitus, hypercholesterolemia, essential hypertension, and congestive heart failure, which are known to be associated with an increased oxidative stress (Ellis et al., 2000). Timmi et al., (1998) reported that the administration of vitamin C restored impaired endothelium-dependent vasodilation in patients who were diagnosed having insulin-dependent diabetes mellitus. Because OSA is linked to oxidative stress (Mathias et al., 2006), antioxidants, like vitamin C, have the potential to attenuate endothelial dysfunction in OSA patients by reducing the production of ROS and decreasing the amount of cellular damage induced by the free radicals (Barcelo, 2006). According to Harris and associates (2009) in healthy individuals, an antioxidant cocktail of vitamins C and E and α-lipoic acid elevated plasma ascorbate levels (~95%) and decreased ROS concentration (~65%) compared to baseline measurements. Mathias et al. (2006) reported that the intravenous injection of the antioxidant vitamin C improved endothelial-dependent vasodilation in

untreated patients with OSA, which supports the hypothesis that endothelial dysfunction in patients with OSA is linked to oxidative stress. Moreover, Taddei et al. (1998) reported that infusion of intrabrachial vitamin C improved endothelial vasodilation in hypertensive patients suggesting that the administration of vitamin C may prove beneficial in patients with OSA and also suffer from CVD.

2.6 Summary

PLM has been shown to be an effective diagnostic tool for endothelia dysfunction, but it has not yet been used as a diagnostic tool for OSA specifically. Moreover, intravenous injections of vitamin C have been shown to reduce oxidative stress in patients with OSA. However, to date, no study has been conducted on the effectiveness of oral administration of vitamin C on endothelial function of OSA patients. To address these gaps in the literature, the study outlined in the following chapter describes the methods used to test the feasibility of using PLM to diagnose OSA as well as the effectiveness of an oral vitamin C intervention in reducing endothelial dysfunction in subjects with OSA.

Chapter 3

Methodology

This chapter explains the methodological details of the study, including subjects, instrumentation, data collection procedures, and data analysis procedures. These methods are designed to answer the research questions, is the PLM approach a technical diagnostic tool to distinguish between healthy subjects and subjects with OSA? The experimental design was also aimed at answering the question, does the oral administration of an acute dosage of vitamin C attenuate endothelial dysfunction in subjects identified as having OSA?

3.1 Subjects

Subjects included two groups: healthy subjects (HEAL) and obstructive sleep apnea subjects (OSA). Thirteen healthy male subjects aged 18-55 years were recruited to participate in this study. Likewise for the OSA group, 13 male subjects 18-55 years old were also recruited to participate in this study. To achieve a sufficient number of participants for this study, the sampling and recruitment method was purposeful and non-random. Subjects for both groups were recruited using flyers placed throughout the main campus of the University of Toledo and by making oral announcements during classroom

visits. Potential subjects were excluded from participating in this study who were matched any of the following criteria including, having any known cardiovascular disease, any underlying metabolic disease, diagnosed with having high blood pressure (defined as a systolic blood pressure of ≥140 mm Hg and/or diastolic blood pressure of ≥90 mm Hg), currently smoke, or having any known pulmonary diseases, besides OSA. Subjects were asked to refrain from performing any moderate to vigorous physical activities while enrolled in this study and to avoid caffeinated beverages on the days they being tested. All subjects were informed of the testing procedures and any risks or discomforts associated with the testing procedures. This study was approved by the Human Subjects Committee of the Institutional Review Board at the University of Toledo, where all testing was conducted. This study was carried out in accordance with the Declaration of Helsinki.

3.2 Experimental Protocol

All testing was performed in a quiet, temperature-controlled room (22-24ºC) with the ambient lighting reduced for optimal visualization of the borders of the arterial walls and the blood velocity profile. Individuals who volunteered to participate in this study reported to the Cardiopulmonary and Metabolism Research Laboratory on the main campus of The University of Toledo and received a summary of the investigation and an explanation of all testing and procedures utilized in this study. Following the overview of the procedures, those individuals willing to participate in the study, reviewed and signed the informed consent form and completed a medical history questionnaire. In

addition, a standardized self-report sleep apnea questionnaire (details provided below) was administered by the same investigator to confirm the presence of OSA.

For each subject, the study visits were divided into two sessions, the preliminary session where the subjects were the study objective and protocols were once again explained to them and then were taken through a shortened practice trial so that the subject could become accustomed to the passive movement protocol. The familiarization visit was followed by the intervention session, where the PLM trials took place under control (CON) conditions and following administration of vitamin C (Vit C) for both the healthy (HEAL) and subjects identified as having obstructive sleep apnea (OSA). This study did not include any exercise, and therefore the time between the familiarization and intervention visits to the laboratory for a given subject varied between 3 and 5 days.

3.2.1 Familiarization Visit

During the familiarization visit, demographic (age, sex, and ethnicity) and anthropometric (height and weight) data were measured and recorded for the HEAL and OSA groups. In addition, resting heart rate, blood pressure and respiratory rate were measured in the HEAL and OSA groups. All of the measurements were collected before and after the administration of Vit C with the exception of demographic data, which was only collected once at the beginning of the study.

Once the demographic and anthropometric data were collected, the subject was seated in the upright position in isokinetic dynamometer that would perform the passive movements of the lower limb. The chair was set in a semi-reclined position to 120° for subject comfort as well as to provide access to the area on the upper leg just distal to the inguinal ligament. The chair was then moved forwards or backwards and the height of

the seat adjusted so that the rotation around the knee joint aligned with the axis of the power head of dynamometer. The position of the seat was recorded for each subject and was returned to same position for subsequent testing for each of the subjects. During the familiarization session, the subjects were taken through a series of short trials so that they would learn to let the dynamometer perform the work of moving their lower limb through the range of motion without providing any force development and/or resistance to the movement during the motion. In addition, the investigator, who did all of the femoral artery blood velocity and diameter measurements for the study, could perform an initial assessment (i.e. probe placement) on each of the subjects. Using this approach, both the subjects and the investigator gained familiarity with the measures being made during the intervention trial and it also ensured that a clear image of the femoral artery border walls and blood velocities could be achieved throughout rest and passive movement phases of the protocol.

3.2.2 Intervention Visit

During the intervention visit, each subject performed two trials, each performed on the same day. The first visit was the pre-intervention trial or CON condition and the second trial was the post-intervention trial or the Vit C condition which was performed 2 hours following the administration of the vitamin C. Upon arrival to the laboratory, the subject was seated on an isokinetic dynamometer (Biodex System 2, Biodex Medical Systems, Shirley, NY) at rest for at least 15 min to ensure blood flow, heart rate, and blood pressure were at baseline values. Once the subject was seated comfortably and rested for an additional 10 to 20 min, femoral artery blood velocity (FABV) and the diameter of the femoral artery (FAD) were measured using Doppler ultrasound. Before

the onset of PLM, stable hemodynamics were documented as well as the measurement of the femoral artery diameter. Following the initial rest period, data collection began; resting measures were recorded for 30 s prior to the onset of PLM which lasted for 2 min followed by 3 min of rest resulting in a total time of 5 min and 30 s for the whole trial.

The PLM was performed using the Biodex dynamometer, which passively flexed and extended the lower limb at the knee through a 90° range of motion. The starting position of the leg was set at 180° of extension of the knee. The first movement served to passively flex the knee from 180° to 90° degrees of motion which was repeated at at 0.5 Hz (30 repetitions/min). Before the start of the trial and throughout the remainder of the protocol, the subjects were reminded to remain relaxed and allow the isokinetic dynamometer to move the lower limb without providing and muscle tension or force development. Also, subjects were notified (at 1 min and 10 s) prior to the onset of the passive movement to minimize an potential for an anticipatory response, but they were not instructed at the moment the passive movement began.

Following the completion of the initial PLM trial, each subject was provided with the equivalent of 1 g of Vit C and were instructed to ingest the capsules with approximately 8-10 oz of water prior to leaving the Cardiopulmonary and Metabolism Research Laboratory. Each subject returned to the laboratory 90 min following ingestion of Vit C and prepared for the second trial which was initiated 120 min after the ingestion of Vit C. All participants were instructed to refrain from any physical activity between the Con and Vit C trials; all subjects either rested in the laboratory or attended lectures during the period between trials. Once the subject returned to the laboratory after ingesting the Vit C, the subjects were seated on isokinetic dynamometer, prepped for data

collection and then rested quietly for no less than 15 min to ensure that any elevations in femoral artery blood flow, heart rate, or blood pressure had returned to baseline values. Following the accommodation period, the subject performed the same PLM protocol as they had performed during the first intervention trial.

3.3 Experimental Measurements

3.3.1 Central Hemodynamic Variables

Resting heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automated finger plethysmography system (Finometer Model 1, Finapres Medical Systems BV, Arnhem, The Netherlands) that continuously measured each variable throughout the duration of the protocol. A small blood pressure cuff was placed around the middle or index finger of the left hand to perform continuous monitoring of the arterial waveform. A blood pressure cuff was placed around the left upper arm for the measuring arterial blood pressure using the traditional approach. The arterial blood pressure waveform measured at the finger was corrected to the blood pressure measured at the brachial artery using the conventional return-to-flow method for establishing systolic and diastolic blood pressures. The difference in the hydrostatic column between the hand and the brachial artery (position at the height of the heart) was automatically corrected by placing a probe at the level of the heart. The beat-by-beat arterial pressure waveform was used to calculate mean arterial pressure (MAP) according to the equation,

$$MAP = DBP + (1/3 \cdot (SBP - DBP))$$

Using the continuous arterial blood pressure waveform, beat-to-beat values for stroke volume (SV) and cardiac output (CO) were simultaneously calculated using the Modelflow software (Beatscope 1.1, Finapress Medical Systems BV, Arnhem, The Netherlands). The Modelflow approach has been reported in detail elsewhere (Wesseling et. al., 1993) but briefly, this approach uses three elements (impedance of the aorta, total arterial compliance, and peripheral vascular resistance) to model aortic input impedance to derive an aortic flow waveform from the arterial pressure wave measures at the finger and corrected to arterial pressure at the level of the heart. Based on the subject's gender, age and a series of complicated calculations, the area under the curve for aortic flow waveform is determined beat-by-beat providing an estimate for left ventricular stroke volume. Thus, CO can be calculated using the product of HR and SV for each minute. Total peripheral resistance (TPR) was calculated as according to the equation,

$$TPR = MAP/CO$$

where MAP is mean arterial pressure, CO is cardiac output.

