Spring 2016

The effect of acute aerobic exercise on hemostasis in obstructive sleep apnea patients

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The Effect of Acute Aerobic Exercise on Hemostasis in Obstructive Sleep Apnea Patients

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A Thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

for the degree of

Master of Science

Department of Kinesiology

May 2016

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Acknowledgements

I would like to thank Dr. Christopher Womack for serving as my thesis chair. Your guidance and patience has proved most influential during my time at James Madison University. This thesis is one of my greatest achievements and greatly thank you for your support.

I would also like to thank Dr. Kent Todd and Dr. Trent Hargens for serving as committee members for my thesis. You both have continued to challenge me and your feedback on this project served invaluable.

Gabrielle Giersch, thank you for your assistance and friendship during the data collection process through the past two years. There are few individuals who can maintain the witty banter and nerdy jargon we continuously exemplify within the laboratory.

Finally, I would like to thank my family for your continuous love and support that have made my goals possible. My hope as I move forward in my professional career is that my successes are also regarded, in part, as your own.

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Abstract

Purpose: To determine the hemostatic response after acute aerobic exercise in Obstructive Sleep Apnea (OSA). **Methods:** Eighteen males were recruited from the university and local community. Individuals who presented evidence of cardiovascular, pulmonary, or metabolic disease were excluded. Apnea-Hypopnea Index (AHI) $\geq$ 5 was criteria for OSA. Subjects performed a treadmill exercise test at 35% and 70% predicted VO_2reserve during the morning hours. Pre exercise blood samples were obtained after 15 minutes supine rest and within two minutes following exercise. Repeated Measures ANOVA were performed for Factor VIII antigen, tPA antigen, tPA activity, and PAI-1 activity across two time points (Pre vs. Post) and between conditions (OSA vs. Controls). Correlational analysis compared all hemostatic factors with age, BMI, and AHI. **Results:** Mean AHI was (13.00 $\pm$ 12.6) indicating mild OSA severity. There were no exercise x condition interaction for any observed hemostatic markers ($P > 0.05$). There was a significant main effect for exercise in Factor VIII, tPA antigen, and tPA activity in both groups ($P < 0.05$). There was no main effect for OSA except an observed trend in PAI-1 which remained elevated after exercise ($P = 0.05$). FVIII:Ag and BMI ($R = .520$), tPA:Ag and AHI ($R = .489$), and tPA:Ag and age ($R = .496$) were positively correlated ($P < 0.05$). **Conclusion:** The hemostatic response after acute aerobic exercise is unaffected in mild OSA although PAI-1 activity seems to be elevated impacting fibrinolytic potential. BMI seems to correlate with FVIII:Ag, while AHI and age correlate with tPA:Ag.

Keywords: Hemostasis, Obstructive Sleep Apnea, acute exercise, coagulation, fibrinolysis

Chapter 1

Introduction

Obstructive Sleep Apnea (OSA) is highly prevalent across populations and appears to affect approximately 2 - 4 % of the middle aged adults (38). An estimated 85% of individuals with clinically significant OSA are undiagnosed with a large impact on males (28, 51). OSA is characterized by repetitive interruptions of ventilation during sleep due to a complete cessation of airflow or a significant decrease in airflow with a concurrent decrease in oxygen saturation (53). Patients with OSA exhibit reductions in the size of the pharyngeal airway directly due to obesity; and/or other obstacles including bone and soft tissues (38, 61). Children may also be affected by this condition due to tonsils and adenoids (53). During wakefulness, greater activation of the pharyngeal dilator muscles results in a patent airway that is less likely to collapse (36). However, during sleep, activation is reduced to the upper airway dilator muscles promoting collapse and consequent arousal (36). Increasing arousal during sleep contributes to excessive daytime sleepiness that could lead to other negative outcomes (53).

OSA has been associated with numerous health conditions and several risk factors may predispose individuals to develop the condition. Risk factors include genetic factors that affect the craniofacial anatomy (35), age, sex, obesity (45), pregnancy, diabetes, and hypothyroidism (52). Many environmental factors also influence this risk such as smoking and alcohol use (45). OSA is a risk factor itself for the development of other diseases such as hypertension, heart failure, arrhythmias, CAD, and stroke (28). Signs of OSA include disturbed sleep (snoring and restlessness), daytime fatigue, and irregular breathing patterns during sleeping hours, choking sensations when wakened, and morning headaches (35, 10).

The diagnosis of OSA is most commonly determined using a multi-channel analysis (polysomnography) utilizing an EEG, EOG, ECG, and oximetry along with an extensive physical and medical history examination (8). The readily accepted diagnosis for the disorder is defined as five or more apnea-hypopneas per hour of sleep ($\geq$ 5 AHI) (43,38,8). Apnea is defined as a complete cessation of airflow greater than or equal to ten seconds ($\geq$ 10 s) while hypopnea is a decrease in airflow of at least 50 %, lasting at least ten seconds ($\geq$ 10 s), and accompanied by at least a 4 % decrease in oxygen saturation (8). Although discrepancies with the definition of a hypopnea and the concomitant decrease in oxygen saturation exist, a reduction of at least 4 % shows the greatest association with cardiovascular disease compared to higher saturations (44).

It has been well established that the presence of OSA is associated with a greater incidence of cardiovascular disease (CVD), which increases with disease severity (40, 50, 30, 63, 55, 41,). Marshall et al. reported moderate to severe OSA to be independently associated with increased risk of all-cause mortality, stroke and cancer after a 20 year follow-up (30). Results also show that efficient treatment of OSA has a beneficial impact on the disease and the subsequent CVD risk (29, 64). Similarly, Marin et al. reported the odds ratio of fatal or non-fatal cardiovascular events with patients under CPAP treatment were not significantly different than simple snorers and mild-moderate untreated persons (29). Previous studies have shown that sleep disordered breathing (SDB) contributes to the initial progression of atherosclerosis, suggesting OSA may be an independent risk factor for CVD incidence (5, 16, 19). In particular, Fox et al. found that increasing OSA severity was linked to a greater carotid intima-media thickness (CIMT) without the presence of

CVD or common risk factors compared to matched controls (19). Therefore, OSA may have a mechanistic role in the progression of cardiovascular disease.

Hemostasis is balanced by two mechanisms: coagulation, otherwise known as blood clotting, and fibrinolysis, the dissolution of a blood clot. Coagulation incorporates two pathways of activation that eventually result in the formation of thrombin and blood clot. Shortly after the Second World War, Davie and Ratnoff discovered a 'waterfall' like sequence of enzymes acting as substrates to other activated enzymes that lead to the formation of a stable fibrin clot (15). This proposed sequence is independent of factors extrinsic to the blood and is known as the intrinsic pathway. The intrinsic pathway is initiated by the activation of Factor XII which sequentially activates Factor XI which further activates Factor IX. In the presence of Factor VIII, Factor IX then activates Factor X which forms a complex with prothrombin generating thrombin (37). The extrinsic pathway is initiated through a subendothelial derived enzyme, Tissue Factor. In the event of vessel damage, Tissue Factor reacts in the presence of Factor V which further activates the reaction of Factor X and prothrombin (37). The common pathway has been described as the pathway where the intrinsic and extrinsic pathways overlap. The activation of Factor X and the subsequent generation of thrombin compose much of the common pathway (Figure 1). Thrombin is a key enzyme that contributes to platelet activation, fibrinogen conversion to fibrin, and amplification (12, 14). Thrombin also causes a feedback loop and activation of Factor XIII which stabilizes the unstable fibrin creating a firm meshwork of fibrin that will compose of much of the clot (37).

Fibrinolysis consists of degradation of fibrin by a serine protease, plasmin. Proteolytic enzymes, known as plasminogen activators, react with plasminogen to generate

plasmin. This most important known activators are tissue-type plasminogen activator (t-PA) and urokinase plasminogen activator (uPA) produced by the endothelium (7). uPA has a lower affinity for plasminogen and therefore much of the process of plasmin formation is performed by tPA (9). These activators have particularly short half-lives (approximately 4 – 8 minutes) as they are either cleared by the liver or inactivated by inhibitors (9). The most common of these inhibitors are Plasminogen Activator Inhibitor-1 (PAI-1), Plasminogen Activator Inhibitor-2 (PAI-2), and α_2-antiplasmin (54). The functional balance between coagulation and fibrinolysis is of the upmost importance so the circulation can remain in a closed circuit without either excessive clotting or bleeding.

