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Lipoprotein associated phospholipase A2 in individuals with obstructive sleep apnea

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Lipoprotein Associated Phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}) in Individuals with Obstructive Sleep Apnea (OSA)

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Abstract

Lipoprotein-associated Phospholipase A2 (Lp-PLA2) is a protein produced by inflammatory cells in circulation and is associated with cardiovascular disease (CVD) risk. Prior research demonstrates that obstructive sleep apnea (OSA) leads to increased inflammation and is also related to CVD. Physical activity is known to reduce inflammation and risk for both CVD and OSA. However, Lp-PLA2 has yet to be examined in individuals with OSA who do not have any other pre-existing conditions nor has it been associated with chronic physical activity. The purpose of this study is to examine if there is a relationship between Lp-PLA2 mass and OSA. A secondary purpose of this study is to determine if physical activity levels have an impact on Lp-PLA2 mass.

A total of 39 subjects with an average BMI of 29.7 (±5.3) were screened for risk of OSA with an at home assessment device and placed into OSA and non-OSA groups. Data collected included anthropometric data, Lp-PLA2 mass, maximal oxygen uptake (VO2max), resting heart rate and blood pressure, heart rate variability, and an assessment of physical activity using an accelerometer. There was no significant difference in Lp-PLA2 mass between OSA and non-OSA groups (mean ± SD: 160.5 ±121.3 vs 124.7 ± 134.4 ng/mL). However, after taking the mean value for Lp-PLA2 mass and dividing subjects up into two groups, one above and one below the mean, the group with the higher Lp-PLA2 had greater waist circumference (87.8 ± 12.2 vs 100.1 ± 11.9 cm; P < 0.05), sedentary minutes (645.4 ± 161.3 vs 937.3 ± 219.1; P < 0.001), and light intensity physical activity minutes (187.9 ± 84.5 vs 94.6 ± 27.0; P = 0.002). There appears to be no relationship between OSA and Lp-PLA2 mass aside from a positive correlation to sedentary minutes. However, when separated by mean Lp-PLA2 mass, it does appear that
Lp-PLA₂ is associated with waist circumference, sedentary time, and time in light activity. This suggests that there could be a relationship between physical activity behavior and Lp-PLA₂, as a result of differing adiposity profile.
Chapter 1

Introduction

Lipoprotein-associated Phospholipase A$_2$

Lipoprotein-associated Phospholipase A$_2$ (Lp-PLA$_2$) is a protein produced by inflammatory cells in circulation and is associated with cardiovascular disease (CVD) risk in individuals with metabolic disorders (Lavi, 2007). After being produced by macrophages in atherosclerotic plaque, Lp-PLA$_2$ is then secreted into the circulatory system with about 80% of it bound to low-density lipoproteins (LDL) and the other 20% bound to high-density lipoproteins (HDL) (Stafforini, 2015). Within the endothelium, LDL becomes oxidized and yields products that are hydrolysed by Lp-PLA$_2$, generating oxidized phospholipids, such as lysophosphatidylcholine (lysoPC) and non-esterified fatty acids (NEFAs) (Benitez, 2004). These products are important in mediating the inflammatory process by upregulating expression of adhesion molecules (Shi, 2007), activating leukocytes (Shi, 2007), and recruiting macrophages and monocytes to the atherosclerotic plaque (Quinn, 1988). This cycle accelerates plaque formation because the increase in inflammation produces even more Lp-PLA$_2$, essentially upregulating itself (Shi, 2007). One study suggests that Lp-PLA$_2$ may be made locally by the macrophages as Lp-PLA$_2$ mRNA transcripts have been found in macrophages (Hakkinen, 1999).

Lp-PLA$_2$ in normal population

Lp-PLA$_2$ measurements can be separated into enzyme mass and activity. Both mass and activity are significantly correlated with each other as well as with very low density lipoprotein (VLDL), LDL, and HDL (Caslake, 2000). Both Lp-PLA$_2$ mass and activity stay relatively consistent in an individual, not being affected by acute
inflammation or infection compared to other markers of inflammation, such as high sensitivity C-reactive protein (hsCRP) (Corson, 2008; Brilakis, 2008; Persson, 2007-epi). This enables better clinical decisions from a single measurement and provides confidence in trends in this inflammatory marker over a period of time (Corson, 2008). One review found a strong positive correlation ($r=0.87$) between Lp-PLA$_2$ mass and activity in plasma samples taken from 22 subjects (Dada, 2002). In a large epidemiologic study, there was a strong correlation between Lp-PLA$_2$ mass and activity, with more variance occurring between the two at higher levels (Persson, 2007-epi). The highest correlations were seen in Lp-PLA$_2$ activity and cholesterol, LDL, and LDL/HDL ratio, with an inverse relationship to HDL. A weaker correlation was seen between these variables and Lp-PLA$_2$ mass (Persson, 2007-epi). In this same study, higher Lp-PLA$_2$ levels were found in smokers compared to non-smokers, individuals with a history of CVD compared to those without, obese individuals compared to non-obese, and diabetics compared to non-diabetics (Persson, 2007-epi); although it should be noted that these differences were not statistically significant. In addition, men tend to have higher levels of Lp-PLA$_2$ mass and activity than women (Brilakis, 2008; Persson, 2007-epi). Lp-PLA$_2$ activity was also lower in African Americans and Hispanics compared to Caucasians with the lowest levels found in African American women and the highest in Caucasian males (Brilakis, 2008).

**Lp-PLA$_2$ & CVD risk**

Many of the common risk factors for atherosclerosis and CVD, such as hypertension, high LDL, smoking, and obesity, are not exhibited in up to 50% of cardiac patients, indicating that there are other potential risk factors that need to be studied further (Sytkowski, 1990; Dada, 2002). Compared to healthy individuals, activity and
mass of Lp-PLA₂ is significantly increased in people with CVD (Cai, 2013). Evidence shows that Lp-PLA₂ plays a crucial role in the pathophysiology of atherosclerosis and is indicative of future cardiovascular disease (Acevedo, 2015). Measuring Lp-PLA₂ levels is useful to evaluate the presence of atherosclerosis, especially in high risk individuals that may have metabolic syndrome (Acevedo, 2015). Originally, >235 ng/mL of Lp-PLA₂ mass was defined as the cutoff for increased risk based off of the 50th percentile of a healthy population (Lanman, 2006). After reviewing many Lp-PLA₂ studies, one consensus panel determined that a clinical cut point of Lp-PLA₂ mass >200 ng/mL would indicate a patient being at higher risk for cardiovascular disease (Davidson, 2008).

Vascular events alter both Lp-PLA₂ mass and activity, which may be due to the alterations in HDL/LDL levels (Delgado, 2012). While Lp-PLA₂ mass and activity are both linked to lipid levels, Lp-PLA₂ mass also has a strong link with cholesterol and triglyceride levels (Delgado, 2012). One study found that Lp-PLA₂ mass was higher in individuals with coronary artery disease (CAD) and subjects post-myocardial infarction compared to a control group (Caslake, 2000). Interestingly, the Lp-PLA₂ mass levels were higher in individuals with CAD even though LDL was not significantly different from the control group. This indicates that plasma Lp-PLA₂ mass was a strong predictor of risk, independent of common risk factors (LDL, HDL, smoking, and BP) in the CAD group (Caslake, 2000). It was speculated that as LDL is cleared from plasma, Lp-PLA₂ would be cleared at the same rate. However, Lp-PLA₂ has also been found bound to a small dense LDL subtype that has a prolonged half-life, which could explain the elevated Lp-PLA₂ mass in these subjects regardless of LDL (Guerra, 1997).
Lp-PLA$_2$ activity and mass has been associated as an independent and significant indicator of metabolic syndrome and inflammation, possibly increasing the risk of CVD (Persson, 2007; Acevedo, 2015). Regardless of metabolic syndrome, higher Lp-PLA$_2$ levels have been found to increase risk of CVD (Persson, 2007). However, high Lp-PLA$_2$ levels in combination with metabolic syndrome has the highest risk for CVD (Persson, 2007). In addition, Lp-PLA$_2$ appears to be influenced by weight, metabolic state and unfavorable circulating lipid profiles (increased oxidized LDL and triglycerides) (Jackisch, 2018). These responses are exacerbated in individuals with type 2 diabetes, which also increases risk for CVD (Jackisch, 2018).