3.3.2 Femoral Artery Blood Flow & Diameter

During each PLM test, femoral artery blood velocity (FBV) and femoral artery diameter were measured using a two-dimensional ultrasound sonography system with a multi-frequency linear array probe (Model L8-2; center frequency of 7.5 MHz) attached to an ultrasound system operating in Duplex mode (z.one ultra, Zonare Medical Systems Inc., Mountain View, CA). The ultrasound probe was placed just distal to the inguinal ligament and proximal to the bifurcation of the superficial and deep femoral arteries. Longitudinal images of the femoral artery diameter were obtained while at rest while blood femoral artery blood velocity (FABV) was measured at rest and during passive

movement of the lower limb. The transducer probe was positioned on the skin over the femoral artery and held in place in order to maintain an angle of insonation of ≤60° while recording femoral artery blood velocities. The gate was adjusted to the width of the artery to ensure the sample volume included both the near and far wall borders and remained centered in the lumen of the vessel. Values for the diameter of the femoral artery were determined from images that had clear borders prior to beginning the passive movement protocol. Femoral artery blood flow (FBF) was calculated according to the following equation,

$$\mathrm{FABF} = \mathrm{MFABV} \cdot \pi\left(\frac{\mathrm{FAD}}{2}\right)^2 \cdot 60$$

where MFABV is mean femoral arterial blood velocity (cm/s), FAD is femoral artery diameter (mm) and 60 s corrects femoral artery blood flow (FABF) to milliliters per minute. Additional measures of the peripheral hemodynamic responses were obtained during PLM trials including, mean femoral artery antegrade blood velocity (FABV), mean femoral artery retrograde blood velocity (FRBV), mean femoral artery blood velocity (MFABV) and femoral artery diameter (FAD).

The images of the femoral artery and the corresponding blood velocity profiles were recorded at a sampling frequency of 30 Hz using a digital imaging frame grabber (DVI2USB 3.0, Epiphan Systems Inc., Palo Alto, CA) and acquisition software system (Cardiovascular Suite 2.1, Quipu srl, Pisa, Italy). The video files, including images of the vessel wall and the blood velocity recordings, were stored on a computer for offline analysis at a later time using software available in (Cardiovascular Suite 2.1, Quipu srl, Pisa, Italy).

3.3.3 Sleep Apnea Questionnaire

To determine the OSA subjects' eligibility, an 8-item, self-report questionnaire on an 8-point type scale called the STOP-BANG sleep apnea questionnaire as previously published was used (Chung et al., 2008). The first four items of the questionnaire were subjective signs and symptoms related to snoring, tiredness, observations, and history of treatment for high blood pressure (STOP). In contrast, the next four items on the questionnaire were objective data that were gathered in the laboratory and include BMI, age, neck circumference, and gender (BANG). The results of a previous study using the same questionnaire (Chung et al., 2008) reported test-retest reliability using Cohen's kappa coefficient on the questionnaire and found 96.4% had the same score upon retesting with a κ coefficient of 0.923 (CI 0.82–1.00). Moreover, compared to polysomnographic diagnosis, the STOP-BANG sleep apnea questionnaire was found to have a good positive predictive value of 75.0% (CI, 57.7–92.3), although the negative predictive value (NPV) was 30% (CI, 6.7–65.3) (Chung et al., 2008). This suggests that the subjects identified as having OSA using the STOP-BANG questionnaire are likely to actually suffer from OSA condition. However, it should be mentioned that while the STOP-BANG questionnaire may not identify some subjects who would be diagnosed as having OSA if more rigorous methods were utilized. For the purposes of this study in determining eligible participants, the predictive value was considered acceptable. The questionnaire was administered to the OSA subjects on the first day of the study following completion of the informed consent form and completion of the medical history questionnaire.

3.4 Statistical Analysis Procedures

Data were analyzed using SPSS and R program. The age distribution of healthy (HEAL) versus the OSA groups was compared using an independent t-test. Anthropmetric and demographic data were compared between the HEAL and OSA subjects using an independent t-test. An analysis of variance (ANOVA) with repeated measures model was used to determine significant differences between main effects (HEAL versus OSA; Vit C versus CON; time) and significant interactions for the primary variables of interest, including femoral artery blood flow (FABF), blood pressure (DBP, SBP, MAP), cardiac output (CO), stroke volume (SV) and total peripheral resistance (TPR). A Student-Neuman Keuls multiple comparison post hoc test was used to identify the specific differences when a significant F ratio was identified for an interaction and/or main effect. Statistical significance was set a priori at $p \leq 0.05$. All results are presented expressed as the group mean ± standard deviation (SD) unless otherwise specified.

Chapter 4

Results

4.1 Subject Characteristics

The healthy group consisted of 11 male and two female subjects (N=13). The group mean age for the HEAL group was 27.1 ± 3.6 (± SD). All were non-smokers, were not on medications known to influence vascular reactivity, and had no signs or symptoms for OSA. The group mean weight of the HEAL group was 77.6 ± 12.3 kg and group mean for height was 178.8 ± 6.5 cm. One participant was a native Brazilian, and the rest were Caucasian Americans. The obstructive sleep apnea patients group originally consisted of 15 male subjects and no females; however, two subjects withdrew from the study (N=13) for reasons unrelated to the study protocol or procedures. All subjects exhibited signs and symptoms associated with OSA and all OSA subjects reported having mild to moderate symptoms (snoring, awakening with a dry mouth or sore throat, morning headache and daytime tiredness). All OSA subjects reported being non-smokers, sedentary, and participating in extended physical activity fewer than once per week. The group mean age for the OSA group was 27.5 ± 2.1. The group mean height was 178.8 ± 7.5 cm and the mean weight was 84.8 ± 17.1 kg. Three participants were

Middle Eastern ancestry, and the rest were Caucasian. See Table 4.1 for a summary of the subjects' demographic variables.

Table 4.1: Demographic variables of the subjects divided into OSA and HEAL groups

Group	Age (yr.)	Height (cm)	Weight (kg)	Gender
OSA				
Subject 1	21	178	90	M
Subject 2	26	175	89	M
Subject 3	22	181	101	M
Subject 4	32	177	77	M
Subject 5	26	172	91	M
Subject 6	24	181	66	M
Subject 7	31	173	86	M
Subject 8	45	176	104	M
Subject 9	25	170	99	M
Subject 10	21	169	99	M
Subject 11	22	168	86	M
Subject 12	23	181	93	M
Subject 13	22	182	70	M
Mean	28	179	89	
HEAL				
Subject 1	30	173	60	M
Subject 2	24	180	87	M
Subject 3	22	170	73	M
Subject 4	21	170	67	M
Subject 5	23	176	86	M
Subject 6	22	181	71	M
Subject 7	29	179	93	M
Subject 8	42	167	77	M
Subject 9	23	166	89	M
Subject 10	19	179	83	M
Subject 11	19	169	62	M
Subject 12	20	168	59	F
Subject 13	21	169	57	F

*No difference between OSA and HEAL groups for age, height or weight.

4.2 Peripheral Blood Flow Responses to PLM

To address whether there was any significant difference between groups (HEAL versus OSA; main effect), conditions (CON versus Vit C; main effect) or interactions (group x time; condition x time) on the femoral blood flow or femoral artery diameter, a repeated measures ANOVA test was used to detect significant main effects and/or interactions. An image of the vascular imaging set-up in provided in Figure 4-1 along the response of the PLM protocol for a representative subject is shown in Figure 4-2.

The results of the repeated measures ANOVA revealed a significant main effect for HEAL and OSA groups as well as a significant interaction for group by time in FABF (Figure 4-3). For both the HEAL and OSA groups, FABF increased immediately from resting values at the onset of PLM in both the CON (HEAL, 142.9 ± 25.0; OSA, 67.7 ± 7.2 ml/min, $p<0.05$) and Vit C (HEAL, 111.2 ± 13.3, OSA, 73.8 ± 8.0 ml/min, $p<0.05$); there was no significant difference in FABF at rest between the HEAL pre- and post Vit C trials or the OSA pre- and post-Vit C trials. For the HEAL group, FABF increased to a similar peak value of 231.8 ± 28.3 ml/min during CON trials compared to 215.4 ± 31.2 in Vit C trials. In the OSA group, FABF increased from resting values to a peak value of 108.8 ± 11.6 ml/min) versus a peak value of 126.7 ± 12.8 ml/min for the Vit C trial. These results suggest that the magnitude of the PLM-induced FABF response may be associated with impaired vascular function in OSA patients compared to the HEAL group.

For each subject and condition, FAD was measured at baseline prior to onset of the PLM. The results of the ANOVA with repeated measures revealed no significant

main effect for group (HEAL vs. OSA), condition (CON vs. Vit C) or interaction (CON HEAL; 6.30 ± 0.26; HEAL Vit C; 6.32 ± 0.25; OSA CON; 5.88 ± 0.21; OSA Vit C; 6.09 ± 0.19 mm).