Much of the clinical evidence suggests greater coagulation potential and lower fibrinolytic activity in individuals with CVD (23, 20, 31). Folsom et al. found that markers of coagulation potential, Factor VIII and von Willebrand Factor (vWF), are associated with cardiovascular disease in both men and women (18). Specifically, increased FVIII and vWF have also been associated with higher incidence of ischemic heart disease and endothelial dysfunction (23, 18, 32, 49, 57). Further, Tracy et al. indicated that Factor VIII was significantly associated with coronary heart disease (CHD) and mortality in men and stroke/transient ischemic attacks in women (57). In the Northwick Park Heart Study, researchers found decreased fibrinolytic activity to be the main determinant of ischemic heart disease in younger men (31). Thogersen et al. showed that elevated plasma levels of PAI-1 and tPA antigen were associated with a first acute myocardial infarction for men and women (56). These studies indicate the clinical importance of hemostatic variables and the role in CVD.

Individuals with OSA exhibit an altered hemostatic balance. Guardiola et al. observed decreased whole blood clotting time in individuals with untreated OSA both before sleep and upon waking (22). The hypoxic environment associated with OSA may explain this prothrombotic state (60). Furthermore, it appears the prothrombotic state is due to a decrease in fibrinolytic activity rather than an increase in coagulation factors (6, 47). However, evidence also suggests that hypoxic conditions result in a shortened coagulation time with a concurrent rise in Factor VIII (6). PAI-1 is positively associated with AHI, and oxygen saturation (SpO_2) suggesting disease severity contributes to reduced fibrinolytic activity (59, 60, 65). Von Kanel et al. reported a higher AHI and increased time spent below 90 % SpO_2 were associated with higher PAI-1 levels in relatively healthy populations (60). A possible mechanism for this relationship is the up regulation of PAI-1 transcription with concomitant increases in PAI-1 mRNA stability during hypoxia, which is exacerbated by low tPA expression (42).

Many instances of myocardial infarction are preceded by physical exertion (21,34). Acil et al. found that persons with coronary artery disease exhibit a hemostatic balance favoring greater coagulation after an acute bout of exercise (1). This could be mechanistically linked with exertion-related ischemic events as occlusive thrombi are responsible for a greater portion of exertion –related events than events triggered by other causes (21). Myocardial infarctions seem to occur more frequently in sedentary individuals after physical exertion, particularly when the activity is strenuous (21, 34). During exercise, both coagulation and fibrinolysis increase proportional to intensity (33). Early research showed an elevation in coagulation potential during exercise, particularly due to an increase in Factor VIII (26, 11, 3, 4). Cohen et al. suggested that the exercise-induced rise

in Factor VIII is partially explained through beta-adrenergic-receptor stimulation (11). There is also a concomitant increase in fibrinolysis, due to increases in tPA and decreases in PAI-1 (26, 11, 3, 17, 4, 24, 48, 27, 43, 61, 13, 62) which change in proportion to the intensity of exercise (24, 48, 62). Following exercise, there is evidence for a prothrombotic state as markers of coagulation potential remain elevated for an hour into the post-exercise period (39), while a significant decrease in fibrinolytic potential occurs within 10 minutes (13, 39).

Although hemostatic responses to exercise are clinically important, these responses are relatively unknown in obstructive sleep apnea patients. Rangemark et al. found that OSA subjects exhibited increases in PAI-1 activity with values remaining significantly elevated before and after 4 minutes of submaximal cycle ergometer exercise compared to matched controls (46). After exercise, tPA showed a four-fold increase but did not significantly differ between groups (46). However, this study did not analyze any factors of coagulation other than platelet activity, which did not significantly change (46). The aim of this study is to determine if acute aerobic exercise increases Factor VIII, tPA antigen and PAI-1 activity with concurrent decreases in tPA activity in obstructive sleep apnea patients.

Chapter 2

Methods

Subjects

All subjects gave informed written consent on the study protocol approved by the James Madison University Institutional Review Board. OSA subjects were recruited from the JMU community and Sentara Rockingham Memorial Hospital (SRMH) Sleep Clinic. All SRMH subjects were diagnosed with sleep apnea and not currently using continuous positive airway pressure (CPAP) as treatment. Eighteen males ($\geq$ 18 years of age) with observed sleep problems or significant snoring were recruited for the present study. Mobility issues, and/or evidence of cardiovascular, pulmonary, and metabolic diseases were criteria for exclusion in both OSA and Non-OSA groups. Demographic data is presented in Table 1 for OSA and Normal individuals.

To establish likelihood of Sleep Apnea and screen for exclusion criteria in community-recruited participants, administration of established sleep surveys including Berlin Questionnaire, Epworth Sleepiness Scale, IPAQ, STOP Questionnaire, a Pre-Screening form, and Medical/Health History were completed prior to participation. Participants deemed ACSM High risk for cardiovascular disease (2), highly active lifestyles, and use of heart rate/blood pressure/ blood thinning medications were criteria for exclusion. Subjects were required to wear an ApneaLink (ResMed; San Diego, CA) device to measure sleep disturbances by thoracic measurements, oxygen saturation (SpO_2), respiratory effort, and airflow during sleep hours. The Apnea-Hypopnea Index (AHI) was used to determine the likelihood for OSA in these subjects. Apneas were defined as a complete cessation in respiration greater than or equal to 10 seconds while hypopnea was

defined as a decrease in ventilation of at least 50 % with a concomitant reduction of oxygen saturation. An AHI $\geq$ 5 is the accepted criteria for diagnosed OSA (53). Subjects recruited at SRMH Sleep Clinic were evaluated for any known diseases and exclusions which were reported to the researcher by the on-site physician. All data was collected in the Human Performance Lab of James Madison University. All written, electronic data and subject information was stored in a locked file cabinet and password locked laptop in a locked file cabinet and room within the laboratory. Each participant entering the study completed the appropriate questionnaires and visited the laboratory three non-consecutive days over the span of approximately 7 – 10 days.

Treadmill Test

Testing was done in the morning following an overnight fast. A resting blood draw was administered after 15 minutes of supine rest. Prior to testing, a warm up of 3 to 4 minutes was performed, after which subjects performed two, five minute stages of exercise at 35% and 70% estimated VO_2 reserve. During the test, walking speed and incline level were adjusted to obtain desired intensity, respectively. Metabolic variables were monitored continuously using a metabolic measurement system (Parvo Medics; Sandy, UT). Heart rate was determined continuously via a Polar® heart rate monitor. Immediately following exercise, a second blood draw was obtained within two minutes of completing the exercise test.

Blood Sampling and Analysis

Blood draws were obtained from an antecubital vein after 15 minutes of supine rest and within two minutes post exercise. Blood was collected in vacutainer tubes containing heparin, sodium citrate, and acidified citrate. Blood collected in heparin vials was immediately analyzed for hematocrit using the micro-hematocrit method. Samples collected in sodium citrate and acidified citrate were spun for 20 minutes at 15000 rpm in a refrigerated centrifuge. Plasma was extracted from spun samples and stored at -80º C until analysis. Blood analysis was performed by standardized Enzyme-Linked Immunosorbent Assay (ELISA) for Factor VIII activity, tPA antigen, tPA activity, and PAI-1 activity. Post exercise values for all of these variables were corrected for plasma volume changes using the method described by Van Beaumont et al. (58).

Statistical Analysis

Factor VIII, tPA antigen and activity, and PAI-1 and activity is analyzed using Repeated Measures Analysis of Variance (ANOVA). OSA and Non-OSA individuals will be compared using time as a within- subject factor and presence of disease as a between subject factor from all samples. *Post hoc* testing was done using paired and independent t-test with a Bonferroni correction factor. Correlation analysis was performed between all hemostatic variables and age, BMI, and disease severity. A priori statistical significance was set at $P < 0.05$. TPA antigen and PAI-1 activity were not normally distributed according to Shapiro-Wilk, so a $\log_{10}$ transformation was performed prior to these ANOVAs.

Chapter 3

Manuscript

The Effect of Acute Aerobic Exercise on Hemostasis in Obstructive Sleep Apnea

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Abstract

Purpose: To determine the hemostatic response after acute aerobic exercise in Obstructive Sleep Apnea (OSA). **Methods:** Eighteen males were recruited from the university and local community. Individuals who presented evidence of cardiovascular, pulmonary, or metabolic disease were excluded. Apnea-Hypopnea Index (AHI) $\geq$ 5 was criteria for OSA. Subjects performed a treadmill exercise test at 35% and 70% predicted VO_2reserve during the morning hours. Pre exercise blood samples were obtained after 15 minutes supine rest and within two minutes following exercise. Repeated Measures ANOVA were performed for Factor VIII antigen, tPA antigen, tPA activity, and PAI-1 activity across two time points (Pre vs. Post) and between conditions (OSA vs. Controls). Correlational analysis compared all hemostatic factors with age, BMI, and AHI. **Results:** Mean AHI was (13.00 $\pm$ 12.6) indicating mild OSA severity. There were no exercise x condition interaction for any observed hemostatic markers ($P > 0.05$). There was a significant main effect for exercise in Factor VIII, tPA antigen, and tPA activity in both groups ($P < 0.05$). There was no main effect for OSA except an observed trend in PAI-1 which remained elevated after exercise ($P = 0.05$). FVIII:Ag and BMI ($R = .520$), tPA:Ag and AHI ($R = .489$), and tPA:Ag and age ($R = .496$) were positively correlated ($P < 0.05$). **Conclusion:** The hemostatic response after acute aerobic exercise is unaffected in mild OSA although PAI-1 activity seems to be elevated impacting fibrinolytic potential. BMI seems to correlate with FVIII:Ag, while AHI and age correlate with tPA:Ag.