The expression of Lp-PLA$_2$ was observed in coronary arteries from 25 subjects who died from sudden cardiac death. Plaque samples with increased Lp-PLA$_2$ were associated with thin, fibrous caps or ruptured plaques compared to more stable lesions that had less Lp-PLA$_2$ (Kolodgie, 2006). An increased Lp-PLA$_2$ mass and activity and lysoPC levels in carotid artery plaques of patients with symptomatic cerebrovascular disease has also been associated with markers of tissue oxidative stress, inflammation, and instability. (Mannheim, 2008) This suggests that there may be a relationship between Lp-PLA$_2$ and plaque instability (Kolodgie, 2006; Mannheim, 2008).

A large portion of patients who suddenly die from CVD present no symptoms prior to their event (Go, 2014). The Framingham Risk Score (FRS) has been used to help detect CVD risk but still many patients are misclassified (NCEP, 2002). In a previous study, many of those who were classified as low risk by the FRS were still classified as high risk by Lp-PLA$_2$ mass (>200 ng/mL) (Hargens, 2014). This suggests that Lp-PLA$_2$ mass may be indicative of CVD independently of other risk factors.
A meta-analysis of 25 clinical trials found elevated Lp-PLA₂ caused an almost two-fold increase in risk for future and recurring CVD events (Corson, 2008). Thus, it was determined that Lp-PLA₂ is a relevant risk factor to identify individuals at a high risk for CVD and those who could benefit from intensive lipid-lowering interventions (Corson, 2008). Another meta-analysis of 32 clinical studies found increased relative risks in individuals with elevated Lp-PLA₂ levels for CHD, ischemic stroke, vascular mortality, and non-vascular mortality (Thompson, 2010). There have also been observed elevations in Lp-PLA₂ in individuals with proven coronary artery disease, independently of LDL, HDL, smoking, and systolic blood pressure (Caslake, 2000). This adds to the evidence that Lp-PLA₂ levels may be a valuable addition to traditional risk factors in assessing CVD risk as been suggested by a consensus panel (Davidson, 2008).

**Obstructive Sleep Apnea (OSA)**

Obstructive Sleep Apnea (OSA) can be described as recurrent episodes of full or partial collapse of the airways during sleep, leading to decreased ventilation, frequent arousals, sleep fragmentation, and oxyhemoglobin desaturation (Somers, 2008). An apnea event can be described as a complete restriction of airflow for greater than 10 seconds while a hypopnea event is defined as a reduction in airflow to less than 50% of normal airflow (Somers, 2008). Diagnosis and determination of severity of sleep apnea is defined by the apnea-hypopnea index (AHI), which is the number of apnea or hypopnea events per hour (mild OSA: ≥ 5 AHI; moderate OSA: ≥ 15 AHI; severe OSA ≥ 30 AHI) (Young, 2002). Recent prevalence estimates of moderate to severe sleep apnea are 10% among 30–49-year-old men; 17% among 50–70-year-old men; 3% among 30–49-year-old women; and 9% among 50–70 year-old women (Peppard, 2013). These estimates
represent a significant increase of 14% to 55% over the last two decades depending on the subgroup (Peppard, 2013). The Wisconsin Sleep Cohort measured AHI over a period of eight years and found the greatest increase in AHI across individuals who were habitual snorers, had a body mass index (BMI) ≥ 30, and those who were 45-60 years of age (Young, 2002). In addition, it is predicted that about 80% of individuals with moderate to severe OSA are undiagnosed (Lee, 2008) and even those who are diagnosed often choose not to accept treatment (Weaver, 2008). When left untreated, OSA can lead to daytime sleepiness, cognitive dysfunction, and decrease in quality of life (Punjabi, 2008).

**Sleep Apnea and CVD**

Interrupted sleep, hypoxemia, and daytime sleepiness due to OSA induces an increase in oxidative stress, inflammation, and endothelial apoptosis, which contributes to endothelial dysfunction, eventually leading to atherosclerosis or CVD if left untreated (Somers, 2008; Atkeson, 2009; Savransky, 2007). Individuals with OSA have been shown to have an increased sympathetic activation, leading to an increased resting heart rate (Narkiewicz, 1998). These individuals have also been seen to have decreased heart rate variability and increased blood pressure variability (Narkiewicz, 1998). In addition, OSA has been associated with insulin resistance, independent of BMI (Punjabi, 2004). Lastly, the forced inspiration during sudden arousals at night creates dramatic pressure change, causing a negative intrathoracic pressure environment and increased stress on the heart (Somers, 2008). About 50% of patients who have OSA also have hypertension (Silverberg, 1998). Even after controlling for age, sex, BMI, and hypertension
medications, the Wisconsin Sleep Cohort study found a dose response relationship between blood pressure and OSA (Peppard, 2000).

One study found that, in middle aged males with OSA, those who were not treated for their condition had a significantly higher CVD incidence (56.8%) compared to those who were treated for their OSA (6.7%) (Peker, 2002). They concluded that individuals with OSA have an increased risk for CVD, independent of age, BMI, blood pressure, and smoking habits. However, those who are treated for their OSA greatly reduced their risk of developing CVD (Peker, 2002).

Due to the metabolic damage caused by apnea events, OSA has also been linked to type 2 diabetes. One study found that sleep disordered breathing was independently related to glucose intolerance and insulin resistance and that the severity of the sleep disordered breathing was also associated with the degree of insulin resistance (Punjabi, 2004). These hypoxic events have been linked to an increase in proinflammatory cytokines, specifically interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), which has been linked to insulin resistance and increased risk for type 2 diabetes (Huiguo, 2000; Punjabi, 2004). Individuals with sleep disordered breathing have also shown to have increased sympathetic neural activity (Peled, 1998), which can increase glycogen breakdown and gluconeogenesis and disrupt glucose homeostasis (Punjabi, 2004). In addition, sleep-disordered patients have increased levels of cortisol (Bratel, 1999), which has been shown to increase glucose levels and insulin concentration (Plat, 1999).

**Weight and Sleep Apnea**
Obesity has consistently been associated with OSA and has even been said to be one of the most important risk factors for sleep disordered breathing (Young, 2002, Wolk, 2003). About 40% of obese individuals have significant sleep apnea and about 70% of individuals with OSA are obese (Vgontzas, 1994). One study found that a 10% increase in weight increased the odds of developing sleep apnea 6-fold (Peppard, 2000). In addition, a 10% decrease in weight was associated with a 26% decrease in AHI (Peppard, 2000). Research suggests that obesity leads to the narrowing and altering of function of the airways (Horner, 1989; Schwartz, 1991). Obese individuals have large fat deposits surrounding the collapsible part of the pharynx, and this is exacerbated in individuals with OSA compared to weight-matched controls without OSA (Horner, 1989). Weight loss has been associated with a decrease in airway collapsibility in individuals who have sleep apnea (Schwartz, 1991). Male patients who have OSA have about 50% higher leptin levels than weight-matched individuals without OSA (Phillips, 2000). Thus, it is likely that leptin resistance is especially increased in individuals with OSA, potentially leading to even greater weight gain and progression of OSA (Wolk, 2003). In healthy individuals who experience weight gain, the possibility of developing OSA is increased. In overweight individuals with OSA, further weight gains accelerate the progression of OSA (Young, 2002; Peppard, 2000). The greater the increase in body weight or waist circumference, the greater the progression of OSA and vice versa (Young, 2002; Peppard, 2000). In addition, individuals who currently have OSA and gain weight experience an increase in OSA severity independent of other factors such as age, body composition, and smoking habits (Peppard, 2000).

Exercise & Sleep Apnea
Similar to baseline weight, habitual physical activity levels predict development of OSA. Awad et al. observed that active individuals exhibited a decreased incidence of OSA in an 8-year follow up compared to sedentary individuals. Furthermore, decreasing exercise over time was associated with an increase in AHI (Awad, 2012). Individuals with OSA tend to be more sedentary regardless of levels of obesity or daytime sleepiness (Hargens, 2019). In addition to just being active, exercise training volume exhibits a dose-response relationship for likelihood of OSA (Peppard, 2004).