According to the results of the ANOVA test, a significant difference was identified for mean femoral artery blood velocity (MFABV) for time ($p < 0.05$) and condition (CON versus Vit C, $p< 0.05$). In addition, there was also a significant interaction effect ($p < 0.05$) for condition x time. Further analysis revealed that MFABV was significantly different between groups prior to PLM. In the HEAL group, pre-vitamin C, the MFABV increased from resting values of 8.14 ± 0.79 cm/s to peak values of 12.50 ± 1.37 cm/s during PLM. Following the ingestion of Vit C in the HEAL group, MFABV increased from 6.04 ± 0.55 cm/s at rest to peak values of 11.38 ± 1.40 cm/s during PLM. In comparison, in the OSA group, pre-vitamin C, the MFABV increased from resting values of 4.64 ± 0.48 cm/s just prior to the onset of PLM to a peak value of (6.84 ± 0.75 cm/s during passive lower limb movement. Following the administration of Vit C, MFABV increased from 4.42 ± 0.52 cm/s to peak values of 7.40 ± 0.80 cm/s during PLM in the OSA group. These results reinforce that notion that the magnitude of the PLM-induced response in blood flow and blood velocity decreased in OSA patients compared to healthy. The results also suggest that the PLM is a good diagnostic tool to distinguish between OSA patients and healthy individuals.

When the femoral artery blood velocities were separated into positive or antegrade velocities (FABV) and negative or retrograde velocities (FRBV), the findings of the ANOVA test revealed a significant difference ($p< 0.05$) in FABV between the groups as well as a significant difference in FABV over time ($p< 0.05$). The interaction

term, time by condition indicated a tendency to increase, but it did not achieve statistical significance. Overall, the significant main effect ($p<0.05$) for FABV was higher for the HEAL group compared to OSA group. The FABV values for the HEAL group with Vit C (10.78 ± 0.86 cm/s) was significantly ($p<0.05$) lower than without Vit C (16.01 ± 1.46 cm/s). However, there was no significant difference in the OSA CON compared to the OSA Vit C conditions for FABV. The results of the ANOVA revealed that there was no significant difference between groups (HEAL versus OSA), conditions (CON versus Vit C), nor was there a significant interaction for FRBV.

4.3 Central Hemodynamic Responses to PLM

The results of the ANOVA test indicated that there was no main effect for group (HEAL versus OSA) or condition (CON versus Vit C) nor was there a significant interaction for HR in response to the PLM protocol. That is, there was no difference in HR at rest between CON conditions HEAL (74 ± 4.21 bpm) and OSA (70 ± 4 bpm) nor was there any difference between Vit C responses for HEAL (68 ± 5 bpm) and OSA (72 ± 3 bpm) at rest. Similarly, the HR response was not altered by the PLM since there was no difference between HEAL and OSA for pre- and post-administration of Vit C conditions at any of the time points examined at rest and during the PLM protocol.

The group mean results for the response for stroke volume (SV) in HEAL and OSA groups before and two hours following Vit C ingestion are shown in Figure 4-4. The results of the ANOVA test revealed that there was a significant main effect for group ($p<0.05$) and but no main effect for condition. Further analysis of the responses for SV indicated that while there was no change in SV as a function of time for the HEAL group

under CON and Vit C conditions. However, there was significant condition by time interaction ($p<0.05$) for the OSA group; there was a significant increase in SV just prior to the onset of PLM during CON (86.7 ± 5.5 ml) to a peak value of 90.8 ± 7.0 ml during PLM. This increase was not evident in the OSA group following Vit C administration.

The findings of the ANOVA results showed a significant main effect for HEAL compared to OSA groups such that cardiac ouput (CO) was lower for HEAL than OSA groups (Figure 4-5). In addition, there was also a significant main effect for CON versus Vit C conditions so that CO was lower following Vit C administration compared to CON conditions. While there was a significant main effect for condition ($p<0.05$) for the HEAL group resulting in a lower CO following Vit C compared to CON, there was no significant change from rest (CON, 5.6 ± 0.5; Vit C, 5.0 ± 0.4 l/min, $p<0.05$) across the protocol. However, in comparison to HEAL, the OSA group demonstrated a significant increase from 6.1 ± 0.4 l/min at rest just prior to the onset of PLM to a peak value of 6.7 ± 0.6 l/min during the PLM protocol under CON conditions. In response to Vit C administration in the OSA group, CO showed a small but significant increase from 5.6 ± 0.4 l/min at rest to a peak value of 5.9 ± 0.3 l/min during PLM.

The group mean results of mean arterial pressure are shown in Figure 4-6. According to the results of the ANOVA test, there was a significant main effect ($p<0.05$) for HEAL compared to OSA but there was no main effect for condition (CON versus Vit C). However, there was a significant interaction ($p<0.05$) for condition by time. In the HEAL group, MAP remained the unchanged from rest (84 ± 4 mm Hg) throughout the protocol, with no change in MAP with PLM. Similarly, MAP remained at resting values (94 ± 6 mm Hg) throughout the PLM protocol following the administration of Vit C in

HEAL. The results of the post-hoc analysis indicated that there was a significant increase in MAP from resting values in CON (rest, 89 ± 4; peak value, 96 ± 6 mm Hg) and following Vit C (rest, 89 ± 5; peak values, 94 ± 4 mm Hg) in the OSA group.

Likewise, both systolic (Figure 4-7) and diastolic (Figure 4-8) blood pressure (SBP and DBP) were significantly different between groups ($p < 0.05$). For the HEAL group, pre-vitamin C, remained almost the same from (63.8) at time 60s to (63.1) at time 90s. However, post -vitamin C, DBP decreased from (74.8) at time 60s to (71.6) at time 90s. Much like DBP, pre-vitamin C, SBP remained the same (122.2) and post- vitamin C, decreased from (134.5) at time 60s to (133.2) at time 90s. In the OSA group, both SBP and DBP increased at the onset of exercise, and continued to increase almost throughout the duration of PLM; however, there was no difference for time. In the OSA group, pre-vitamin C, the DBP increased from (71.3) at time 60s to (76.1) at time 90s. In addition, post- vitamin C, DBP increased from (72.3) at time 60s to (73.3) at time 90s. Moreover, in the OSA group, pre-vitamin C, the SBP increased from (122.1) at time 60s to (133.9) at time 90s. Post- vitamin C, SBP increased from (124.2) at time 60s to (130.1) at time 90s.

Lastly, the results of the analysis for total peripheral resistance (TPR) demonstrated a significant main effect for condition ($p<0.05$) between HEAL CON compared to HEAL Vit C in that HEAL CON was lower compared to HEAL Vit C. In addition, there was no main effect for group nor was there any significant interactions between group by condition identified for TPR (Figure 4-9).

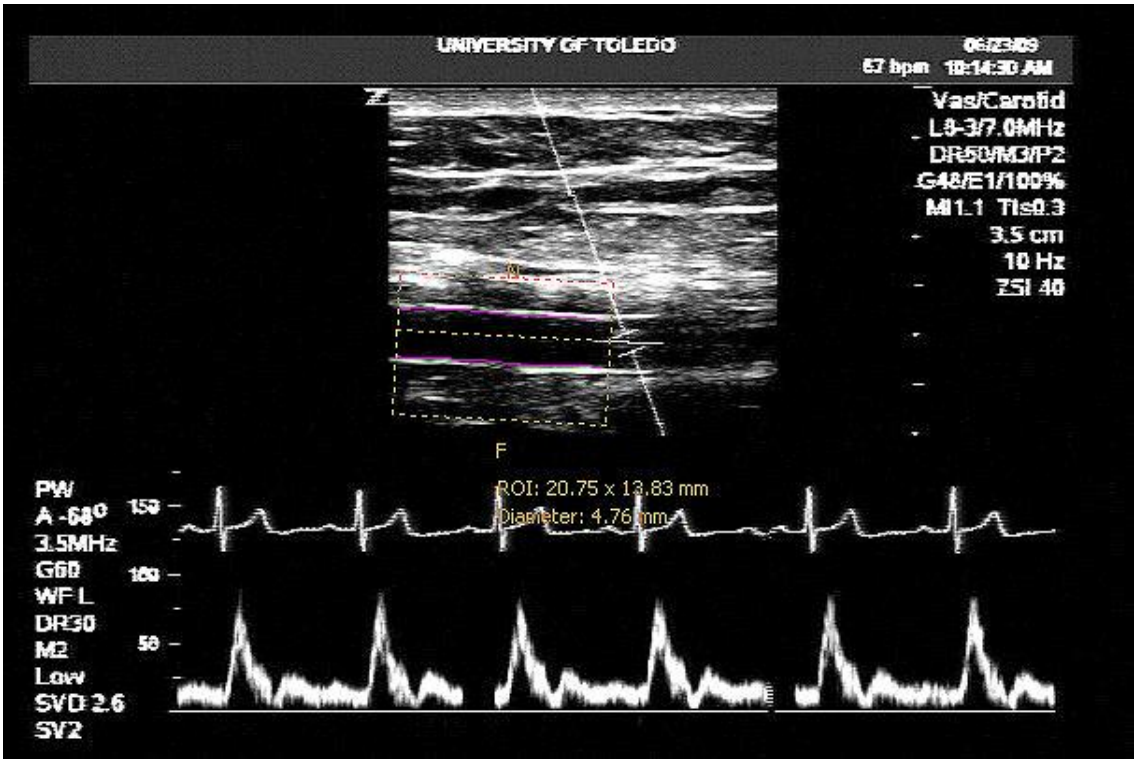

Figure 4-1. An image obtained using ZONARE ultrasound system. A linear probe operating at an imaging frequency of 7-10 MHz and a Doppler frequency of 4-6 MHz. The femoral artery of the left leg was measured distal to the inguinal ligament and proximal to the bifurcation of the superficial and deep femoral artery. Vessel diameter was measured perpendicular to the scanned area.