Keywords: Hemostasis, Obstructive Sleep Apnea, acute exercise, coagulation, fibrinolysis

Introduction

OSA is characterized by continuous episodes of arousal during sleep due to complete or partial cessation of airflow from pharyngeal collapse (30, 36, 22) and affects 2 – 4% of the middle-aged population (22). Studies have determined that mortality risk, with fatal and non-fatal events (i.e. traffic accidents), directly increases with AHI severity in sleep disordered breathing (40, 16). Observational studies show a possible causal relationship between OSA and atherosclerosis (4, 8, 10). One such study reported that middle-aged participants without diagnosed cardiovascular disease portrayed early indications of atherosclerosis (8). Therefore, OSA may have a mechanistic role in the progression of cardiovascular disease.

Much evidence suggests that an increase in blood coagulation, accompanied with decreased fibrinolysis, is associated with an increased risk for cardiovascular disease. The ARIC study found that markers of coagulation potential, Factor VIII and von Willebrand Factor (vWF), are associated with cardiovascular disease in both men and women (9). Similarly, the Cardiovascular Heart Study showed Factor VIII is independently associated with stroke/transient ischemic attacks and coronary heart disease (32). In the Northwick Park Heart Study, researchers found decreased fibrinolytic activity to be the main determinant of ischemic heart disease in younger men (18).

Individuals with OSA exhibit an altered hemostatic balance. Specifically, markers of coagulation are elevated and potential for fibrinolysis is decreased. Von Kanel et al. observed that plasminogen activator inhibitor 1 (PAI-1), which inhibits tissue plasminogen activator (tPA), the main stimulus of the fibrinolytic process, correlated with AHI and oxygen desaturations in subjects with sleep disruptions (35). Furthermore, mice exposed to

hypoxic conditions experience a greater PAI-1 gene expression with a concomitant increase in PAI-1 mRNA stability, resulting in greater lung fibrin deposition (23). It has also been observed that untreated OSA subjects have a shortened whole blood clotting time, suggesting that OSA is also accompanied with global hypercoagulability (12). Evidence also suggests that hypoxic conditions result in a shortened coagulation time with a concurrent rise in Factor VIII (5).

Many instances of myocardial infarction are preceded by physical exertion (11, 21). During exercise, both coagulation and fibrinolysis increase proportional to intensity (14). Acil et al. found that persons with coronary artery disease exhibit a hemostatic balance favoring greater coagulation after an acute bout of exercise (1). This could be mechanistically linked with exertion-related ischemic events as occlusive thrombi are responsible for a greater portion of exertion-related events than events triggered by other causes (11). Although hemostatic responses to exercise are clinically important, these responses are relatively unknown in obstructive sleep apnea patients. Rangemark et al. found that OSA subjects exhibited increases in PAI-1 activity with values remaining significantly elevated before and after 4 minutes of submaximal cycle ergometer exercise compared to matched controls (24). After exercise, tPA showed a four-fold increase but did not significantly differ between groups (24). However, this study did not analyze any factors of coagulation other than platelet activity, which did not significantly change (24). The aim of this study is to determine if obstructive sleep apnea impacts the Factor VIII, tPA and PAI-1 response to acute exercise.

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concomitant reduction of oxygen saturation. An AHI $\geq$ 5 is the accepted criteria for diagnosed OSA (30). Subjects recruited at SRMH Sleep Clinic were evaluated for any known diseases and exclusions which were reported to the researcher by the on-site physician. All data was collected in the Human Performance Lab of James Madison University. All written, electronic data and subject information was stored in a locked file cabinet and password locked laptop in a locked file cabinet and room within the laboratory. Each participant entering the study completed the appropriate questionnaires and visited the laboratory three non-consecutive days over the span of approximately 7 – 10 days.

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heparin, sodium citrate, and acidified citrate. Blood collected in heparin vials was immediately analyzed for hematocrit using the micro-hematocrit method. Samples collected in sodium citrate and acidified citrate were spun for 20 minutes at 15000 rpm in a refrigerated centrifuge. Plasma was extracted from spun samples and stored at -80° C until analysis. Blood analysis was performed by standardized Enzyme-Linked Immunosorbent Assay (ELISA) for Factor VIII activity, tPA antigen, tPA activity, and PAI-1 activity. Post exercise values for all of these variables were corrected for plasma volume changes, which were estimated using the method described by Van Beaumont et al. (33).

Statistical Analysis

Factor VIII, tPA antigen and activity, and PAI-1 activity were analyzed using Repeated Measures Analysis of Variance (ANOVA). Disease status (OSA vs. Non-OSA) was the between-subjects factor and exercise (Pre vs. Post) was the within-subject factor. *Post hoc* testing was done using paired and independent t-tests with a Bonferroni correction factor. Correlation analysis was performed between all hemostatic variables and age, BMI, and disease severity. A priori statistical significance was set at $P < 0.05$. TPA antigen and PAI-1 activity were not normally distributed according to Shapiro-Wilk, so a $\log_{10}$ transformation was performed prior to analysis.

Results

Eighteen subjects were recruited for the present study. Normal and OSA individuals were similar in age, height, weight, and BMI. The Apnea-Hypopnea Index was significantly higher ($P < 0.05$) in OSA (13.00 $\pm$ 12.6) than controls (1.44 $\pm$ 1.33) respectively indicating mild disease severity in OSA (30). Table 2 presents average VO_2 and heart rate values during exercise at 70% predicted VO_2 reserve. Oxygen consumption reached similar levels ($P > 0.05$) between OSA individuals and controls (28.97 $\pm$ 7.99 ml/kg/min vs. 25.29 $\pm$ 5.19, respectively). There were no differences ($P > 0.05$) in heart rates during exercise between controls (136.83 $\pm$ 23.48 bpm) and OSA (128.83 $\pm$ 19.35 bpm).

Figure 2 displays mean Factor VIII antigen responses to exercise. Factor VIII antigen significantly ($P < 0.05$) increased from pre to post exercise in controls (0.84 $\pm$ 0.30 IU • ml^{-1} to 1.13 $\pm$ 0.42 IU • ml^{-1}) and OSA (1.06 $\pm$ 0.37 IU • ml^{-1} to 1.15 $\pm$ 0.31 IU • ml^{-1}). There was no main effect for condition or an exercise x condition interaction.

Mean values for tPA antigen in response to exercise are displayed in Figure 3. Post exercise values were significantly ($P < 0.05$) increased in controls (1.18 $\pm$ 0.81 ng • ml^{-1} to 1.91 $\pm$ 1.36 ng • ml^{-1}) and OSA (1.74 $\pm$ 1.89 ng • ml^{-1} to 2.87 $\pm$ 2.87 ng • ml^{-1}). There was no main effect for condition or an exercise x condition interaction.

Figure 4 displays mean tPA activity responses to exercise. There was no main effect for condition or an exercise x condition interaction. tPA activity significantly ($P < 0.05$) increased post exercise similarly in controls (0.08 $\pm$ 0.06 IU • ml^{-1} to 0.78 $\pm$ 0.35 IU • ml^{-1}) and OSA (0.07 $\pm$ 0.43 IU • ml^{-1} to 0.77 $\pm$ 0.47 IU • ml^{-1}).

Mean values for PAI-1 activity in response to exercise are displayed in Figure 5. A trend ($P = 0.05$) was observed for a main effect of OSA compared to controls (21.35 ± 10.65 U • ml^{-1} to 24.85 ± 18.83 U • ml^{-1} vs. 8.70 ± 9.11 U • ml^{-1} to 8.15 ± 7.90 U • ml^{-1}, respectively). There was no main effect for exercise; nor was there an exercise x condition interaction ($P > 0.05$).

Figure 6 displays the correlation between post exercise FVIII antigen and BMI. There was a significant positive correlation between post exercise FVIII antigen and BMI ($R = .520$; $P < 0.05$). Correlations for pre exercise tPA antigen are presented in Figures 7 and 8. Pre exercise tPA antigen was significantly correlated with AHI ($R = .489$; $P < 0.05$) and age ($R = .496$; $P < 0.05$), respectively. No other hemostatic variables were significantly correlated with AHI, BMI, or age.

Discussion

The primary finding of the present study is Obstructive Sleep Apnea (OSA) does not appear to have an effect on the hemostatic response to exercise compared to healthy individuals. However, OSA may have an effect on resting PAI-1 levels.