Exercise is also beneficial in the reduction of obesity, blood pressure, and CVD, which are all associated with OSA (Young, 2002). In addition to these benefits, AHI levels may be reduced by up to 50% following chronic exercise training in those who have sleep apnea (Norman, 2000; Sengul, 2011). This may be due to general improvements in strength and fatigue resistance of the airways (Vincent, 2002). In sedentary, obese individuals with untreated moderate to severe sleep apnea, significant improvements in AHI were observed following an exercise training program, even without a significant reduction in body weight (Kline, 2011). There were also improvements in both objective and subjective sleep quality, suggesting that exercise training may be beneficial (Kline, 2011). Similarly, another study found that exercise training led to improvements in AHI, sleep efficiency, and daytime sleepiness, independent of changes in BMI (Iftikhar, 2014). A similar study in OSA patients found that regular exercise training improved BMI, along with aerobic capacity and quality of life (Norman, 2000). A meta-analysis of exercise intervention studies also observed a reduction in AHI regardless of types, durations, or frequencies of exercise or continuous positive airway pressure (CPAP) use (Aiello, 2016).
Chronic exercise is associated with an increase in slow wave sleep (SWS), total sleep time; and decreases in rapid eye movement (REM) sleep, sleep onset latency (SOL), and wake after sleep onset (WASO) (Kubitz, 1996). In previously sedentary individuals, a two month exercise intervention increased vagal activity and decreased sympathetic activity during sleep (Pichot, 2002). The exact physiological mechanisms of how exercise improves OSA are unknown and more research is needed to determine how exercise improves OSA independent of weight loss.

Exercise intensity is also associated with prevalence and severity of OSA. Vigorous physical activity for >3 hours has been associated with a larger decrease in the prevalence of OSA than the same duration of exercise performed at a moderate intensity (Quan, 2007). These outcomes were more pronounced in males and obese individuals (Quan, 2007). These findings were similar to another study who also observed increased planned exercise associated with less severe OSA (Peppard, 2004). Both studies determined that benefits were seen with about 3-6 hours of exercise per week or at least 3 hours of vigorous intensity, independent of weight loss and daytime sleepiness (Quan, 2007; Peppard, 2004).

**OSA & Lp-PLA₂**

In recent years research has evaluated the relationship between OSA and Lp-PLA₂ concentration. In one study looking at CVD risk among individuals with OSA, COPD, or overlap syndrome (having both OSA and COPD), they found that Lp-PLA₂ mass was elevated in all 3 groups, with the highest concentration in the overlap syndrome group, followed by the OSA group (Badr, 2014). In a similar study, Lp-PLA₂ mass was elevated in individuals with OSA and in individuals with metabolic syndrome, but those
who had OSA and metabolic syndrome had the highest concentration of Lp-PLA₂ (Moise, 2018). Another study separated the OSA subjects into mild, moderate, and severe categories and found that Lp-PLA₂ mass increased with OSA severity and also increased with the presence of CVD in addition to OSA (Xu, 2020). Lastly, a study that solely studied OSA and its correlation to Lp-PLA₂ mass, separated 50 male patients diagnosed with OSA into quartiles based on their number of arousals. They found that those in the highest quartile had the highest Lp-PLA₂ (Bekci, 2011).

Thus, it is apparent that there is a relationship between Lp-PLA₂ and OSA. It is also known that Lp-PLA₂ concentration and presence of OSA are typically higher in individuals who are at risk for CVD and in individuals who are overweight. In addition to these risk factors, OSA has been shown to be both more prevalent and more severe in those who lead a sedentary lifestyle.

Purpose

It has not been examined if chronic physical activity influences Lp-PLA₂ concentration. Additionally, there needs to be more research in the relationship between Lp-PLA₂ and individuals with OSA who have no other pre-existing conditions. Therefore, the primary purpose of this study is to determine whether there is an increase in Lp-PLA₂ concentration in individuals with OSA who have no other chronic conditions. In addition, we will determine if there is a relationship between Lp-PLA₂ concentration and physical activity. We hypothesize that individuals with OSA will have higher Lp-PLA₂ concentrations. In addition, individuals Lp-PLA₂ concentration will be associated with chronic physical activity, with more active individuals exhibiting a lower concentration of Lp-PLA₂.
Limitations

One limitation in this study includes using an at home screening device to diagnose OSA. However, despite this being an at-home screening tool, it has been validated against hospital based polysomnography (Ng, 2009). We are assuming that the equipment used in this study provides valid assessments of the variables we are observing and that the participants understand our instructions and are knowledgeable in how to properly assemble the at home screening device. The questionnaires utilized for this study are also assuming that participants are disclosing accurate information about their health history and physical activity. Additionally, we are limiting our subjects to individuals who do not have other pre-existing chronic diseases. Lastly, placing an age range on our subjects means that we will not be able to generalize our results to individuals who are outside that age range.
Subjects

Participants were recruited through bulk email requests to all JMU faculty and students. Thirty-nine subjects were recruited for the study (11 individuals with OSA and 28 control subjects). Inclusion criteria for both the experimental and control groups included subjects that have been cleared to exercise by the Physical Activity Questionnaire-Plus (PAR-Q+) and are between the ages of 18 and 57 years old. Individuals in the experimental group had an AHI of at least 5. Individuals in the control group had an AHI of <5. Exclusion criteria included participants who had a current diagnosis of any cardiovascular, metabolic, or pulmonary disease as well as participants who were pregnant (due to the low dose of radiation from DEXA).

Experimental methods

This study was part of a larger study that looked at airway responsiveness and nitric oxide (NO) exhalation in individuals with OSA following a maximal exercise bout. Subjects visited the laboratory a total of 2 times. During the first visit, after explaining and signing a subject consent form, subjects completed a series of questionnaires, including a PAR-Q+, International Physical Activity Questionnaire (IPAQ), Epworth Sleepiness Scale (ESS), and Berlin Questionnaire. The IPAQ is validated in adults to assess moderate to vigorous physical activity (MVPA) per week both in leisure time and work related physical activity (Craig, 2003). The PAR-Q+ is a validated, self-guided physical activity readiness questionnaire (Riebe, 2018). If the participant reported that they do not regularly participate in planned exercise, they must also have no signs or
symptoms of cardiovascular disease, metabolic or renal disease. If they did have signs
and symptoms or were asymptomatic but have previously been diagnosed with the
aforementioned diseases, they were excluded from the study. Participants that engaged in
regular physical activity, were asymptomatic and had never been diagnosed with renal,
metabolic or cardiovascular diseases were cleared by the American College of Sports
Medicine (ACSM) guidelines to undergo the incremental exercise test to exhaustion to
determine peak oxygen uptake (VO$_{2peak}$), even if they have sleep apnea. The Berlin
Questionnaire and ESS assessed the subjects snoring habits and daytime sleepiness
(Kang, 2013; Johns, 1992). Anthropometric variables were then obtained including,
height, weight, and waist/neck circumferences. Cardiorespiratory fitness was determined
using a graded cycle ergometer VO$_{2max}$ test. Subjects were then sent home with an
unattended, home sleep evaluation using a type III validated device (ApneaLink™ Plus,
RedMed Corp., San Diego, CA) to assess for the likely presence of OSA (Ng, 2009).
Research staff instructed the patient on the proper setup and use of the at-home screening
device. The device is composed of a pulse oximeter, which was worn on the end of an
index finger, and a nasal cannula, which was worn over the face, and into the nose to
measure airflow. This device is harmless and painless to wear. The results of this test will
objectively divide the subjects into OSA and non-OSA control groups. Subjects were also
sent home with an accelerometer (Actigraph GT3X+, Actigraph Corp., Pensacola, FL) to
assess their 7-day physical activity. Subjects were asked to wear the device on their waist
at all times, except while sleeping and showering. Accelerometers have been validated to
assess MVPA per week (Kelly, 2013). On their second visit, a fasted, resting venous
blood draw was taken. This was followed by a heart rate variability assessment that also
included a resting blood pressure measurement in the supine position. Lastly, a Dual-energy X-ray absorptiometry (DEXA) analysis using the General Electrics (GE) Lunar iDEXA was performed to assess body composition (body fat percentage and lean mass).

**Fasting blood collection**

10 ml of blood was drawn from an antecubital vein using clean venipuncture. Blood samples were immediately centrifuged for 20 min at 1,500xg and 4°C to obtain platelet-poor plasma. Plasma aliquots were frozen and stored at -20°C until assayed. These samples were used to assess Lp-PLA₂ mass.