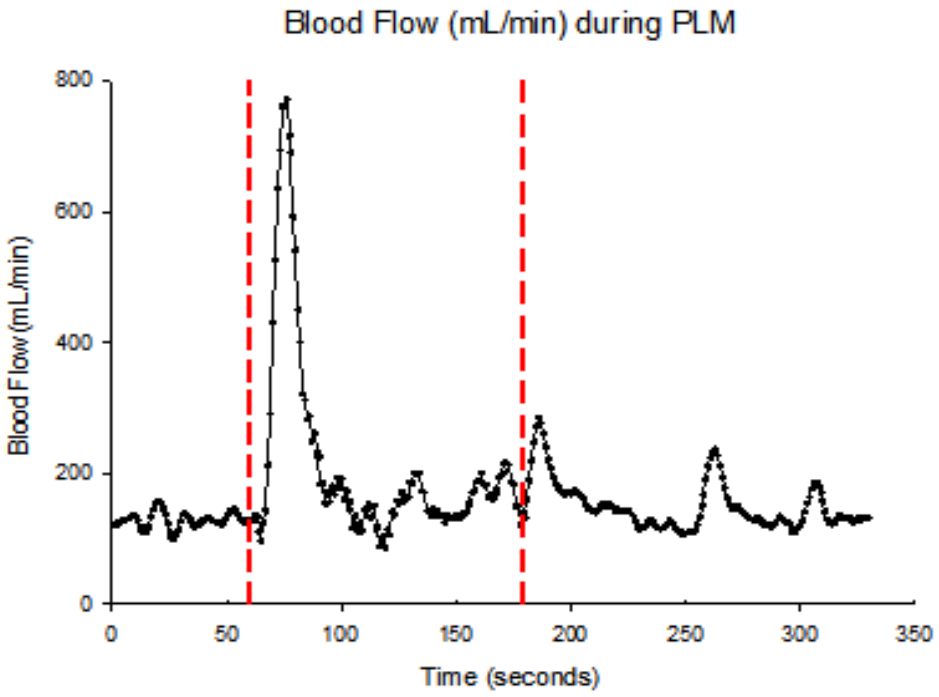

Figure 4-2. Blood flow response during PLM. The first 60s is baseline. At the start of passive leg movement, there was a significant increase in blood flow leading to a transient peak. PLM was performed for two continuous minutes, and then during the recovery (180s – 330s), blood flow returned to baseline levels

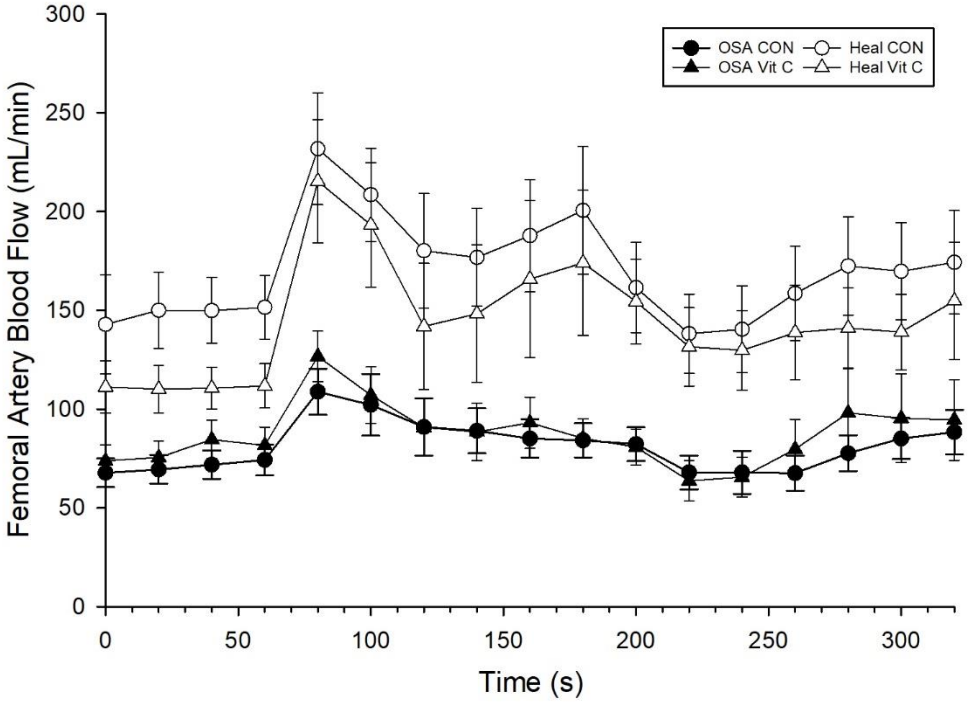

Figure 4-3. The time influence of FBF was assessed in OSA with VC (N=13) and Healthy with VC (N=13).
*: Significant difference between OSA and H ($P<0.05$).
Data was represented by mean ± SD.

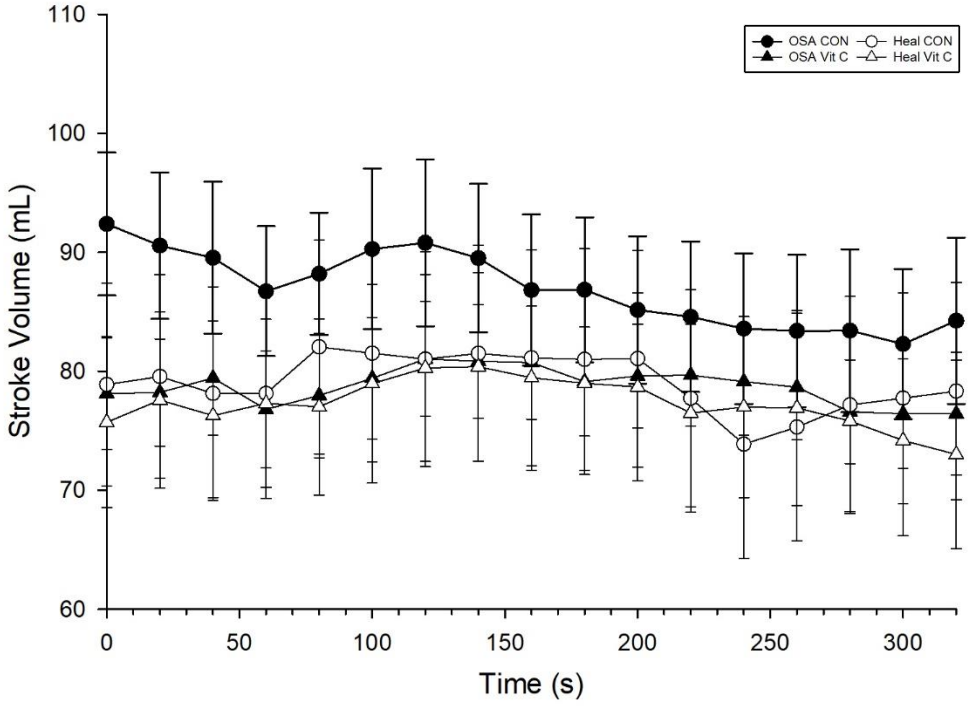

Figure 4-4. The time influence of stroke volume was assessed in OSA with VC (N=13) and Healthy with VC (N=13).
*: Significant difference between OSA and H ($P<0.05$).
Data was represented by mean ± SD.

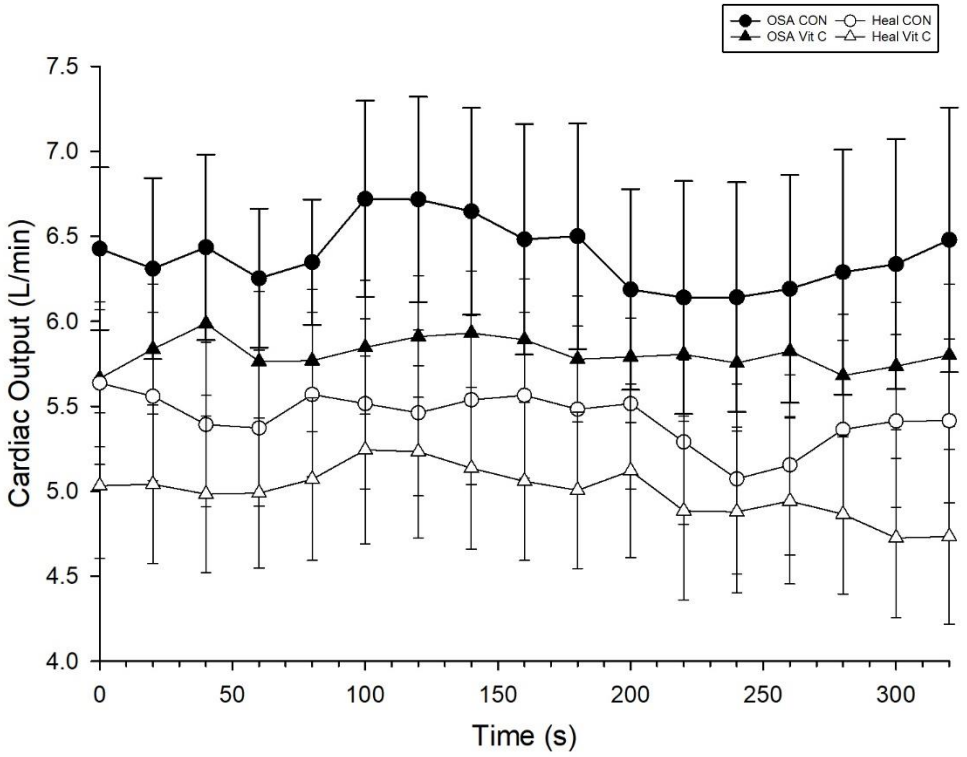

Figure 4-5. The time influence of cardiac output was assessed in OSA with VC (N=13) and Healthy with VC (N=13).
*: Significant difference between OSA and H ($P<0.05$).
Data was represented by mean ± SD.