Under normal conditions, FVIII increases during exercise in direct proportion to exercise intensity (14, 6, 3, 37, 20). Because FVIII acts as a coenzyme in relatively small amounts along the coagulation cascade, an increase in concentration appears to have an exponential effect in coagulation potential making it a primary variable for study (6). Further, elevations in FVIII has been associated with increased risk for cardiovascular disease (CVD) making its evaluation of clinical importance (19, 27, 31). Our data show similar increases in FVIII antigen in both OSA and normal individuals after acute aerobic exercise. Currently, there are no known studies describing the coagulation potential after exercise in OSA. Bartsch et al. showed an increase in FVIII in fifteen army pilots after hypoxia exposure (5). However, the hypoxia exposure and subsequent oxygen desaturation levels may not be comparable to OSA. Although there appears to be no differences in the exercise response of FVIII, an increased coagulation potential may still occur among other factors along the coagulation cascade. Additionally, post exercise FVIII antigen was positively correlated with BMI, although there appeared to be no differences in BMI between groups. Activation of the sympathetic nervous system in larger individuals may account for this association (6). Indeed, BMI is a risk factor for OSA development (30). Further, if left untreated, OSA individuals have increased risk of cardiovascular disease (29, 16, 40, 17). The association of CVD and FVIII levels in OSA should further be described.

Recently, Obstructive Sleep Apnea appears to be accompanied with an altered hemostatic balance at rest (12, 34, 35). Guardiola et al. demonstrated shortened clotting times in OSA individuals (12) suggesting a pro coagulant imbalance. However, limited evidence exists for the hemostatic response to exercise in OSA. During exercise, fibrinolytic potential increases in parallel with coagulation potential (3, 26, 37, 7, 38, 20). Specifically, increases in tPA antigen and activity are readily observed (7, 38, 20), likely due to increased sympathetic nervous system activity (6). Our findings suggest that mild OSA has a similar tPA antigen and activity response to exercise as healthy individuals. Further, increases in tPA activity after exercise exists in OSA similar to normal subjects, confirming previous reports (24). Although there are no known studies evaluating tPA antigen and OSA, it appears that the exercise response is unaffected.

Pre exercise tPA antigen levels were positively associated with OSA severity and age. Age and tPA antigen have been previously shown to be correlated (28), however the relationship in OSA is less described. The data suggest OSA severity positively impacts tPA antigen. Elevations in tPA antigen have been indicated to increase risk of cardiovascular disease and acute cardiovascular events (31). Although, individuals in the present study were classified as having mild disease severity ($\geq$ 5 - < 15 AHI) (30), we speculate that this association may contribute to the observed elevated CVD risk at increasing disease severity (16). The current findings are novel in describing the relationship of tPA antigen and OSA.

Perhaps the greatest impact on hemostasis in OSA is due to PAI-1 (34, 35, 41). Von Kanel et al. found elevated PAI-1 levels in OSA (34) with others confirming similar findings (41). PAI-1 activity also seems to remain elevated after exercise (24). Our findings

are similar to that of Rangemark et al. (24) showing elevated PAI-1 activity levels in OSA, unaffected by exercise. Together, mild OSA appears to have an independent effect on PAI-1 activity levels potentially lowering fibrinolytic capacity. Acute exercise has been suggested to temporarily lower PAI-1 in clinical populations thus enhancing chronically diminished fibrinolytic potential (39, 15). However, this does not appear to occur in low-moderate intensity exercise in mild OSA. Possible mechanisms may exist that explain the differences in PAI-1 activity. Pinsky et al. found elevated PAI-1 mRNA in mice accompanied with greater PAI-1 mRNA stability after sustained exposure to hypoxia (23). Indeed, Obstructive Sleep Apnea exhibits several short duration episodes of hypoxia during supine rest (30), however these mechanistic avenues should be further explored. Contrary to previous findings, there was no observed correlation between PAI-1 activity and AHI (34). OSA individuals in the present study were classified as mild disease severity, which may account for the unobserved relationship. Therefore moderate (AHI 15 - $\leq$ 30) or severe (AHI $\geq$ 30) individuals may need to be examined to clarify this association. It is possible, that greater disease severity accompanied with greater hypoxia exposure, produces this observed association.

Altered hemostatic balance has been associated with increased risk in developing cardiovascular disease (13, 9, 18, 31, 32). Specifically, FVIII appears to be elevated in CVD and is now regarded as a possible risk factor (27, 32). Rumley et al. found increases in relative odds of ischemic heart disease with increasing concentrations of Factor VIII (27). Previous studies have shown similar associations (9). Further, impaired fibrinolytic activity is also associated with increased risk (18). Therefore, the hemostatic variables evaluated in the present study are of clinical relevance.

Additionally, many cardiovascular events seem to occur after a period of physical exertion (21, 11). These events are due mostly to occlusive thrombus formation (3, 20). Because individuals with OSA already exhibit a hemostatic imbalance (34, 35), the exercise response is of clinical importance. It appears that mild OSA does not influence the hemostatic response to low-intensity exercise. However, it is possible fibrinolytic potential may become compromised either during higher intensity activity (11) or increasing disease severity (35).

Several limitations exist that may have impacted our findings. Firstly, exercise intensities were selected based on predictions of VO_2 reserve with BMI and self-reported activity levels (data not shown). Because maximal aerobic capacity was not determined, variation in predictions could have influenced the current findings. Factor VIII increases directly proportional to exercise intensity with greater levels achieved during vigorous exercise (3, 38) while fibrinolytic potential increases even at modest intensity (26). However, because responses were observed in both groups, the exercise stimulus might have been sufficient for appropriate comparisons. Additionally, the prescribed exercise was similar to previous reports, further supporting the current findings (24). Obstructive Sleep Apnea appeared to have no impact on coagulation potential after exercise. Although FVIII is unaffected, other factors along the coagulation cascade could be influenced. Robinson et al. showed no differences in FVIII levels in OSA individuals compared to normal controls while increases in resting levels of factors XII, VII, and Thrombin anti-thrombin complex were evident (25). Therefore, future studies will need to evaluate other potential candidates. The current sample size may also be insufficient, therefore increases in sample size and disease severity may provide responses or relationships unclear in the current study.

In conclusion, the hemostatic response after acute exercise is well established. However evidence of this response in Obstructive Sleep Apnea is limited. Primarily, the hemostatic changes from acute aerobic exercise are not affected in mild OSA. Rather, PAI-1 activity seems to be elevated which remains after exercise showing greater influence of OSA on fibrinolytic than coagulation potential. Additionally, it appears disease severity, BMI, and age have some influence on select hemostatic factors in mild OSA individuals. Currently, the present study adds to the limited data of hemostatic changes and the first to describe coagulation potential after exercise in Obstructive Sleep Apnea.

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Figure Legend

Figure 1. Overview of Coagulation Cascade

Figure 2. Mean (± SE) Factor VIII antigen (IU • ml^{-1}) pre exercise and post exercise for OSA (*circles)* and controls (*squares).* * Main effect for exercise (Post > Pre).

Figure 3. Mean (± SE) tPA antigen (ng • ml^{-1}) pre exercise and post exercise for OSA (*circles)* and controls (*squares).* * Main effect for exercise (Post > Pre).

Figure 4. Mean (± SE) tPA activity (IU • ml^{-1}) pre exercise and post exercise for OSA (*circles)* and controls (*squares).* * Main effect for exercise (Post > Pre).

Figure 5. Mean (± SE) PAI-1 activity (AU • ml^{-1}) pre exercise and post exercise for OSA (*circles)* and controls (*squares).* † Main effect for OSA ($P = 0.05$).

Figure 6. Post Exercise Factor VIII antigen (IU • ml^{-1}) and BMI (kg/m^2). *Significant correlation ($P < 0.05$).

Figure 7. Pre Exercise tPA antigen (ng • ml^{-1}) and AHI. *Significant correlation ($P < 0.05$).

Figure 8. Pre Exercise tPA antigen (ng • ml^{-1}) and Age (yrs). *Significant correlation ($P < 0.05$).

	OSA	Controls
Age (yrs)	45 ± 13	38 ± 12
Height (cm)	177.87 ± 7.18	179.64 ± 4.70
Weight (kg)	92.75 ± 14.39	88.20 ± 10.1
BMI (kg/m^2)	29.35 ± 3.73	27.39 ± 3.24
AHI	13.00 ± 12.6*	1.44 ± 1.33

Table 1. Subject Demographics. * Significantly different than Controls ($P < 0.05$).

	VO2 (ml/kg/min)	Heart Rate (bpm)
OSA	25.29 ± 5.19	128.83 ± 19.35
Controls	28.97 ± 7.99	136.83 ± 23.48

Table 2. Average VO_2 and Heart Rate during Predicted 70% VO_2 Reserve.