**Blood Analysis**

An Enzyme-Linked Immunosorbent Assay (ELISA) kit was used to determine Lp-PLA₂ mass. Plasma samples were pipetted into the wells and the target protein becomes bound to the wells by an immobilized antibody after sitting for 2.5 hours. Cells are then washed four times to remove any unbound antibodies. The wells are then washed with another antibody, sandwiching the Lp-PLA₂ molecules between the two antibodies after sitting for 1 hour. Cells are then washed four times again to remove any unbound antibodies. A HRP-streptavidin solution was then pipetted into the wells with the absorbance of this enzyme being directly proportional to the Lp-PLA₂ concentration in the sample. After 45 minutes, the wells were washed for a third time. A TMB substrate is then added to the wells and a color develops, proportional to the concentration of Lp-PLA₂. A Stop solution is then added and the color turns from blue to yellow with the intensity of the color being measured at 450 nm. (Sigma-Aldrich ELISA Kit Information; Dada, 2002)

**Graded Exercise Test**
A maximal ramped (15 Watts per minute ramp rate) exercise test was completed on an electronically braked, cycle ergometer. Metabolic responses were measured continuously using a metabolic cart (Parvo Medics TrueOne 2400, Parvo Medics, Salt Lake City, UT). Heart rate was measured continuously using a Polar V800 watch and Polar H10 heart rate chest strap (Polar Electro). Rating of perceived exertion was measured every minute using the Borg 6-20. Dyspnea ratings were also recorded every minute using a dyspnea scale from 1-10. Blood pressure was taken manually every 3 minutes during the test. Subjects were instructed to keep their revolutions per minute (RPM) above 50 and the test was terminated either by the subject voluntarily or if 50 RPMs could not be maintained.

**Statistical Analysis**

All statistical analyses were performed using IBM SPSS Statistics (version 26.0). Independent sample $t$ tests were used to assess group differences in Lp-PLA$_2$ mass. Pearson correlations were calculated to determine relationships between Lp-PLA$_2$, OSA, physical activity and sedentary behavior. A value of $P < 0.05$ was considered statistically significant *a priori*. 
Chapter 3
Manuscript

Lipoprotein Associated Phospholipase A2 (Lp-PLA₂) in Individuals with Obstructive Sleep Apnea (OSA)

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Abstract

**Introduction:** Lipoprotein-associated Phospholipase A₂ (Lp-PLA₂) is a protein produced by inflammatory cells in circulation and is associated with cardiovascular disease (CVD) risk. Prior research demonstrates that obstructive sleep apnea (OSA) leads to increased inflammation and is also related to CVD. Physical activity is known to reduce inflammation and risk for both CVD and OSA. However, Lp-PLA₂ has yet to be examined in individuals with OSA who do not have any other pre-existing conditions nor has it been associated with chronic physical activity. The purpose of this study was to examine if there is a relationship between Lp-PLA₂ mass and OSA. A secondary purpose of this study was to determine if physical activity levels have an impact on Lp-PLA₂ mass.

**Methods:** A total of 39 subjects with an average BMI of 29.7 ±5.3 were screened for risk of OSA with an at home assessment device and placed into OSA and non-OSA groups. Data collected included anthropometric data, Lp-PLA₂ mass, maximal oxygen uptake (VO₂max), resting heart rate and blood pressure, heart rate variability, and an assessment of physical activity using an accelerometer.

**Results:** There was no significant difference in Lp-PLA₂ mass between OSA and non-OSA groups (160.5 ±121.3 vs 124.7 ± 134.4 ng/mL). However, after taking the mean value for Lp-PLA₂ mass and dividing subjects up into two groups, one above and one below the mean, the group with the higher Lp-PLA₂ had greater waist circumference (87.8 ± 12.2 vs 100.1 ± 11.9 cm; P < 0.05), sedentary minutes (645.4 ± 161.3 vs 937.3 ± 219.1; P < 0.001), and light intensity physical activity minutes (187.9 ± 84.5 vs 94.6 ± 27.0; P = 0.002).
**Conclusions:** There appears to be no relationship between OSA and Lp-PLA₂ mass aside from a positive correlation to sedentary minutes. However, when separated by mean Lp-PLA₂ mass, it does appear that higher Lp-PLA₂ is associated with waist circumference, sedentary time, and time in light activity. This indicates that there could be a relationship between physical activity behavior and Lp-PLA₂, as a result of differing adiposity profile.
Introduction

Lipoprotein-associated Phospholipase A₂ (Lp-PLA₂) is a protein produced by inflammatory cells in circulation and is associated with increased cardiovascular disease (CVD) risk in individuals with metabolic disorders (Lavi, 2007). This biomarker is bound primarily to low density lipoprotein (LDL) and causes an acceleration in plaque formation, increasing inflammation and essentially upregulating itself (Stafforini, 2015; Shi, 2007). Lp-PLA₂ concentration can be separated into enzyme mass and activity, which are both associated with very low density lipoprotein (VLDL), LDL, and high density lipoprotein (HDL), with Lp-PLA₂ mass also being correlated with triglyceride and cholesterol levels (Caslake, 2000; Delgado, 2012). Lp-PLA₂ concentration stays relatively consistent, as it is not affected by acute inflammation compared to other markers of inflammation, such as high sensitivity C-reactive protein (hsCRP) (Corson, 2008; Brilakis, 2008; Persson, 2007-epi). However, Lp-PLA₂ concentration can be influenced by chronic inflammation. Higher levels of the biomarker are found in men, smokers and individuals with a history of CVD, obesity, diabetes, or metabolic syndrome (Persson, 2007-epi; Persson, 2007; Brilakis, 2008; Jackisch, 2018) and has also been associated with plaque instability (Kolodgie, 2006; Mannheim, 2008). Elevated Lp-PLA₂ increases risk for developing CVD or experiencing recurring CVD events, even in those determined to be low risk (Thompson, 2010, Corson, 2008, Hargens, 2014). This adds to the evidence that Lp-PLA₂ levels may be a valuable prognostic tool for CVD events in addition to traditional risk factors.

With this research, it seems that Lp-PLA₂ is associated with many risk factors and conditions that often lead to or are caused by chronic inflammation. Obstructive Sleep
Apnea (OSA), which can be described as recurrent episodes of full (apnea) or partial (hypopnea) collapse of the airways during sleep, results in systemic inflammation (Somers, 2008; Atkeson, 2009; Savransky, 2007). Apneas and hypopneas lead to decreased ventilation, frequent arousals, sleep fragmentation, and oxyhemoglobin desaturation (Somers, 2008). Diagnosis and severity of sleep apnea is determined by the apnea-hypopnea index (AHI), which is the number of apneas and hypopneas per hour of sleep. Interrupted sleep due to OSA induces an increase in oxidative stress, inflammation, and endothelial apoptosis, which contributes to endothelial dysfunction, eventually leading to atherosclerosis or CVD if left untreated (Atkeson, 2009; Savransky, 2007).

The link between OSA, inflammation, and CVD suggests a possible role of Lp-PLA₂. This has been demonstrated in recent studies finding that Lp-PLA₂ mass is higher in individuals with OSA, and increased with OSA severity (Badr, 2014; Moise 2018; Xu, 2020, Bekci, 2011). However, many of these studies examined an older population with OSA and other co-morbid conditions.

While OSA may increase inflammatory markers, including Lp-PLA₂, habitual physical activity is anti-inflammatory and has been associated with a decrease in traditional risk factors (Kokkinos, 2012). In regard to OSA, individuals with the condition tend to be more sedentary, regardless of BMI or daytime sleepiness (Hargens, 2019). However, active individuals exhibited a decreased incidence of OSA in an 8-year follow up compared to sedentary individuals, while individuals who decreased exercise over time exhibited a significant increase in AHI (Awad, 2012). Since physical activity reduces indicators of inflammation, the impact that physical activity may have on Lp-PLA₂ has not been explored.
Therefore, the primary purpose of this study is to determine whether there is an increase in Lp-PLA\(_2\) concentration in individuals with OSA who have no other chronic conditions. In addition, we will determine if there is a relationship between Lp-PLA\(_2\) concentration and physical activity. It is hypothesized that individuals with OSA will have higher Lp-PLA\(_2\) concentration. In addition, more active individuals will exhibit a lower concentration of Lp-PLA\(_2\).