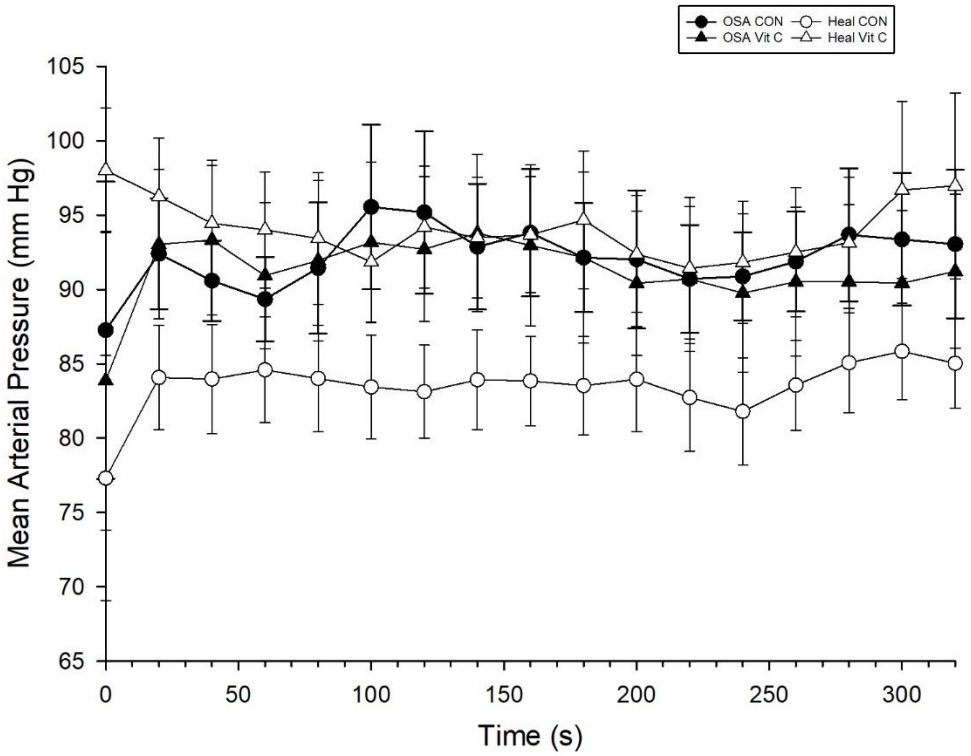

Figure 4-6. The time influence of mean arterial pressure was assessed in OSA with VC (N=13) and Healthy with VC (N=13).
*: Significant difference between OSA and H ($P<0.05$).
Data was represented by mean ± SD.

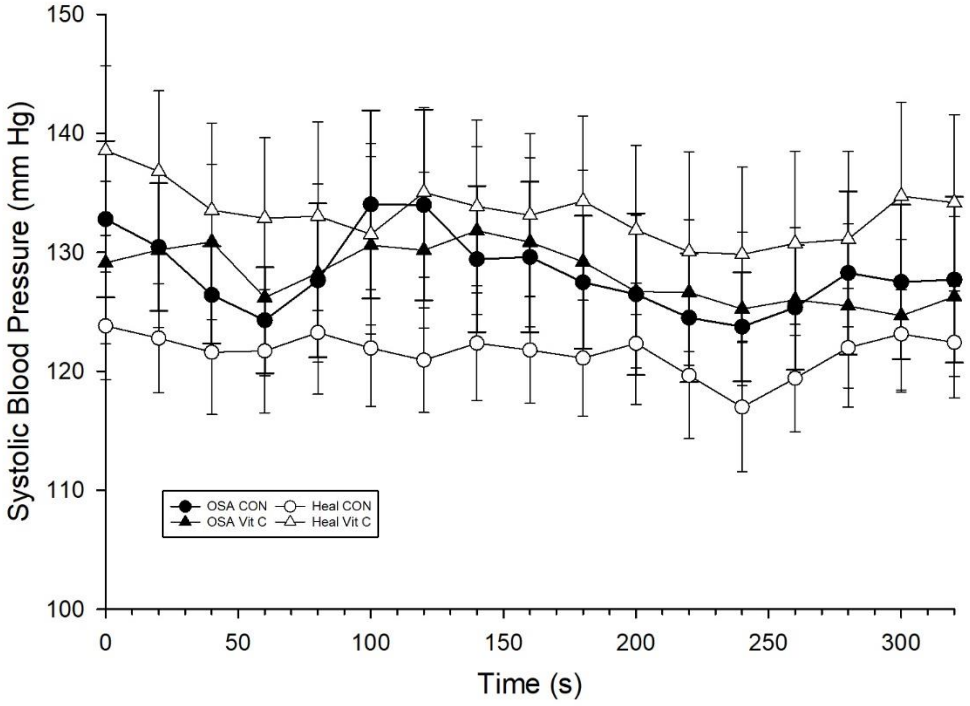

Figure 4-7. The time influence of systolic blood pressure was assessed in OSA with VC (N=13) and Healthy with VC (N=13).
*: Significant difference between OSA and H ($P<0.05$).
Data was represented by mean ± SD.

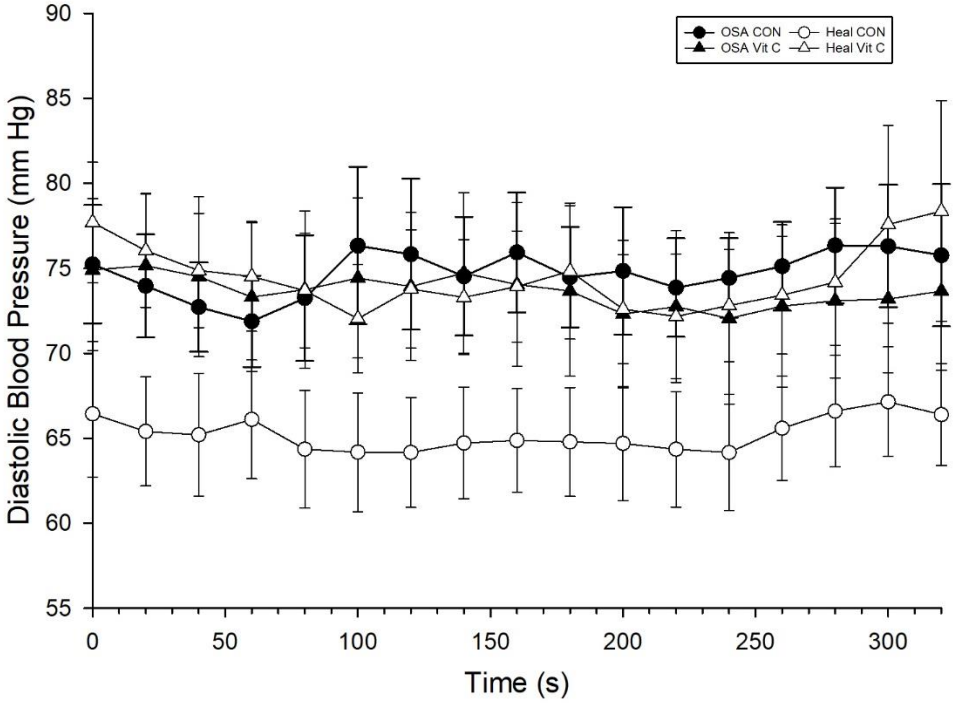

Figure 4-8. The time influence of diastolic blood pressure was assessed in OSA with VC (N=13) and Healthy with VC (N=13).
*: Significant difference between OSA and H ($P<0.05$).
Data was represented by mean ± SD.

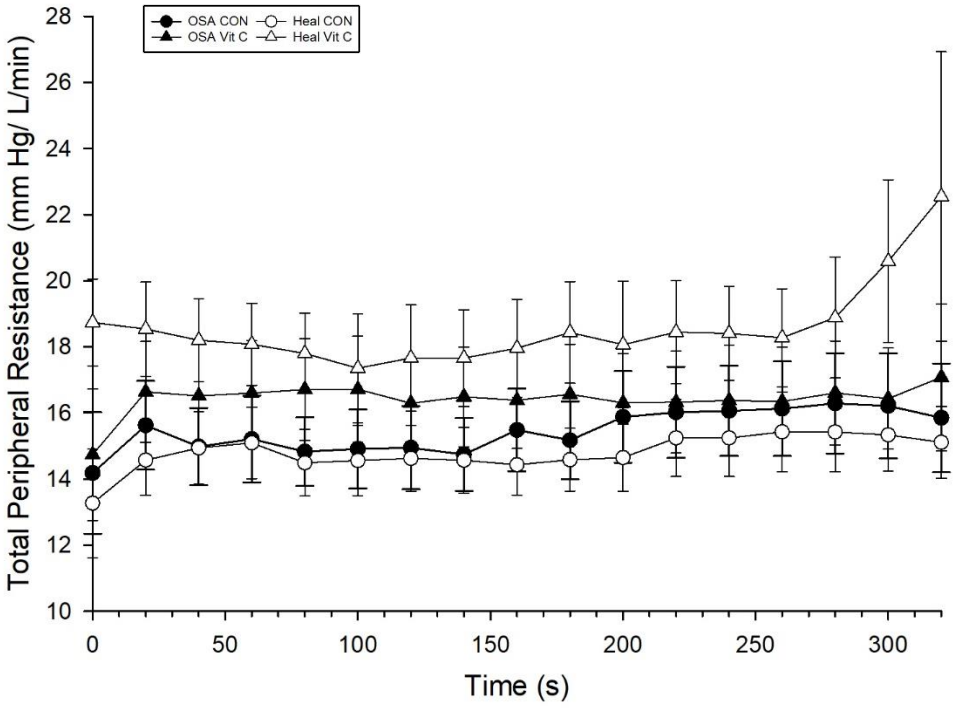

Figure 4-9. The time influence of total peripheral resistance was assessed in OSA with VC (N=13) and Healthy with VC (N=13).
*: Significant difference between OSA and H ($P<0.05$).

Chapter 5

Discussion

This chapter includes a discussion of the results and their broader significance in the area of OSA research. In particular, the results of femoral blood flow response to PLM, central hemodynamic, and the effect of vitamin C on OSA patients are highlighted in relation to previous research. Additionally, the clinical implications, limitations, recommendations, and conclusions are discussed

5.1 Femoral Blood Flow Response to the PLM

The results of this study demonstrated a significant difference between the OSA and healthy groups in their femoral blood flow response to the PLM. The significantly higher response of femoral blood flow to PLM among the health group aligns with findings in Groot et al. (2015), who found higher response of femoral blood flow to PLM among health subjects. Moreover, the results of this study support previous literature demonstrating attenuated femoral blood flow response to PLM among patients with endothelial dysfunction (Mortensen et al.2012). Since the sample in this dissertation included subjects with OSA, which is known to be associated with endothelial dysfunction, this result confirms previous research on the use of PLM as a diagnostic tool

to distinguish between healthy and diseased population. The difference between the OSA and healthy groups in this study adds to the literature that PLM can be a useful non-invasive diagnostic tool for OSA.