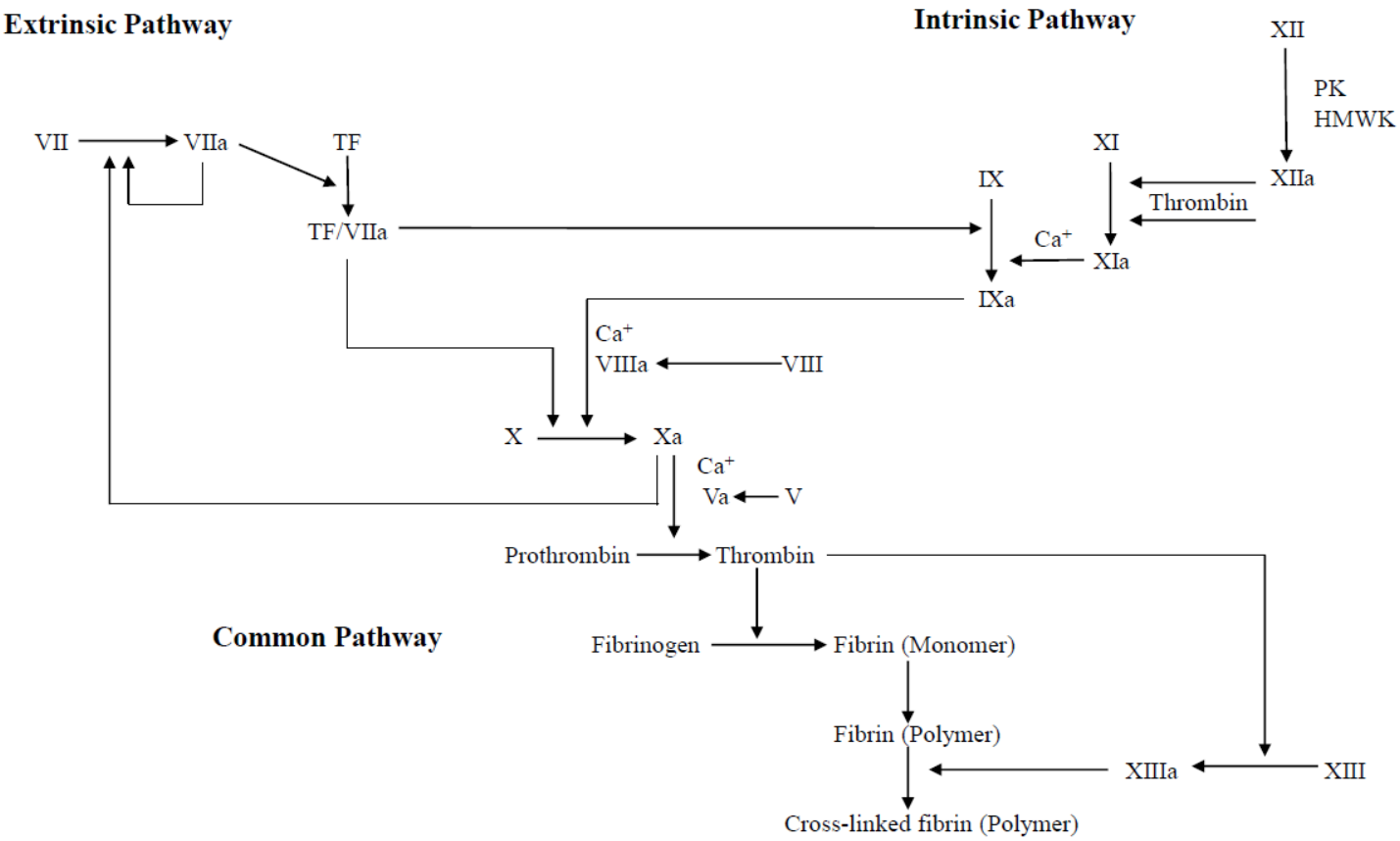
Extrinsic Pathway
Intrinsic Pathway
XII
PK
HMWK
VII
VIIa
TF
XI
IX
XIIa
Thrombin
TF/VIIa
Ca+
XIa
IXa
Ca+
VIIIa
VIII
X
Xa
Ca+
Va
V
Prothrombin
Thrombin
Common Pathway
Fibrinogen
Fibrin (Monomer)
Fibrin (Polymer)
XIIIa
XIII
Cross-linked fibrin (Polymer)

Figure 1.

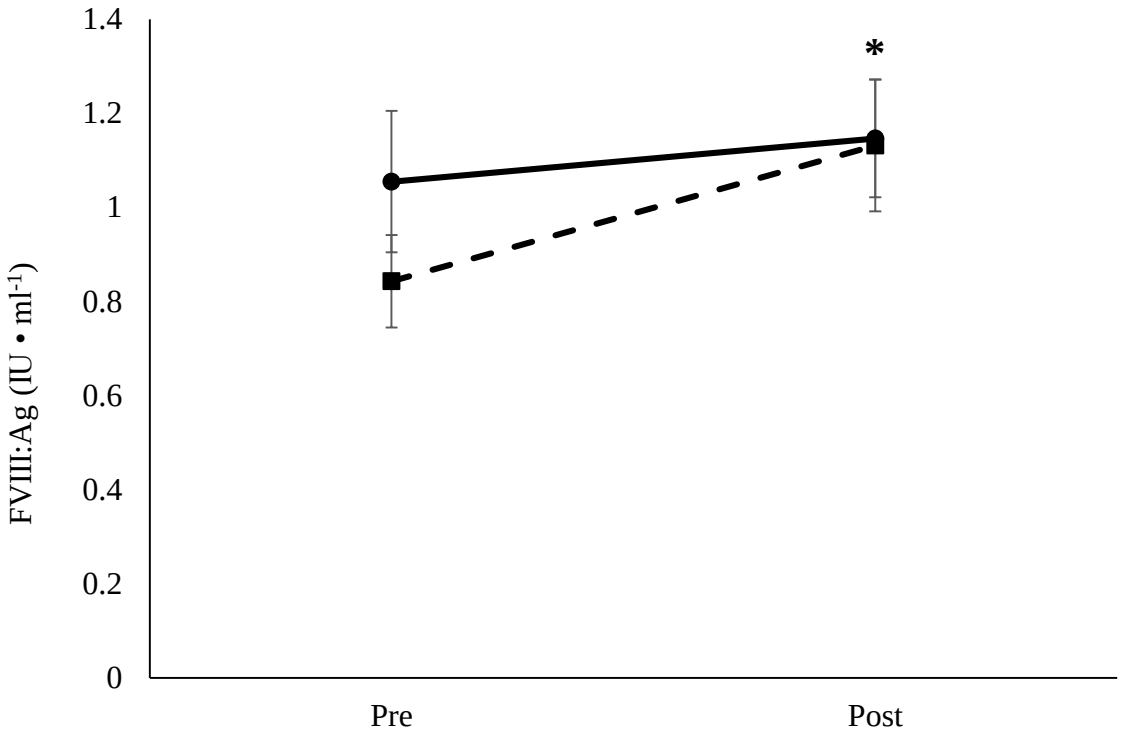

Figure 2.

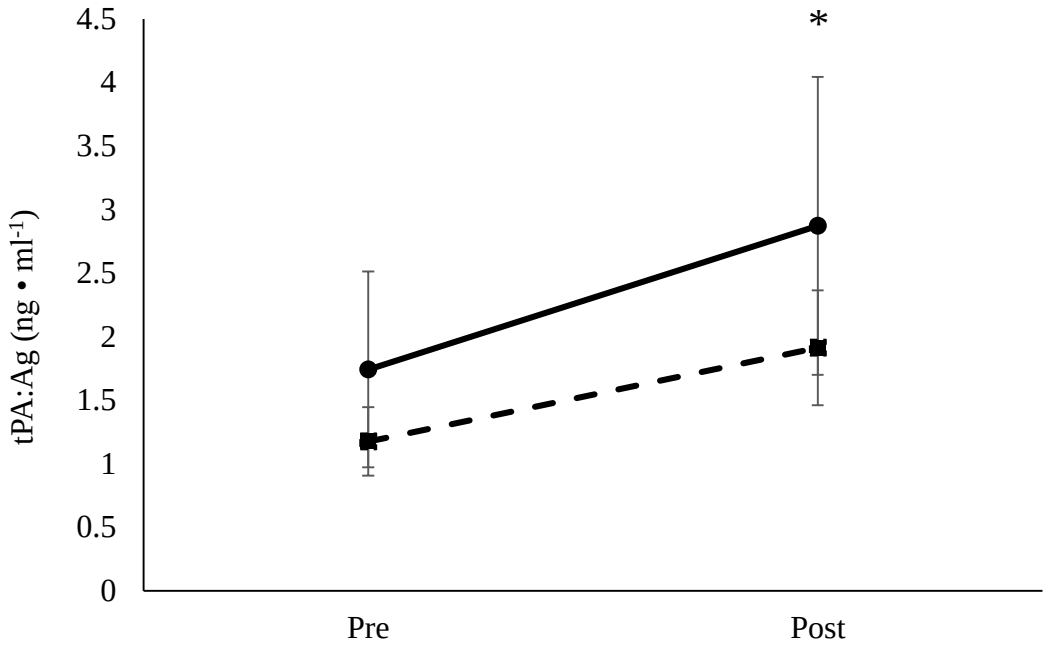

Figure 3.