**Methodology**

*Subjects*

Participants were recruited through bulk email requests to all JMU faculty and students. In addition to subjects recruited through these methods, previous data was added to the sample pool (6 individuals with OSA and 10 control subjects) from an unpublished study using identical methods. Thirty-nine subjects (18-57 years of age) were used in the analysis for this study (11 individuals with OSA and 28 control subjects). Inclusion criteria for both the experimental and control groups included subjects that have been cleared to exercise by the Physical Activity Questionnaire-Plus (PAR-Q+). Individuals in the experimental group had an AHI of at least 5. Individuals in the control group had an AHI of <5. Exclusion criteria included participants with known cardiovascular, metabolic, or pulmonary disease as well as participants who were pregnant (due to the low dose of radiation from DEXA).

*Experimental methods*

This study was part of a larger study evaluating airway responsiveness and nitric oxide (NO) exhalation in individuals with OSA following a maximal exercise bout. Subjects visited the laboratory a total of 2 times. During the first visit, subjects completed
the informed consent and initial questionnaires, including a PAR-Q+, International Physical Activity Questionnaire (IPAQ), Epworth Sleepiness Scale (ESS), and Berlin Questionnaire. The IPAQ is validated in adults to assess moderate to vigorous physical activity (MVPA) per week both in leisure time and work related physical activity (Craig, 2003). The PAR-Q+ is a validated, self-guided physical activity readiness questionnaire (Riebe, 2018). The Berlin Questionnaire and ESS assessed the subjects snoring habits and daytime sleepiness (Kang, 2013; Johns, 1992). Anthropometric variables were then obtained including, height, weight, and waist/neck circumferences. Cardiorespiratory fitness was determined using a graded cycle ergometer maximal oxygen uptake (VO₂ max) test. Subjects were then sent home with an unattended, home sleep evaluation using a type III validated device (ApneaLink™ Plus, RedMed Corp., San Diego, CA) to assess for the likely presence of OSA (Ng, 2009). Research staff instructed the patient on the proper setup and use of the at-home screening device. The device is composed of a pulse oximeter, which was worn on the end of an index finger, and a nasal cannula, which was worn over the face, and into the nose to measure airflow. The results of this test were used to objectively divide the subjects into OSA and non-OSA control groups. Subjects were also sent home with an accelerometer (Actigraph GT3X+, Actigraph Corp., Pensacola, FL) to assess their 7-day physical activity. Subjects were asked to wear the device on their waist at all times, except while sleeping and showering. Accelerometers have been validated to assess MVPA per week (Kelly, 2013). On their second visit, a fasted, resting venous blood draw was taken. This was followed by a Dual-energy X-ray absorptiometry (DEXA) analysis using the General Electrics (GE) Lunar iDEXA to assess body composition (body fat percentage and lean mass).
Fasting blood collection

10 ml of blood was drawn from an antecubital vein using clean venipuncture. Blood samples were immediately centrifuged for 20 min at 1,500xg and 4°C to obtain platelet-poor plasma. Plasma aliquots were frozen and stored at -20°C until assayed. These samples were used to assess Lp-PLA₂ mass.

Blood Analysis

An Enzyme-Linked Immunosorbent Assay (ELISA) kit was used to determine Lp-PLA₂ mass (Sigma-Aldrich). All samples were measured in duplicate at minimum.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (version 26.0). Independent sample t tests were used to assess group differences in Lp-PLA₂ mass. Pearson correlations were calculated to determine relationships between Lp-PLA₂, AHI, physical activity, and sedentary behavior variables. A value of P < 0.05 was considered statistically significant a priori.

Results

By design, the OSA group had a significantly higher AHI than the non-OSA group. Subject characteristics for the OSA and non-OSA groups are listed in Table 1. The OSA group was older than the non-OSA group (P = 0.001). There were no group differences in any body composition variable, however there was a trend for a greater neck circumference in the OSA group (P = 0.05). There was no correlation between Lp-PLA₂ mass and age (r=0.104, P=0.536) or AHI (r=0.122, P=0.458). Independent sample t-test revealed no difference in Lp-PLA₂ mass between the OSA and non-OSA groups,
which did not change when age was used as a covariate in a one way analysis of variance (P=0.45). (Figure 1)

*Lp-PLA*₂ *Mass & Physical Activity*

There was a positive correlation between Lp-PLA₂ mass and sedentary minutes per day (*r*=0.405, *P*<0.05), based on an analysis of 25 subjects, as those with insufficient accelerometer data were not included in this analysis. (Figure 2) Physical activity between groups did not differ between the OSA and non-OSA groups, with the exception of the OSA group having fewer moderate intensity minutes per day (*P* = 0.039). However, when controlled for age this difference was no longer significant. (Table 2)

Due to the positive association seen between sedentary time and Lp-PLA₂ in the entire sample, we sought to explore the relationship between sedentary behavior and Lp-PLA₂ further. The sample of 25 subjects were split into two groups based on whether they were above or below the mean value for Lp-PLA₂ mass (134.7 ng/mL). The mean was chosen due to the fact that our subjects were healthy and relatively young, which resulted in overall lower Lp-PLA₂ compared to other studies, and only four subjects with an Lp-PLA₂ mass over 200 ng/mL. The two groups did not differ in age, AHI, BMI, or % body fat. Waist circumference was greater in the higher Lp-PLA₂ group compared to the lower Lp-PLA₂ group (*P*<0.05). (Table 3) Those in the higher Lp-PLA₂ group had a greater number of sedentary minutes per day (<0.001) and fewer light intensity PA minutes per day compared to the lower Lp-PLA₂ group (*P*=0.002). Total moderate to vigorous intensity physical activity and average step count per day did not differ between groups. (Table 4)
Discussion

Results from this study suggest that OSA may not impact Lp-PLA₂ mass in a younger, healthier cohort, as we found no difference in Lp-PLA₂ in those at high likelihood for OSA compared to those screened as negative for likely presence of OSA. It is important to note that our OSA group had a mean OSA severity rated as “mild”, with only four reaching a moderate severity and none reaching severe. Of these four moderate OSA subjects, the mean Lp-PLA₂ was 182 ng/mL and only three of them exceeded 200 ng/mL. This may have impacted our ability to observe a relationship between OSA severity and Lp-PLA₂. In addition, our sample was relatively young, with ages in the OSA group ranging from 21-57. Thus, some subjects may have only been exposed to the potential inflammatory effects of OSA for a short period of time.

In previous clinical studies, Lp-PLA₂ was typically evaluated in relation to OSA and another chronic condition, such as CVD, COPD, and metabolic syndrome (Xu, 2020; Badr, 2014; Moise, 2018). In each of these studies, Lp-PLA₂ was found in the greatest amount in the groups diagnosed with OSA in addition to another condition. In studies that split OSA groups into severities, the most severe group had the greatest increase in Lp-PLA₂ (Xu, 2020; Bekci, 2011). These previous studies used subjects with moderate to severe sleep apnea, which would also lead us to expect greater Lp-PLA₂ concentrations than the present study (Moise, 2018; Badr, 2014; Bekci, 2011) as Xu et al. (2020) have observed that Lp-PLA₂ mass is only increased in individuals with moderate and severe sleep apnea. The present study sought to evaluate the effects of OSA independent of other chronic conditions that increase inflammation, such as CVD. Thus, prior findings may have been influenced by comorbid conditions and OSA per se may not have a large
impact on Lp-PLA$_2$. Additionally, our OSA group was younger (44.1 ± 12.9 years) than all of the aforementioned studies with our control group being even younger (28.6 ± 10.8 years), which could have contributed to the similar Lp-PLA$_2$ between groups.

The interesting finding of the current study was the relationship between Lp-PLA$_2$ and sedentary behavior, independent of OSA. We observed that in those with greater sedentary behavior, Lp-PLA$_2$ was higher. Additionally, those with higher Lp-PLA$_2$ had fewer minutes of light intensity PA and greater waist circumference. While both groups would be classified as being “low active” based on step counts (Tudor-Locke, 2013), and are meeting current physical activity recommendations in terms of MVPA (Riebe, 2018), the group with higher Lp-PLA$_2$ had a lower overall physical activity profile with less light activity minutes and greater sedentary minutes. This decrease in overall activity profile may contribute to the greater waist circumference observed, which may correlate to the greater Lp-PLA$_2$.