However, this study found no significant effect of vitamin C on neither OSA nor healthy groups. The lack of change in femoral blood flow response to the PLM among the OSA group pre- and post-VC means this particular protocol did not effectively reduce oxidative stress or vascular inflammation, but at the same time, it did not exacerbate them. The lack of effect of oral vitamin C in blood flow response on OSA subjects disagrees with findings from the literature. Thomas et al. (1996) found that infused vitamin C into the brachial artery markedly improves endothelium-dependent responses in chronic smokers. Moreover, Both Barcelo´ et al. (2006) and Mathias et al. (2006) found that infused antioxidants like vitamin C could attenuate the endothelial dysfunction in OSA patients by diminishing radical oxygen species and decreasing their cellular damage. Mathias et al. (2006) intravenously injected 0.5 g of vitamin C, using sever OSA conditions. The present study was designed to non-invasively examine the effect of oral vitamin C on mild to medium conditions. This suggests administration method of vitamin C and severity of disease are important factors in determining the effectiveness of vitamin C in reducing oxidative stress and vascular inflammation in OSA patients.

5.2 Central Hemodynamic Variables

The results of this dissertation showed significant difference between the healthy and OSA group in mean arterial pressure, systolic and diastolic blood pressure. However, there was no significant difference in variable for time. The results of this study support

previous literature demonstrating that OSA patients have higher blood pressure than healthy (Cano-Pumarega et al. 2011). Cano-Pumarega et al. (2011) recruited 1889 participants for 12.2 years of median follow identified an increased blood pressure in patients with OSA compared to control subjects and stated that obstructive sleep apnea disorder and high blood pressure association remained independent of confounders including age and obesity. Moreover, Roche et al. (2012) showed OSA patients was associated with a significant 5 mmHg increase in systolic pressure, and with 3 mmHg increase in diastolic pressure. Furthermore, Norman et al. (2006) explained that the amount of oxygen desaturation associated with apnea has been recognised as the pathophysiological cornerstone for the development of high blood pressure.

The lack of effect of oral vitamin C intervention in blood pressure on OSA subjects disagrees with findings from the literature. Ettarh et al. (2002) found vitamin C intake for 6 weeks lowers systolic and diastolic blood pressure. Moreover, Stephen et al. (2012) showed 500 mg a day of oral vitamin C for 8 weeks lowers both systolic and diastolic blood pressure. This suggests the dosage and the duration of vitamin C intake are important variables in determining the effectiveness of vitamin C in reducing oxidative stress, vascular inflammation, and enhancing endothelial function in OSA patients. In other words, the longer the treatment and the higher the dosage, the greater effect vitamin C interventions seem to have.

5.3 Muscle Blood Flow Measures

The results of this study showed no significant difference between healthy and OSA group in femoral artery diameter. The result differs from the findings of previous

literature. Thomas et al. (2016) found severe OSA have increased intima- media thickness and increased arterial diameter. Moreover, Ramzi et al. (2005) showed that arteries' intima-media increased in patients with severe OSA conditions, which predispose the patients to cerebrovascular disease. These findings suggest that severity of the disease is an important factor to see a change in the arterial diameter.

5.4 Clinical Implications

One of the growing areas of research in diagnosing endothelial function is the use of PLM as a diagnostic tool to assess vascular function to predict cardiovascular disease risk. The appeal of PLM as a diagnostic tool for OSA is its non-invasiveness compared the standard FMD approach. The current findings support the clinical use of PLM since the healthy and OSA groups differed significantly on this measure. It appears PLM can diagnose endothelial function and distinguish between healthy and OSA, although the measures must still be interpreted cautiously. It is also possible that PLM can help provide an additional definition of severity of OSA. In addition, the findings of this study also have implications for the comfort and safety of PLM compared to FMD. It is clear that PLM is a non-invasive and was well tolerated by all subjects and so it can be implemented with no risk to subjects. This study reinforces earlier findings that PLM can be used as a diagnostic tool to assess endothelial function to predict cardiovascular disease risk. Moreover, it must be emphasized that the current findings provide the first-known evidence to support the use of PLM as a potential diagnostic tool to distinguish between healthy and OSA patients.

5.5 Limitations

The major limitations of this study concern the protocol and the sample. First of all, the protocol was probably too short. One day is very brief and in most cases not long enough to see significant changes, especially only one dosage of 1000 mg vitamin C, although we did see some significant changes. Most previous studies on the effect of vitamin C on OSA have used protocol that last for at least six weeks and often even longer. The only studies that have shown significant changes over time among OSA groups preformed intravenous injection and other invasive approaches, and no studies have attempted such a short protocol with PLM technique. Likewise, the dosage and duration of the vitamin C protocol applied to the both group was probably lower and shorter than necessary. Strictly speaking, some may not even consider it as a treatment. The initial concern was for the safety of the subjects, but with the findings showing no adverse reactions, a higher dosage and longer duration ware probably safe and could have been more effective.

The sample suffered from a few issues that likely limited the significance and generalizability of the findings. One issue is the small sample size. With only 13 OSA patients and 13 healthy subjects, it prevented the use of t-tests to determine change over time on specific variables in each group. The small sample size also resulted in a large standard of error, which could have been reduced by a larger sample. A larger sample would also address a second issue, which was the mixing of mild and medium OSA conditions. More OSA subjects can allow the OSA group to be divided into subgroups of mild and medium OSA. Without a large sample size, these groups were combined while the effect of vitamin C on each type might actually differ. A third

limitation with the sample of OSA was the fact that all subjects fell somewhere within the mild-to-moderate severity range, and none of the subjects fit the definition of severe OSA. Previous findings have shown that severity makes a difference on the effectiveness of antioxidants supplements (more severe, greater the effect). Moreover, the low severity of the sample makes it not completely representative of the population of OSA patients that fit within the entire range of severity. A fourth and final limitation with the sample was the lack of females in OSA group. When it comes to physical traits, men and women differ greatly, so the findings of this study cannot be generalized to women.

5.6 Recommendations

Based on the findings and limitations, a few important future concerns and recommendations arise. What is most needed to build from the current study is an extension of the protocol and changing the administration method of vitamin C. The kind of physiological changes needed to make oral vitamin C an effective preventative treatment for endothelial dysfunction in OSA patients did not occur in a brief time, although either a longer duration or administration method of vitamin C, or both, could make a difference. All of the protocols in the previous literature have found stronger results with either longer duration or alternative administration methods. At the time of designing this current study, safety was a concern for the treatment of the OSA group, so a non-invasive method was chosen. However, the findings of this study in combination with previous literature shows a higher dosage can be safe. It is also possible that such a study could produce more pronounced changes in endothelial dysfunction levels not found in the current study because of brevity and administration method.

In addition to duration of protocol and administration method of vitamin C, severity of OSA seems to play a crucial role in how effective vitamin C interventions can be. In the current study, most of the OSA subjects appeared to have mild to medium OSA based on their medical questionnaire measurements, while previous studies have found greater effects of antioxidant supplements with more severe conditions. To help with accurately defining the severity of OSA and to determine the appropriate duration and dosage of vitamin C intake based on the condition, future studies in this area should rely on thorough health records and polysomnography sleep study results as much as possible. Doing so would avoid the problem of self-reported severity that can be affected by poor memory or ulterior motives of the subjects.

Future studies should also avoid mixing mild with medium OSA and clearly distinguish the results of each. It is possible that oral vitamin C intake can have different effects on each degree, although more research into this comparison is needed. To get a better idea of how oral vitamin C and antioxidants supplements in general might affect various degrees of OSA differently, future studies should implement longer protocol for the three degrees of OSA and analyze differences in femoral blood flow response, central hemodynamics, and arterial diameter changes. In order to do such a comparison and with generalizability, a bigger sample is needed.

5.7 Conclusions

PLM is a non-invasive procedure, does not have any known discomforts measurement whereas during the FMD technique and is preferred over the FMD approach. Additionally, PLM is performed on the lower limb, which is at a greater

predisposition to develop vascular disease. When taken together, these factors make the PLM approach more accessible and could make it very practical test for both clinical and research settings. Moreover, PLM has shown to be a good diagnostic tool to distinguish between healthy and OSA patients. The findings of this study are very important because individuals with OSA can be asymptomatic for long periods before the onset of signs and symptoms and this result in a delayed diagnosis and treatment for the disease. PLM can be a useful diagnostic tool to assess endothelial dysfunction in OSA, which would help in early diagnosis before disease progress. Early diagnosis is important because if endothelial dysfunction is allowed to progress, it may lead to serious life threating conditions like coronary artery disease and peripheral arterial disease.

Oral vitamin C did not make a difference in either OSA or healthy groups. These findings challenge the hypothesis that the vitamin C would result in an improved blood flow response during PLM. An increased response to blood flow was expected due to the positive effects that a vitamin C has on endothelial dysfunction due to reducing oxidative stress, vascular inflammation, and enhancing endothelial function in OSA patients that other studies have shown as a result of the vitamin C. Although the study did not identify a difference with oral vitamin C in OSA, it is possible that vitamin C could still be effective if different administration methods, higher dosages, or more severe conditions were used. Despite these findings, research in the areas of alternative treatment methods for OSA that are less invasive and more time-efficient remains important as the incidence of OSA continues to increase and adherence to current treatments is inconsistent among many sufferers.

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Appendix A

Adult Research Subject Information & Consent Form

Passive leg movement and nitric oxide-mediated vascular function: the impact of obstructive sleep apnea (OSA)

Principal Investigator: Barry W. Scheuermann, Ph.D.