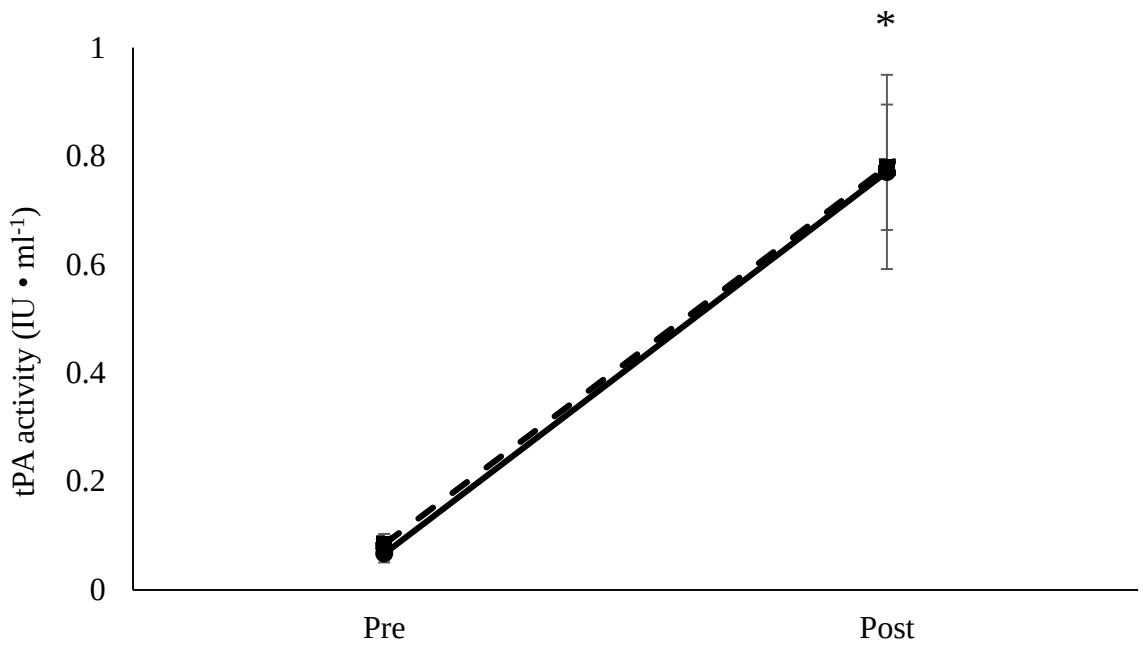

Figure 4.

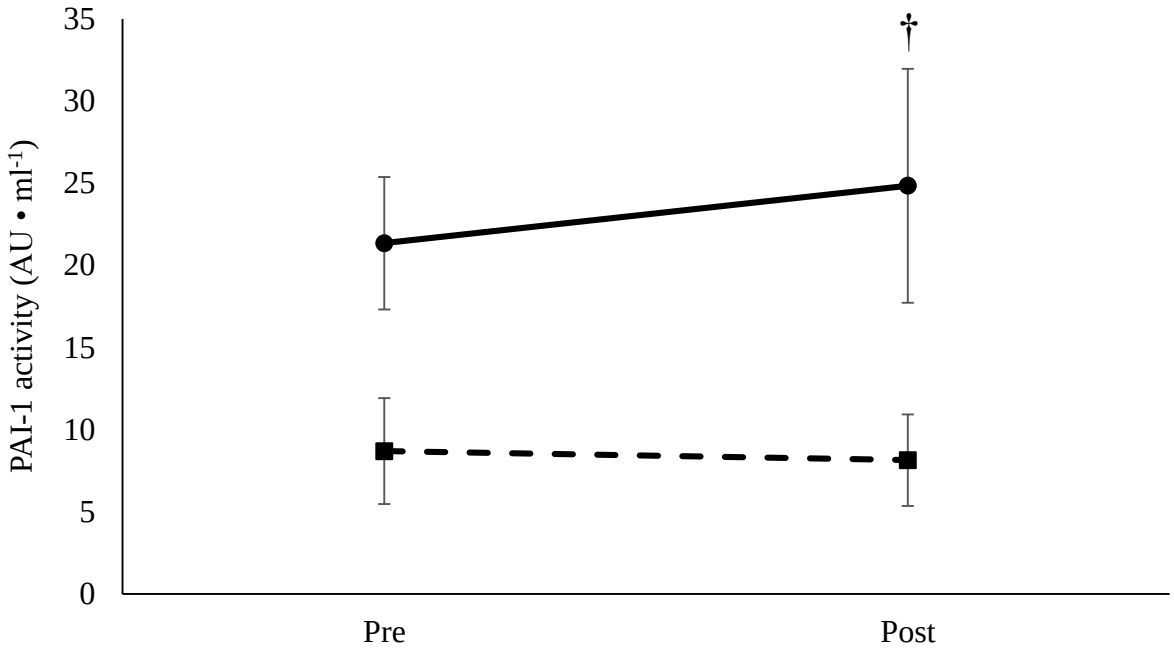

Figure 5.

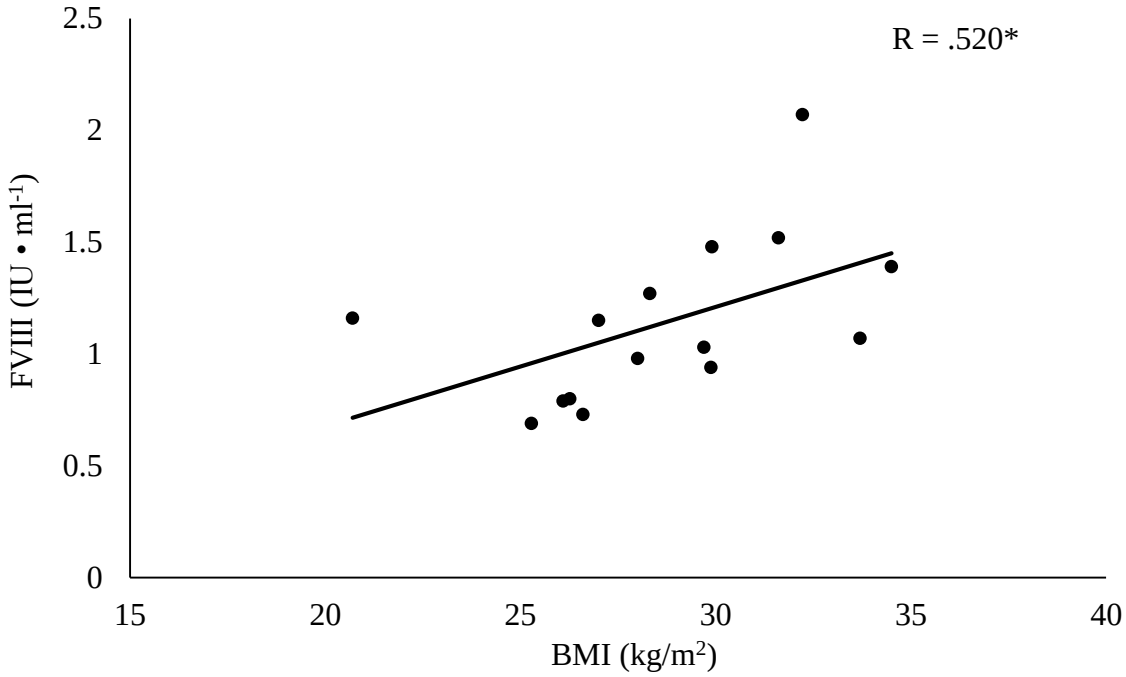

Figure 6.