Habitual physical activity is anti-inflammatory and has been associated with a decrease in traditional risk factors (Kokkinos, 2012). Data from the Harvard Alumni Health Study suggests that while light intensity activities are not associated with reduced mortality rates, moderate intensity is somewhat beneficial, and vigorous intensity significantly reduces mortality rates (Lee, 2000). In contrast, one systematic review found that while moderate to vigorous intensity activities provide more benefits per unit of time, light intensity physical activity offers some reduction of cardiovascular risk and all-cause mortality (Chastin, 2019). Sedentary time, independent of total activity levels, is also an important risk factor for CVD and all-cause mortality with more than 2.5 times greater risk in those who accumulate 10 hours/day compared to 6.5 hours/day (Dohrn, 2018).
Even intermittent breaks in sedentary time is beneficially correlated to waist circumference and cardiometabolic biomarkers (Healy, 2011). In the present study, both groups exceeded 10 hours/day of sedentary time on average, regardless of if groups were separated based on presence of OSA (13.6 hours vs 12.6 hours) or concentration of Lp-PLA₂ (10.8 hours vs 15.6 hours).

With much of the research and data published, including the national guidelines, focusing on the importance of MVPA, this somewhat novel information on sedentary time and light intensity PA is an important contribution to this area of literature. With many Americans struggling to even meet the guidelines it is important to research how our sedentary time is impacting our mortality risk and what even the smallest bit of activity can do to help.

Physical activity has also been a predictor of waist circumference with those who engage in higher levels of physical activity typically having lower abdominal adiposity (Ekelund, 2011). We found that, in addition to a lack of light PA and an excess of sedentary time, the high Lp-PLA₂ group also appeared to have a larger waist circumference, regardless of BMI or body fat percent. Although BMI is typically used to determine health risks, waist circumference may be a better determinant of predicting obesity-related health risks, with most individual risk factors for CVD having a higher correlation to waist circumference than BMI (Janssen, 2004; Zhu, 2002). In addition to obesity and visceral adiposity, inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and C-reactive protein (CRP) have been associated with waist circumference, indicating that an increase in waist circumference can create an increase in inflammation (Park, 2005). When comparing normal weight women to
overweight/obese women, the overweight/obese group had significant increases in waist circumference and Lp-PLA₂ in addition to other indicators of oxidative stress (Paik, 2015). A separate study observed an increase in waist circumference, inflammatory biomarkers, including Lp-PLA₂, and a decrease in physical activity among men and women who developed CVD across a mean follow-up of 10 years compared to those who did not (Rana, 2011). One study indicates that Lp-PLA₂ is actively produced in adipose tissue and is also expressed in greater amounts specifically in abdominal subcutaneous tissue (Jackisch, 2018). Weight loss interventions resulting in a reduction of waist circumference have also been associated with a reduction in Lp-PLA₂ (Tzotzas, 2008). Our findings indicate similar results that individuals with greater waist circumferences exhibited greater Lp-PLA₂ concentrations. This adds to the evidence that location of adipose tissue may contribute greater risk for obesity related conditions.

One limitation of the present study is that there was a relatively small sample size with only 39 individuals used for the analysis of Lp-PLA₂ mass, only 11 of which were classified as having sleep apnea. Additionally, those that we did identify as having sleep apnea, only exhibited a mild form of it with the average AHI being 12.4 ± 7.3. However, it is informative that Lp-PLA₂ levels are not altered in individuals with mild OSA. Lastly, our subjects were recruited from a college campus, which likely led to the discrepancy in age between the OSA and non-OSA groups (44.1 ± 12.9 years vs 28.6 ± 10.8 years).

In conclusion, our results showed no difference in Lp-PLA₂ mass between OSA and non-OSA subjects. When split up based on mean Lp-PLA₂ mass, there was a significant difference in waist circumference, sedentary time, and time spent in light intensity physical activity, with MVPA and step count remaining the same between
groups. This suggests that abdominal fat as well as activity and sedentary time outside of MVPA may be important in determining Lp-PLA\textsubscript{2} concentration.
Manuscript References


Ng SSS, Chan TO, To KW, et al. Validation of a portable recording device (ApneaLink) for identifying patients with suspected obstructive sleep apnoea syndrome. *Internal medicine journal.* 2009;39(11):757-762.


Persson M, Hedblad B, Nelson JJ, Berglund G. Elevated Lp-PLA2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events.


<table>
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<th></th>
<th>OSA (n=11)</th>
<th>Non-OSA (n=28)</th>
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<tbody>
<tr>
<td>Age</td>
<td>44.1 ± 12.9*</td>
<td>28.6 ± 10.8</td>
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<tr>
<td>AHI</td>
<td>12.4 ± 7.3</td>
<td>1.5 ± 1.2</td>
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<tr>
<td>Weight (kg)</td>
<td>95.4 ± 15.6</td>
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<tr>
<td>Height (cm)</td>
<td>175.3 ± 9.6</td>
<td>169.7 ± 10.5</td>
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<tr>
<td>BMI</td>
<td>31.0 ± 4.7</td>
<td>29.7 ± 6.0</td>
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<tr>
<td>Waist (cm)</td>
<td>99.6 ± 12.1</td>
<td>91.5 ± 13.4</td>
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<tr>
<td>Neck (cm)</td>
<td>39.1 ± 4.5</td>
<td>36.0 ± 4.1</td>
</tr>
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<td>HR rest</td>
<td>68.2 ± 8.0</td>
<td>69.0 ± 11.0</td>
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*P=0.001 when compared to Non-OSA group

<table>
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<th>OSA (n=8)</th>
<th>Non-OSA (n=17)</th>
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<tr>
<td>Sedentary</td>
<td>815.3 ± 260.5*</td>
<td>754.3 ± 230.2</td>
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<td>Light</td>
<td>123.9 ± 59.7</td>
<td>157.6 ± 87.5</td>
</tr>
<tr>
<td>Moderate</td>
<td>29.4 ± 9.7</td>
<td>42.7 ± 20.7</td>
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<tr>
<td>Vigorous</td>
<td>1.1 ± 2.4</td>
<td>3.6 ± 8.5</td>
</tr>
<tr>
<td>Very Vigorous</td>
<td>0.4 ± 0.5</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>Total MVPA</td>
<td>32.9 ± 15.5</td>
<td>42.4 ± 26.5</td>
</tr>
<tr>
<td>Step Count</td>
<td>6303.1 ± 1440.9</td>
<td>7505.5 ± 2391.9</td>
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*P<0.05 when compared to Non-OSA group
Table 3. Subject characteristics between high and low Lp-PLA₂ groups

<table>
<thead>
<tr>
<th></th>
<th>Low Lp-PLA₂ (n=20)</th>
<th>High Lp-PLA₂ (n=19)</th>
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<tbody>
<tr>
<td>Age</td>
<td>31.6 ± 12.9</td>
<td>34.7 ± 14.0</td>
</tr>
<tr>
<td>AHI</td>
<td>3.4 ± 4.7</td>
<td>5.9 ± 7.6</td>
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<tr>
<td>BMI</td>
<td>28.5 ± 5.5</td>
<td>31.8 ± 5.4</td>
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<tr>
<td>Waist (cm)</td>
<td>87.8 ± 12.2</td>
<td>100.1 ± 11.9*</td>
</tr>
<tr>
<td>Total % Fat</td>
<td>32.7 ± 11.1</td>
<td>36.9 ± 11.9</td>
</tr>
</tbody>
</table>

*P<0.05 when compared to low Lp-PLA₂ group

Table 4. Physical activity minutes between high and low Lp-PLA₂ groups

<table>
<thead>
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<th>Low Lp-PLA₂ (n=14)</th>
<th>High Lp-PLA₂ (n=11)</th>
</tr>
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<tbody>
<tr>
<td>Sedentary</td>
<td>645.4 ± 161.3</td>
<td>937.3 ± 219.1*</td>
</tr>
<tr>
<td>Light</td>
<td>187.9 ± 84.5</td>
<td>94.6 ± 27.0*</td>
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<tr>
<td>Moderate</td>
<td>35.1 ± 17.7</td>
<td>42.6 ± 20.1</td>
</tr>
<tr>
<td>Vigorous</td>
<td>4.1 ± 9.3</td>
<td>1.1 ± 1.7</td>
</tr>
<tr>
<td>Total MVPA</td>
<td>39.4 ± 24.7</td>
<td>43.9 ± 20.7</td>
</tr>
<tr>
<td>Step Counts</td>
<td>7175.4 ± 2310.3</td>
<td>7051.1 ± 2108.4</td>
</tr>
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*P<0.005 when compared to low Lp-PLA₂ group
Figure 1. Lp-PLA$_2$ mass values for the OSA and Non-OSA groups

Figure 2. Lp-PLA$_2$ in relation to number of sedentary minutes per day.
Appendix A. Informed Consent

Project Title: Does an Acute Bout of Exercise Reduce Exhaled Nitric Oxide Levels in Individuals with Obstructive Sleep Apnea?