Other Staff (identified by role): Zakaria Alyousif, MS

Contact Phone number(s): (419) 530-2692 Office

(419) 530-2058 Lab

What you should know about this research study:

- We give you this consent/authorization form so that you may read about the purpose, risks, and benefits of this research study. All information in this form will be communicated to you verbally by the research staff as well.
- Routine clinical care is based upon the best-known treatment and is provided with the main goal of helping the individual patient. The main goal of research studies is to gain knowledge that may help future patients.
- We cannot promise that this research will benefit you. Just like routine care, this research can have side effects that can be serious or minor.
- You have the right to refuse to take part in this research, or agree to take part now and change your mind later.
- If you decide to take part in this research or not, or if you decide to take part now but change your mind later, your decision will not affect your routine care.

- Please review this form carefully. Ask any questions before you make a decision about whether or not you want to take part in this research. If you decide to take part in this research, you may ask any additional questions at any time.
- Your participation in this research is voluntary.

PURPOSE (WHY THIS RESEARCH IS BEING DONE)

You are being asked to take part in a research study examining the effects of oral vitamin C in terms of whether it attenuates endothelial dysfunction in non-compiant patient with Obstrictive Sleep Apnea (OSA) to provide an alternative and affordable approach in order to prevent cardiovascular diseases among this population. . Previous studies have shown that the intravenous injection of vitamin C improves endothelial dysfunction. Also to examine whether the PLM approach is a technical diagnostic tool to distinguish between healthy subjects and subjects with OSA.The results of the present study may provide further knowledge and better understanding of PLM test as an alternative approach to distinguish between healthy subjects and subjects with OSA.

You were contacted since you expressed an interest in taking part in this investigation. A maximum of 30 volunteers (15 in each of two groups) will take part in this investigation.

To participate in this study, you must either be a healthy individual between the age of 18 and 65 years old and be free of any known cardiovascular, pulmonary, metabolic or musculoskeletal (muscle or joint) diseases as determined by the medical history questionnaire.

OR

An individual who has OSA between the age of 18 and 65 years old, and be free of any known cardiovascular or any musculoskeletal (muscle or joint) injuries as determined by the medical history questionnaire.

Individuals who currently smoke or quit smoking less than 6 months ago will be excluded from participating in this study. If you do not meet these criteria, we greatly appreciate your willingness to volunteer but unfortunately, you will not be able to participate in this study.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT

If you decide to take part in this study, you will be asked to vist the Cardiopulmonary and Metabolism Research Laboratory (room 1407, Health and Human Service Building, University of Toledo) on 3 separate occasions over a two days period. Each of the visits will take 45 minutes of your time.

Study Design and Protocol:

The schedule of the study will be divided into two sessions: preliminary session and intervention session, which will take a total of two days.

Visit 1: (Preliminary Visit)
During the preliminary visit subjects age, gender, body weight, height, and blood pressure will be recorded. Then the subject will rest in the upright position in the Biodex isokinetic machine, semi-reclined to 120 degrees for subject comfort. Chair measurements will be recorded. PLM and Doppler ultrasound will be performed to provide the subjects with familiarity of the measures and to ensure that a clear image of the femoral artery can be achieved throughout rest and movement.

Intervention Visits
The intervention stage will include two visits. The first visit is the pre-intervention stage before the vitamin C intervention. The second visit is the post-intervention stage two hours after the vitamin C has been taken.

Visit 2:
The subject will return to the laboratory, subjects will be seated on Biodex dynamometer for 30 minutes to ensure blood flow, heart rate, and blood pressure returned to baseline values. Then LBF and the diameter of the femoral artery will be measured by Doppler ultrasound. Before the onset of PLM, stable hemodynamics will be documented as well as the measurement of the femoral artery diameter. The PLM will be performed by Biodex dynamometer, which will passively move the leg through a 90-degree range. The starting position of the leg will be 180-degree extension of the knee. The first movement will serve to passively flex the knee from 180 to 90 degrees of motion at 0.5 H. The protocol will include 30 minutes of seated comfortably followed by two minutes of PLM. Before the start and throughout the protocol subjects will be encouraged to remain passive. Also, subjects will be aware that PLM will take place in 1 minute, but to minimize the chance of an anticipatory response, they will not be informed when exactly the PLM will begin.

Vitamin C intervention:
Following the completion of the control PLM test, subjects will be instructed to take 1 g of oral vitamin C before they leave the Cardiopulmonary and Metabolism Research Laboratory and they will be asked to come back after two hours.

Visit 3:
Upon their arrival two hours after taking vitamin C, subjects will be seated on Biodex dynamometer for 30 minutes to ensure blood flow, heart rate, and blood pressure returned to baseline values. Then the same procedure that was done in visit one will be

RISKS AND DISCOMFORTS YOU MAY EXPERIENCE IF YOU TAKE PART IN THIS RESEARCH

Risks and Discomforts Associated with PLM
Previous research has shown that the risks associated with this protocol are no more than minimal. Individuals who participate in this study will have their heart rate closely monitored throughout each PLM test. Heart rate will be monitored during the two PLM sessions and will be supervised by a registered respiratory therapist who is also trained in first aid and CPR.

POSSIBLE BENEFIT TO YOU IF YOU DECIDE TO TAKE PART IN THIS RESEARCH

We cannot and do not guarantee or promise that you will receive any benefits from this research study. The benefit of participating in this study is to help further research regarding the effects of oral vitamin C in terms of whether it attenuates endothelial dysfunction in non-complaint patient with OSA. Also, examine whether the PLM approach is a technical diagnostic tool to distinguish between healthy subjects and subjects with OSA.

COST TO YOU FOR TAKING PART IN THIS STUDY

You are not directly responsible for making any type of payment to take part in this research study. However, you are responsible for providing your own means of transportation to and from the Cardiopulmonary and Metabolism Research Laboratory at The University of Toledo's main campus in the Health and Human Services Building (room 1407). You will not be compensated for gas, travel, or any other expenses to participate in this research study.

PAYMENT OR OTHER COMPENSATION TO YOU FOR TAKING PART IN THIS RESEARCH

No compensation including money, free treatment, free medications, or free transportation will be provided for this study.

ALTERNATIVE(S) TO TAKING PART IN THIS RESEARCH

There are no alternatives to taking part in this research study. Exclusion from the study, however, will not affect the quality of care you may receive at the sports medicine/physical therapy facility, doctor's office, and/or other medical facilities.

CONFIDENTIALITY - (USE AND DISCLOSURE OF YOUR PROTECTED HEALTH INFORMATION)

By agreeing to take part in this research study, you give to The University of Toledo (UT), the Principal Investigator and all personnel associated with this research study your permission to use or disclose health information that can be identified with you that we obtain in connection with this study. We will use this information for the purpose of conducting the research study as described in the research consent/authorization form.

Under some circumstances, the Institutional Review Board, or the Research and Sponsored Programs of the University of Toledo may review your information for compliance audits. If you receive any payments for taking part in this study, your personal information and limited information about this study will be given to The University of Toledo's accounts payable department as necessary to process payment to you. We may also disclose your protected health information when required by law, such as in response to judicial orders.

The University of Toledo is required by law to protect the privacy of your health information, and to use or disclose the information we obtain about you in connection with this research study only as authorized by you in this form. There is a possibility that the information we disclose may be re-disclosed by the persons we give it to, and no longer protected. However, we will encourage any person who receives your information from us to continue to protect and not re-disclose the information.

Your permission for us to use or disclose your protected health information as described in this section is voluntary. However, you will not be allowed to participate in the research study unless you give us your permission to use or disclose your protected health information by signing this document.

You have the right to revoke (cancel) the permission you have given to us to use or disclose your protected health information at any time by giving written notice to Barry Scheuermann, PhD, or Zakaria Alyousif, B.S., 2801 W. Bancroft, Mail Stop #119, Toledo, OH 43606. However, a cancellation will not apply if we have acted with your permission, for example, information that already has been used or disclosed prior to the cancellation. Also, a cancellation will not prevent us from continuing to use and disclose information that was obtained prior to the cancellation as necessary to maintain the integrity of the research study.

Except as noted in the above paragraph, your permission for us to use and disclose your protected health information will stop at the end of the research study**.**

A more complete statement of University of Toledo's Privacy Practices is set forth in its Joint Notice of Privacy Practices. If you have not already received this Notice, a member of the research team will provide this to you. If you have any further questions concerning privacy, you may contact the University of Toledo's Privacy Officer at 419-383-6933.

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov*, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

IN THE EVENT OF A RESEARCH-RELATED INJURY

In the event of injury resulting from your taking part in this study, treatment can be obtained at a health care facility of your choice. You should understand that the costs of such treatment will be your responsibility. Financial compensation is not available through The University of Toledo or The University of Toledo Medical Center.

In the event of an injury, you may contact at any time, day or night:
Dr. Barry W. Scheuermann (419-530-2692 - office) or (567-288-9732 - available 24 hrs)

VOLUNTARY PARTICIPATION

Taking part in this study is voluntary. You may refuse to participate or discontinue participation at any time without penalty or a loss of benefits to which you are otherwise entitled. If you decide not to participate or to discontinue participation, your decision will not affect your future relations with the University of Toledo or The University of Toledo Medical Center.

NEW FINDINGS

You will be notified of new information that might change your decision to be in this study if any becomes available.

OTHER IMPORTANT INFORMATION

There is no additional information.

ADDITIONAL ELEMENTS

There are no additional elements to this study.

OFFER TO ANSWER QUESTIONS

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over. If you have

questions regarding the research at any time before, during, or after the study, you may contact Barry Scheuermann, PhD at (567) 288-9732,

If you have questions beyond those answered by the research team or your rights as a research subject or research-related injuries, please feel free to contact the Chairperson of the University of Toledo Biomedical Institutional Review Board at 419-383-6796.

SIGNATURE SECTION (Please read carefully)

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ THE INFORMATION PROVIDED ABOVE, YOU HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND YOU HAVE DECIDED TO TAKE PART IN THIS RESEARCH.

BY SIGNING THIS DOCUMENT YOU AUTHORIZE US TO USE OR DISCLOSE YOUR PROTECTED HEALTH INFORMATION AS DESCRIBED IN THIS FORM.