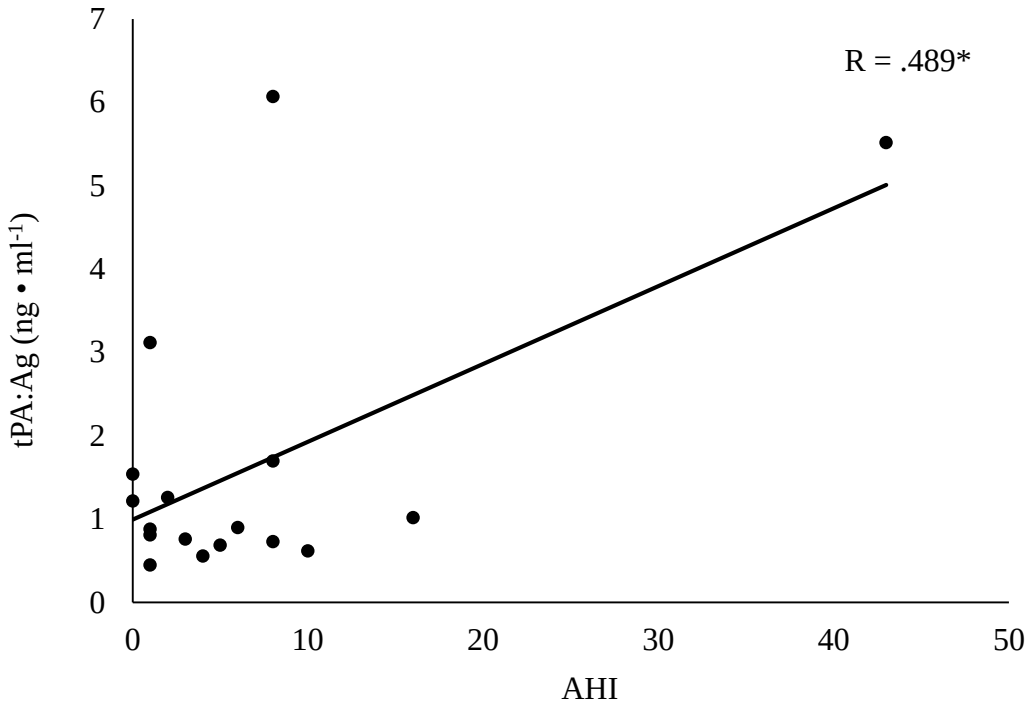

Figure 7.

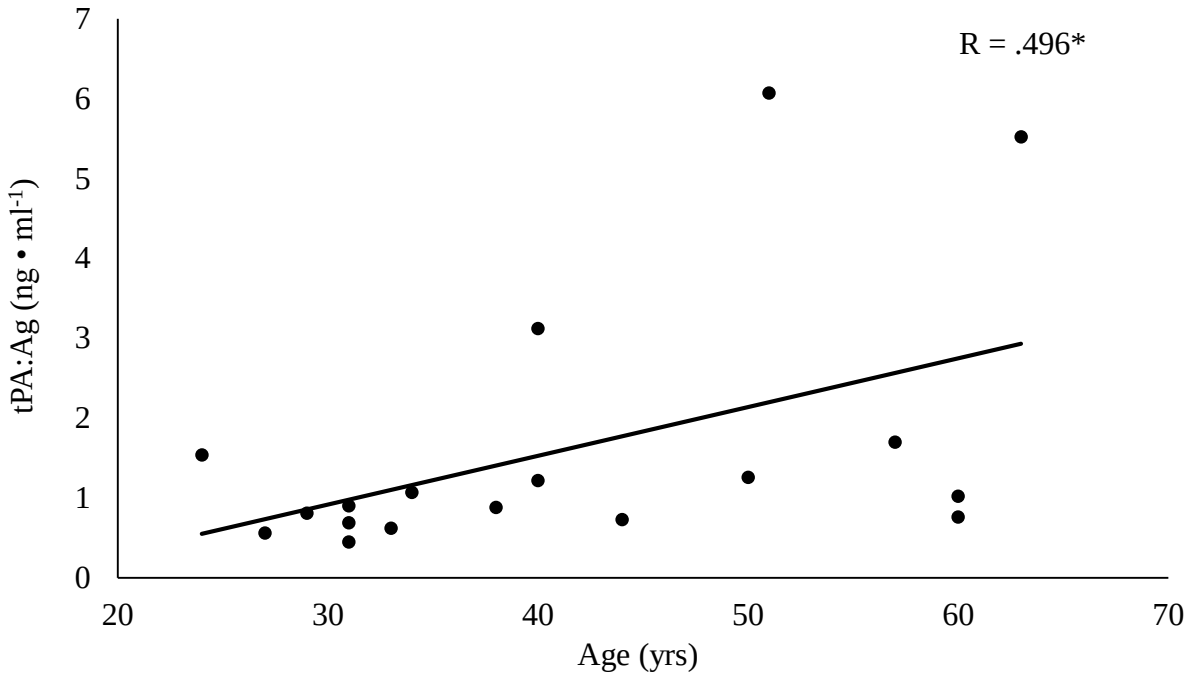

Figure 8.

Appendices

James Madison University

Department of Kinesiology

Informed Consent

Purpose

You are being asked to volunteer for a research project conducted by Dr. Trent Hargens from James Madison University entitled, "The effects of obstructive **SLEEP** apnea on **AUTONOMIC** and cardiovascular **FUNCTION** during steady-state **EXERCISE**", or the **SAFE** study.

The primary goal of this study is to examine whether individuals who are newly identified as high risk for, or diagnosed with, obstructive sleep apnea (OSA) show altered heart rate and cardiovascular variables during sub-maximal exercise. This may provide a clearer picture into why OSA increases the risk for other chronic health problems.

Experimental Procedures

You will be asked to visit the Human Performance Laboratory (HPL) in Godwin Hall 3 times over the course of about 7 – 10 days. Your total time commitment for participation in this study will be approximately 2 and a half hours. In addition, you will be asked to wear (for one night) an at-home sleep testing device (the ApneaLink) to screen you for possible OSA. It is equipped with straps, a finger probe, and a nasal cannula. It measures your breathing activity, heart rate, and blood oxygen levels, and is harmless to wear. If you have previously been diagnosed with OSA through Sentara RMH, then you will not have to wear the ApneaLink device. Also, you will be asked to wear a device (an accelerometer) on your waist during the day for a period of 4 days, including 1 weekend

day, while wearing the same device on your wrist at night while you sleep. Detailed information on each visit is provided below:

Visit 1

Before any test is given, you will be asked to complete a screening form and an informed consent, to insure that you meet the study criteria, that you do not have any factors that would disqualify you from participation. Upon completion of the informed consent, you will be asked to complete a short health history questionnaire providing information about your characteristics and health. You will then be asked complete 3 standardized questionnaires about snoring, the quality of your sleep, daytime sleepiness, and risk for OSA.

You will then have your height, weight, waist circumference and neck circumference measured. After that, your body composition will be analyzed via a Dual-energy x-ray absorptiometer (DEXA). The DEXA scan will allow us to measure your percent body fat and the mineral content and density of your bones. The DEXA is much like an X-ray machine. The DEXA will scan your entire body very slowly; so, you will need to lie on a table without moving for almost 10 minutes, while the DEXA is passed over your entire body. You will feel no discomfort associated with this test.

At the end of this first visit you will also be instructed on the proper use procedures for wearing the ApneaLink device for your one-night sleep assessment, as well as instructions on wearing the accelerometer. An accelerometer is a small device that is to be worn on your waist during the day and on your wrist while in bed.

Following the DEXA scan, we will ask that you wear a heart rate monitor while lying down in a darkened room for 15 minutes to get measures of your heart rate. We will also obtain your resting blood pressure.

Visit 2

The following day after Visit 1, you will be asked to return to the HPL to return the ApneaLink device. Following this, we will ask that you wear a heart rate monitor while lying down in a darkened room for 15 minutes to get measures of your heart rate. We will also obtain your resting blood pressure. This visit should take about 30 minutes.

Visit 3

Approximately 5 – 6 days later, you will be asked to return to the HPL for your final visit. For this visit you will undergo a submaximal treadmill exercise test. You will be asked to walk on the treadmill for a total of about 15 minutes, which includes a 3 – 4 minute warm-up, and 2 – 3 minute cool-down. In between the warm-up and cool-down you will be asked to walk at 2 separate workloads, 1 fairly easy and 1 somewhat hard, for 5 minutes at each workload. During this test we will monitor the electrical output of your heart by placing electrodes directly on your skin across your chest, stomach and back. During the test, we will measure the electrical activity of your heart, your heart rate, blood pressure, your effort, and how much oxygen your body is using. To see how much oxygen you use, we will ask you to breathe into a rubber mouthpiece. During the test, you will breathe only through the mouthpiece and may experience some dryness in your mouth. Prior to your arrival to the HPL for this visit, you will be asked to consume a normal meal (breakfast or lunch) and not to come to the HPL with a full stomach. Caffeine should be avoided the day of your exercise trial. We will obtain about 10 ml of

blood (about 2 teaspoons) prior to and immediately after the exercise trial in order to determine the potential of your blood to coagulate. These blood samples will be obtained from an arm vein.

Follow-up testing

If you are shown to have OSA, and you undergo treatment for OSA through continuous positive airway pressure treatment (CPAP), you will be asked to undergo repeat testing of all previously described tests (except for the ApneaLink device) after 6 months of treatment. If you are shown to not have OSA, follow-up testing will not be done.