Consent to Participate in Research

Identification of Investigators & Purpose of Study

You are being asked to participate in a research study conducted by Drs. Stephanie Kurti and Trent Hargens from James Madison University. To participate, you must be in good health and not diagnosed with any cardiovascular, pulmonary, or metabolic diseases (with the exception of Obstructive Sleep Apnea (OSA)).

Previous research suggests that a bout of nighttime exercise was effective in reducing exhaled nitric oxide levels in inactive asthmatic patients, which translated to improvements in pulmonary outcomes. However, there is no literature that has shown if an acute bout of exercise lowers airway and systemic inflammation, and improves vascular health in individuals with OSA. Therefore, the purpose of this study is to determine whether an acute bout of exercise will reduce exhaled nitric oxide levels in individuals with OSA. This study will contribute to the knowledge of both practitioners and clinicians, and may provide an important public health message in providing exercise recommendations for those with elevated airway inflammation, particularly in individuals with OSA. Should you decide to participate in this research study, you will be asked to sign this consent form once all your questions have been answered to your satisfaction.

Research Procedures

This study consists of four questionnaires, a body composition scan, an incremental exercise test to exhaustion on a cycle ergometer, a home sleep evaluation device, a 7-day physical activity recording device, and various physiological tests administered to individual participants in the Human Performance Laboratory at James Madison University. The total time required for participation in the research study is outlined on the following page (time required section).

Your will visit the laboratory a total of two times. The specific procedures during each visit are included below:

**Initial visit:** On the first visit to the laboratory, you will be briefed on the study and asked to read and sign a consent form and complete a health history questionnaire, as well as questionnaires assessing your physical activity habits, daytime sleepiness, and snoring habits. If you’d like to see any of these questionnaires prior to consenting, please ask a researcher and we’ll be happy to provide one for you.

After completion of the questionnaire, height, weight, and neck/waist circumference measurements will be taken. We will then perform a fasted blood draw for Lp-PLA2. Following the blood draw, you will complete two non-invasive breathing tests that will assess pulmonary function and airway inflammation. Next, you will be asked to complete a maximal ramping exercise test, in which the resistance on the bike will increase steadily until you say that you can no longer continue or cannot keep up, which should take around 8-15 minutes. We will be measuring some of your physiological responses continuously throughout this test. At the end of this visit, you will be instructed how to properly set up and wear a home sleep evaluation device to assess OSA. You will be asked to return with the device one day later.

**Second visit:** After returning with the sleep evaluation device one day after the initial visit, we’ll perform a blood draw for Lp-PLA2. Then, we will perform a DEXA scan to assess your body composition (how much of your body is fat vs. lean mass). Next, we will record your resting heart rate, heart rate variability, and blood pressure, which will be obtained in the supine position. You will then be instructed how to properly wear an accelerometer device (Actigraph GT-3X) to assess your 7-day physical activity. You will return 8 days later for your control (CON) or Nighttime Exercise (NE) session.
Participation in this study will require approximately 2 hours of your time in the lab over the course of 2 separate visits. The visits are outlined below:

**Initial Visit**: On the first visit to the laboratory, you will be asked to complete the required questionnaires and have height, weight, and neck/waist circumference measurements taken. We will then complete a blood draw and two non-invasive breathing tests. Also during the initial visit, you will be asked to complete the incremental test to exhaustion. The entire testing day should take approximately 90 minutes.

**Visit 2**: You will be asked to return an ApneaLink device one day after your initial visit. Resting heart rate and blood pressure and heart rate variability will be assessed followed by a DEXA scan. During this time, you will also be instructed how to properly wear an accelerometer device. This visit should take approximately 30 minutes.

**Risks**
Participation in this study does have some risks, although they are small. The risk of any serious event during this study is very small. Possible risks include:

**DEXA**: The DEXA scan entails a low dose of radiation equivalent to approximately one transatlantic flight (0.015 mSv= millisievert). While there is no validated questionnaire to define extensive exposure, radiation exposure is cumulative (200 DEXA scans is equal to the cumulative exposure of living at sea level for a year (3 mSv). DEXA scans carry minimal X-ray exposure. To minimize exposure, the DEXA scan will only be performed once. All body composition assessment will be performed according to the American College of Sports Medicine guidelines for body composition assessment).

**Blood sampling**: The risks of blood sampling using venipuncture include possible mild bruising, and the risk of transfer of blood-borne pathogens, as well as possible risks of infection or skin irritation. These risks are considered to be minimal, and all safety precautions for handing blood samples will be followed according to Occupational Safety and Health Administration (OSHA) protocols.

**Benefits**
By participating in this study, you may benefit by gaining knowledge of inflammatory markers, airway inflammation (exhaled nitric oxide) and airway hyperresponsiveness, which provide important information about your current cardiovascular and pulmonary health. You will learn your VO2peak, body composition, and pulmonary function scores (forced expiratory flow in 1-sec, forced vital capacity, forced expiratory flow at 25-75% of vital capacity). Society will benefit from more knowledge about how acute and chronic exercise may benefit vascular and pulmonary outcomes in individuals with OSA.

**Confidentiality**
The results of this research will be presented at the American College of Sports Medicine Annual meeting as well as the American Society of Nutrition annual conferences. The results of this project will be coded in such a way that the respondent’s identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. While individual responses are confidential, aggregate data will be presented representing averages or generalizations about the responses as a whole. All data will be stored in a secure location accessible only to the JMU researchers. Upon completion of the study, all information that matches up individual respondents with their answers will be destroyed.

**Participation & Withdrawal**
Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.
Questions about the Study
If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact:

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Telephone: 540-568-3947

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Telephone: 540-568-5844

Questions about Your Rights as a Research Subject
Dr. Taimi Castle
Chair, Institutional Review Board
James Madison University
(540) 568-5929
castletl@jmu.edu

Giving of Consent
I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

__________________________________________
Name of Participant (Printed)

__________________________________________  
Name of Participant (Signed)  Date

__________________________________________
Name of Researcher (Signed)  Date
Appendix B. PAR-Q+

2017 PAR-Q+
The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly; check YES or NO.

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Has your doctor ever said that you have a heart condition □ OR high blood pressure □?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? PLEASE LIST CONDITION(S) HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) Are you currently taking prescribed medications for a chronic medical condition? PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer NO if you had a problem in the past, but it does not limit your current ability to be physically active. PLEASE LIST CONDITION(S) HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7) Has your doctor ever said that you should only do medically supervised physical activity?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If you answered NO to all of the questions above, you are cleared for physical activity.
Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.
- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:
- You have a temporary illness such as a cold or fever, it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

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01-01-2017
# 2017 PAR-Q+

## FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. **Do you have Arthritis, Osteoporosis, or Back Problems?**
   - If the above condition(s) is/are present, answer questions 1a-1c
   - If NO go to question 2
   - **1a.** Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments) **YES ☐ NO ☐
   - **1b.** Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylosis/pars defect (a crack in the bony ring on the back of the spinal column)? **YES ☐ NO ☐
   - **1c.** Have you had steroid injections or taken steroid tablets regularly for more than 3 months? **YES ☐ NO ☐

2. **Do you currently have Cancer of any kind?**
   - If the above condition(s) is/are present, answer questions 2a-2b
   - If NO go to question 3
   - **2a.** Does your cancer diagnosis include any of the following types: lung/brethogenic, multiple myeloma (cancer of plasma cells), head, and/or neck? **YES ☐ NO ☐
   - **2b.** Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)? **YES ☐ NO ☐

3. **Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm**
   - If the above condition(s) is/are present, answer questions 3a-3d
   - If NO go to question 4
   - **3a.** Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments) **YES ☐ NO ☐
   - **3b.** Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction) **YES ☐ NO ☐
   - **3c.** Do you have chronic heart failure? **YES ☐ NO ☐
   - **3d.** Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months? **YES ☐ NO ☐

4. **Do you have High Blood Pressure?**
   - If the above condition(s) is/are present, answer questions 4a-4b
   - If NO go to question 5
   - **4a.** Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments) **YES ☐ NO ☐
   - **4b.** Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure) **YES ☐ NO ☐