The date you sign this document to enroll in this study, that is, today's date, MUST fall between the dates indicated on the approval stamp affixed to the bottom of each page. These dates indicate that this form is valid when you enroll in the study but do not reflect how long you may participate in the study. Each page of this Consent/Authorization Form is stamped to indicate the form's validity as approved by the UT Biomedical Institutional Review Board (IRB).

Appendix B

Curriculum Vita for Zakaria A. Alyousif

Zakaria A. Alyousif
Graduate Teaching Assistant

University of Toledo

Contact Information

Office Address: 1407 Cardio-pulmonary and Metabolism Lab
Health and Human Services Building
University of Toledo
Toledo, OH 43606-3390

Office email: zakaria.alyousif@rockets.utoledo.edu

Personal Address: 2919 Densmore Dr
Toledo, OH, 43606
Personal Cell: 419-208-4188
Personal Email: alyousef_z@hotmail.com

Profile

As my educational achievements and work experiences attest to, I am hardworking, motivated, creative and imaginative. I thrive on challenging experiences and love tackling problems logically. I thoroughly enjoy participating in hospital or academic activities. Moreover, I have the confidence to give excellent presentations, which is a necessary quality in the medical field, as demonstrated by my experiences teaching students who have come to the hospital on clinical rotations to learn from our experience. At the University of Toledo, I have been a teaching assistant for five years and I have also filled in for my colleagues whenever I am needed. I have also assisted my peers in

their research, particularly with blood sample collection, equipment preparation, and general data collection. As an instructor, I approach education from a student-centered perspective, and strive to involve students as much as possible through interaction, small-group discussions, and hands-on laboratory activities. As a researcher, I am interested in discovering more about the relationship between the cardiovascular and pulmonary systems in populations with chronic respiratory diseases.

Education

Ph.D. in Exercise Science Expected May 2020

University of Toledo, Toledo, OH, U.S.A.
Cardiopulmonary and Metabolism Track
Dissertation: *Passive leg movement and nitric oxide-mediated vascular function: the impact of Obstructive Sleep Apnea (OSA)*

Master of Science in Exercise Science August 2014
University of Toledo, Toledo, OH, U.S.A.
Cardiopulmonary and Metabolism Track
Master thesis: *The Effects of High Intensity Interval Training on Asthmatic Adult Males*

Bachelor of Science in Respiratory Therapy May 2010
University of Toledo, Toledo, OH, U.S.A.

Nursing Diploma November 2005
Arab Development Institute, Dammam, KSA

Licenses & Certifications

Registered Respiratory Therapist (RRT) by the NBRC, OH, USA

Certified Respiratory Therapist (CRT) by the NBRC, OH, USA

Advanced Cardiac Life Support (ACLS) from OH, USA

Basic Life Support Instructor (BLSA) from OH, USA

Certified Nurse Technician by Saudi Council for Health Specialties

Experience

Registered Respiratory Therapist

- The University of Toledo Medical Center (UTMC)

Part-time Teaching

- RCBS3300-Advanced Cardiac Life Support Present -
- RCBS4800-Issues in Professional Practice Present -
- RCBS4700-Research Analysis (3 credit hours) Fall 2019
- RCBS4700-Research Analysis (3 credit hours) Fall 2018
- RCBS3020-Respiratory care Practice I Lab (4 credit hours) Summer 2018
- RCBS4160-Clinical Assessment (3 hours credit hours) Fall 2016/2017

Volunteer Teaching Assistant

- RCBS3120 – Respiratory care Practice II Lab (3 credit hours) Summer 2016
- RCBS3020 – Respiratory care Practice I Lab (4 credit hours) Summer 2015

Graduate Teaching Assistant at the University of Toledo Fall 2014-2015

- KINE 2460 – Anatomy and Physiology I Lab (3 credit hours)
- KINE 4830 – Principles of Endurance Conditioning (3 credit hours)

Critical Care Respiratory Therapist in AICU 2010-2011

Dammam Medical Complex, KSA

- Reviewed current respiratory care orders & initiated plan of providing care as per established standard of patient care.
- Conducted diagnostic & bedside techniques, including include arterial/venous blood gas sampling, electrocardiogram, peak flow, spirometry, and vital capacity measurement.
- Assembled, assured function, and conducted routine & non-routine maintenance of equipment.
- Conducted therapeutic procedures such as invasive & non-invasive ventilation, pulmonary function test, oxygen therapy, bronchodilator therapy & airway management.
- Provided staff, patient & family respiratory care education.
- Delivered clinical instruction for students from Dammam University.

Student Respiratory Therapist in Toledo, OH, USA 2008-2010

Toledo Children's Hospital, St. Anne Mercy Hospital, St Vincent Mercy Hospital,

The University of Toledo Medical Center, St Luke's Hospital, and Regency Hospital

Publications & Presentations

Khan, Z., **Alyousif, Z.,** Srour, K., Khan, M.S., Assaly, R.A. The Impact of Obesity on Mortality and Disease Severity in Patients with Pulmonary Embolism in Unites States. A 13 Years National Analysis. **2019**, May; Pub Status: (**Submitted**)

Khan, Z., **Alyousif, Z.,** Darr, U., Javaid, T., Hasan, S., Alastal, Y., Siddiqui, N., Sodeman, T., Nawras, A..Epidemiology of Opportunistic Fungal Infections in Patients of Inflammatory Bowel Disease: National Inpatient Sample Analysis 2002-2014. TGH **2019** (**Accepted**)

Khan, Z., **Alyousif, Z**., Khurshid, T. Trends in the Incidence and Outcomes of Clostridium difficile Colitis in Hospitalized Cirrhotic Patients With Hepatic Encephalopathy: Nationwide Analysis. **2019**, January; Pub Status: (**Submitted**)

Khan, Z., **Alyousif, Z**., Alastal, Y., Khan, M.A., Nawras, A Gastrointestinal Bleeding in Patients with Mechanical Ventilation: Insights from the Healthcare Utilization Project. ACG **2018**. (**Published**)

Khan, Z., Darr, U., **Alyousif, Z**., Baig, M.A., T., Nawras, A Smartphone App Based Tool to Counteract Over-prescription of Proton Pump Inhibitors Upon Discharge from Academic Medicine Services: A Quality Improvement Project. ACG **2018**. (**Published**)

Khan, Z., **Alyousif, Z**., Khurshid, T., Fahim, A., Khan, M.S. Obstructive Jaundice secondary to intrahepaticcholestasis by diffuse metastatic liver disease as an initial presentation of small cell lung cancer. Case Reports in Hepatology. **2018**, May; Pub Status: (**Published**)

Garmyn, EC, **Alyousif, ZA**, Silette, CR, Scheuermann, BW. The Consumption of a High Fat Meal and its Effects on Endothelial Function in the Upper and Lower Extremities in Individuals with a Family History of Type II Diabetes Mellitus. (Unpublished manuscript)

Monahan, MO, **Alyousif, ZA**, Silette, CR, Scheuermann, BR, Rotarius, TI, Scheuermann, BW. Effect of High Fat Meal on Blood Flow and Endothelial Function During Passive Leg Movement. (**Published abstract**)

Sugiura, S, **Alyousif, ZA**, Silette, CR, Garmyn, EC, Scheuermann, BW. The Effects of High Intensity Interval Training (HIIT) with L-arginine versus HIIT on Cardiovascular Function. Journal of Athletic Training. **2015**; 50(6): S-223. (**Published abstract**)

Sugiura, S, **Alyousif, ZA**, Scheuermann, BW. The Effects of High Intensity Interval Training (HIIT) on Insulin Sensitivity in a Family History of Type 2 Diabetes Mellitus (T2DM). (Unpublished manuscript)

Alyousif, ZA. The Effects of High Intensity Interval Training on Asthmatic Adult Males. A Poster Presentation at the Annual Kinesiology Graduate Student Research Symposium at the University of Toledo. (May 1, 2015)

Alyousif, ZA. The Effects of High Intensity Interval Training on Asthmatic Adult Males. Presentation at the 6th Annual CHS Undergraduate and Graduate Research Forum at the University of Toledo (April 10, 2015)

Alyousif, ZA. The Effects of High Intensity Interval Training on Asthmatic Adult Males. OhioLINK 2014. (**Published**)

Research in Progress

Mckenzie, NI, **Alyousif ZA**, Hernandez, DA. The effects of short-term use of electronic cigarettes on respiratory physiology in smokers without respiratory disorders and in healthy nonsmokers

Conferences, Symposia & Workshops Attended

- American Association of Respiratory Care (AARC) conference in Las Vegas (Dec 4-7, 2018)
- Sleep Apnea Symposium at the University of Toledo Medical Center (August 24, 2015)
- 2014 Annual Meeting of the American College of Sport Medicine in Orlando, Florida (May 27–31)
- High Frequency Oscillatory Ventilation (HFOV Workshop) in King Fahad Specialist Hospital -Dammam (September 28 – 30, 2011)
- Saudi Thoracic Society (Against Tuberculosis & Lung Disease (IUATLD) & Recent advances in respiratory care symposium) (March 20 – 22, 2011).
- Respiratory Care Program in King Faisal University (Symposium & Workshop on Respiratory Care & Mechanical Ventilation) (September 13 – 15, 2010).

Workshops, Training & Clinical Instruction Conducted

- Clinical instructor respiratory students Dammam Medical Complex (January–December 2011)
    - Twice per week for 10 hours/day for two semesters
- Asthma Crash Course Program (July 30, 2011)

- CPR Instructor Certified by Saudi Heart Association (former ABC guidelines) (2011)
- Mechanical Ventilation Course & Workshop in King Fahad Specialist Hospital – Dammam (speaker) (December 22, 2010).

Professional & Academic Membership

- Member at American College of Sport Medicine
- Member at Golden Key International Honor Society
- Successfully completed the requirements for Vapotherm Precision Flow online course
- Member of American Association of Respiratory Care (AARC)

Community Service

- CedarCreek Church Community Clinic
- Ahlul Bayt Center of Toledo
- Soccer Coach at Center of Toledo
- Board of education at the Islamic School of Greater Toledo