Risks

There are no risks associated with wearing an accelerometer. Also, there is no risk associated with heart rate, blood pressure, height, weight, and waist and neck circumference measures. You will not be asked to change any of your personal habits during the course of the study. Measurements with associated risks include: the DEXA scan and treadmill exercise test.

The amount of radiation that you will receive in the DEXA exam is less than the amount you will receive during a transatlantic flight, and is equal to about 1/20 of a chest x-ray. You should not be pregnant for this study because of risks from the DEXA scan radiation to the embryo or fetus. If you feel that you might be pregnant, inform the research staff immediately.

There is a risk of abnormal changes during the submaximal treadmill exercise test. These changes may include abnormal blood pressure, fainting, heart rhythm disorders, stroke, heart attack, and death. The chance of serious heart problems during maximal exercise among adults is very small (less than 1/10,000 maximal exercise tests). _ENREF_10 The

exercise trial for this proposed study is sub-maximal vs. maximal, so the risk for complications is even less. Every effort will be made to minimize risks of an abnormal response by reviewing you health history and providing adequate supervision of the exercise test. All staff are certified by the American Heart Association in BLS (Basic Life Support).

With the blood draw, there is the risk of discomfort, bruising, and, in rare instances, infection, lightheadedness, and fainting. We will use sterile procedures and trained personnel to minimize discomfort and complications.

Benefits

Participation may include knowledge about your health status. You will receive information on your body composition, including percent body fat and bone mineral density, an assessment of your sleep quality and risk for sleep apnea, an assessment of your physical activity level, and cardiovascular fitness. In addition, a $50 stipend is provided for subjects who complete the study protocol. Indirect benefits of participating in this study will be helping the researchers better understand the relationship between sleep apnea and cardiovascular disease risk.

Inquiries

If you have any questions or concerns or you would like to receive a copy of the final aggregate results of this study, please contact Dr. Trent Hargens at hargenta@jmu.edu or (540) 568-5844.

Questions about Your Rights as a Research Subject

Dr. David Cockley	Dr. Stewart Pollock
Chair, Institutional Review Board	Chair, Institutional Review Board

James Madison University
(540) 568-2834
cocklede@jmu.edu

Sentara RMH Medical Center
(540) 689-1000
SGPOLLO3@SENTARA.com

Confidentiality

All data and results will be kept confidential. You will be assigned an identification code. At no time will your name be identified with your individual data. The researcher retains the right to use and publish non-identifiable data. All data will be kept secured in a locked cabinet. All electronic data will be kept on a password-protected computer. Final aggregate results will be made available to participants upon request.

Freedom of Consent

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.

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 Subject (Printed)	Name of Researcher (Printed)
_____	_____
Name of Subject (Signed)	Name of Researcher (Signed)
_____	_____
Date	Date

Medical and Health History Form

Name: _____ Date of Birth: _____

Ethnicity: _____

Height: _____ ft Weight: _____ pounds

Gender: Female_____ Male_____

Campus Address: _____

Campus Telephone Number: _____ Campus Email Address: _____

Address for Permanent Residence: _____

Person to contact in case of emergency: _____

Relationship: _____ Daytime Telephone: _____ Home Telephone: _____

Primary Care Physician: _____ Telephone: _____

Medical History

Please indicate any current or previous conditions or problems you have experienced or have been told by a physician you have had:

	Yes	**No**
Heart disease or any heart problems:	_____	_____
Rheumatic fever:	_____	_____
Respiratory disease or breathing problems:	_____	_____

Circulation problems:	_____	_____
Kidney disease or problems:	_____	_____
Urinary problems:	_____	_____
Reproductive problems:	_____	_____
Musculoskeletal problems:	_____	_____
Fainting or dizziness, especially with exertion:	_____	_____
Neurological problems/disorders:	_____	_____
High blood pressure:	_____	_____
Low blood pressure:	_____	_____
High blood cholesterol:	_____	_____
Diabetes:	_____	_____
Thyroid problems:	_____	_____
Eating disorders (bulimia, anorexia):	_____	_____
Allergies:	_____	_____

If "yes" to any of the above please indicate the date, explain, and describe:

_____

_____

_____

Please list any hospitalizations/operations/recent illnesses (Type/Date):_____

_____

_____

_____

Do you ever feel faint, short of breath, or chest discomfort with exertion? Yes: _____

No: _____

If "yes", please explain : _____

_____

_____

Are there any orthopedic limitations you have that may restrict your ability to perform hard running exercise or intense strength-type exercises? (back, hips, knees, ankles)

Yes _____ No _____

If "yes" please explain: _____

_____

_____

_____

Family Health History

Has anyone in your family (blood relatives only) been diagnosed or treated for any of the following?

	Yes	**No**	**Relationship**	**Age**
Heart attack	_____	_____	_____	_____
Heart disease	_____	_____	_____	_____
High blood pressure	_____	_____	_____	_____
Stroke	_____	_____	_____	_____
Kidney disease	_____	_____	_____	_____
Diabetes	_____	_____	_____	_____

Health Habits

Do you add salt to your food? Yes ___ No ___ Are you on any special type of diet? Yes ___ No ___

If "yes" please describe _____

_____

_____

Do you drink caffeinated beverages? Yes _____ No _____ How many cups per day?

Do you drink alcoholic beverages? Yes _____ No _____ How many drinks per week? _____

What is the average number of drinks that you consume on the weekend? _____

Did you use tobacco products in the past (more than 12 months ago)? Yes _____ No _____

Sleep Habits Evaluation

Do you have episodes of parasomnias (disorders such as sleep walking, sleep talking, night terrors, body rocking, bedwetting that will cause partial or full awakening?) Yes_ No_____

Do you show signs of sleep disturbances (such as insomnia, daytime sleepiness) when you are anxious, stressed? Yes_____ No_____

Do you have difficulties to fall asleep if a certain object or a certain situation is absent such as listening to the radio, watching the television, etc? Yes_____ No_____

Do you have difficulties to fall asleep earlier or later of your usual bedtime? Yes_____ No_____

Do you wake up at night to get a little snack? Yes__ No_____

If "yes", do you think that the snack is helping you to go back to sleep? Yes_____ No___

_____

Do you have hallucinations (vivid images that look like dreams occurring when you sleep) or find yourself physically weak or paralyzed for a few seconds? Yes_____

No_____

Tonsils and Adenoids evaluation questionnaire

Do you have a history of recurrent tonsillitis which is an inflammation of the tonsils (clusters of tissue that lie in bands on both sides of the back of the throat) caused by an infection? In tonsillitis, the tonsils are enlarged, red, and often coated either partly or entirely? Yes_____ No_____

Did you ever have inflammation of the adenoids (single clump of tissue in the back of the nose) causing a **blockage of the back of the nose, chronic and recurrent fluid or infections of your ears, or chronic or recurrent sinus infections?** Yes

_____ No_____

Did you have tonsillectomy (tonsils removed) or adenoidectomy (adenoids removed)? Yes

_____ No_____

Medications

Please list all medications (prescription and over-the-counter) you are currently taking or have taken in the past week: _____

_____

_____

_____

_____

_____

_____

_____

Please sign to indicate the above information is correct:

_____ _____

Print Name Signature Date

Follow Up Review and Interview by:

_____

Signature of Project Staff Member Date

Subject Prescreening Information

Please complete the Following:

Age (yrs):

Height (inches):

Weight (lbs):

Average Exercise Habits over the Past 2 Months:

Avg. # days of exercise per week:

Avg. # of days of aerobic exercise per week:

Avg. # of days of resistance exercise per week:

Do you currently use medications of any kind?

Have you previously been diagnosed with a heart or lung disease?

Do you have diabetes?

Have you previously been diagnosed with Obstructive Sleep Apnea?

If yes, are you currently being treated for it?

Snoring

Do you snore?

If yes, do you snore: (circle one)

1 Day per week

2-4 Days per week

5 or greater Days per week

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** 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. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ days per week

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent 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.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

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

SAFE Study Exercise Protocol Data Sheet

Subject ID_____

Age _____ Age pred. max HR _____ 85% APMHR _____

35% of Predicted VO_2 Reserve_____ 70% of Predicted VO_2Reserve_____

Ht (in) _____ Wt (kg) _____ (lbs) _____ Resting HR _____(seated) _____ (standing)

Resting BP: Seated:_____
Standing:_____

Time	Speed/Grade	HR (bpm)	BP mmHg	RPE	Signs/Symptoms
Warm-up 1	2.0 / 0%				
Warm-up 2	2.0 / 0%				
Stage 1 1	/				
2	/		*		
3	/				
4	/		*		
5	/				
Stage 2 6	/				
7	/		*		
8	/				
9	/		*		
10	/				
Recovery					
1	2.0 / 0%				
2					
3					
4					
5					

Comments:

VO_2 Stage 1: _____ mL/kg/min **VO_2 Stage 2: _____ mL/kg/min**
METs Stage 1: _____ **METs Stage 2: _____**

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