5. **Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes**
   - If the above condition(s) is/are present, answer questions 5a-5e
   - If NO go to question 6
   - **5a.** Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies? **YES ☐ NO ☐
   - **5b.** Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness. **YES ☐ NO ☐
   - **5c.** Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, or the sensation in your toes and feet? **YES ☐ NO ☐
   - **5d.** Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)? **YES ☐ NO ☐
   - **5e.** Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future? **YES ☐ NO ☐
2017 PAR-Q+

6. Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer’s, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome
   If the above condition(s) is/are present, answer questions 6a-6b
   If NO go to question 7
   6a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)
   YES □ NO □
   6b. Do you have Down Syndrome AND back problems affecting nerves or muscles?
   YES □ NO □

7. Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure
   If the above condition(s) is/are present, answer questions 7a-7d
   If NO go to question 8
   7a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)
   YES □ NO □
   7b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?
   YES □ NO □
   7c. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?
   YES □ NO □
   7d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?
   YES □ NO □

8. Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia
   If the above condition(s) is/are present, answer questions 8a-8c
   If NO go to question 9
   8a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
   YES □ NO □
   8b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?
   YES □ NO □
   8c. Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?
   YES □ NO □

9. Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event
   If the above condition(s) is/are present, answer questions 9a-9c
   If NO go to question 10
   9a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)
   YES □ NO □
   9b. Do you have any impairment in walking or mobility?
   YES □ NO □
   9c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?
   YES □ NO □

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?
    If you have other medical conditions, answer questions 10a-10c
    If NO read the Page 4 recommendations
    10a. Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?
    YES □ NO □
    10b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?
    YES □ NO □
    10c. Do you currently live with two or more medical conditions?
    YES □ NO □

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE:

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.
2017 PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 5-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the ePARmed-X+ at www.eparmedx.com and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.

- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

NAME ____________________________________________ DATE ____________
SIGNATURE ____________________________________________ WITNESS ____________________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

The PAR-Q+ was created using the evidence-based AGREE process [1] by the PAR-Q+ Collaboration chaired by Dr. Darren E. F. Warburton with Dr. Norman Geddes, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie OQ. Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Citation for PAR-Q+

Key References

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Appendix C. IPAQ

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
(October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

**Background on IPAQ**
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

**Using IPAQ**
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

**Translation from English and Cultural Adaptation**
Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

**Further Developments of IPAQ**
International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

**More Information**
INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous and moderate activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home? Yes

No Skip to PART 2: TRANSPORTATION

☐ Yes

☐ No Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the last 7 days as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, heavy construction, or climbing up stairs as part of your work? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

☐ No vigorous job-related physical activity Skip to question 4

3. How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?

_____ hours per day

_____ minutes per day
4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads as part of your work? Please do not include walking.

_____ days per week

☐ No moderate job-related physical activity

Skip to question 6

5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?

_____ hours per day

_____ minutes per day

6. During the last 7 days, on how many days did you walk for at least 10 minutes at a time as part of your work? Please do not count any walking you did to travel to or from work.

_____ days per week

☐ No job-related walking

Skip to PART 2: TRANSPORTATION

7. How much time did you usually spend on one of those days walking as part of your work?

_____ hours per day

_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the last 7 days, on how many days did you travel in a motor vehicle like a train, bus, car, or tram?

_____ days per week

☐ No traveling in a motor vehicle

Skip to question 10
9. How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle?

_____ hours per day
_____ minutes per day

Now think only about the bicycling and walking you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the last 7 days, on how many days did you bicycle for at least 10 minutes at a time to go from place to place?

_____ days per week
No bicycling from place to place Skip to question 12

11. How much time did you usually spend on one of those days to bicycle from place to place?

_____ hours per day
_____ minutes per day

12. During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place?

_____ days per week
No walking from place to place Skip to question 12

13. How much time did you usually spend on one of those days walking from place to place?

_____ hours per day
_____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.
14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ days per week

[ ] No vigorous activity in garden or yard → **Skip to question 16**

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ hours per day

_____ minutes per day

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ days per week

[ ] No moderate activity in garden or yard → **Skip to question 18**

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ hours per day

_____ minutes per day

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping inside your home?

_____ days per week

[ ] No moderate activity inside home → **Skip to PART 4:**

**RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY**
19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ hours per day

_____ minutes per day

**PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY**

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ days per week

[ ] No walking in leisure time → **Skip to question 22**

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ hours per day

_____ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ days per week

[ ] No vigorous activity in leisure time → **Skip to question 24**

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

 _____ hours per day

 _____ minutes per day
24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in your leisure time?

_____ days per week

No moderate activity in leisure time

25. How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?

_____ hours per day

_____ minutes per day

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the last 7 days, how much time did you usually spend sitting on a weekday?

_____ hours per day

_____ minutes per day

27. During the last 7 days, how much time did you usually spend sitting on a weekend day?

_____ hours per day

_____ minutes per day

This is the end of the questionnaire, thank you for participating.
Appendix D. ESS

Epworth Sleepiness Scale

SubjectID______Name___________________________DateCompleted_____/_____/____

This questionnaire asks you to indicate the chances of you becoming drowsy during hours of the day that you are not in bed sleeping. “How likely are you to doze off or fall asleep in the following situations?”

Use the following scale and indicate the most appropriate number for each situation.

0 = would never doze
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing

<table>
<thead>
<tr>
<th>Situation</th>
<th>Chance of dozing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sitting and reading</td>
<td>____</td>
</tr>
<tr>
<td>2. Watching T.V.</td>
<td>____</td>
</tr>
<tr>
<td>3. Sitting, inactive in a public place (ex. Theatre or meeting)</td>
<td>____</td>
</tr>
<tr>
<td>4. As a passenger in a car for an hour without a break</td>
<td>____</td>
</tr>
<tr>
<td>5. Lying down to rest in the afternoon when circumstances permit</td>
<td>____</td>
</tr>
<tr>
<td>6. Sitting and talking with someone</td>
<td>____</td>
</tr>
<tr>
<td>7. Sitting quietly after a lunch without alcohol</td>
<td>____</td>
</tr>
<tr>
<td>8. In a car, while stopped for a few minutes in the traffic</td>
<td>____</td>
</tr>
</tbody>
</table>

Sum of Scores, items 1-8 (staff use only) ______/24
Appendix E. Berlin Questionnaire

Berlin Questionnaire

Subject ID______ Name________________________ Date Completed ____/____/____

Height (cm) ___________Weight (kg) _________ Age _____

Please choose the correct response to each question.

**Category 1**

1. Do You Snore?
   - □a. Yes
   - □b. No
   - □c. Don’t know

*If you snore:*

2. Your snoring is:
   - □a. Slightly louder than breathing
   - □b. As loud as talking
   - □c. Louder than talking
   - □d. Very loud—can be heard in adjacent rooms

3. How often do you snore?
   - □a. Nearly every day
   - □b. 3-4 times a week
   - □c. 1-2 times a week
   - □d. 1-2 times a month
   - □e. Never or nearly never

4. Has your snoring every bothered other people?
   - □a. Yes
   - □b. No
   - □c. Don’t Know

5. Has anyone noticed that you quit Breathing during your sleep?
   - □a. Nearly every day
   - □b. 3-4 times a week
   - □c. 1-2 times a week
   - □d. 1-2 times a month
   - □e. Never or nearly never

**Category 2**

6. How often do you feel tired or fatigued after you sleep?
   - □a. Nearly every day
   - □b. 3-4 times a week
   - □c. 1-2 times a week
   - □d. 1-2 times a month
   - □e. Never or nearly never

7. During your waking time, do you feel tired, fatigued or not up to par?
   - □a. Nearly every day
   - □b. 3-4 times a week
   - □c. 1-2 times a week
   - □d. 1-2 times a month
   - □e. Never or nearly never

8. Have you ever nodded off or fallen asleep while driving a vehicle?
   - □a. Yes
   - □b. No

*If yes:*

9. How often does this occur?
   - □a. Nearly every day
   - □b. 3-4 times a week
   - □c. 1-2 times a week
   - □d. 1-2 times a month
   - □e. Never or nearly never

**Category 3**

10. Do you have high blood pressure?
    - □a. Yes
    - □b. No
    - □c. Don’t Know
References


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Silverberg DS, Oksenberg A. Are sleep-related breathing disorders important contributing factors to the production of essential hypertension?. *Current Hypertension Reports.* 2001;3(3):209-215.


